SOCIETY FOR NEUROSCIENCE

ABSTRACTS

VOLUME 22, PART 2

26TH ANNUAL MEETING

WASHINGTON, D.C.

NOVEMBER 16–21, 1996

1996 © Society for Neuroscience

Made in the United States of America.
International Standard Book Numbers:
Part 1 ISBN 0-916110-48-6
Part 2 ISBN 0-916110-49-4
Part 3 ISBN 0-916110-50-8
All parts ISSN 0190–5295
Library of Congress Catalog Card Number 75-7761

Proper citation form for this volume:
Soc. Neurosci. Abstr., Vol. 22, Part 2, p. xxx, 1996.

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

CONTENTS—PART 2

*12,537 volunteer abstracts, 18 symposia abstracts, 24 history of neuroscience abstracts, and 58 teaching of neuroscience abstracts.

1996 Program Committee

Clifford B. Saper, M.D., Ph.D.
Chairperson
Harvard Medical School, Beth Israel Hospital

Arthur P. Arnold, Ph.D.
University of California, Los Angeles

Jocelyne Bachevalier, Ph.D.
University of Texas Health Science Center, Houston

Jeffery L. Barker, M.D.
National Institutes of Health/NINDS

M. Catherine Bushnell, Ph.D.
McGill University

Catherine E. Carr, Ph.D.
University of Maryland

Raymond J. Dingledine, Ph.D.
Emory University School of Medicine

Stephen S. Easter, Jr., Ph.D.
University of Michigan

David A. Greenberg, M.D., Ph.D.
University of Pittsburgh School of Medicine

Steven E. Hyman, M.D.
National Institute of Mental Health, NIH

Leonard K. Kaczmarek, Ph.D.
Yale University School of Medicine

Ned H. Kalin, M.D.
University of Wisconsin Medical School

David M. Katz, Ph.D.
Case Western Reserve University School of Medicine

Pat Levitt, Ph.D.
UMDNJ–Robert Wood Johnson Medical School

Arthur D. Loewy, Ph.D.
Washington University School of Medicine

Paul J. Lombroso, M.D.
Yale University

Marla B. Luskin, Ph.D.
Emory University School of Medicine

Amy B. MacDermott, Ph.D.
Columbia University, College of Physicians and Surgeons

Rodney K. Murphey, Ph.D.
University of Massachusetts

Karen L. O'Malley, Ph.D.
Washington University School of Medicine

Marcus E. Raichle, M.D.
Washington University School of Medicine

Bryan L. Roth, M.D., Ph.D.
Case Western Reserve University School of Medicine

Mark A. Segraves, Ph.D.
Northwestern University

Barry E. Stein, Ph.D.
Bowman Gray School of Medicine, Wake Forest University

Peter L. Strick, Ph.D.
Syracuse VA Medical Center, SUNY–HSC at Syracuse

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

R. Suzanne Zukin, Ph.D.
Albert Einstein College of Medicine

Pasko Rakic, M.D., D.Sc., *ex officio*
Yale University School of Medicine

Bruce S. McEwen, Ph.D., *ex officio*
The Rockefeller University

Michael J. Zigmond, Ph.D., *ex officio*
University of Pittsburgh

Society for Neuroscience Policies on the Use of Animals and Humans in Neuroscience Research

Policy on the Use of Animals in Neuroscience Research

The Policy on the Use of Animals in Neuroscience Research affects a number of the Society's functions that involve making decisions about animal research conducted by individual members. These include the scheduling of scientific presentations at the Annual Meeting, the review and publication of original research papers in *The Journal of Neuroscience*, and the defense of members whose ethical use of animals in research is questioned by antivivisectionists. The responsibility for implementing the policy in each of these areas will rest with the relevant administrative body (Program Committee, Publications Committee, Editorial Board, and Committee on Animals in Research, respectively), in consultation with Council.

Introduction

The Society for Neuroscience, as a professional society for basic and clinical researchers in neuroscience, endorses and supports the appropriate and responsible use of animals as experimental subjects. Knowledge generated by neuroscience research on animals has led to important advances in the understanding of diseases and disorders that affect the nervous system and in the development of better treatments that reduce suffering in humans and animals. This knowledge also makes a critical contribution to our understanding of ourselves, the complexities of our brains, and what makes us human. Continued progress in understanding how the brain works and further advances in treating and curing disorders of the nervous system require investigation of complex functions at all levels in the living nervous system. Because no adequate alternatives exist, much of this research must be done on animal subjects. The Society takes the position that neuroscientists have an obligation to contribute to this progress through responsible and humane research on animals.

Several functions of the Society are related to the use of animals in research. A number of these involve decisions about research conducted by individual members of the Society, including the scheduling of scientific presentations at the Annual Meeting, the review and publication of original research papers in *The Journal of Neuroscience*, and the defense of members whose ethical use of animals in research is questioned by antivivisectionists. Each of these functions, by establishing explicit support of the Society for the research of individual members, defines a relationship between the Society and its members. The purpose of this document is to outline the policy that guides that relationship. Compliance with the following policy will be an important factor in determining the suitability of research for presentation at the Annual Meeting or for publication in *The Journal of Neuroscience*, and in situations where the Society is asked to provide public and active support for a member whose use of animals in research has been questioned.

General Policy

Neuroscience research uses complicated, often invasive methods, each of which is associated with different problems, risks, and specific technical considerations. An experimental method that would be deemed inappropriate for one kind of research may be the method of choice for another kind of research. It is therefore impossible for the Society to define specific policies and procedures for the care and use of all research animals and for the design and conduct of every neuroscience experiment.

The U.S. *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (PHS Policy) and the *Guide for the Care and Use of Laboratory Animals* (the Guide) describe a set of general policies and procedures designed to ensure the humane and appropriate use of live vertebrate animals in all forms of biomedical research. The Society finds the policies and procedures set forth in the PHS Policy and the Guide to be both necessary and sufficient to ensure a high standard of animal care and use and adopts them as its official "Policy on the Use of Animals in Neuroscience Research" (Society Policy). All Society members are expected to conduct their animal research in compliance with the Society Policy and are required to verify that they have done so when submitting abstracts for presentation at the Annual Meeting or manuscripts for publication in *The Journal of Neuroscience*. Adherence to the Society Policy is also an important step toward receiving help from the Society in responding to questions about a member's use of animals in research. A complete description of the Society's policy and procedures for defending members whose research comes under attack is given in the Society's *Handbook for the Use of Animals in Neuroscience Research.*

Local Committee Review

An important element of the Society Policy is the establishment of a local committee that is charged with reviewing and approving all proposed animal care and use procedures. In addition to scientists experienced in research involving animals and a veterinarian, the membership of this local committee should include an individual who is not affiliated with the member's institution in any other way. In reviewing a proposed use of animals, the committee should evaluate the adequacy of institutional policies, animal husbandry, veterinary care, and the physical plant. Specific attention should be paid to proposed procedures for animal procurement, quarantine and stabilization, separation by species, disease diagnosis and treatment, anesthesia and analgesia, surgery and postsurgical care, and euthanasia. The review committee also should ensure that procedures involving live vertebrate animals are designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society. This review and approval of a member's use of live vertebrate animals in research by a local committee is an essential component of the Society Policy. Assistance in developing appropriate animal care and use procedures and establishing a local review committee can be obtained from the documents listed below and from the Society.

Other Laws, Regulations, and Policies

In addition to complying with the policy described above, Regular Members (i.e., North American residents) of the Society must also adhere to all relevant national, state, or local laws and/or regulations that govern their use of animals in neuroscience research. Thus, U.S. members must observe the U.S. Animal Welfare Act (as amended in 1985) and its implementing regulations from the U.S. Department of

Agriculture. Canadian members must abide by the *Guide to the Care and Use of Experimental Animals*, and members in Mexico must comply with the *Reglamento de la Ley General de Salud en Materia de Investigacion para la Salud of the Secretaria de Salud* (published on Jan. 6, 1987). Similarly, in addition to complying with the laws and regulations of their home countries, Foreign Members of the Society should adhere to the official Society Policy outlined here.

Recommended References

"Anesthesia and paralysis in experimental animals." *Visual Neuroscience*, 1:421–426. 1984.

The Biomedical Investigator's Handbook for Researchers Using Animal Models. 1987. Foundation for Biomedical Research, 818 Connecticut Ave., N.W., Suite 303, Washington, D.C. 20006.

Guide for the Care and Use of Laboratory Animals, 7th edition. 1996. NRC (National Research Council), Institute of Laboratory Animal Resources, National Academy of Sciences, 2101 Constitution Ave., N.W., Washington, D.C. 20418.

Guide to the Care and Use of Experimental Animals. vol. 1, 2nd edition, 1993. Canadian Council on Animal Care, 350 Albert St., Suite 315, Ottawa, Ontario, Canada K1R 1B1.

Handbook for the Use of Animals in Neuroscience Research. 1991. Society for Neuroscience, 11 Dupont Circle, N.W., Suite 500, Washington, D.C. 20036.

OPRR Public Health Service Policy on Humane Care and Use of Laboratory Animals (revised Sept. 1986). Office for Protection from Research Risks, NIH, 6100 Executive Blvd., Suite 3B01-MSC 7507, Rockville, MD 20892-7507.

Preparation and Maintenance of Higher Mammals During Neuroscience Experiments. Report of a National Institutes of Health Workshop. NIH Publication No. 91-3207, March 1991. National Eye Institute, Bldg. 31, Rm. 6A47, Bethesda, MD 20892.

The following principles, based largely on the PHS *Policy on Humane Care and Use of Laboratory Animals*, can be a useful guide in the design and implementation of experimental procedures involving laboratory animals.

Animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results.

Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain, when consistent with sound scientific practices, is imperative.

Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

Postoperative care of animals shall be such as to minimize discomfort and pain and, in any case, shall be equivalent to accepted practices in schools of veterinary medicine.

Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure. If the study requires the death of the animal, the animal must be killed in a humane manner.

Living conditions should be appropriate for the species and contribute to the animals' health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, appropriate veterinary care shall be provided.

Exceptions to these principles require careful consideration and should only be made by an appropriate review group such as an institutional animal care and use committee.

Policy on the Use of Human Subjects in Neuroscience Research

Experimental procedures involving human subjects must have been conducted in conformance with the policies and principles contained in the Federal Policy for the Protection of Human Subjects (United States Office of Science and Technology Policy) and in the Declaration of Helsinki. When publishing a paper in *The Journal of Neuroscience* or submitting an abstract for presentation at the Annual Meeting, authors must sign a statement of compliance with this policy.

Recommended References

Declaration of Helsinki. (Adopted in 1964 by the 18th World Medical Assembly in Helsinki, Finland, and revised by the 29th World Medical Assembly in Tokyo in 1975.) In: *The Main Issue in Bioethics Revised Edition*. Andrew C. Varga, ed. New York: Paulist Press, 1984.

Federal Policy for the Protection of Human Subjects; Notices and Rules. *Federal Register*, vol. 56, no. 117 (June 18, 1991), pp. 28002–28007.

Policy on Ethics

It is expected that authors submitting papers or abstracts will have conducted their work in strict accordance with the following statement on ethics approved by the Society for Neuroscience in November 1989 and amended in November 1993.

The Society for Neuroscience believes that progress in understanding the nervous system materially benefits human welfare. It recognizes that such progress depends on the honest pursuit of scientific research and the truthful representation of findings. While recognizing that both scientific error and differences of interpretation are a natural part of the creative process, the Society affirms that misconduct, in the form of fabrication, falsification, or plagiarism, jeopardizes the success of the entire scientific endeavor. Members of the Society assume an obligation to maintain the highest level of integrity in all scientific activities.

The primary responsibility for considering and resolving allegations of scientific misconduct lies within the individual academic communities and institutions where scientific work is carried out. The Society for Neuroscience therefore supports the principle that academic institutions should develop and have in place procedures to deal with allegations of scientific misconduct. However, the Society has a special responsibility and interest surrounding those scientific activities for which it is directly responsible, e.g., publication of *The Journal of Neuroscience* and the presentations at the Annual Meeting.

Every author of articles or abstracts submitted for publication in *The Journal of Neuroscience* or the neuroscience *Abstracts* agrees to assume full responsibility, within the limits of his or her professional competence, for the accuracy of the report. In the case of multiple-authored papers, each author should have made a significant intellectual or practical contribution to the scientific work; "honorary authorship," i.e., the granting of authorship to persons who have made no substantive contribution to a scientific report, is not appropriate.

Scientists must have access to their original research results. The retention of accurately recorded and retrievable results is essential for the progress of scientific inquiry. Moreover, errors may be mistaken for misconduct when primary results are unavailable. Primary data should remain in the laboratory and should be preserved as long as there may be a reasonable need to refer to them.

Authors submitting articles or abstracts do so with the understanding that reports have not been submitted elsewhere. An abstract is a proper forum for rapid communication of work that will subsequently appear as a full-length article. However, submission of abstracts reporting already published work or publication of multiple similar manuscripts or abstracts, i.e., duplicate publication, is improper. When previously published data are presented as part of a new manuscript or abstract, as in a gradually developing longitudinal data set, or if a subject group or condition is included again for comparison purposes, citations to the previously published work should appear explicitly in the new report.

Scientific publication is an important part of the process by which priority is established for experimental work and ideas. Duplicating without citation of text previously published by others or expropriating the experimental findings of others without attribution, i.e., plagiarism, is unethical. When authors of articles or abstracts have prepublication access to related work of others, as in a review process, care must be taken to avoid the appearance that priority is being claimed for work already done by others.

It is the responsibility of the authors, therefore, and not of the Society or the Editorial Board of *The Journal of Neuroscience*, to ensure that relevant prior discoveries are appropriately acknowledged in manuscripts that are submitted to the *Journal* for publication.

Questions raised about the conduct of experiments or their presentation will be evaluated preliminarily by the Editor of *The Journal of Neuroscience* (in the case of an article in the *Journal*) or by the Chair of the Program Committee (in the case of an abstract), in consultation with the Chair of the Publications Committee and the Secretary of the Society. If possible, the matter may be resolved informally at this level. However, if deemed appropriate, the matter will be referred to the institution where the scientific work in question was done. There, it would be expected that the matter would be reviewed in accordance with institutional procedures for handling allegations of misconduct. At all stages, every effort should be made to ensure that the process is fair and just, both for those who are accused of misconduct and for those who have raised the issue of scientific misconduct.

Based on their own findings or those of the institution, the *Journal* Editor or the Chair of the Program Committee, in consultation with the Chair of the Publications Committee and the Secretary of the Society, may recommend action to the Publications Committee. The Publications Committee will then decide on appropriate action, including, for example, retracting a published report. The Council of the Society and the relevant institution will be informed of any action that is taken. Council retains the right to consider additional action. In accordance with the Bylaws, this action could include, for example, expulsion from the Society. If it is found that allegations were not made in good faith, or were maliciously motivated, action may be recommended for those responsible.

In the event that a published article or abstract is to be retracted, a statement of retraction will be published in *The Journal of Neuroscience* or in the *Abstracts* for the next Annual Meeting.

CHRONOLOGICAL LIST OF SESSIONS

(See page xxi for Thematic List of Sessions)

Session Number & Title — **Page**

(History and Teaching Posters will be posted the entire week.)

Symposia—1:00 p.m.

Special Lecture—1:00 p.m.

Presidential Special Lecture—4:15 p.m.

Session Number & Title — **Page**

Slide Sessions—1:00 p.m.

Poster Sessions—1:00 p.m.

MONDAY, NOV. 18

TUESDAY, NOV. 19

WEDNESDAY, NOV. 20

THURSDAY, NOV. 21

THEMATIC LIST OF SESSIONS

(Includes slide and poster sessions and symposia only.)

Session Number	Session Title	Type	Day and Time Sun.	Mon.	Tue.	Wed.	Thu.
	THEME A: DEVELOPMENT AND REGENERATION						
492.	Aging processes	Poster			Tue PM		
491.	Aging processes: hippocampus	Poster			Tue PM		
307.	Aging processes: neuronal alterations	Poster		Mon PM			
593.	Aging processes: toxicity, inflammation, and non-neuronal cells	Poster				Wed AM	
386.	Axon guidance mechanisms and pathways I	Slide			Tue AM		
765.	Axon guidance mechanisms and pathways II	Poster					Thu AM
676.	Axon guidance mechanisms and pathways: cell adhesion molecules	Poster				Wed PM	
585.	Axon guidance mechanisms and pathways: collapsins and semaphorins	Poster				Wed AM	
584.	Axon guidance mechanisms and pathways: neurotrophins, netrins, and Eph family	Poster				Wed AM	
677.	Axon guidance mechanisms and pathways: outgrowth patterns	Poster				Wed PM	
281.	**Cell Cycle Regulation and CNS Development: Is One Division Like Any Other?**	SYMP		**Mon PM**			
22.	Cell differentiation and migration I	Poster	Sun AM				
119.	Cell differentiation and migration II	Poster	Sun PM				
120.	Cell differentiation and migration III	Poster	Sun PM				
216.	Cell differentiation and migration IV	Poster		Mon AM			
217.	Cell differentiation and migration V	Poster		Mon AM			
393.	Cell differentiation and migration VI	Poster			Tue AM		
394.	Cell differentiation and migration VII	Poster			Tue AM		
479.	Cell differentiation and migration VIII	Slide			Tue PM		
582.	Cell differentiation and migration IX	Poster				Wed AM	
674.	Cell differentiation and migration X	Poster				Wed PM	
21.	Cell lineage and determination I	Poster	Sun AM				
205.	Cell lineage and determination II	Slide		Mon AM			
215.	Cell lineage and determination III	Poster		Mon AM			
392.	Cell lineage and determination IV	Poster			Tue AM		
403.	Cerebral cortex and limbic system	Poster			Tue AM		
774.	Cerebral cortex and limbic system: function	Poster					Thu AM
773.	Cerebral cortex and limbic system: molecular expression patterns	Poster					Thu AM
404.	Development of visual cortex I	Poster			Tue AM		
682.	Development of visual cortex II	Poster				Wed PM	
211.	Developmental genetics I	Poster		Mon AM			
212.	Developmental genetics II	Poster		Mon AM			
381.	Developmental genetics III	Slide			Tue AM		
25.	Formation and specificity of synapses I	Poster	Sun AM				
122.	Formation and specificity of synapses II	Poster	Sun PM				
219.	Formation and specificity of synapses III	Poster		Mon AM			
485.	Formation and specificity of synapses IV	Poster			Tue PM		
586.	Formation and specificity of synapses V	Poster				Wed AM	
665.	Formation and specificity of synapses VI	Slide				Wed PM	
766.	Formation and specificity of synapses VII	Poster					Thu AM
118.	Genesis of neurons and glia	Slide	Sun PM				
213.	Genesis of neurons and glia: EGF and FGF effects	Poster		Mon AM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
390.	Genesis of neurons and glia: adult	Poster			Tue AM		
214.	Genesis of neurons and glia: mechanisms and kinetics	Poster		Mon AM			
391.	Genesis of neurons and glia: regulation	Poster			Tue AM		
128.	Glia and other non-neuronal cells I	Poster	Sun PM				
488.	Glia and other non-neuronal cells II	Poster			Tue PM		
577.	Glia and other non-neuronal cells III	Slide				Wed AM	
680.	Glia and other non-neuronal cells IV	Poster				Wed PM	
772.	Glia and other non-neuronal cells V	Poster					Thu AM
486.	Hormones and development	Poster			Tue PM		
302.	Hormones and development: sex steroids	Poster		Mon PM			
226.	Hormones and development: sexual differentiation	Poster		Mon AM			
395.	Morphogenesis	Poster			Tue AM		
230.	Motor systems: development and regeneration I	Poster		Mon AM			
489.	Motor systems: development and regeneration II	Poster			Tue PM		
26.	Neuronal death I	Poster	Sun AM				
27.	Neuronal death II	Poster	Sun AM				
127.	Neuronal death III	Poster	Sun PM				
284.	Neuronal death IV	Slide		Mon PM			
679.	Neuronal death V	Poster				Wed PM	
228.	Neuronal death: ICE—related responses	Poster		Mon AM			
590.	Neuronal death: calcium and potassium	Poster				Wed AM	
678.	Neuronal death: culture systems	Poster				Wed PM	
589.	Neuronal death: excitotoxicity	Poster				Wed AM	
227.	Neuronal death: intracellular signals	Poster		Mon AM			
126.	Neuronal death: lesions	Poster	Sun PM				
588.	Neuronal death: oxidative stress	Poster				Wed AM	
229.	Neuronal death: p53 and Bcl family	Poster		Mon AM			
221.	Neurotransmitter systems and channels: development of excitatory and inhibitory receptors	Poster		Mon AM			
220.	Neurotransmitter systems and channels: development of intrinsic cellular properties—ionic currents and synaptogenesis	Poster		Mon AM			
209.	Neurotrophic factors: biologic effects	Slide		Mon AM			
396.	Neurotrophic factors: biologic effects—BDNF and NT-4 I	Poster			Tue AM		
397.	Neurotrophic factors: biologic effects—BDNF and NT-4 II	Poster			Tue AM		
769.	Neurotrophic factors: biologic effects—CNTF, LIF, and interleukins	Poster					Thu AM
768.	Neurotrophic factors: biologic effects—EGF, FGF, IGF, and TGF	Poster					Thu AM
767.	Neurotrophic factors: biologic effects—GDNF	Poster					Thu AM
300.	Neurotrophic factors: biologic effects—NGF I	Poster		Mon PM			
301.	Neurotrophic factors: biologic effects—NGF II	Poster		Mon PM			
398.	Neurotrophic factors: biologic effects—NT-3	Poster			Tue AM		
770.	Neurotrophic factors: biologic effects—neurotransmitters	Poster					Thu AM
299.	Neurotrophic factors: biologic effects—novel or uncharacterized factors	Poster		Mon PM			
123.	Neurotrophic factors: expression and regulation—development and aging	Poster	Sun PM				
124.	Neurotrophic factors: expression and regulation—physiologic and pathophysiologic mechanisms I	Poster	Sun PM				
222.	Neurotrophic factors: expression and regulation—physiologic and pathophysiologic mechanisms II	Poster		Mon AM			
223.	Neurotrophic factors: expression and regulation—physiologic and pathophysiologic mechanisms III	Poster		Mon AM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
125.	Neurotrophic factors: expression and regulation—synthesis, expression, and transport	Poster	Sun PM				
224.	Neurotrophic factors: receptors and cellular mechanisms I	Poster		Mon AM			
225.	Neurotrophic factors: receptors and cellular mechanisms II	Poster		Mon AM			
399.	Neurotrophic factors: receptors and cellular mechanisms III	Poster			Tue AM		
400.	Neurotrophic factors: receptors and cellular mechanisms IV	Poster			Tue AM		
401.	Neurotrophic factors: receptors and cellular mechanisms V	Poster			Tue AM		
402.	Neurotrophic factors: receptors and cellular mechanisms VI	Poster			Tue AM		
482.	Neurotrophic factors: receptors and cellular mechanisms VII	Slide			Tue PM		
587.	Neurotrophic factors: receptors and cellular mechanisms VIII	Poster				Wed AM	
660.	**Neurotrophins and Synaptic Plasticity**	SYMP				**Wed PM**	
771.	Nutritional and prenatal factors: dietary and environmental factors	Poster					Thu AM
487.	Nutritional and prenatal factors: malnutrition	Poster			Tue PM		
290.	Parkinson's disease	Slide		Mon PM			
23.	Patterning I	Poster	Sun AM				
484.	Patterning II	Slide			Tue PM		
121.	Patterning and gene expression	Poster	Sun PM				
24.	Process outgrowth, growth cones, and sprouting I	Poster	Sun AM				
218.	Process outgrowth, growth cones, and sprouting II	Poster		Mon AM			
296.	Process outgrowth, growth cones, and sprouting III	Poster		Mon PM			
297.	Process outgrowth, growth cones, and sprouting IV	Poster		Mon PM			
298.	Process outgrowth, growth cones, and sprouting V	Poster		Mon PM			
583.	Process outgrowth, growth cones, and sprouting VI	Poster				Wed AM	
675.	Process outgrowth, growth cones, and sprouting VII	Poster				Wed PM	
757.	Process outgrowth, growth cones, and sprouting VIII	Slide					Thu AM
108.	Regeneration I	Slide	Sun PM				
305.	Regeneration II	Poster		Mon PM			
130.	Regeneration and degeneration	Poster	Sun PM				
405.	Regeneration: altered gene expression—CNS	Poster			Tue AM		
231.	Regeneration: altered gene expression—PNS	Poster		Mon AM			
591.	Regeneration: functional recovery	Poster				Wed AM	
490.	Regeneration: influence of substrate	Poster			Tue PM		
681.	Retinal development I	Poster				Wed PM	
775.	Retinal development II	Poster					Thu AM
129.	Sensory systems: auditory and olfactory	Poster	Sun PM				
303.	Sensory systems: somatosensory	Poster		Mon PM			
304.	Subcortical visual development	Poster		Mon PM			
28.	Transplantation I	Poster	Sun AM				
306.	Transplantation II	Poster		Mon PM			
481.	Transplantation III	Slide			Tue PM		
592.	Transplantation: Parkinson's disease—related	Poster				Wed AM	
131.	Transplantation: Parkinson's disease—retina	Poster	Sun PM				
232.	Transplantation: functional	Poster		Mon AM			
406.	Transplantation: mostly spinal cord	Poster			Tue AM		
114.	Visual system: development I	Slide	Sun PM				
477.	Visual system: development II	Slide			Tue PM		

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
THEME B:CELL BIOLOGY							
30.	Blood-brain barrier I	Poster	Sun AM				
308.	Blood-brain barrier II	Poster		Mon PM			
777.	Cytoskeleton and membrane composition	Poster					Thu AM
778.	Cytoskeleton	Poster					Thu AM
133.	Gene structure and function I	Poster	Sun PM				
378.	Gene structure and function II	Slide			Tue AM		
685.	Gene structure and function: expression	Poster				Wed PM	
235.	Gene structure and function: promoter analysis	Poster		Mon AM			
673.	Membrane composition	Slide				Wed PM	
29.	Neuroglia and myelin I	Poster	Sun AM				
234.	Neuroglia and myelin II	Poster		Mon AM			
407.	Neuroglia and myelin III	Poster			Tue AM		
594.	Neuroglia and myelin IV	Poster				Wed AM	
684.	Neuroglia and myelin V	Poster				Wed PM	
776.	Neuroglia and myelin VI	Poster					Thu AM
132.	Staining, tracing, and imaging techniques I	Poster	Sun PM				
233.	Staining, tracing, and imaging techniques II	Poster		Mon AM			
493.	Staining, tracing, and imaging techniques III	Poster			Tue PM		
683.	Staining, tracing, and imaging techniques IV	Poster				Wed PM	
3.	**Tyrosine Phosphorylation Pathways in Neuronal Signalling**	SYMP	**Sun AM**				
THEME C: EXCITABLE MEMBRANES AND SYNAPTIC TRANSMISSION							
9.	Calcium channel structure, function, and expression I	Slide	Sun AM				
495.	Calcium channel structure, function, and expression II	Poster			Tue PM		
139.	Calcium channels: physiology, pharmacology, and modulation I	Poster	Sun PM				
140.	Calcium channels: physiology, pharmacology, and modulation II	Poster	Sun PM				
286.	Calcium channels: physiology, pharmacology, and modulation III	Slide		Mon PM			
688.	Calcium channels: physiology, pharmacology, and modulation IV	Poster				Wed PM	
689.	Calcium channels: physiology, pharmacology, and modulation V	Poster				Wed PM	
690.	Calcium channels: physiology, pharmacology, and modulation VI	Poster				Wed PM	
752.	**Caught in the Act: Structural Changes Associated with Channel Gating**	SYMP					**Thu AM**
781.	Ligand-gated ion channels	Poster					Thu AM
138.	Ligand-gated ion channels: glutamate, GABA, and glycine receptors	Poster	Sun PM				
137.	Ligand-gated ion channels: nicotinic acetylcholine and P2X receptors	Poster	Sun PM				
135.	Long-term potentiation: pharmacology I	Poster	Sun PM				
136.	Long-term potentiation: pharmacology II	Poster	Sun PM				
210.	Long-term potentiation: physiology I	Slide		Mon AM			
389.	Long-term potentiation: physiology II	Slide			Tue AM		
580.	Long-term potentiation: physiology III	Slide				Wed AM	
596.	Long-term potentiation: physiology IV	Poster				Wed AM	
597.	Long-term potentiation: physiology V	Poster				Wed AM	
598.	Long-term potentiation: physiology VI	Poster				Wed AM	

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
599.	Long-term potentiation: physiology VII	Poster				Wed AM	
600.	Long-term potentiation: physiology VIII	Poster				Wed AM	
601.	Long-term potentiation: physiology IX	Poster				Wed AM	
310.	Mechanisms of neurotransmitter release I	Poster		Mon PM			
311.	Mechanisms of neurotransmitter release II	Poster		Mon PM			
780.	Mechanisms of neurotransmitter release III	Poster					Thu AM
408.	Other ion channels I	Poster			Tue AM		
409.	Other ion channels II	Poster			Tue AM		
313.	Postsynaptic mechanisms: Ach, ATP and peptide signal	Poster		Mon PM			
316.	Postsynaptic mechanisms: Ca^{2+} signalling	Poster		Mon PM			
314.	Postsynaptic mechanisms: GABA signals	Poster		Mon PM			
200.	Postsynaptic mechanisms: chemical excitability	Slide		Mon AM			
315.	Postsynaptic mechanisms: dendritic functions	Poster		Mon PM			
387.	Postsynaptic mechanisms: electrical excitability and Ca^{2+} signalling	Slide			Tue AM		
317.	Postsynaptic mechanisms: glutamate signals	Poster		Mon PM			
312.	Postsynaptic mechanisms: morphological and molecular correlates	Poster		Mon PM			
687.	Postsynaptic mechanisms: network activity and models	Poster				Wed PM	
691.	Potassium channels: expression	Poster				Wed PM	
473.	Potassium channels: function and expression	Slide			Tue PM		
497.	Potassium channels: modulation I	Poster			Tue PM		
498.	Potassium channels: modulation II	Poster			Tue PM		
142.	Potassium channels: pharmacology	Poster	Sun PM				
141.	Potassium channels: physiology	Poster	Sun PM				
572.	Potassium channels: physiology, pharmacology, and modulation	Slide				Wed AM	
496.	Potassium channels: structure and function	Poster			Tue PM		
31.	Presynaptic mechanisms I	Poster	Sun AM				
206.	Presynaptic mechanisms II	Slide		Mon AM			
686.	Presynaptic mechanisms III	Poster				Wed PM	
779.	Presynaptic mechanisms IV	Poster					Thu AM
494.	Presynaptic mechanisms: calcium and release	Poster			Tue PM		
134.	Presynaptic mechanisms: modulation—plasticity	Poster	Sun PM				
309.	Presynaptic mechanisms: neuromuscular junction	Poster		Mon PM			
595.	Presynaptic mechanisms: release and recycling	Poster				Wed AM	
32.	Sodium channels: expression and cloning	Poster	Sun AM				
33.	Sodium channels: pharmacology	Poster	Sun AM				
34.	Sodium channels: physiology, structure, and function	Poster	Sun AM				
483.	Sodium channels: synaptic transmission and disease	Slide			Tue PM		

THEME D: NEUROTRANSMITTERS, MODULATORS, TRANSPORTERS, AND RECEPTORS

526.	5-HT_{1A} receptors: binding	Poster			Tue PM		
527.	5-HT_{1A} receptors: pharmacology	Poster			Tue PM		
528.	5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F} receptors	Poster			Tue PM		
500.	Acetylcholine receptors: muscarinic—structure/function I	Poster			Tue PM		
692.	Acetylcholine receptors: muscarinic—structure/function II	Poster				Wed PM	
110.	Acetylcholine receptors: nicotinic	Slide	Sun PM				
504.	Acetylcholine receptors: nicotinic—binding	Poster			Tue PM		
501.	Acetylcholine receptors: nicotinic—molecular biology and knock-out	Poster			Tue PM		

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
502.	Acetylcholine receptors: nicotinic—pharmacology I	Poster			Tue PM		
602.	Acetylcholine receptors: nicotinic—pharmacology II	Poster				Wed AM	
503.	Acetylcholine receptors: nicotinic—physiology	Poster			Tue PM		
603.	Acetylcholine receptors: nicotinic—recombinant	Poster				Wed AM	
411.	Acetylcholine receptors: nicotinic—regulation of gene expression	Poster			Tue AM		
410.	Acetylcholine: structure/function I	Poster			Tue AM		
499.	Acetylcholine: structure/function II	Poster			Tue PM		
617.	Adenosine and ATP as neurotransmitters	Poster				Wed AM	
530.	Behavioral pharmacology	Poster			Tue PM		
622.	Behavioral pharmacology: drugs of abuse	Poster				Wed AM	
623.	Behavioral pharmacology: serotonin	Poster				Wed AM	
609.	CRF receptors: structure and function	Poster				Wed AM	
202.	Catecholamine receptors	Slide		Mon AM			
697.	Catecholamine receptors: anti-psychotic/ nervous system disorders	Poster				Wed PM	
523.	Catecholamine receptors: knock-outs and behavior	Poster			Tue PM		
329.	Catecholamine receptors: localization and structure	Poster		Mon PM			
330.	Catecholamine receptors: molecular biology and receptor binding	Poster		Mon PM			
696.	Catecholamine receptors: pharmacology	Poster				Wed PM	
788.	Catecholamine receptors: regulation of gene expression	Poster					Thu AM
522.	Catecholamine receptors: second messenger signal transduction	Poster			Tue PM		
614.	Catecholamines: biosynthetic enzymes	Poster				Wed AM	
145.	Catecholamines: dopamine I	Poster	Sun PM				
331.	Catecholamines: dopamine II	Poster		Mon PM			
524.	Catecholamines: dopamine III	Poster			Tue PM		
240.	Catecholamines: norepinephrine	Poster		Mon AM			
620.	Dopamine transporters	Poster				Wed AM	
35.	Excitatory amino acid receptors: physiology, pharmacology, and modulation—NMDA I	Poster	Sun AM				
36.	Excitatory amino acid receptors: physiology, pharmacology, and modulation—NMDA II	Poster	Sun AM				
693.	Excitatory amino acid receptors: physiology, pharmacology, and modulation—NMDA III	Poster				Wed PM	
239.	Excitatory amino acid receptors: physiology, pharmacology, and modulation—mGluR I	Poster		Mon AM			
322.	Excitatory amino acid receptors: physiology, pharmacology, and modulation—mGluR II	Poster		Mon PM			
606.	Excitatory amino acid receptors: physiology, pharmacology, and modulation I	Poster				Wed AM	
607.	Excitatory amino acid receptors: physiology, pharmacology, and modulation II	Poster				Wed AM	
608.	Excitatory amino acid receptors: physiology, pharmacology, and modulation III	Poster				Wed AM	
671.	Excitatory amino acid receptors: physiology, pharmacology, and modulation IV	Slide				Wed PM	
236.	Excitatory amino acid receptors: structure, function, and expression—functional properties	Poster		Mon AM			
413.	Excitatory amino acid receptors: structure, function, and expression—metabotropic glutamate receptor	Poster			Tue AM		
237.	Excitatory amino acid receptors: structure, function, and expression—receptor assembly	Poster		Mon AM			
238.	Excitatory amino acid receptors: structure, function, and expression—regulation of expression	Poster		Mon AM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
292.	Excitatory amino acid receptors: structure, function, and expression I	Slide		Mon PM			
782.	Excitatory amino acid receptors: structure, function, and expression II	Poster					Thu AM
143.	Excitatory amino acids: anatomy and physiology I	Poster	Sun PM				
144.	Excitatory amino acids: anatomy and physiology II	Poster	Sun PM				
318.	Excitatory amino acids: excitotoxicity I	Poster		Mon PM			
319.	Excitatory amino acids: excitotoxicity II	Poster		Mon PM			
320.	Excitatory amino acids: excitotoxicity III	Poster		Mon PM			
321.	Excitatory amino acids: excitotoxicity IV	Poster		Mon PM			
478.	Excitatory amino acids: excitotoxicity V	Slide			Tue PM		
505.	Excitatory amino acids: excitotoxicity VI	Poster			Tue PM		
506.	Excitatory amino acids: excitotoxicity VII	Poster			Tue PM		
604.	Excitatory amino acids: pharmacology I	Poster				Wed AM	
605.	Excitatory amino acids: pharmacology II	Poster				Wed AM	
412.	Excitatory amino acids: pharmacology—metabotropic receptors	Poster			Tue AM		
507.	Excitatory amino acids: pharmacology—modulation	Poster			Tue PM		
508.	Excitatory amino acids: pharmacology—synaptic receptors	Poster			Tue PM		
514.	GABA: GAD, GAT and GABA studies	Poster			Tue PM		
511.	$GABA_A$ receptors: anesthetics	Poster			Tue PM		
509.	$GABA_A$ receptors: benzodiazepines	Poster			Tue PM		
762.	$GABA_A$ receptors: cellular and molecular studies	Slide					Thu AM
510.	$GABA_A$ receptors: ethanol	Poster			Tue PM		
327.	$GABA_A$ receptors: knock-outs and knock-downs	Poster		Mon PM			
325.	$GABA_A$ receptors: microanatomy	Poster		Mon PM			
323.	$GABA_A$ receptors: native functions	Poster		Mon PM			
324.	$GABA_A$ receptors: native modulation studies	Poster		Mon PM			
326.	$GABA_A$ receptors: recombinant studies and molecular mapping	Poster		Mon PM			
512.	$GABA_A$ receptors: steroids	Poster			Tue PM		
513.	$GABA_B$ and $GABA_C$ receptors	Poster			Tue PM		
618.	Glutamate transporters I	Poster				Wed AM	
619.	Glutamate transporters II	Poster				Wed AM	
470.	**Glutamatergic Transmission: A View from the Dendrite**	SYMP			**Tue PM**		
789.	Histamine	Poster					Thu AM
610.	NPY-like receptors	Poster				Wed AM	
38.	Neurotransmitter interactions I	Poster	Sun AM				
39.	Neurotransmitter interactions II	Poster	Sun AM				
40.	Neurotransmitter interactions III	Poster	Sun AM				
616.	Nitric oxide and other modulators	Poster				Wed AM	
701.	Novel 5HT receptors: $5HT_6$, $5HT_7$, and others	Poster				Wed PM	
37.	Opioid receptors I	Poster	Sun AM				
328.	Opioid receptors II	Poster		Mon PM			
380.	Opioid receptors III	Slide			Tue AM		
695.	Opioid receptors IV	Poster				Wed PM	
786.	Opioid receptors V	Poster					Thu AM
787.	Opioid receptors: sigma receptors	Poster					Thu AM
519.	Opioids: anatomy, physiology, and behavior—anatomy	Poster			Tue PM		
521.	Opioids: anatomy, physiology, and behavior—behavior	Poster			Tue PM		

Session Number	Session Title	Type	Day and Time				
			Sun.	Mon.	Tue.	Wed.	Thu.
520.	Opioids: anatomy, physiology, and behavior—physiology	Poster			Tue PM		
615.	Other neurotransmitters	Poster				Wed AM	
790.	Other peptide neurotransmitters	Poster					Thu AM
661.	Peptide receptor molecular biology	Slide				Wed PM	
515.	Peptide receptor structure and function I	Poster			Tue PM		
516.	Peptide receptor structure and function II	Poster			Tue PM		
517.	Peptide receptor structure and function III	Poster			Tue PM		
611.	Peptides: anatomy and physiology I	Poster				Wed AM	
612.	Peptides: anatomy and physiology II	Poster				Wed AM	
613.	Peptides: anatomy and physiology III	Poster				Wed AM	
783.	Peptides: anatomy and physiology IV	Poster					Thu AM
784.	Peptides: anatomy and physiology V	Poster					Thu AM
785.	Peptides: anatomy and physiology VI	Poster					Thu AM
518.	Peptides: biosynthesis, metabolism, and biochemical characterization—opiates	Poster			Tue PM		
694.	Peptides: biosynthesis, metabolism, and biochemical characterization I	Poster				Wed PM	
754.	Peptides: biosynthesis, metabolism, and biochemical characterization II	Slide					Thu AM
41.	Receptor modulation: up- and down-regulation I	Poster	Sun AM				
415.	Receptor modulation: up- and down-regulation II	Poster			Tue AM		
531.	Receptor modulation: up- and down-regulation III	Poster			Tue PM		
246.	Regional localization of receptors and transmitters I	Poster		Mon AM			
414.	Regional localization of receptors and transmitters II	Poster			Tue AM		
621.	Regional localization of receptors and transmitters III	Poster				Wed AM	
107.	Second messengers and phosphorylation I	Slide	Sun PM				
152.	Second messengers and phosphorylation II	Poster	Sun PM				
153.	Second messengers and phosphorylation III	Poster	Sun PM				
247.	Second messengers and phosphorylation IV	Poster		Mon AM			
151.	Second messengers: cAMP	Poster	Sun PM				
150.	Second messengers: kinases	Poster	Sun PM				
385.	Serotonin receptors	Slide			Tue AM		
699.	Serotonin receptors: $5HT_2$ I	Poster				Wed PM	
700.	Serotonin receptors: $5HT_2$ II	Poster				Wed PM	
698.	Serotonin receptors: $5HT_2$—anatomy and behavior	Poster				Wed PM	
525.	Serotonin receptors: electrophysiology	Poster			Tue PM		
245.	Serotonin transporters	Poster		Mon AM			
334.	Serotonin/catecholamine interactions	Poster		Mon PM			
243.	Serotonin: behavior	Poster		Mon AM			
242.	Serotonin: general	Poster		Mon AM			
571.	Serotonin: pharmacology	Slide				Wed AM	
241.	Serotonin: tryptophan hydroxylase	Poster		Mon AM			
244.	Serotonin: uptake and release	Poster		Mon AM			
154.	Signal transduction I	Poster	Sun PM				
155.	Signal transduction II	Poster	Sun PM				
372.	**The Synaptic Vescile Neurotransmitter Transporters: Chemical Coding at Central and Peripheral Synapses**	SYMP			**Tue AM**		
529.	Transmitters in invertebrates	Poster			Tue PM		
146.	Transmitters in invertebrates: monoamines	Poster	Sun PM				
332.	Transmitters in invertebrates: neuropeptides I	Poster		Mon PM			
333.	Transmitters in invertebrates: neuropeptides II	Poster		Mon PM			
147.	Transmitters in invertebrates: nitric oxide	Poster	Sun PM				
148.	Transporters I	Poster	Sun PM				
207.	Transporters II	Slide		Mon AM			
295.	Transporters III	Slide		Mon PM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
702.	Transporters IV	Poster				Wed PM	
703.	Transporters V	Poster				Wed PM	
149.	Transporters: structure/activity	Poster	Sun PM				
	THEME E: ENDOCRINE AND AUTONOMIC REGULATION						
338.	Cardiovascular regulation: blood pressure regulation	Poster		Mon PM			
44.	Cardiovascular regulation: brainstem mechanisms	Poster	Sun AM				
157.	Cardiovascular regulation: forebrain mechanisms	Poster	Sun PM				
251.	Cardiovascular regulation: nucleus tractus solitarius	Poster		Mon AM			
416.	Cardiovascular regulation: peripheral autonomic control	Poster			Tue AM		
158.	Cardiovascular regulation: sympathetic preganglionic neurons	Poster	Sun PM				
337.	Cardiovascular regulation: ventral medulla I	Poster		Mon PM			
377.	Cardiovascular regulation: ventral medulla II	Slide			Tue AM		
159.	Gastrointestinal regulation: CNS control	Poster	Sun PM				
417.	Gastrointestinal regulation: peripheral mechanism	Poster			Tue AM		
201.	Hypothalamic-pituitary-adrenal regulation I	Slide		Mon AM			
335.	Hypothalamic-pituitary-adrenal regulation II	Poster		Mon PM			
532.	Hypothalamic-pituitary-adrenal regulation III	Poster			Tue PM		
791.	Hypothalamic-pituitary-adrenal regulation IV	Poster					Thu AM
42.	Hypothalamic-pituitary-gonadal regulation I	Poster	Sun AM				
379.	Hypothalamic-pituitary-gonadal regulation II	Slide			Tue AM		
533.	Hypothalamic-pituitary-gonadal regulation III	Poster			Tue PM		
624.	Hypothalamic-pituitary-gonadal regulation IV	Poster				Wed AM	
704.	Hypothalamic-pituitary-gonadal regulation V	Poster				Wed PM	
43.	Neural-immune interactions: CNS mechanisms	Poster	Sun AM				
336.	Neural-immune interactions: cytokines I	Poster		Mon PM			
537.	Neural-immune interactions: cytokines II	Poster			Tue PM		
536.	Neural-immune interactions: depression and stress	Poster			Tue PM		
706.	Neural-immune interactions: inflammation	Poster				Wed PM	
578.	Neural-immune interactions: other	Slide				Wed AM	
705.	Neural-immune interactions: pathology	Poster				Wed PM	
626.	Neuroendocrine regulation: estrogen	Poster				Wed AM	
534.	Neuroendocrine regulation: growth hormone and somatostatin	Poster			Tue PM		
625.	Neuroendocrine regulation: other	Poster				Wed AM	
156.	Neuroendocrine regulation: paraventricular hypothalamic nucleus	Poster	Sun PM				
535.	Neuroendocrine regulation: prolactin	Poster			Tue PM		
250.	Neuroendocrine regulation: supraoptic nucleus	Poster		Mon AM			
249.	Osmotic regulation	Poster		Mon AM			
101.	**Pain and the Cerebral Cortex**	SYMP	**Sun PM**				
628.	Respiratory regulation: chemoreception and hypoxic responses	Poster				Wed AM	
629.	Respiratory regulation: integrative mechanisms	Poster				Wed AM	
627.	Respiratory regulation: pattern generation and motoneuron	Poster				Wed AM	
248.	Thermal regulation	Poster		Mon AM			
45.	Urogenital regulation: bladder	Poster	Sun AM				
418.	Urogenital regulation: sexual organs	Poster			Tue AM		
	THEME F: SENSORY SYSTEMS						
425.	Auditory systems: central anatomy—forebrain	Poster			Tue AM		
57.	Auditory systems: central anatomy—hindbrain	Poster	Sun AM				

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
161.	Auditory systems: central physiology—birds and bats	Poster	Sun PM				
256.	Auditory systems: central physiology—brainstem	Poster		Mon AM			
424.	Auditory systems: central physiology—forebrain	Poster			Tue AM		
716.	Auditory systems: central physiology—hearing loss	Poster				Wed PM	
350.	Auditory systems: central physiology—midbrain	Poster		Mon PM			
637.	Auditory systems: central physiology—primate cortex	Poster				Wed AM	
636.	Auditory, vestibular, and lateral line: development and regeneration	Poster				Wed AM	
423.	Auditory, vestibular, and lateral line: hair cell properties	Poster			Tue AM		
715.	Auditory, vestibular, and lateral line: integration	Poster				Wed PM	
106.	Chemical senses	Slide	Sun PM				
718.	Gustatory sensation	Poster				Wed PM	
373.	**Molecular Biology of Perception**	SYMP			**Tue AM**		
257.	Olfactory receptors: cell physiology	Poster		Mon AM			
258.	Olfactory receptors: development and specificity	Poster		Mon AM			
427.	Olfactory systems: invertebrates	Poster			Tue AM		
426.	Olfactory systems: olfactory bulb anatomy	Poster			Tue AM		
794.	Olfactory systems: olfactory bulb pharmacology	Poster					Thu AM
793.	Olfactory systems: olfactory bulb physiology	Poster					Thu AM
717.	Olfactory systems: olfactory responses	Poster				Wed PM	
208.	Pain modulation	Slide		Mon AM			
55.	Pain modulation: anatomy and physiology—behavior	Poster	Sun AM				
53.	Pain modulation: anatomy and physiology—higher centers I	Poster	Sun AM				
54.	Pain modulation: anatomy and physiology—higher centers II	Poster	Sun AM				
342.	Pain modulation: anatomy and physiology—neuropathic pain	Poster		Mon PM			
711.	Pain modulation: anatomy and physiology—receptors and nerves	Poster				Wed PM	
343.	Pain modulation: anatomy and physiology—spinal cord I	Poster		Mon PM			
344.	Pain modulation: anatomy and physiology—spinal cord II	Poster		Mon PM			
539.	Pain modulation: pharmacology—GABA and NMDA receptors	Poster			Tue PM		
346.	Pain modulation: pharmacology—amines, purines, cannabinoids	Poster		Mon PM			
542.	Pain modulation: pharmacology—amino acids, anesthetics, antidepressants	Poster			Tue PM		
712.	Pain modulation: pharmacology—inflammation and hyperalgesia	Poster				Wed PM	
345.	Pain modulation: pharmacology—neuropeptides and capsaicin	Poster		Mon PM			
540.	Pain modulation: pharmacology—opiates I	Poster			Tue PM		
541.	Pain modulation: pharmacology—opiates II	Poster			Tue PM		
383.	Pain pathways	Slide			Tue AM		
52.	Pain pathways: behavior	Poster	Sun AM				
51.	Pain pathways: higher centers	Poster	Sun AM				
341.	Pain pathways: spinal cord and brainstem	Poster		Mon PM			
199.	Retina and photoreceptors I	Slide		Mon AM			
792.	Retina and photoreceptors II	Poster					Thu AM
630.	Retinal anatomy	Poster				Wed AM	
713.	Retinal intracellular signalling	Poster				Wed PM	
56.	Retinal physiology	Poster	Sun AM				
347.	Retinal receptors and channels	Poster		Mon PM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
4.	**Sensorimotor Integration in Superior Colliculus: What Does the Colliculus Control?**	SYMP	Sun AM				
339.	Sensory systems: spinal cord I	Poster		Mon PM			
340.	Sensory systems: spinal cord II	Poster		Mon PM			
707.	Somatic and visceral afferents—visceral afferents I	Poster				Wed PM	
708.	Somatic and visceral afferents—visceral afferents II	Poster				Wed PM	
710.	Somatic and visceral afferents: mechanoreceptors	Poster				Wed PM	
709.	Somatic and visceral afferents: nociceptors	Poster				Wed PM	
16.	Somatosensory cortex and thalamocortical relationships I	Slide	Sun AM				
48.	Somatosensory cortex and thalamocortical relationships II	Poster	Sun AM				
49.	Somatosensory cortex and thalamocortical relationships III	Poster	Sun AM				
50.	Somatosensory cortex and thalamocortical relationships IV	Poster	Sun AM				
419.	Somatosensory cortex and thalamocortical relationships V	Poster			Tue AM		
420.	Somatosensory cortex and thalamocortical relationships VI	Poster			Tue AM		
538.	Somatosensory cortex and thalamocortical relationships VII	Poster			Tue PM		
46.	Subcortical somatosensory pathways I	Poster	Sun AM				
47.	Subcortical somatosensory pathways II	Poster	Sun AM				
252.	Subcortical visual pathways I	Poster		Mon AM			
253.	Subcortical visual pathways II	Poster		Mon AM			
574.	Subcortical visual pathways III	Slide				Wed AM	
631.	Subcortical visual pathways IV	Poster				Wed AM	
714.	Subcortical visual pathways V	Poster				Wed PM	
475.	Visual cortex: extrastriate—attention	Slide			Tue PM		
288.	Visual cortex: extrastriate—dorsal stream I	Slide		Mon PM			
635.	Visual cortex: extrastriate—dorsal stream II	Poster				Wed AM	
666.	Visual cortex: extrastriate—dorsal stream III	Slide				Wed PM	
160.	Visual cortex: extrastriate—functional organization I	Poster	Sun PM				
422.	Visual cortex: extrastriate—functional organization II	Poster			Tue AM		
421.	Visual cortex: extrastriate—mapping	Poster			Tue AM		
634.	Visual cortex: extrastriate—ventral stream	Poster				Wed AM	
760.	Visual cortex: extrastriate—ventral stream/mapping	Slide					Thu AM
117.	Visual cortex: striate I	Slide	Sun PM				
198.	Visual cortex: striate II	Slide		Mon AM			
254.	Visual cortex: striate III	Poster		Mon AM			
255.	Visual cortex: striate IV	Poster		Mon AM			
376.	Visual cortex: striate V	Slide			Tue AM		
632.	Visual cortex: striate VI	Poster				Wed AM	
633.	Visual cortex: striate VII	Poster				Wed AM	
672.	Visual cortex: striate VIII	Slide				Wed PM	
111.	Visual psychophysics and behavior I	Slide	Sun PM				
348.	Visual psychophysics and behavior II	Poster		Mon PM			
349.	Visual psychophysics and behavior III	Poster		Mon PM			
	THEME G: MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION						
804.	Animal locomotion	Poster					Thu AM
164.	Basal ganglia: anatomy I	Poster	Sun PM				
352.	Basal ganglia: anatomy II	Poster		Mon PM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
432.	Basal ganglia: dopamine	Poster			Tue AM		
165.	Basal ganglia: function I	Poster	Sun PM				
353.	Basal ganglia: function II	Poster		Mon PM			
431.	Basal ganglia: function III	Poster			Tue AM		
797.	Basal ganglia: function IV	Poster					Thu AM
798.	Basal ganglia: models	Poster					Thu AM
162.	Basal ganglia: striatum I	Poster	Sun PM				
163.	Basal ganglia: striatum II	Poster	Sun PM				
659.	**The Cellular Bases of Functional Brain Imaging**	SYMP				**Wed PM**	
203.	Cerebellum	Slide		Mon AM			
640.	Cerebellum: anatomy	Poster				Wed AM	
476.	Cerebellum: basal ganglia	Slide			Tue PM		
638.	Cerebellum: behavior and pharmacology	Poster				Wed AM	
639.	Cerebellum: clinical, development, genetic models	Poster				Wed AM	
433.	Cerebellum: physiology, models	Poster			Tue AM		
802.	Control of posture and movement: development	Poster					Thu AM
170.	Control of posture and movement: hand movement	Poster	Sun PM				
643.	Control of posture and movement: kinematics	Poster				Wed AM	
354.	Control of posture and movement: motor learning	Poster		Mon PM			
264.	Control of posture and movement: motor units	Poster		Mon AM			
169.	Control of posture and movement: sensory control of reaching	Poster	Sun PM				
265.	Control of posture and movement: spinal cord	Poster		Mon AM			
13.	Cortex: animal studies	Slide	Sun AM				
430.	Cortex: connectivity	Poster			Tue AM		
260.	Cortex: human studies I	Poster		Mon AM			
351.	Cortex: human studies II	Poster		Mon PM			
576.	Cortex: human studies III	Slide				Wed AM	
796.	Cortex: premotor	Poster					Thu AM
795.	Cortex: sensorimotor	Poster					Thu AM
719.	Cortex: transformations	Poster				Wed PM	
727.	Effects of injury and disease I	Poster				Wed PM	
803.	Effects of injury and disease II	Poster					Thu AM
58.	Exercise and therapy	Poster	Sun AM				
726.	Human locomotion	Poster				Wed PM	
641.	Human posture	Poster				Wed AM	
642.	Mechanics and dynamics	Poster				Wed AM	
59.	Motor systems and sensorimotor integration: circuitry and pattern generation I	Poster	Sun AM				
543.	Motor systems and sensorimotor integration: circuitry and pattern generation II	Poster			Tue PM		
544.	Motor systems and sensorimotor integration: circuitry and pattern generations III	Poster			Tue PM		
569.	Motor systems and sensorimotor integration: circuitry and pattern generation IV	Slide				Wed AM	
644.	Motor systems and sensorimotor integration: circuitry and pattern generation V	Poster				Wed AM	
805.	Motor systems and sensorimotor integration: circuitry and pattern generation VI	Poster					Thu AM
259.	Motor systems and sensorimotor integration: invertebrate sensory and motor systems I	Poster		Mon AM			
428.	Motor systems and sensorimotor integration: invertebrate sensory and motor systems II	Poster			Tue AM		
429.	Motor systems and sensorimotor integration: invertebrate sensory and motor systems III	Poster			Tue AM		
436.	Muscle	Poster			Tue AM		

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
801.	Oculomotor system: accomodation, vergence, eye blink, and muscle	Poster					Thu AM
800.	Oculomotor system: behavioral studies, coordinate frames, and models	Poster					Thu AM
263.	Oculomotor system: brainstem	Poster		Mon AM			
166.	Oculomotor system: cortex	Poster	Sun PM				
664.	Oculomotor system: human studies	Slide				Wed PM	
382.	Oculomotor system: pursuit, 3-D stimuli, head movements	Slide			Tue AM		
579.	Oculomotor system: saccades	Slide				Wed AM	
167.	Oculomotor system: smooth pursuit	Poster	Sun PM				
262.	Oculomotor system: superior colliculus	Poster		Mon AM			
721.	Oculomotor system: vestibulo-ocular and optokinetic systems	Poster				Wed PM	
670.	Reaching and posture	Slide				Wed PM	
435.	Reflex function: animal studies	Poster			Tue AM		
168.	Reflex function: human studies	Poster	Sun PM				
722.	Spinal cord and brainstem: anatomic organization	Poster				Wed PM	
723.	Spinal cord and brainstem: plasticity and integrative mechanisms	Poster				Wed PM	
725.	Spinal cord and brainstem: properties of motoneurons and interneurons	Poster				Wed PM	
724.	Spinal cord and brainstem: responses to injury	Poster				Wed PM	
799.	Thalamus	Poster					Thu AM
113.	Vestibular system	Slide	Sun PM				
720.	Vestibular system: anatomy and pharmacology	Poster				Wed PM	
434.	Vestibular system: behavioral studies	Poster			Tue AM		
261.	Vestibular system: physiology and behavior	Poster		Mon AM			
THEME H: OTHER SYSTEMS OF THE CNS							
356.	Association cortex and thalamocortical relations	Poster		Mon PM			
7.	Brain metabolism and blood flow I	Slide	Sun AM				
268.	Brain metabolism and blood flow II	Poster		Mon AM			
357.	Brain metabolism and blood flow III	Poster		Mon PM			
437.	Brain metabolism and blood flow IV	Poster			Tue AM		
438.	Brain metabolism and blood flow V	Poster			Tue AM		
267.	Comparative neuroanatomy: higher vertebrates	Poster		Mon AM			
60.	Comparative neuroanatomy: lower vertebrates	Poster	Sun AM				
564.	**Gene Transfer: Applications of Viral Vectors for the Study and Treatment of CNS Disorders**	SYMP				**Wed AM**	
171.	Limbic system and hypothalamus I	Poster	Sun PM				
266.	Limbic system and hypothalamus II	Poster		Mon AM			
355.	Limbic system and hypothalamus III	Poster		Mon PM			
806.	Limbic system and hypothalamus IV	Poster					Thu AM
THEME I: NEURAL BASIS OF BEHAVIOR							
77.	Aging behavior	Poster	Sun AM				
459.	Aging: memory	Poster			Tue AM		
740.	Aging: other	Poster				Wed PM	
65.	Biological rhythms and sleep: circadian rhythms I	Poster	Sun AM				
451.	Biological rhythms and sleep: circadian rhythms II	Poster			Tue AM		
551.	Biological rhythms and sleep: circadian rhythms III	Poster			Tue PM		
807.	Biological rhythms and sleep: circadian rhythms IV	Poster					Thu AM
808.	Biological rhythms and sleep: circadian rhythms V	Poster					Thu AM

Session Number	Session Title	Type	Day and Time				
			Sun.	Mon.	Tue.	Wed.	Thu.
272.	Biological rhythms and sleep: circadian rhythms and sleep	Poster		Mon AM			
20.	Biological rhythms and sleep: sleep I	Slide	Sun AM				
64.	Biological rhythms and sleep: sleep II	Poster	Sun AM				
273.	Biological rhythms and sleep: sleep III	Poster		Mon AM			
565.	**Bird Song: Twenty Years of Progress**	SYMP				**Wed AM**	
669.	Cognition: attention I	Slide				Wed PM	
729.	Cognition: attention II	Poster				Wed PM	
172.	Cognition: disorders	Poster	Sun PM				
439.	Cognition: frontal/prefrontal	Poster			Tue AM		
10.	Cognition: functional neuroimaging	Slide	Sun AM				
291.	Cognition: language I	Slide		Mon PM			
440.	Cognition: language II	Poster			Tue AM		
384.	Cognition: memory I	Slide			Tue AM		
441.	Cognition: memory II	Poster			Tue AM		
730.	Cognition: other	Poster				Wed PM	
728.	Cognition: sensory	Poster				Wed PM	
185.	Drugs of abuse: alcohol, barbiturates, and benzodiazepines I	Poster	Sun PM				
755.	Drugs of abuse: alcohol, barbiturates, and benzodiazepines II	Slide					Thu AM
815.	Drugs of abuse: amphetamine—neurotoxicity	Poster					Thu AM
187.	Drugs of abuse: amphetamines I	Poster	Sun PM				
279.	Drugs of abuse: amphetamines II	Poster		Mon AM			
457.	Drugs of abuse: amphetamines III	Poster			Tue AM		
362.	Drugs of abuse: cocaine I	Poster		Mon PM			
363.	Drugs of abuse: cocaine II	Poster		Mon PM			
364.	Drugs of abuse: cocaine III	Poster		Mon PM			
365.	Drugs of abuse: cocaine IV	Poster		Mon PM			
737.	Drugs of abuse: cocaine V	Poster				Wed PM	
738.	Drugs of abuse: cocaine VI	Poster				Wed PM	
756.	Drugs of abuse: cocaine VII	Slide					Thu AM
758.	Drugs of abuse: cocaine VIII	Slide					Thu AM
361.	Drugs of abuse: cocaine—DA-5-HT interactions	Poster		Mon PM			
739.	Drugs of abuse: cocaine—fetal and neonatal effects	Poster				Wed PM	
278.	Drugs of abuse: cocaine—glutamatergic influences	Poster		Mon AM			
186.	Drugs of abuse: ethanol, barbiturates, and benzodiazepines I	Poster	Sun PM				
277.	Drugs of abuse: ethanol, barbiturates, and benzodiazepines II	Poster		Mon AM			
456.	Drugs of abuse: ethanol, barbiturates, and benzodiazepines III	Poster			Tue AM		
813.	Drugs of abuse: ethanol, barbiturates, and benzodiazepines IV	Poster					Thu AM
814.	Drugs of abuse: ethanol, barbiturates, and benzodiazepines V	Poster					Thu AM
70.	Drugs of abuse: nicotine	Poster	Sun AM				
72.	Drugs of abuse: opioids I	Poster	Sun AM				
73.	Drugs of abuse: opioids II	Poster	Sun AM				
74.	Drugs of abuse: opioids III	Poster	Sun AM				
458.	Drugs of abuse: opioids IV	Poster			Tue AM		
71.	Drugs of abuse: other I	Poster	Sun AM				
662.	Drugs of abuse: other II	Slide				Wed PM	
78.	Epilepsy: human studies and animal models—human studies	Poster	Sun AM				
67.	Hormonal control of reproductive behavior I	Poster	Sun AM				

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
276.	Hormonal control of reproductive behavior II	Poster		Mon AM			
360.	Hormonal control of reproductive behavior III	Poster		Mon PM			
454.	Hormonal control of reproductive behavior IV	Poster			Tue AM		
557.	Hormonal control of reproductive behavior V	Poster			Tue PM		
180.	Ingestive behavior: behavioral analysis	Poster	Sun PM				
663.	Ingestive behavior: central mechanisms	Slide				Wed PM	
555.	Ingestive behavior: forebrain mechanisms	Poster			Tue PM		
556.	Ingestive behavior: hypothalamus and brainstem	Poster			Tue PM		
182.	Ingestive behavior: other mediators	Poster	Sun PM				
181.	Ingestive behavior: peptide mediators	Poster	Sun PM				
15.	Ingestive behavior: regulators of ingestion	Slide	Sun AM				
12.	Invertebrate learning and behavior I	Slide	Sun AM				
275.	Invertebrate learning and behavior II	Poster		Mon AM			
553.	Invertebrate learning and behavior III	Poster			Tue PM		
554.	Invertebrate learning and behavior IV	Poster			Tue PM		
573.	Invertebrate learning and behavior V	Slide				Wed AM	
61.	Learning and memory: pharmacology I	Poster	Sun AM				
62.	Learning and memory: pharmacology II	Poster	Sun AM				
63.	Learning and memory: pharmacology III	Poster	Sun AM				
173.	Learning and memory: pharmacology IV	Poster	Sun PM				
174.	Learning and memory: pharmacology V	Poster	Sun PM				
446.	Learning and memory: pharmacology VI	Poster			Tue AM		
447.	Learning and memory: pharmacology VII	Poster			Tue AM		
358.	Learning and memory: physiology I	Poster		Mon PM			
359.	Learning and memory: physiology II	Poster		Mon PM			
548.	Learning and memory: physiology III	Poster			Tue PM		
549.	Learning and memory: physiology IV	Poster			Tue PM		
734.	Learning and memory: physiology V	Poster				Wed PM	
735.	Learning and memory: physiology VI	Poster				Wed PM	
759.	Learning and memory: physiology VII	Slide					Thu AM
116.	Learning and memory: systems and functions I	Slide	Sun PM				
269.	Learning and memory: systems and functions II	Poster		Mon AM			
270.	Learning and memory: systems and functions III	Poster		Mon AM			
289.	Learning and memory: systems and functions IV	Slide		Mon PM			
442.	Learning and memory: systems and functions V	Poster			Tue AM		
443.	Learning and memory: systems and functions VI	Poster			Tue AM		
444.	Learning and memory: systems and functions VII	Poster			Tue AM		
445.	Learning and memory: systems and functions VIII	Poster			Tue AM		
545.	Learning and memory: systems and functions IX	Poster			Tue PM		
546.	Learning and memory: systems and functions X	Poster			Tue PM		
547.	Learning and memory: systems and functions XI	Poster			Tue PM		
575.	Learning and memory: systems and functions XII	Slide				Wed AM	
645.	Learning and memory: systems and functions XIII	Poster				Wed AM	
646.	Learning and memory: systems and functions XIV	Poster				Wed AM	
731.	Learning and memory: systems and functions XV	Poster				Wed PM	
732.	Learning and memory: systems and functions XVI	Poster				Wed PM	
733.	Learning and memory: systems and functions XVII	Poster				Wed PM	
280.	**Modulation of Neuronal Excitability and Behavior**	**SYMP**		**Mon PM**			
68.	Monoamines and behavior I	Poster	Sun AM				
69.	Monoamines and behavior II	Poster	Sun AM				
810.	Monoamines and behavior III	Poster					Thu AM
811.	Monoamines and behavior IV	Poster					Thu AM
271.	Motivation and emotion: brain stimulation	Poster		Mon AM			
449.	Motivation and emotion: drugs	Poster			Tue AM		
176.	Motivation and emotion: humans	Poster	Sun PM				
177.	Motivation and emotion: lesions	Poster	Sun PM				

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
450.	Motivation and emotion: other	Poster			Tue AM		
175.	Neural plasticity I	Poster	Sun PM				
448.	Neural plasticity II	Poster			Tue AM		
550.	Neural plasticity III	Poster			Tue PM		
736.	Neural plasticity IV	Poster				Wed PM	
469.	**The Neurobiology of OB Protein (Leptin): A Peripheral Signal Acting on Central Neural Networks to Regulate Body Energy Balance**	SYMP			**Tue PM**		
179.	Neuroethology: electroreception	Poster	Sun PM				
452.	Neuroethology: invertebrates	Poster			Tue AM		
178.	Neuroethology: other systems	Poster	Sun PM				
66.	Neuroethology: songbirds I	Poster	Sun AM				
274.	Neuroethology: songbirds II	Poster		Mon AM			
552.	Neuroethology: songbirds III	Poster			Tue PM		
184.	Neuropeptides and behavior I	Poster	Sun PM				
455.	Neuropeptides and behavior II	Poster			Tue AM		
812.	Neuropeptides and behavior III	Poster					Thu AM
11.	Neurotoxicity: excitotoxic injury	Slide	Sun AM				
76.	Psychopharmacological agents: antidepressants	Poster	Sun AM				
75.	Psychopharmacological agents: antipsychotics I	Poster	Sun AM				
189.	Psychopharmacological agents: antipsychotics II	Poster	Sun PM				
188.	Psychopharmacological agents: other	Poster	Sun PM				
102.	**Single Nerve Cells as Complex Computing Devices: Integrating Experimental and Computational Approaches**	SYMP	**Sun PM**				
183.	Stress I	Poster	Sun PM				
453.	Stress II	Poster			Tue AM		
474.	Stress III	Slide			Tue PM		
809.	Stress IV	Poster					Thu AM
THEME J: DISORDERS OF THE NERVOUS SYSTEM							
86.	Alzheimer's disease: ApoE	Poster	Sun AM				
87.	Alzheimer's disease: anatomical specificity	Poster	Sun AM				
831.	Alzheimer's disease: biochemistry	Poster					Thu AM
741.	Alzheimer's disease: cell biology	Poster				Wed PM	
88.	Alzheimer's disease: immune mechanisms	Poster	Sun AM				
105.	Alzheimer's disease: mechanisms of cellular injury	Slide	Sun PM				
14.	Alzheimer's disease: pathological mechanisms	Slide	Sun AM				
293.	Alzheimer's disease: presenilin cell biology	Slide		Mon PM			
653.	Alzheimer's disease: presenilin cell biology	Poster				Wed AM	
652.	Alzheimer's disease: presenilin gene expression	Poster				Wed AM	
568.	Alzheimer's disease: presenilin localization	Slide				Wed AM	
388.	Alzheimer's disease: tau and ApoE	Slide			Tue AM		
559.	Alzheimer's disease: tau and neurofibrillary degeneration	Poster			Tue PM		
194.	**Central Nervous System Autoimmunity in Human Diseases**	SYMP		**Mon AM**			
83.	Degenerative disease: Alzheimer's—cognitive function I	Poster	Sun AM				
463.	Degenerative disease: Alzheimer's—cognitive function II	Poster			Tue AM		
829.	Degenerative disease: Alzheimer's—cognitive function III	Poster					Thu AM
84.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters I	Poster	Sun AM				

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
85.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters II	Poster	Sun AM				
830.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters III	Poster					Thu AM
668.	Degenerative disease: Alzheimer's-beta-amyloid—ApoE	Slide				Wed PM	
461.	Degenerative disease: Alzheimer's-beta-amyloid—accumulation and aggregation	Poster			Tue AM		
19.	Degenerative disease: Alzheimer's-beta-amyloid—animal models	Slide	Sun AM				
828.	Degenerative disease: Alzheimer's-beta-amyloid—apolipoproteins	Poster					Thu AM
81.	Degenerative disease: Alzheimer's-beta-amyloid—glial interactions	Poster	Sun AM				
826.	Degenerative disease: Alzheimer's-beta-amyloid—membrane interactions	Poster					Thu AM
462.	Degenerative disease: Alzheimer's-beta-amyloid—neuropathology	Poster			Tue AM		
82.	Degenerative disease: Alzheimer's-beta-amyloid—neuroprotection	Poster	Sun AM				
827.	Degenerative disease: Alzheimer's-beta-amyloid—neurotoxicity	Poster					Thu AM
480.	Degenerative disease: Alzheimer's-beta-amyloid—pathogenesis	Slide			Tue PM		
80.	Degenerative disease: Alzheimer's-beta-amyloid—processing I	Poster	Sun AM				
115.	Degenerative disease: Alzheimer's-beta-amyloid—processing II	Slide	Sun PM				
191.	Degenerative disease: Alzheimer's-beta-amyloid—protein interactions I	Poster	Sun PM				
204.	Degenerative disease: Alzheimer's-beta-amyloid—protein interactions II	Slide		Mon AM			
651.	Degenerative disease: Alzheimer's-beta-amyloid—therapeutic approaches I	Poster				Wed AM	
764.	Degenerative disease: Alzheimer's-beta-amyloid—therapeutic approaches II	Slide					Thu AM
834.	Degenerative disease: miscellaneous	Poster					Thu AM
837.	Degenerative disease: other—ALS	Poster					Thu AM
836.	Degenerative disease: other—ataxias and dementias	Poster					Thu AM
835.	Degenerative disease: other—metabolic and inflammatory	Poster					Thu AM
285.	Degenerative disease: other—molecular biology	Slide		Mon PM			
92.	Degenerative disease: other—movement disorders	Poster	Sun AM				
18.	Developmental disorders I	Slide	Sun AM				
190.	Developmental disorders II	Poster	Sun PM				
460.	Developmental disorders III	Poster			Tue AM		
825.	Epilepsy: anti-convulsant drugs—other	Poster					Thu AM
824.	Epilepsy: anti-convulsant drugs—transmitter-related	Poster					Thu AM
570.	Epilepsy: basic mechanisms—cellular and molecular studies	Slide				Wed AM	
818.	Epilepsy: basic mechanisms—molecular studies	Poster					Thu AM
819.	Epilepsy: basic mechanisms—morphological studies	Poster					Thu AM
820.	Epilepsy: basic mechanisms—other	Poster					Thu AM
822.	Epilepsy: basic mechanisms—physiological studies I	Poster					Thu AM
823.	Epilepsy: basic mechanisms—physiological studies II	Poster					Thu AM

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
821.	Epilepsy: basic mechanisms—transmitters and second messengers	Poster					Thu AM
817.	Epilepsy: human studies and animal models	Poster					Thu AM
650.	Epilepsy: human studies and animal models—alterations in glutamate receptors	Poster				Wed AM	
558.	Epilepsy: human studies and animal models—cellular mechanisms	Poster			Tue PM		
79.	Epilepsy: human studies and animal models—limbic seizures I	Poster	Sun AM				
649.	Epilepsy: human studies and animal models—limbic seizures II	Poster				Wed AM	
753.	**Genes in Ischemia**	SYMP					**Thu AM**
816.	Genetic models	Poster					Thu AM
647.	Genetic models: natural mutants	Poster				Wed AM	
648.	Genetic models: transgenes	Poster				Wed AM	
367.	Infectious diseases: HIV	Poster		Mon PM			
844.	Infectious diseases: other	Poster					Thu AM
761.	Ischemia: animal models	Slide					Thu AM
464.	Ischemia: apoptosis	Poster			Tue AM		
839.	Ischemia: behavioral, clinical, and imaging studies	Poster					Thu AM
742.	Ischemia: enzymes and metabolism	Poster				Wed PM	
655.	Ischemia: gene expression	Poster				Wed AM	
560.	Ischemia: glia and edema	Poster			Tue PM		
561.	Ischemia: glucose, pH and, temperature	Poster			Tue PM		
366.	Ischemia: glutamate	Poster		Mon PM			
838.	Ischemia: inflammation and coagulation	Poster					Thu AM
841.	Ischemia: ionic mechanisms	Poster					Thu AM
581.	Ischemia: mechanisms	Slide				Wed AM	
8.	Ischemia: mediators	Slide	Sun AM				
840.	Ischemia: models	Poster					Thu AM
667.	Ischemia: molecular biology	Slide				Wed PM	
287.	Ischemia: neuroprotection	Slide		Mon PM			
563.	Ischemia: neurotransmitters	Poster			Tue PM		
562.	Ischemia: oxidative injury	Poster			Tue PM		
656.	Ischemia: tolerance and stress proteins	Poster				Wed AM	
654.	Ischemia: trophic factors, peptides, and hormones	Poster				Wed AM	
195.	**Mitochondrial Involvement in Neuronal Degeneration**	SYMP		**Mon AM**			
369.	Neuro-oncology: tumor biology	Poster		Mon PM			
370.	Neuro-oncology: treatment and diagnosis	Poster		Mon PM			
94.	Neuromuscular diseases I	Poster	Sun AM				
763.	Neuromuscular diseases II	Slide					Thu AM
95.	Neuropsychiatric disorders I	Poster	Sun AM				
96.	Neuropsychiatric disorders II	Poster	Sun AM				
368.	Neuropsychiatric disorders: depression	Poster		Mon PM			
109.	Neuropsychiatric disorders: imaging I	Slide	Sun PM				
468.	Neuropsychiatric disorders: imaging II	Poster			Tue AM		
294.	Neuropsychiatric disorders: postmortem I	Slide		Mon PM			
658.	Neuropsychiatric disorders: postmortem II	Poster				Wed AM	
467.	Neuropsychiatric disorders: schizophrenia I	Poster			Tue AM		
657.	Neuropsychiatric disorders: schizophrenia II	Poster				Wed AM	
748.	Neurotoxicity: dopaminergic and sympathomimetic agents	Poster				Wed PM	
747.	Neurotoxicity: environmental and therapeutic agents	Poster				Wed PM	
746.	Neurotoxicity: glutamatergic agents	Poster				Wed PM	
745.	Neurotoxicity: metabolic poisons	Poster				Wed PM	

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
751.	Neurotoxicity: metals	Poster				Wed PM	
749.	Neurotoxicity: other	Poster				Wed PM	
750.	Neurotoxicity: oxidants	Poster				Wed PM	
112.	Neurotoxicity: oxidative and other injury	Slide	Sun PM				
832.	Parkinson's disease: animal models I	Poster					Thu AM
833.	Parkinson's disease: animal models II	Poster					Thu AM
90.	Parkinson's disease: neurotoxicity	Poster	Sun AM				
91.	Parkinson's disease: pathophysiology	Poster	Sun AM				
89.	Parkinson's disease: pharmacology and therapy	Poster	Sun AM				
17.	Trauma I	Slide	Sun AM				
93.	Trauma II	Poster	Sun AM				
465.	Trauma III	Poster			Tue AM		
466.	Trauma IV	Poster			Tue AM		
743.	Trauma V	Poster				Wed PM	
744.	Trauma VI	Poster				Wed PM	
842.	Trauma VII	Poster					Thu AM
843.	Trauma VIII	Poster					Thu AM

OTHER

97.	History of neuroscience	Poster	Sun AM				
98.	Teaching of neuroscience: computers, World Wide Web, and multimedia	Poster	Sun AM				
99.	Teaching of neuroscience: curricular innovations	Poster	Sun AM				
100.	Teaching of neuroscience: laboratory exercises	Poster	Sun AM				

328.19

DEVELOPMENTAL EXPRESSION OF THE MU, KAPPA AND DELTA OPIOID RECEPTORS IN THE MOUSE Y. Zhu*, M. Hsu, J. E. Pintar. Dept. Neurosci. & Cell Biol., UMDNJ Robert Wood Johnson Med. Sch., Piscataway, NJ 08854

Previously we have reported the distribution of the μ, κ and δ opioid receptor mRNAs during late gestational and neonatal mouse development. The present study extends previous results to earlier embryonic stages and additional peripheral structures. Significant levels of κ mRNA were detected in the gut epithelium as early as e9.5 and by e11.5, κ receptor mRNA is expressed in not only the epithelium but also some scattered cells, presumably migrating neural crest cells, surrounding the gut wall. At the same stage, μ receptor mRNA begins to be expressed in the ventral spinal cord and the vagal ganglion, in addition to the facial(VII) and vestibulocochlear(VIII) ganglia described before. At e13.5, positive hybridization signals of μ receptor are readily detected in the mantle layer of the hindbrain, tectum and tegmentum of the midbrain and pons, as well as in the thalamus and hypothalamus, while κ receptor mRNA is also observed in the dorsal thalamus and tegmentum, which are comparable to the μ expression sites. Cells expressing κ receptor mRNA are also located in peripheral tissues including rostral pial progenitors and mesenchyme just beneath the dorsal limb ectoderm (both sites of dynorphin mRNA expression), and the atrium of the heart. Interestingly, low levels of δ receptor mRNA are also detected in the heart at this age, though in the ventricle instead of the atrium. We have also begun to examine the expression pattern of opioid receptor mRNAs in the placenta and uterus. At e7.5, the earliest stage that has been examined so far, striking expression of δ receptor mRNA is observed in the trophoblast giant cells, which remains until at least e18. Cells expressing both μ and κ receptor mRNAs are located in the peripheral uterus, while κ mRNA expression is also detected in the decidual basalis, which remains a major site of expression throughout post-implantation stages. These results described above suggest that early developmental events in neural and non-neural tissues may be mediated by opioid receptors and provide the first cellular localization of opioid receptor gene expression in the placenta and uterus (Supported by DA-09040).

328.20

μ-OPIATE RECEPTOR DISTRIBUTION IN TRANSGENIC SUPEROXIDE DISMUTASE AND INBRED MICE. G.I. Elmer*[1], B. Ladenheim[1], L.S. Sharpe[1], S.R.Goldberg[1], C.J. Epstein[2], J.L. Cadet[1]. [1]Div. Intramural Research, NIDA, NIH, 4940 Eastern Ave, Balt., MD, 21224, [2]Dept Pediatrics, Univ. of Calif., San Francisco, CA 94143

μ-Opiate receptors (μ-OR) are thought to be the primary pharmacological mechanism by which opioids produce analgesia and reinforcement. The purpose of this study was to map the neuroanatomical distribution of μ-ORs in six genotypes that differ significantly in opioid-related phenotypes. Previous studies have demonstrated as much as a 30- and 10-fold difference in the analgesic potency of morphine and behavior maintained by morphine injections, respectively. ^{125}I-DAMGO (42nM) autoradiography was used to label μ-ORs in six genotypes: 2 inbred (C57BL/6J, BALB/ByJ), 2 recombinant inbred (CXBH/ByJ, CXBK/ByJ) and 1 set of transgenic mice in which insertion of the superoxide dismutase (SOD) gene has resulted in a selective increase in SOD and the μ-OR. Significant genotype, region and genotype x region differences were found. μ-OR concentration was consistently lower in all regions in the CXBK/ByJ mice. μ-OR concentration differed the greatest between the CXBK/ByJ and SOD-Tg mice; the differences were 200, 214, and 385% in the ventral tegmental area (VTA), nucleus accumbens (NAc) and dorsal central grey, respectively. Preliminary multiple correlation analysis suggests that the VTA, cortical amygdala and NAc shell are significantly associated with the efficacy of morphine as a reinforcer. These data will contribute to identifying neural regions which account for a significant portion of the reinforcing and analgesic properties of morphine. (NIH Intramural Funding)

CATECHOLAMINE RECEPTORS: LOCALIZATION AND STRUCTURE

329.1

RECONSTITUTION OF FUNCTIONAL DOPAMINE RECEPTORS BY COEXPRESSION OF N- AND C-TERMINAL RECEPTOR DOMAINS P. Barbier, R. Maggio*, A. Colelli, F. Vaglini, F. Fornai and G.U. Corsini Institute of Pharmacology, School of Medicine, University of Pisa (Italy)

An N-terminal D2 dopamine receptor clone was constructed and coexpressed in cell cultures possessing a separate gene fragment encoding the C-terminal sequence of the D2 receptor. The truncated receptor (referred to as D2-*trunc*) contained transmembrane domains (TMD) I-V and the N-terminal portion of the third cytoplasmic loop (i3), whereas the C-terminal receptor fragment (referred to as D2-*tail*) possessed TMD VI and VII and the adjacent intra- and extracellular sequences. Expression in COS-7 cells of any of these two polypeptides alone did not result in any detectable [^{3}H]spiperone binding activity. However, specific [^{3}H]spiperone binding could be observed after coexpression of the D2-*trunc* and D2-*tail* gene constructs. Initial pharmacological characterization indicated that the reconstituted receptor behaved in a similar manner to the wild type D2 receptor. This data raises the intriguing possibility that dopaminergic receptors can behave in a fashion analogous to multiple subunit receptors. It appears that the i3 loop can function as a linker between the two functional domains. Our findings confirm and extend analogous data for muscarinic and adrenergic receptors indicating that this phenomenon could be of general importance for the entire superfamily of G protein-coupled receptors.

This work was supported by Consiglio Nazionale delle Ricerche (contribute number 95.02903.CT14)

329.2

DOPAMINE D1, D2, β_2-ADRENERGIC AND 5-HT$_{1B}$ SEROTONIN RECEPTORS EXIST AS DIMERS. G.Y.K. Ng, S.P. Lee, G. Varghese, Z. Xie, L. Grupp*, M.R. Brann, B. O'Dowd, and S.R. George. Depts. of Pharmacology and Medicine, Univ. of Toronto, Toronto, ON, M5S 1A8, the Addiction Research Foundation, M5S 2S1, Toronto, ON, Canada, and the Depts. of Psychiatry and Pharmacology, Univ. of Vermont School of Medicine, Burlington, VT, 05405

We provide direct physicochemical evidence that dopamine D2 and D1, β_2-adrenergic and 5-HT$_{1B}$ serotonin receptors exist as dimers in crude membranes from cultured cells, and following solubilization and immunoprecipitation of these receptors. Intriguingly, immunoblots of crude membranes from human caudate nucleus revealed that the D2 dimer was the predominant species. The G-protein coupled receptor (GPCR) dimers were stable in SDS and under reducing conditions indicating that dimerization was not attributed to covalent disulphide bonds. Incubation of the GPCR dimers with receptor-specific peptides, under high temperatures, or low pH resulted in the dissociation of dimers to monomers. Exposure of living D2 expressing cells (D2/cells) to glutaraldehyde resulted in a conversion of receptor monomers to dimers due to the irreversible cross-linking of receptor monomers. Using the D2 receptor as the model for other GPCRs, we investigated the function of dimers. D2 dimers and monomers were labeled by benzamide antagonists whereas only monomers were labeled by butyrophenone antagonists, which explains the discrepancy in receptor densities estimated by these ligands in PET studies. Further, dopamine exposure of D2/cells mediated an increase in cell surface D2 monomers and dimers involving the translocation of intracellular receptors. We conclude that D2 and other G protein-coupled receptors exist as receptor dimers and that dimers play a functional role in the response to agonist exposure. This work was supported by the Medical Research Council of Canada, National Institute of Drug Abuse, and the Ontario Ministry of Health.

329.3

CHARACTERIZATION OF ANTIBODIES TO DOPAMINE D4 RECEPTORS IN RAT BRAIN. R. E. Harlan*, M. M. Garcia and R. J. Webber. Tulane Medical School, New Orleans, LA (REH, MMG) and Research & Diagnostic Antibodies, Berkeley, CA (RJW).

Antibodies were generated in rabbits against synthetic peptides corresponding to amino acids 16-30 or 176-185 of the cloned human dopamine D4 receptor. Sera were screened with ELISA against the cognate peptide and related peptides. Sera demonstrating specific binding were used in western blots against whole rat brain homogenates. These blots revealed single bands at an apparent molecular weight of 51 kDa. Immunocytochemistry was performed in rat brain sections fixed with either 3% neutral-buffered paraformaldehyde or PLP. Essentially identical results were obtained with both fixatives and with antibodies directed against either peptide. In the olfactory bulb, glomeruli were well labeled with diffuse and punctate immunoreactivity, while numerous fine dots were observed in the external plexiform layer, sometimes arranged along apical dendrites, and on the surface of cells in the mitral and internal granular layers. A prominent band of immunoreactivity was observed in layer 4 of cerebral cortex, especially in sensory areas. Immunoreactivity consisted of a meshwork of fine dots in layer 4, with a somewhat decreased intensity in layers 1-3. Organization into barrels was evident, but not striking. In layer 5, small intense puncta were observed along apical dendrites of many neurons. In the caudate-putamen, scattered labeled neurons were observed amidst a moderately-intense neuropil consisting of a multitude of fine dots. In the hippocampal formation, diffuse immunoreactivity was observed in the molecular layer of the dentate gyrus, with fine dots associated with many neurons in the granule cell layer. Supported by LEQSF RDA-29 to MMG.

329.4

MONOCLONAL ANTIBODIES AGAINST HUMAN α_2-ADRENOCEPTOR SUBTYPE C2: CHARACTERIZATION AND PRODUCTION. S. Liitti, M.-T. Matikainen, H. Närvä, M. Koulu* and M. Jalkanen. *Dept. of Pharmacology and Clinical Pharmacology and Centre for Biotechnology, Univ. of Turku P.O.B 123, FIN-20521, Turku, Finland

We have raised and characterized monoclonal antibodies (Mabs) against the human α_2-adrenergic receptor subtype C2 (α_2C2) in mice. The Mabs were raised against a bacterially expressed fusion protein which consisted of sequence from the putative third intracellular loop (amino acids 213-343) of human α_2C2 and GST.

Production of Mabs was first detected with Western blotting. Membranes prepared from α_2C2-transfected mammalian Shionogi 115 (S115) and *Saccharomyces cerevisiae* yeast cells were used as antigens. Membranes from S115 cell lines expressing the α_2-adrenergic subtypes C4 and C10 and wild type *Saccharomyces cerevisiae* yeast cells were used as negative controls. The α_2C2 receptor antibody production of the hybridomas was also screened with TR-IFMA (Time Resolved ImmunoFluoroMetric Assay). Ten out of forty-four positive clones were recloned and retested. Three α_2C2 specific monoclonal cell lines were expanded to *in vitro* production in hollow fiber systems. The specificity of the Mabs was further determined by immunoprecipitation of receptors solubilized from S115 cells expressing only one cloned α_2-adrenergic receptor gene product.

Scatchard analysis of Europium-labelled antigen (amino acids 213-343 of α_2C2) revealed binding affinity constants of 0.4, 0.7 and 1.6 nM^{-1} for the three Mabs and K_ds of 2.6, 1.4 and 0.6 nM, respectively.

This study was funded by grants from the Technology Development Centre of Finland.

329.5

α_2-ADRENOCEPTORS IN GUINEA-PIG AND HUMAN CORTEX: α_{2D} VERSUS α_{2A}. S.C. Cheetham*, E. O'Brien, R.W. Horton[1] & D.J. Heal. Knoll Pharmaceuticals Research and Development, Nottingham, NG2 3AA, UK and [1]Department of Pharmacology, St. George's Hospital Medical School, London, SW17 ORE, UK.

Based on molecular biological and radioligand binding studies, four subtypes of α_2-adrenoceptors have been identified; α_{2A}, α_{2B}, α_{2C} and α_{2D} (Bylund et al., 1994, Pharmacol. Rev., 46, 121-136). We have determined the subtype of α_2-adrenoceptors in guinea-pig cortex and human temporal cortex (Brodmann area 38) using radioligand binding techniques. Inhibition constants for a range of agonists and antagonists which distinguish between the known α_2-adrenoceptor subtypes were obtained by displacement of [^{3}H]RX 821002 binding from cortical membranes. There was a highly significant correlation between the pK_i values of the ligands for α_2-adrenoceptors in guinea-pig cortex and the α_{2A} (r=0.864; P< 0.001) and the α_{2D} (r=0.945; P<0.001) subtypes, with much weaker correlations with the α_{2B} (r=0.614; P<0.01) and the α_{2C} (r=0.667; P<0.05) subtypes, indicating that the α_2-adrenoceptors are of the α_{2A} or α_{2D} subtypes. Similar results were obtained for human temporal cortex; α_{2A} (r=0.919; P<0.001), α_{2D} (r=0.864; P<0.001), α_{2B} (r=0.130; P=0.686) and α_{2C} (r=0.698; P=0.054). Although the α_{2A} and α_{2D} subtypes exhibit similar affinities for several structural classes of compound, there are ligands which can distinguish between these α_2-adrenoceptor sub-populations, including rauwolscine, yohimbine, WB4101, fluparoxan and prazosin (Renouard et al., 1994, J. Pharmacol. Exp. Ther., 3, 946-957). These compounds gave K_i values for α_2-adrenoceptors in guinea-pig cortex comparable to those for the α_{2D}, but not the α_{2A} subtype. The converse was true for α_2-adrenoceptors in human temporal cortex. These data argue strongly that α_2-adrenoceptors in guinea-pig cortex are predominately of the α_{2D} subtype, whilst those in human temporal cortex are of the α_{2A} subtype.

Financial support provided by Knoll Pharmaceuticals Research and Development.

329.6

AGONIST-INDUCED MORPHOLOGICAL CHANGES IN D_{1A} DA RECEPTOR DISTRIBUTION PATTERNS. D.R. Sibley*, C.E. Sortwell[1], K.A. Altemus[2], M.S. Levine[2], and M.A. Ariano[1]. Molecular Neuropharmacology, ETB, NINDS, Bethesda, MD 20892; [1]Neuroscience, FUHS/CMS, North Chicago, IL 60064; [2]MRRC, UCLA, Los Angeles, CA 90024.

The distribution of the D_{1A} DA receptor was assessed using subtype specific anti-receptor antisera after acute DA exposure. The immunofluorescent staining of the D_{1A} receptor was examined in: 1) stably transfected Chinese Hamster Ovary (CHO) cells, 2) primary striatal cultures, and 3) rat striatal brain slices. Following agonist exposure as brief as 2-min and as long as 60-min, profound loss of cellular D_{1A} receptor staining occurred in each paradigm. In the slice, immunofluorescent neuropil staining for the D_{1A} receptor also was attenuated. The altered staining patterns were blocked by the DA antagonist (+)-butaclamol or the D_1-selective antagonist, SCH 23390. Receptor expression patterns reverted back to the control pattern 15-min after removing the agonist from the bath. Immunofluorescence for cyclic AMP increased at all DA exposure times in the experimental paradigms, was blocked by receptor antagonists, and decreased to basal staining levels after brief recovery periods. These results demonstrated the functional integrity of the D_{1A} receptor in target cells. Pretreatment with concanavalin A blocked the immunofluorescent decrease in receptor staining, but not the elevation of cyclic AMP, indicating a morphological separation of these two events, parallel to other biochemical reports. The data suggest that the anatomical basis of acute homologous D_{1A} receptor desensitization may be transposition of membrane-surface receptors to a transiently unavailable, intracellular compartment in transfected CHO cells and neurons from rat striatal cultures and brain slices. Supported by USPHS grant NS 32277 (MAA) and NS 33538 (MSL).

329.7

DIFFERENTIAL ROLE OF GLYCOSYLATION IN D1 AND D5 DOPAMINE RECEPTOR LOCALIZATION AND FUNCTION. C. Bergson* (1), R. Levenson (1), and M. Lidow (2) (1) Pennsylvania State College of Medicine, Hershey, PA 17033; (2) Yale School of Medicine, New Haven, CT 06510.

Previous studies revealed that D1 and D5 receptors are co-expressed in many cortical projection neurons, but suggested that they may be carrying out specialized functions by virtue of their different subcellular localization within dendritic spines and shafts (Bergson et al., (1995) J. Neurosci. *15*, 7821-7836). *N*-linked glycosylation is a prominent post-translational modification of both D1 and D5 receptors expressed in brain and transfected cells. We have investigated the possibility that *N*-linked sugars play a role in regulating the normal localization and/or function of either the D1 or D5 subtypes. Initial studies of receptors expressed in African Green monkey fibroblast (CV-1) cells have revealed that *N*-linked glycosylation is essential for proper D5 receptor localization in the plasma membrane as well as for its ability to bind D1-like receptor ligands. For example, specific binding of the D1 antagonist SCH23390 is abrogated when receptors are expressed in the presence of 0.5 µg/ml tunicamycin, an inhibitor of protein glycosylation. Further, confocal immunofluorescence reveals that unglycosylated D5 receptors do not reach the plasma membrane, but accumulate in a perinuclear compartment. In contrast, similar studies of D1 receptors suggest that its subcellular localization is not regulated by glycosylation. This information could provide critical insight into the differential regulation of D1 and D5 receptor folding, transport, and function in vivo, as well as help explain the physiological requirements for multiple D1-like subtypes. Supported by NARSAD Young Investigator Award (C.B.) and NIMH grant MH44866-05.

329.8

DIFFERENTIAL EXPRESSION OF D-2 RECEPTOR SUBTYPES IN RAT CHROMAFFIN CELLS AND SYMPATHETIC NEURONS. S. Sigala*, M. Monselice, V. Pasotti, C. Missale, P.F. Spano. Div. of Pharmacology, Dept. of Biomed. Sci. and Biotec., Brescia University Medical School, Via Valsabbina 19, 25123 Brescia, Italy.

Dopamine (DA) is involved in the inhibition of secretion from both neurons and endocrine cells. In chromaffin cells as well as in sympathetic nerve endings, DA receptors belonging to the D-2 family inhibit catecholamine secretion. Chromaffin cells and sympathetic neurons arise from a common bipotential progenitor under the influence of different factors such as Nerve Growth Factor (NGF), that promotes the differentiation into the neuronal phenotype. On this basis, we established primary cultures of newborn rat chromaffin cells, differentiating them into sympathetic neurons with NGF. Using RT-PCR followed by Southern blot, we studied D-2-like receptors expression in adult chromaffin cells and in sympathetic neurons. Our results show that, while D-3 receptor messenger RNA (mRNA) is not present in any cell type examined, adult chromaffin cells express D-4 and both isoforms of the D-2 receptor. Interestingly, the mRNA encoding the shorter D-2 receptor form (D-2s) was expressed at approximately 4-fold higher levels than the mRNA for the longer form (D-2l). PCR amplification of mRNA obtained from sympathetic neurons shows that this phenotype selectively express the mRNA encoding for the D-2s isoform. No signal for D-2l, D-3 or D-4 receptors was found.

Taking together, these data suggest that 1): NGF and glucocorticoid hormones play a role in regulating D-2 family receptor expression. 2) NGF seems to be involved in the regulation of splicing mechanism, since NGF-directed differentiation of newborn chromaffin cells inhibits the expression of the D-2l mRNA. 3) Sympathetic neurons selectively express the D-2s receptor; thus suggesting a specific and different role of the D-2s and D-2l isoforms in the control of catecholamine release. *This work was supported by the MURST 60% grant.*

329.9

D1 AND D2 DOPAMINE RECEPTOR PRUNING IN THE ADOLESCENT FEMALE RAT. S.L. Andersen*, M. Rutstein, J. Benzo, J.H. Hostetter, and M.H. Teicher. Dept. of Psychiatry, Harvard Medical School, Laboratory for Developmental Psychopharmacology, McLean Hospital, Belmont, MA 02178.

We have previously shown that D1 and D2 dopamine receptors are overproduced and eliminated by a minimum of 35% in the striatum, but not in the nucleus accumbens of male rats during the periadolescent period (Teicher et al 1995). In this study we determined whether this same dramatic pattern was followed in the striatum and nucleus accumbens of female rats the ages of 40, 60, 80, 100, and 120 days. All tissue was harvested on the second day of diestrous to control for hormonal cycling. The density of D1 and D2 receptors was based on quantitative autoradiography with Scatchard analysis of 7 concentrations (0.01-4 nM) of 3H-SCH-23390 and 3H-YM-09151-2, respectively. Data were then analyzed with LIGAND. In contrast to marked receptor pruning observed in male rats across the adolescent period, D1 and D2 receptor decline only slightly in the striatum. Between 40 and 120 days of age, D1 density dropped 20%, while D2 receptor density was attenuated by 10%. Slight pruning was also observed in the nucleus accumbens, with both receptor subtypes declining by 10-20% between 40 and 120 days. We hypothesize that this difference between male and females may reflect differences in the amount of overproduction, rather than elimination of these receptor subtypes and studies are underway to determine if this is the case. These findings may help explain why Tourette's Syndrome and ADHD are much more prevalent in males than females and wane in severity during adolescence.

Supported by NIMH RO1-43743 (to MHT) and the Scottish Rite Foundation (to SLA).

329.10

QUANTITATIVE ANALYSIS OF DOPAMINE RECEPTOR TRANSCRIPTS IN D_2 AND D_4 RECEPTOR-DEFICIENT MICE. G. Zhang*, J.R. Bunzow, J.L. Larson, M.A. Kelly, M.J. Low and D.K. Grandy. Vollum Institute, OHSU, Portland, OR 97201.

Brain dopamine receptors play an important role in movement initiation, congnition, affect and neuroendocrine function. To better understand the contributions that each of the various dopamine receptor subtypes make to behavior and physiology we generated transgenic mice that lack either the D_2 or D_4 receptor. In the present study transcripts from the mutated D_2 and D_4 receptor genes were quantitated. For this analysis a sensitive ribonuclease protection assay (RPA) was designed for D_2 and D_4 dopamine receptor subtypes and cyclophilin (the internal standard). This assay was used to survey a number of different mouse tissues. By RPA mouse retina was found to be a rich source of D_4 receptor mRNA. Interestingly, receptor-deficient animals expressed ~10% of wild type (+/+) levels of D_4 mRNA, even though the gene's promoter had not been mutated. A similar reduction in D_2 receptor transcripts was also observed in the D_2 knockout mice (-/-): mutant D_{2L} and D_{2S} transcripts were expressed at ~30% and ~4% of wild type levels ($p < 0.05$), respectively. Currently mouse D_1, D_{1b} and D_3 receptor mRNAs are being evaluated by RPA to determine whether any compensatory changes may have occurred in response to the absence of functional D_2 or D_4 receptors during development. This work was supported by NIDA grant DA0962 (DKG).

329.11

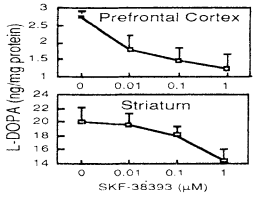
CEREBROCORTICAL EXPRESSION OF THE D_1 DOPAMINE RECEPTOR AND DARPP-32 IN RAT BRAIN. K.C. Langley*, C. Bergson‡ and C.C. Ouimet. Program in Neuroscience, Florida State University, Tallahassee, FL 32306, ‡Department of Pharmacology, Pennsylvania State College of Medicine, Hershey, PA 17033

The D_1 dopamine receptor is known to regulate DARPP-32 phosphorylation in the caudate-putamen, a region in which almost all neurons contain DARPP-32. In the cerebral cortex, however, the distribution of DARPP-32-containing neurons is restricted to specific laminae. The extent of D_1 receptor overlap with this distribution is not known. This light microscopic study localizes the laminar distribution of the D_1 receptor and DARPP-32 in cerebral cortex via double-label immunocytochemistry. The D_1 receptor was localized primarily to dendritic processes and fine puncta, whereas DARPP-32 was localized to both neuronal somata and dendrites. Dendrites expressing both the D_1 receptor and DARPP-32 were observed. Within most cortical areas, occasional cell bodies were outlined with D_1 reaction product and these cells invariably contained DARPP-32. Throughout the cortical areas examined, layer VI contained the heaviest dendritic labelling for the D_1 receptor and the strongest neuronal/dendritic labelling for DARPP-32. The colocalization of the D_1 receptor and DARPP-32 in dendrites and certain somata indicates that cortical D_1/DARPP-32 mechanisms may mirror those of the basal ganglia.

329.12

TRANSIENT D1 SYNTHESIS MODULATING AUTORECEPTOR IN THE PREFRONTAL CORTEX AND STRIATUM. N.L. Dumont, S.L. Andersen, S.K. Yeghiayan*, and M.H. Teicher. Dept. of Psychiatry, Harvard Medical School, Laboratory of Developmental Psychopharmacology, McLean Hospital, Belmont, MA 02178.

Previous research (Teicher et al, 1990) has provided evidence for a D1 transient synthesis-modulating autoreceptor in rat prefrontal cortex and striatum utilizing the in vivo GBL model. This autoreceptor -like effect was apparent at postnatal (P) day 15, waned by P22 and was absent by P60. In order to clarify whether the effect was due to an autoreceptor or a heteroreceptor, we utilized an in vitro slice model. 250μM sections of the prefrontal cortex and striatum were incubated at 37°C in Kreb's-Ringers buffer with the D1 agonist SKF-38393(.01μm-10.0μm); after 20 minutes, the decarboxylase inhibitor NSD-1015 was added and the reaction was terminated 20 minutes later. In a dose-dependent manner, SKF-38393 inhibited L-DOPA accumulation at P15 (see Figure).



This effect persisted in the striatum until P40, but L-DOPA was uneffected by SKF-38393 in the prefrontal cortex at this age. Research is underway to examine the effect of a D1 antagonist and TTX to determine if this is mediated by a heteroreceptor. Supported by NIMH RO1-43743 to MHT.

329.13

DIFFERENTIAL EXPRESSION OF MULTIPLE β1-ADRENERGIC RECEPTOR TRANSCRIPTIONAL START SITE CLUSTERS IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM. Y.-F. Yang[1], H. Nichols[1], and C. A. Machida[1,2]*. [1]Div. of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006 and [2]Graduate Program in Neuroscience, Oregon Health Sciences University, Portland, OR 97201.

In a previous report [Machida, C. A. et al. (1995). Soc. Neurosci. Abs. 21:90], we determined that the rat β1-adrenergic receptor (β1-AR) mRNA transcript has multiple start sites, occurring primarily in two major clusters centered at bases -280 and -250, relative to the start site of translation. RNase protection analyses indicated that the two major β1-AR transcriptional start site clusters were preferentially utilized within the peripheral nervous system (PNS), and that extreme 5' start sites, upstream from the two major clusters, were preferentially selected in the central nervous system (CNS). To more precisely identify the upstream β1-AR transcriptional start sites, we synthesized a series of oligonucleotides, representing β1-AR sequences spanning positions -495 to -2264. These oligonucleotides served as primers for reverse transcription from mRNA templates obtained from tissues/cell lines, which were either abundant or deficient in β1-ARs. Interestingly, one primer (representing β1-AR sequence -1743 to -1764) produced a series of extension products whose 5' ends were delimited within the region -2163 to -1911. These extension products were highly prevalent in cortex, a CNS tissue abundant in β1-ARs, and were almost nonexistent in heart, a PNS tissue containing β1-ARs. In addition, the extension products were faintly detectable in C6 glioma cells, a cell line originating from a brain tumor and abundant in β1-ARs, and absent in L6 cells and liver, sources devoid of β1-ARs. The differential expression of β1-AR transcripts in the CNS will help define the β1-AR gene and identify potential CNS-selective β1-AR promoter elements and enhancers. *(CAM is supported by NIH grants RR00163 and HL 42358, and by an American Heart Association Established Investigatorship).*

329.14

D_{1A} RECEPTOR-DEFICIENT TRANSGENIC MICE REVEAL D_{1B} (D_5) RECEPTORS IN HIPPOCAMPUS. D.M. Montague, J.S. Overcash, C.D. Striplin[1], J. Drago[1], R.B. Mailman[1]* and C.P. Lawler. Neuroscience Center, Univ. of North Carolina, Chapel Hill, NC 27599-7250 and [1]Neuroscience Unit, Monash University, Clayton 3168 Australia

The dopamine D_{1A} receptor is the predominant "D_1-like" subtype expressed in the mammalian brain. The targeting of the D_{1A} dopamine receptor gene in mice with a mixed C57BL/6 and 129/Sv genetic background resulted in mice that exhibited essentially normal locomotor responses (*PNAS* 91:12564). This unexpected finding suggested that some mechanism might be compensating for the loss of the D_{1A} receptor. The D_{1A} knockout model was used in the present study to: i) observe possible density changes in specific receptor populations that may be associated with a compensation for D_{1A} deficiency; and, ii) confirm the presence and location of the rare D_{1B} (D_5) receptor subtype, which is normally masked by much higher D_{1A} densities. Quantitative receptor autoradiography studies were performed to determine the densities of D_1-like receptors ([^{3}H]-SCH23390) and D_2-like ([^{125}I]-sulpiride; [^{3}H]N-n-propylnorapomorphine) receptors in various brain regions of wildtype, heterozygote, and homozygous transgenic mice. As expected, the data indicated a near-complete absence of D_1-like receptors in homozygous (-/-) mice and a drastic loss in heterozygotes. Surprisingly, D_2 receptor densities were similar among the three genotypes. This evidence suggests that alterations in D_2 receptor density are not a factor involved in D_{1A} receptor compensation. Finally, our studies have also provided the first localization and quantification of the less prevalent "D_1-like" subtype, the D_{1B}. Autoradiographic data, supported by homogenate binding studies, demonstrated the presence of D_{1B} receptors throughout the hippocampal complex of homozygous mice. Low densities of [^{3}H]-SCH23390-labeled sites were also detected in the parafesicular nucleus of the thalamus in these mice. These findings are consistent with earlier data localizing the mRNA for this receptor to these regions. (MH42705, MH40537, HD03110, MH33127, and GM07040)

329.15

SUBCELLULAR DISTRIBUTION AND PHARMACOLOGY OF [^{3}H]QUINPIROLE AND [^{3}H]SPIPERONE BINDING SITES IN RAT STRIATUM. B. Levant* and G.N. Bancroft. Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66160-7417

[^{3}H]Quinpirole is an ergoline dopamine agonist with high affinity for the D_2 and D_3 receptor subtypes. Surprisingly, MAO inhibitors inhibit the binding of [^{3}H]quinpirole, but not [^{3}H]spiperone or [^{3}H](-)NPA, in rat striatal membranes by a competitive mechanism that does not appear to involve the enzymatic activity of MAO (Levant et al., EJP, 246:171, 1993). To further characterize this observation, the density and pharmacology of [^{3}H]quinpirole and [^{3}H]spiperone binding sites were compared in subcellular fractions of rat striatum. Total binding activity of both ligands was enriched in the synaptosomal fraction (P_{2B}); however, there was significantly less [^{3}H]quinpirole than [^{3}H]spiperone binding in the P_3 and P_4 fractions. [^{3}H]Quinpirole binding was inhibited by the MAO inhibitor Ro 41-1049, as well as by spiperone, in all fractions. The densities of MAO inhibitor-displaceable [^{3}H]quinpirole binding in each fraction were not significantly different from those inhibited by spiperone. In contrast, [^{3}H]spiperone binding was not inhibited by Ro 41-1049 in any fraction. Quinpirole had significantly lower affinity for [^{3}H]spiperone-labeled sites in the P_3 and P_4 fractions than the affinity of [^{3}H]quinpirole in these fractions. These data demonstrate that [^{3}H]quinpirole labels a membrane-associated site and suggest that [^{3}H]quinpirole and [^{3}H]spiperone may interact with distinct binding sites in certain subcellular fractions.

Supported by NARSAD

329.16

SIMULTANEOUS COMPARISON OF D_2/D_3 RECEPTOR AFFINITY OF NOVEL D_3-SELECTIVE DRUGS IN RAT BRAIN. R.J. Flietstra*, B. Levant. Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66160.

Several dopaminergic drugs have recently been shown to exhibit higher affinity for D_3 receptors than for D_2 receptors in transfected cell lines. This study used quantitative autoradiography to simultaneously evaluate the D_2/D_3 selectivities of novel D_3-selective compounds in rat brain. Slide-mounted sagittal sections were incubated with [^{3}H]quinpirole and various concentrations of competing drug. The ability of each drug to inhibit [^{3}H]quinpirole binding in cerebellar lobule X, associated with D_3 mRNA, was used as a measure of D_3 affinity; inhibition of [^{3}H]quinpirole binding in the caudate/putamen, primarily associated with D_2 mRNA, estimated D_2 affinity. PD 128907, a dopamine agonist, was more potent in cerebellum than in caudate/putamen, indicating selectivity for the D_3 receptor. In contrast, risperidone and domperidone were more potent in the caudate/putamen, indicating a greater affinity for the D_2 receptor. The D_2/D_3 selectivities of other compounds, including dopamine and UH 232, were also evaluated.

Supported by NIMH MH52839.

330.1

MULTIPLE ^{3}H-NEMONAPRIDE BINDING SITES IN HUMAN AND COW BRAIN. D.M. Helmeste*, H. Fang, M. Li and S.W. Tang, Depts. Psychiatry and Pharmacology, University of California, Irvine, Irvine, CA 92717.

Tritiated nemonapride has been used to label dopamine D_4 receptors. We reported that a substantial proportion of ^{3}H-nemonapride binding in striatum, frontal cortex and cerebellum in both human and bovine brains represents sigma sites. Using haloperidol (DA and sigma ligand) to define specific ^{3}H-nemonapride binding yielded biphasic Scatchard plots in human striatum (Kds: 0.03 and 7.2 nM). In human frontal cortex and cerebellum, haloperidol (1 μM) also defined more than one ^{3}H-nemonapride site by Scatchard analysis. The dopamine and sigma components of ^{3}H-nemonapride binding can be differentially shown by (+) and (-) butaclamol displacement in striatum, frontal and cerebellar cortex. The sigma site labeled by ^{3}H-nemonapride had a sigma-1 profile [IC50s (nM): haloperidol=5.9, PPAP=45, (±) pentazocine=84, progesterone=400, (-) butaclamol=99, (+)butaclamol=515]. When dopaminergic and sigma sites were blocked with sulpiride and pentazocine, respectively, haloperidol revealed a third component of ^{3}H-nemonapride binding. Spiperone, (+) butaclamol, loxapine and thioridazine but not clozapine, mianserin, (-) butaclamol, sertraline, DOI, (-) PPP, atropine, diphenhydramine or econazole compete for this third component. This suggests an additional dopamine site labeled by ^{3}H-nemonapride, not previously described. (Human tissue from NNRSB, VAMC, LA).

(Non-commercial/non-grant funding sources)

330.2

EXCITOTOXIC LESIONS OF THE MEDIAL PREFRONTAL REDUCE DOPAMINE D1-LIKE BINDING SITES IN THE VENTRAL TEGMENTAL AREA. K. Dewar [1], P.-P. Rompré[1], J. Stewart[2] and R.A Warren[1]* [1]Psychiatry Department, University of Montreal and [2]Psychology Department, Concordia University, Montreal (Québec), Canada.

Repeated exposure to amphetamine (AMPT) results in a progressive increase in its locomotor activating effect, a phenomenon known as sensitization. Although the exact mechanism(s) involved in the development of AMPT sensitization remain(s) unclear, several studies suggest that activation of dopamine D1-like receptors in, or near, the ventral tegmental area (VTA) is important. The localization of tegmental D1 receptors on afferent terminals is suggested by the absence of D1 mRNA in the VTA. Since the mPFC neurons not only project to the VTA but also contain a significant amount of D1 mRNA, D1 receptors may be localized on these mPFC terminals. In this study, we investigated whether excitotoxic lesions of the mPFC reduced D1-like binding sites in the VTA. Male Wistar rats were anesthetized with pentobarbital and ibotenic acid (2 μg/μl or 10 μg/μl) or its vehicle was microinjected at three anterior-posterior placements the left mPFC (between 3.2 and 4.5 mm from bregma, 1 μl per placement). Ten days after surgery, animals were sacrificed, their brains removed, and divided into midbrain and forebrain blocks. The forebrain region was sectioned and stained with formal-thionin to verify the nature and the extent of the lesion, while the midbrain block was used for autoradiographic quantification of D1-like receptors using the selective D1 receptor antagonist [^{125}I]SCH23938. Small excitotoxic lesions (low dose) of the mPFC resulted in a significant loss in D1-like binding sites in the ipsi- (42%) and contralateral (15%) VTA compared with vehicle control. The larger, non-specific lesions (high dose) resulted in a similar ipsilateral (43%) but a larger contralateral loss (38%) in D1-like binding sites. These findings demonstrate that a significant amount of D1-like receptors are located on mPFC efferent to the VTA.

330.3

INTERACTION OF HUMAN D3 DOPAMINE RECEPTORS EXPRESSED IN Sf-9 CELLS WITH ENDOGENOUS G-PROTEINS AS DETECTED WITH GTP-γ-S BINDING. J.F.Pregenzer, G.L. Alberts and W.B.Im*. CNS Diseases Research, Pharmacia & Upjohn, Inc. Kalamazoo, MI 49001.

The human D3 dopamine receptor was expressed in Sf-9 cells, using the recombinant baculovirus carrying the receptor cDNA. With cell harvesting at various post infection times, we obtained membranes with different receptor densities of 0.73, 9.2, 26 and 52 pmol/mg protein, as measured with [^{3}H]spiperone binding. The dissociation constant for the ligand was 0.5 nM. The effect of GTP-γ-S on [^{3}H]dopamine binding was variable, depending on the receptor density. The nucleotide at a concentration of 10 μM reduced [^{3}H]dopamine binding by 52%, 13% and 2% with receptor densities of 0.73, 9.2, and 26 pmol/mg protein, respectively. This suggests that the fraction of the D3 receptor coupled to endogenous G-proteins in Sf-9 cells progressively decreased as the receptor density increased. On the other hand, Na^+ (200 mM), an allosteric modulator, reduced [^{3}H]dopamine binding by 20% to 30 %, regardless of receptor density. Agonist-induced enhancement of GTP-γ-[^{35}S] binding was also observed in membranes of low and high receptor density. A number of agonists for the D2-like receptors enhanced GTP-γ-[^{35}S] binding, whereas antagonists did not. It appears that GTP-γ-[^{35}S] binding is useful for characterizing ligand functionality in recombinant human D3 dopamine receptors in Sf-9 cells.

330.4

GTP SENSITIVITY OF DOPAMINE D3 RECEPTORS IN THE RAT BRAIN. J. M. Vessotskie*, M.-P. Kung, S. Chumpradit and H. F. Kung, Departments of Pharmacology and Radiology, University of Pennsylvania, Philadelphia, PA 19104.

Dopamine D3 receptors are members of the D2-like dopamine receptor family. It is well documented that agonist binding to dopamine D2 receptors in rat brain is GTP and pertussis toxin sensitive (Olianas et al., J Neurochem 48:1443, 1987). The results of experiments to examine the GTP sensitivity of agonist binding to D3 receptors in native state are ambiguous because appropriate tools are not available. Recently, a radioiodinated ligand, S(-)-5-OH-PIPAT (5-hydroxy-2-(N-n-propyl-N-3'-iodo-2'-propenyl)aminotetralin), was shown to bind with high affinity (K_d = 0.1 nM) to dopamine D3 receptors in molecular layers 9 and 10 of rat cerebellum using autoradiographic techniques (Vessotskie, Abs Neurosci 341.2, 1995). This brain region contains high densities of D3 receptors with minimal contamination of D2 or D4 receptors (based on mRNA expression, Bouthenet et al., Brain Res 564:203, 1991; Van Tol et al., Nature 350:610, 1991). A series of autoradiographic experiments was completed to examine the effect of GTP on D3 receptors. Increasing concentrations of GTP, but not ATP, decreased [^{125}I]S(-)-5-OH-PIPAT (putative agonist) binding to D3 receptors in the cerebellum by 60%, but did not influence [^{125}I]NCQ298 (putative antagonist) binding. Analysis of competition experiments of S(-)5-OH-PIPAT for [^{125}I]]NCQ298 binding revealed that a two site model best fit the data. Additional competition experiments were performed, using dopamine, the endogenous neurotransmitter, and [^{125}I]NCQ298, to insure that the effect was not a ligand characteristic. The presence of 100 μM Gpp(NH)p caused a rightward shift of the dopamine inhibition curve. Autoradiography experiments of D3 receptors in the Major Island of Calleja showed the presence of 300 μM GTP decreased [^{125}I]S(-)-5-OH-PIPAT binding by 27% without influencing [^{125}I]NCQ298 binding. These experiments suggest that D3 receptors in the rat brain are GTP sensitive. The pertussis toxin sensitivity of D3 receptors is currently under investigation. (Supported by MH-51880)

330.5

BINDING CHARACTERISTICS OF IODINATED LIGANDS FOR DOPAMINE D4 RECEPTORS. H. F. Kung*, M.-P. Kung, D. A. Stevenson, Z.-P. Zhuang, J. M. Vessotskie, S. Chumpradit, X.-M. Sun. Departments of Radiology and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Binding characteristics of two iodinated compounds, *trans*-5-hydroxy-2-(N-n-propyl-N-3'-iodo-2'-propenyl)aminotetralin (*(S)*-5-OH-PIPAT), and 3-[4-(4-iodophenyl)piperazin-1-yl]methyl-1H-pyrrolo(2,3-b)pyridine (IPMPP), were evaluated using human D4 (D4.2 variant) receptors expressed in CHO cells. Saturation analysis revealed high-affinity-site binding for both [^{125}I]*(S)*-5-OH-PIPAT (K_d = 0.36 ± 0.08 nM) and [^{125}I]IPMPP (K_d = 0.39 ± 0.18 nM). The number of D4 receptor sites labeled with [^{125}I]IPMPP at 25 °C was four times higher than that labeled with [^{125}I]*(S)*-5-OH-PIPAT (572 fmol/mg protein vs. 124 fmol/mg protein). A significant decrease of the number of binding sites was observed with [[^{125}I]*(S)*-5-OH-PIPAT when assays were carried out at 37 °C. In contrast to [^{125}I]*(S)*-5-OH-PIPAT, [^{125}I]IPMPP labeled more D4 sites at 37 °C. The magnesium ion stimulated the binding of [^{125}I]*(S)*-5-OH-PIPAT, but it showed no effect on [^{125}I]IPMPP binding. Gpp(NH)p significantly inhibited specific binding of [^{125}I]*(S)*-5-OH-PIPAT (60% reduction at 100 μM concentration); however, [^{125}I]IPMPP binding was not affected. These data strongly suggest that [^{125}I]*(S)*-5-OH-PIPAT is an agonist radioligand and [^{125}I]IPMPP is an antagonist radioligand for labeling dopamine D4 receptors expressed in CHO cells. In addition, results of measuring D4 receptor-stimulated [^{35}S]GTPγS binding in CHO cells lend support to the agonist and antagonist activity for *(S)*-5-OH-PIPAT and IPMPP, respectively. Unfortunately, neither radioligand is adequate for visualizing D4 receptors in rat brain sections due to the lack of dopamine subtype selectivity of [^{125}I]*(S)*-5-OH-PIPAT and the high non-specific binding of [^{125}I]IPMPP. A more desirable radioiodinated ligand with better subtype selectivity and lower non-specific binding should be developed in the future for the characterization of D4 receptor binding. (Supported by NS-24538)

330.6

LOCALIZATION OF THE IMIDAZOLINE / GUANIDINIUM BINDING DOMAIN ON MONOAMINE OXIDASE B. R. Raddatz* and S.M. Lanier Dept. of Pharmacology, Medical University of South Carolina, Charleston, SC 29425. Two members of the family of imidazoline binding proteins were recently identified as monoamine oxidase A and B (MAO-A and B). Although some imidazoline/guanidinium derivatives inhibit enzyme activity, the imidazoline binding domain on MAO appears to be distinct from the enzyme active site suggesting an additional motif for regulating enzyme function. To identify the imidazoline binding domain on MAO-B, a radiolabeled photolabile imidazoline derivative was used to covalently label the protein. The photolabeled MAO-B was isolated by SDS-PAGE, the gel slice containing radioactivity was excised and the protein was digested to completion with CNBr (70% formic acid, 24 hours at 37° C). The resulting peptide fragments were separated on a 15.5% acrylamide gel using a Tricine buffer system and radiolabeled peptides detected by autoradiography. Radiolabeled peptides of M, ~13,000 for rat MAO-B and M, ~8,000 for human MAO-B were identified. Based on the species differences in the primary sequence of rat and human MAO-B and the M, of labeled peptides following CNBr digestion, the site of photoincorporation of the imidazoline ligand is apparently localized to a 73 amino acid fragment (residues 149 - 222) of MAO-B in these two species. In contrast to the site of action of various mechanism-based MAO inhibitors that interact with the FAD cofactor at Cys-397, the imidazoline binding domain represents a potentially novel site for enzyme regulation. (Supported by NS 24821/CTR 2235, RR was supported by 5-T32-HL07260-18)

330.7

FACTORS INFLUENCING SELECTIVITY FOR D_{1A} VS. D_{1B} (D_5) DOPAMINE RECEPTORS: BT Hoffman*, MA Mayleben, DE Nichols, SD Wyrick and RB Mailman. Univ. of North Carolina, Chapel Hill, NC 27599, & Purdue University, W. Lafayette IN 47907

We have tested the hypothesis that the ligand binding domain of the D_{1A} and D_{1B} receptors are similar except for subtle differences in the accessory hydrophobic region, and in the orientation of the amine binding region. We have used and/or synthesized several chemical classes, including hexahydrobenzophenanthridine, phenyltetrahydrobenzazepine, tetrahydroisoquinoline (THIQ) and aminodihydroxytetrahydronapthalene (ADTN) derivatives. Drugs were tested in CHO cells transfected with either of the human isoforms. None of this wide range of compounds showed even as much as a ten-fold selectivity for either isoform. For example, 7-chloro-8-hydroxy-N-methyl-1-phenyl-1,2,3,4-THIQ and analogs with phenyl ring substituents (R= Cl or CH_3) displayed similar affinity for D_{1A} and D_{1B}. While *o*-substitution (regardless of the electronic character of the substituent) doubled the preference of THIQs for D_{1B} receptors, this was still a rather subtle change. This area was probed further by the synthesis of a series of phenyl-substituted compounds. Whereas the *o*-,*o*-dimethyl substituted THIQ analog had a 2.7-fold preference for the D_{1B} receptor, an *o*-isopropyl substitution decreased ligand affinity for D_{1B} receptors, without changing the affinity for D_{1A} receptors. These data suggest that D_{1A} and D_{1B} receptors have somewhat similar steric pockets. In the absence of predictive molecular models of the D_{1A} and D_{1B} receptors, we believe that development of a selective ligand should utilize a pharmacophore that incorporates a subset of the D_1 pharmacophore (e.g., catechol and nitrogen) incorporated into a novel structural backbone. (Supported by MH42705, MH40537, HD03110, MH33127, DA07244 and a grant from Hoechst Marion Roussel.)

330.8

RESIDUES IN THE SEVENTH MEMBRANE-SPANNING SEGMENT OF THE DOPAMINE D2 RECEPTOR ACCESSIBLE IN THE BINDING-SITE CREVICE. J. A. Javitch*, D. Fu, J.A. Ballesteros, and J. Chen. Center for Molecular Recognition & Psychiatry, Columbia University; NYS Psychiatric Institute, NY, NY 10032; Physiology & Biophysics, Mt. Sinai School of Medicine, NY, NY 10029.

The binding site of the dopamine D2 receptor, like that of other homologous G protein-coupled receptors, is contained within a water-accessible crevice formed among its seven membrane-spanning segments. Using the substituted-cysteine accessibility method, we are mapping all the residues exposed in this binding-site crevice. We mutate individual residues in the membrane-spanning segments to cysteine, express the cysteine-substitution mutants in HEK 293 cells, and examine antagonist binding to mutant receptor in intact cells. If mutant receptors are near-normal in their binding, we determine whether the substituted cysteine is at the water-accessible surface of the binding site by testing its accessibility to small, charged hydrophilic, lipophobic sulfhydryl-specific derivatives of methanethiosulfonate (MTS). These reagents react covalently with cysteine, and if the engineered cysteine faces into the binding-site crevice, the reaction will block antagonist binding irreversibly and will be retarded in the presence of ligand. We previously mapped the residues that form the surface of the binding-site crevice in the third and fifth membrane-spanning segments. We have now mutated to cysteine, one at a time, twenty-six consecutive residues in and flanking the seventh membrane-spanning segment (M7). Nine of these mutants reacted with the MTS reagents added extracellularly and were protected from reaction by a reversible dopamine antagonist, sulpiride. Thus, we infer that the side chains of these nine residues are in the water-accessible surface of the binding-site crevice. The pattern of accessibility of the cysteine substitution mutants is consistent with M7 being a kinked α-helix but not an ideal α-helix. *Supported by MH01030, MH54137, NS07258, NARSAD, the Scottish Rite, the Mathers Charitable Trust and DA09083.*

330.9

RESIDUES IN THE FIFTH MEMBRANE-SPANNING SEGMENT OF THE β_2 ADRENERGIC RECEPTOR ACCESSIBLE IN THE BINDING-SITE CREVICE. D. Fu*, and J. A. Javitch. Center for Molecular Recognition & Dept. of Psychiatry, Columbia University; NYS Psychiatric Institute, NY, NY 10032.

Using the substituted-cysteine accessibility method, we previously mapped the residues in the fifth membrane-spanning segment (M5) that are accessible in the binding-site crevice of the dopamine D2 receptor (Javitch et al., Biochem. 34:16433, 1995). We mutated twenty-four consecutive residues in and flanking M5 to cysteine and expressed the mutant receptors in HEK 293 cells. Thirteen of these mutants reacted with charged polar sulfhydryl-specific derivatives of methanethiosulfonate (MTS) added extracellularly, and were protected from reaction by a reversible dopamine antagonist. Of the thirteen accessible residues, ten were consecutive, from Phe189 to Phe198. This pattern of accessibility is inconsistent with the expectation that M5, like M3, forms a fixed α-helix, one side of which is accessible in the binding-site crevice. Six of the ten consecutive accessible residues are identical to the aligned residues in the β_2 adrenergic receptor. We have now mutated to cysteine twelve consecutive residues in M5 of the β_2 receptor. MTSethylammonium (MTSEA) did not inhibit antagonist binding to wild type β_2 receptor but did inhibit binding to seven of the twelve β_2 cysteine mutants. Binding to three cysteine mutants, the aligned residues of which were accessible to MTSEA in the D2 receptor, was unaffected by MTSEA at 2.5 mM. Furthermore, MTSEA did not change the IC_{50} for isoproterenol competition with the antagonist [^{3}H]CGP-12177 in these mutants. It appears, therefore, that these three residues in the β_2 receptor are not accessible to reaction with MTSEA. This may reflect a difference in the structures and/or packing of membrane-spanning segments in the two receptors. Alternatively, the conformational changes involved in receptor activation may differ. The pattern of accessibility is consistent with M5 in β_2 receptor being an α-helix, one side of which is accessible in the binding-site crevice. *Supported by NS07258.*

330.10

IN VIVO BINDING OF [^{3}H]SCH 23390: EVALUATION OF THE IMPORTANCE OF REBINDING TO RECEPTORS IN SLOWING THE APPROACH TO EQUILIBRIUM. A.N. Gifford*, S.J. Gatley and N.D. Volkow. Medical Dept., Brookhaven Natl. Lab., Upton, NY 11973.

The importance of rebinding to receptors in influencing the kinetics of the *in vivo* binding of a high affinity radioligand was examined by comparing the time for striatal [^{3}H]SCH 23390 binding to reach equilibrium in tissue homogenates with that in brain slices and *in vivo*. In homogenates [^{3}H]SCH 23390 binding reached equilibrium in approximately 60 min. By contrast, in rat striatal slices incubated in [^{3}H]SCH 23390 the radioactivity levels in the slice continuously increased over a 4 h period, with no indication of an approach to equilibrium at the time of termination of the experiment. However, radioactivity in the slice reached a steady state in less than an hour when D_1 receptors were blocked by inclusion of a high concentration of the selective D_1 antagonist, SCH 39166, in the incubation medium. In the *in vivo* experiments [^{3}H]SCH 23390 was given to mice both as a bolus injection and as a constant intravenous infusion over a 4 h time period. Following the bolus injection striatal and cerebellar radioactivity levels reached a peak only 15 min after the injection. However, following a constant infusion striatal levels of radioactivity continued to increase in an almost linear fashion throughout the 4 h infusion period whereas cerebellar levels of radioactivity and serum levels of [^{3}H]SCH 23390 reached a steady state within 15 min. Calculations based on the slice experiments suggested that a primary factor accounting for failure of [^{3}H]SCH 23390 levels to reach equilibrium *in vivo* is its hindered diffusion through receptor rich tissue as a result of repeated rebinding to receptors. These observations have important implications for the widely used compartmental models for the *in vivo* binding of PET and SPECT ligands.
Supported by NIDA grant DA 06891

330.11

DOPAMINE D_3 RECEPTORS IN BASAL GANGLIA/MESOLIMBIC AREAS DETECTED BY ^{3}[H]-PRAMIPEXOLE RECEPTOR BINDING STUDIES. M.Camacho-Ochoa*, E.L.Walker, D.L.Feldpausch, and M.F.Piercey. Pharmacia & Upjohn Inc., Kalamazoo, MI, USA 49001.

Although there are 5 dopamine (DA) receptor subtypes, little knowledge exists concerning their distribution. mRNA distributions suggest D_3 receptors located mostly in mesolimbic regions, but D_3 receptor antibody binding sites (Brain Res 649:95, 1994) and some D_3 mRNA have also been found in caudate (Brain Res 564:203, 1991). We have now used 8 concentrations of ^{3}[H]-pramipexole (PPx), a D_3-preferring DA agonist useful in Parkinson's Disease (PD) to extimate K_ds by saturation analsis in various brain regions of coronal sections of rat brain. In clonal cell lines expressing rat receptors, ^{3}[H]-PPx has a D_3 K_i of 0.37 nM and a D_2 K_i of 7.2 nM at high affinity binding sites relevant for agonist activity (Eur J Pharmacol 290:29, 1995). In anterior brain slices, ^{3}[H]-PPX bound.with affinities comparable to D_3 high affinity sites (0.2 - 0.4 nM) in Islets of Calleja (ICj), n accumbens, olfactory tubercle and caudate and with lower affinities (1.5-10 nM) in L septum, M septum (MS), and diagonal band (DBB). Binding in more caudal segments had higher K_d's, suggesting possible mixed D_2/D_3 receptor populations for both caudate and nucleus accumbens shell and core, and D_2 receptors for n basalis. However, ICj K_ds were still in the D_3 high affinity range. It is concluded that D_3 receptor distribution is broader than suggested by mRNA studies and that PPx's efficacy in treating PD could, in part, be due to stimulation of basal ganglia D_3 receptors. DA receptor populations suggested for MS, DBB, and n basalis are consistent with known DA neuron projections into forebrain cholinergic areas (Prog. Brain Res 98:31, 1993).

330.12

DESTRUCTION OF SEROTONERGIC NEURONS DOES NOT ALTER BINDING OF THE D3 LIGAND [^{125}I]-7-OH-PIPAT IN THE MEDIAN AND PARAMEDIAN RAPHE NUCLEI OF THE RAT. G.D. Stanwood*, R.P. Artymyshyn, M.P. Kung, R.K. Raghupathi, A. Singh, I. Lucki, and P. McGonigle. Institute of Neurological Sciences and Department of Phamacology, University of Pennsylvania, Philadelphia, PA 19104.

Recent morphological and microdialysis studies suggest that the ascending serotonergic projections from the raphe nuclei to the forebrain are regulated by dopamine D2-like (D2, D3, D4) receptors. The expression of dopamine D2 and D3 receptors was therefore examined at the level of the raphe nuclei in the rat brain using quantitative autoradiography. The D2/D3 ligand [^{125}I]-NCQ 298 labeled a moderate density of sites in the dorsal, median and paramedian raphe nuclei and the anterior, ventral and dorsal tegmental nuclei. [^{125}I]-7-OH-PIPAT (0.2 nM), which has been shown to be D3-selective in the presence of GTP (300 μM) and DTG (1 μM), labeled a low density of sites in the median and paramedian raphe nuclei as well as in the tegmental nuclei. No labeling was detected in the dorsal raphe nucleus. Binding of [^{125}I]-7-OH-PIPAT was unaltered by the addition of the 5-HT_{1A}-selective antagonist MPPF (100 nM) to the assay buffer indicating that it does not label 5-HT_{1A} receptors under these conditions. A second D3 ligand, [^{125}I]-5-OH-PIPAT (0.25 nM), produced an identical pattern of labeling. Animals were lesioned with the neurotoxin 5,7 DHT to determine whether the receptors labeled by [^{125}I]-7-OH-PIPAT are located on serotonin containing cells in the raphe nuclei. Extensive lesion of serotonergic neurons did not alter [^{125}I]-7-OH-PIPAT binding, suggesting that D3 receptors in the median and paramedian raphe are not located on serotonin-containing neurons. D3 receptor expression in the nucleus accumbens and substantia nigra was also unchanged following 5,7 DHT lesion. (Supported by NS-18591, MH-48125, and MH-51880.)

330.13

FUNGAL TRIPRENYL PHENOLS SHOW AFFINITY TO THE BRAIN DOPAMINE D_1 RECEPTOR *IN VITRO*. K. Dekermendjian[1], M.R. Witt[1], O. Sterner[2] and M. Nielsen[1]*. Research Institute of Biological Psychiatry, St. Hans Hospital, DK-4000 Roskilde, Denmark.

From extracts of the mushroom *Albatrellus ovinus* we isolated the four known triprenyl phenols scutigeral, ilicicolin B, neogrifolin and grifolin as well as the two new triprenyl phenols metabolites which we named ovinal and ovinol. All six triprenyl phenols competitively inhibited the *in vitro* binding of ^{3}H-SCH 23390 (R-(+)--7 chloro-8 hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydrol-1H-3-benzazepin-7-ol) to the dopamine D_1 receptor subfamily in rat striatal brain membranes. The IC_{50} values were in the range of 2.6 - > 50 μM. Grifolin additionally inhibits the specific binding of ^{3}H-spiperone (IC_{50} 20.2 μM) to the dopamine D_2 receptor subfamily and is therefore not subfamily specific. Ovinal, ovinol, scutigeral, neogrifolin and ilicicolin B had no effect on ^{3}H-spiperone binding suggesting that these compounds selectively interact with the dopamine D_1 receptor subfamily. The structure of the compounds was determined by NMR spectroscopy.

330.14

HIGH AFFINITY AGONIST BINDING TO THE α_{2C}- ADRENOCEPTOR IN THE OPOSSUM KIDNEY CELL LINE. A. L. KIRIFIDES*, M.A. LEWIS and E. E. CODD. Drug Discovery, The R.W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477.

Human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors (AR) belong to the family of membrane bound G-protein coupled receptors regulated by mono and divalent cations and guanine nucleotides. Whereas many previously published radioligand binding assays used conditions conducive to formation of the agonist low affinity state of the receptor, the present study was performed using 50 mM Tris buffer in the presence of 5 mM $MgCl_2$ to promote formation of the agonist high affinity state. We previously studied human cloned receptors under these conditions and were able to differentiate agonists from antagonists by their binding characteristics. The present study used an OK cell line derived from an opossum kidney which expresses the α_{2C}-AR. Saturation studies using the α_2-antagonist [^{3}H]-MK-912 fit a single site model with a K_d value of 0.044 nM. Similarly, competition curves by several antagonists were best fit by a one site model. In contrast, competition curves by several agonists were best fit by a two site model. Agonist inhibition curves conducted in the presence of GTPγS (G-protein uncoupling agent) were best fit by a single site model with affinities similar to the low affinity site found in the presence of $MgCl_2$ (high affinity binding conditions). Comparison of these results with those obtained from the human cloned α_{2C}-AR produced similar results. In both studies, antagonist inhibition curves were best fit by a single site model whereas agonist data were best fit by a two site model. These and related data suggest that the present assay conditions can be used to distinguish agonist from antagonist binding to α_{2C}-AR in the OK cell line.

330.15

HIGH AFFINITY ^{3}[H]-MK-912 BINDING TO α_{2B}-ADRENOCEPTORS IN NEONATAL RAT LUNG . M.A. LEWIS*, A.L.KIRIFIDES and E. E. CODD. Drug Discovery Research, The R.W. Johnson Pharmaceutical Research Institute, Welsh and McKean Roads, Spring House, PA 19477-0776.

We used the potent and highly selective α_2-adrenoceptor antagonist ^{3}[H]-MK-912 to characterize high affinity binding to the α_{2B}-adrenoceptor subtype in neonatal rat lung. Many previously published radioligand binding assays used conditions that prevent the formation of the agonist high affinity state of the receptor. The present study was performed using 50 mM Tris buffer in the presence of 5 mM $MgCl_2$ to promote formation of the agonist high affinity state of the receptor. Saturation experiments using ^{3}[H]-MK-912 under these conditions fit a single-site model with a K_d value of 0.97 nM. Agonist competition curves better fit a two-site model with approximately 30% of labeled sites in the high affinity state. Agonist competition experiments conducted in the presence of GTPγS and/or NaCl shifted curves to the right and fit a single-site model indicating the uncoupled or low affinity state of the receptor. Antagonist competition curves yielded a high affinity component comprising approximately 90% of the sites with the remaining 10% yielding a tissue unspecific low affinity (>μM) component. These results indicate that this assay, under conditions promoting the agonist high affinity state, may be able to differentiate agonist from antagonist binding to the α_{2B}-adrenoceptor.

330.16

ZINC ALLOSTERICALLY MODULATES ANTAGONIST BINDING TO CLONED D_{1A} DOPAMINE RECEPTORS EXPRESSED IN CHO CELLS. John A. Schetz* and David R. Sibley. Molecular Neuropharmacology Section, ETB/NINDS/NIH, 9000 Rockville Pike, Bethesda, MD 20892.

Zinc ions were found to inhibit antagonist binding to the cloned rat D_{1A} dopamine receptor expressed in Chinese Hamster Ovary (CHO) cells. The nature of zinc's action was characterized by measuring the radioligand binding properties of [^{3}H]-SCH23390 in the presence or absence of zinc ions. Zinc exerts a low affinity (micromolar Ki value), dose-dependent inhibition of the binding of [^{3}H]-SCH23390 primarily by decreasing the ligand's affinity for the D_{1A} receptor. Zinc inhibition is completely reversed by addition of the divalent cation chelator EDTA and not mimicked by an equal concentration of manganese (a cation of similar size and equal charge). This effect is specific for zinc ions as various zinc salts produce identical effects. The mechanism of zinc inhibition appears to be allosteric modulation of the dopamine receptor protein since zinc increases the K_D of ligand binding and Schild plots of zinc inhibition reach a plateau. Moreover, zinc accelerates the dissociation of [^{3}H]-SCH23390 from its receptor. Here we report the inhibition of antagonist binding by zinc, and demonstrate that the mechanism of zinc inhibition is a dose-dependent, reversible and allosteric modulation of the D_{1A} dopamine receptor protein. (Supported by NINDS/NIH).

330.17

EFFECTS OF MEDIAL PREFRONTAL CORTICAL, NIGRAL, AND STRIATAL LESIONS ON STRIATOLIMBIC D_2 AND D_4 DOPAMINE RECEPTORS. F.I. Tarazi*, S.K. Yeghiayan, A. Campbell, and R.J. Baldessarini. Psychiatry & Neuroscience Depts, Harvard Medical School; Mailman Research Center, Belmont, MA 02178.

We examined changes in striatolimbic D_2 and D_4 dopamine (DA) receptor binding following: [1] unilateral surgical ablation of anterior medial prefrontal cortex (MPC) to remove descending glutamatergic projections mainly to the nucleus accumbens (ACC), [2] unilateral injections of 6-hydroxydopamine into substantia nigra (SN) to destroy ascending DA projections, [3] intrastriatal (STR) injections of kainic acid to degenerate intrinsic neurons locally. Rats were sacrificed one week after lesioning; contralateral striata served as controls. D_2 receptors (D_2Rs) were quantified autoradiographically with the D_2-like ($D_2/D_3/D_4$) ligand ^{3}H-emonapride, while D_4Rs were quantified with ^{3}H-nemonapride plus 300 nM S(–)-raclopride to occlude D_2/D_3 receptors, as well as 100 nM pindolol and 500 nM 1,3-ditolylguanidine (DTG) to mask $5HT_{1A}$ and sigma sites. Nigral lesions was verified by the loss of DA transporters (>85%) measured by *in vitro* autoradiography with ^{3}H-GBR-12953. Ablation of MPC and neurotoxin lesioning of SN did not change D_2R or D_4R binding in STR. However, D_4R, but not D_2R binding was significantly decreased (–27%) in ACC after MPC ablation. Kainate injection in STR produced large losses of D_2R (–50%) and D_4R (–22%). These results suggest that the majority of D_2Rs and a portion D_4Rs in STR are on intrinsic neurons postsynaptic to DA afferents, and that some D_4Rs, at least in ACC, may be located presynaptically on glutamatergic afferents.

Supported by MH-34006, MH-31154, MH-47370, and B.J. Anderson Foundation.

330.18

AFFINITY AND INTRINSIC ACTIVITY OF DOPAMINE AGONISTS AND PARTIAL AGONISTS AT THE CLONED hD_{2i}, $hD_{4.4}$, $hD_{4.7}$ RECEPTORS. C.A. Tamminga*, A. Mutin*, E.V. Cochrane*; and R.A. Lahti*. *Maryland Psychiatric Research Center, Univ. of Maryland School of Medicine, Baltimore, MD, USA.

The dopamine D_2-family of receptors is made up of the D_2, D_3 and D_4 receptors (Civelli et al., 1991). Dopamine agonists and partial agonists have been evaluated in the treatment of schizophrenia (Tamminga et al., 1978, 1986, 1992). In this study using receptor state (high- and low-affinity agonist states) binding affinities (Lahti et al, 1992) and hD_{2i}, $hD_{4.4}$, and $hD_{4.7}$ membranes (cells courtesy M. Caron and H.VanTol) the affinity and intrinsic activity of dopamine agonists and partial agonists were determined. For the hD_{2i} receptor affinities at the low-affinity agonist state were determined using [^{3}H]-raclopride + GTP and at the high-affinity agonist state [^{3}H]-U-86170 was used. [^{3}H]-YM-09151-2 + GTP and [^{3}H]-N-0437 were used at both the $hD_{4.4}$ and $hD_{4.7}$ receptor. (+)-3-PPP has high intrinsic activity at both the hD_{2i} and $hD_{4.4}$ receptors. (-)-3-PPP appears to have higher intrinsic activity at the $hD_{4.4}$ (89%) and $hD_{4.7}$ (94%) receptor, than the D_2 (46%). (-)-NPA has higher intrinsic activity at the hD_{2i} (100%) than at the $hD_{4.4}$ (54%). Both (-)-N-0437 and (-)-3-PPP caused substantial releases of [^{3}H]-arachidonic acid from the CHO-$hD_{4.4}$ cells and the effect was significantly reduced by a dopamine antagonist, suggesting that an agonist/antagonist combination may have clinical use as a designed functional partial agonist. These findings may also be of interest in light of the relationship between novelty seeking behavior in humans and the presence of the $hD_{4.7}$ receptor. Results show that some agonists have different affinities and intrinsic activities among the D_2-family of receptors.

Supported by NIH Grant MH-49667-01

330.19

HIGH RESOLUTION SCATCHARD ANALYSIS OF THE D_1 RECEPTOR PYRAMIDAL (PN) AND NONPYRAMIDAL (NP) NEURONS IN THE RAT MEDIAL PREFRONTAL CORTEX (mPFCx). S.A. Davidoff, H.-M. Chu* and F.M. Benes. Laboratory for Structural Neuroscience, McLean Hospital, Belmont, MA; Department of Psychiatry and Program in Neuroscience, Harvard Medical School, Boston, MA.

The results of a recent colocalization study employing fluorescently labelled ligands suggested that D_1 receptors may be preferentially localized on NPs in rat mPFCx. To explore whether the binding affinity (K_d) and/or maximal receptor density (B_{max}) of D_1 receptors may differ with respect to PNs, NPs or in neuropil of rat mPFCx, a Scatchard analytic technique has been adapted to high resolution autoradiography to determine the specific K_d and B_{max} values. Frozen sections of mPFCx were incubated for 45 min at 20°C in five concentrations of tritiated SCH23390 (1-12nM) in the presence or absence of the competitive inhibitor SKF38393 (100nM). The D_1 antagonist was found to bind with a similar affinity to PNs (K_d=1.7nM) and NPs (K_d=2.7nM), as well as in neuropil (K_d=2.2nM) in layer VI of the rat mPFCx. The K_d values obtained were similar to those reported by other groups. It is noteworthy that the B_{max} values for PNs (4.8 grains/100μm^2) and NPs (5.7 grains/100μm^2) were also quite similar. These results are consistent with the idea that D_1 receptors may be broadly distributed in several neuronal compartments of rat mPFCx. Accordingly, Scatchard analyses will provide a useful approach to determining whether various neurotransmitter receptors differ with respect to K_d or B_{max} on PNs, NPs and/or NPL in patients with schizophrenia and other neuropsychiatric disorders. Supported by NARSAD, MH00423 and MH31154.

330.20

INTRINSIC ACTIVITY DETERMINATIONS FOR VARIOUS DOPAMINERGIC AGENTS AT HUMAN D_{2S}, D_{2L}, D_3, AND $D_{4.2}$ RECEPTORS USING [^{35}S]GTPγS BINDING. T. P. Wasik, M. I. Cockett, R. Ochalski and T. H. Andree*. Wyeth-Ayerst Research Inc., CN 8000, Princeton, N.J. 08543.

Agonist-induced stimulation of [^{35}S]GTPγS binding is becoming increasingly valuable as a tool for assessing intrinsic activity at a wide variety of receptors. The present study examined the effects of various dopamine (D_2 family) agonists, partial agonists and antagonists to stimulate or block [^{35}S]GTPγS binding at D_{2S}, D_{2L}, D_3 and $D_{4.2}$ cloned human receptors. In addition, the relative affinity of these compounds for the high affinity (^{3}H-quinpirole) vs the low affinity (^{3}H-spiperone + GppNH) state of the D_2 receptor in rat striatal tissue was determined. Maximal responses obtained with [^{35}S]GTPγS binding in CHO cells with human D_{2S} and D_{2L} receptors correlated well with Ki ratios of antagonist/agonist binding (r = 0.88 and 0.89 for D_{2S} and D_{2L}, respectively) in rat striatal tissue. These results support and extend the results obtained by Tamminga et al. (Soc. Neurosc. 21: #252.9, 1995) and Lahti et al. (Mol. Pharmacol. 42:432, 1992) in characterizing intrinsic activity levels of agonists at the D_{2L} and $D_{4.4}$ receptors. The present results demonstrate the value of [^{35}S]GTPγS binding in determining the intrinsic activity of agents for a variety of dopaminergic receptors and should be of value in discovering new dopaminergic agents for treatment of dopamine-related disorders such as schizophrenia and Parkinson's disease.

330.21

CONSTITUTIVE ACTIVITY OF A CHIMERIC DOPAMINE RECEPTOR L.B.Kozell* and K.A.Neve. Dept of Physiology & Pharmacology, Oregon Health Sciences University & VAMC, Portland OR., 97201.

Chimeric D1/D2 dopamine receptors facilitate the identification of structural determinants of ligand binding and effector coupling. Our previous work has led us to several hypotheses. First, transmembrane region VII (TMVII) is important in the selective potency of several D1-selective ligands. Second, determinants of drug efficacy are dissociable from those for drug affinity at D2 receptors, with TMVII important in selective binding of some agonists, and TMVI important for efficacy. Third, incompatibilities between TMI and TMII from D2, and TMVII from D1 receptors result in non-functional chimeras. To expand upon and test these hypotheses further, we constructed chimeric D1/D2 receptors with D2 receptor sequence at the amino and carboxy terminal ends, and expressed them in HEK293 cells. One of these chimeras, CH6*, with D1 receptor sequence only from TMV through TMVI, had higher affinity for non-selective and most D2-selective agonists, than either D1 or D2 wildtype receptors. The affinity of CH6* for most antagonists and D1-selective agonists fell between D1 and D2 receptor affinities. CH6* coupled to Gs, as was indicated by stimulation of adenylate cyclase by most dopaminergic agonists, although the ability of CH6* to activate adenylate cyclase was reduced when compared to D1 receptors. Thus, the region around and including the 3rd intracellular loop of the D1 receptor was sufficient for coupling to this effector. Several observations suggested that CH6* was a constitutively active receptor. CH6* had increased affinity for most dopamine agonists. In the absence of agonist stimulation, cells expressing CH6* had elevated basal levels of cAMP, which could be inhibited by several dopamine antagonists, including spiperone and clozapine. The D1-selective antagonist, SCH23390, was able to stimulate adenylate cyclase slightly, suggesting that SCH23390 has intrinsic activity at CH6*. [MH45372 and VA Merit Review Program]

CATECHOLAMINES: DOPAMINE II

331.1

DENSITY OF DA NERVE TERMINALS AS ESTIMATED FROM CONCENTRATION-RESPONSE CURVES E. Agneter, M.R. Emmerling*, W. Sauermann, E.A. Singer, T.J. Feuerstein. Neuropharmacology Units, Univ. of Vienna (A-1090, Austria), Parke-Davis (MI 48105Ann Arbor, USA), and Freiburg (D-79106, Germany)

The present study tries to establish a link between functional data on presynaptic autoinhibition of [^{3}H]-DA release and the density of DA nerve terminals in slices of rabbit prefrontal cortex (PFC) and striatum (STR), respectively. Superfusion experiments have shown previously that the behavior of mesocortical and striatal DA terminals during single and repetitive electrical stimulation is different (Agneter et al. 1994, JPET 269:470). The disinhibition of [^{3}H]-DA release by blockade of D_2-autoreceptors with sulpiride allowed to estimate the biophase concentration of endogenous DA. Estimation was based on the finding of a direct proportionality between occupancy of D_2-autoreceptors and resulting inhibition of [^{3}H]-DA release in both tissues. Congruent concentration-inhibition curves and their identical antagonist-induced shifts to the right proved the identity of release-modulating D_2-autoreceptors in PFR and STR. The disinhibition of [^{3}H]-DA release over the frequency range used for electrical stimulation (0.05-100 Hz) was paralleled by the estimated increase in the endogenous DA concentration, being highest at 1 - 10 Hz. The highest DA biophase concentration induced by electrical stimulation was about 7 times higher in the STR than in the PFC. A space parameter λ was defined reflecting the mean distance of all the autoreceptors from all the release sites within the tissue to assess a mutual inhibition of DA terminals by released DA, assuming an exponential decay of the DA biophase concentration with time and distance. The estimate of λ in PFC was about 4 times larger than that in STR.

Autoreceptors on a single STR terminal are not only activated by DA released from this very same terminal, but are also activated by DA released from neibourghing terminals. Therefore, autoinhibition of DA release is more pronounced in the STR than in the PFC were, due to the lower density of DA terminals, such an intense mutual inhibition of DA terminals does not exist.

The study was supported by grants from the Ministry of Sience to E.A, and T.J.F.

331.2

BLOCKADE OF 5-HT_3 RECEPTORS INCREASES DOPAMINE RELEASE IN NEOSTRIATUM. AN IN VIVO MICRODIALYSIS STUDY IN CONSCIOUS RATS. X.-M. Li*, K. W. Perry, D. Wong and R. W. Fuller. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana

The in vivo modulation of dopamine (DA) release in rat neostriatum by one of serotonin (5-HT) receptor subtypes 5-HT_3 receptor, has been studied by means of a microdialysis analysis performed in conscious rats. The results indicate a possible inhibitory regulation of DA release by 5-HT_3 receptors in the neostriatum according to the following findings: (1). A 5-HT_3 antagonist, zatosetron (LY277359), perfused into neostriatum significantly increased extracellular DA level in the neostriatum in a dose-dependent way with a peak increase found at 50 μM; (2). While LY306801, an inactive isoform of zatosetron, at same dose (50 μM) did not change the DA level; (3). Consistently another 5-HT_3 antagonist, LY278584 which has an affinity for 5-HT_3 receptors about 1/3 of that of zatosetron, at 150 μM also caused a significant increase in the DA level; (4). In the presence of 50 μM, but not 5 μM zatosetron a further increase in the DA level was achieved when the extracellular 5-HT level was risen by systemic co-administration of a 5-HT uptake inhibitor, fluoxetine, and the 5-HT precursor, 5-HTP.

The present studies demonstrating a specific and selective pharmacological action of zatosetron at 50 μM on DA release, indicate that 5-HT_3 receptors probably mediate the negative regulation of DA release in neostriatum.

331.3

SPATIAL MAPPING OF EVOKED DOPAMINE RELEASE IN STRIATUM WITH MICROELECTRODE ARRAYS, S.F. Dressman, A.C. Michael*, Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260.

Electrical stimulation of the medial forebrain bundle in rats causes dopamine release in striatum that can be monitored by cyclic voltammetry at microelectrodes constructed from 7-μm diameter carbon fibers. Stimulation responses recorded with cylindrical electrodes, 200-400 μm long, depend very little on the exact placement of the electrode in striatum, which implies that cylindrical electrodes measure a spatially averaged response. The responses recorded at disk electrodes, prepared so that only the cross section of the fiber serves as the active region of the electrode, are highly sensitive to the location of the electrode: movements of the electrode over distances as small as 40 μm can cause dramatic changes in the stimulation response. This implies that stimulated dopamine release is heterogeneous on a micrometer scale. When disk electrodes are used, the change in the stimulation response induced by nomifensine administration (20 mg/kg i.p.) is also highly dependent on the specific features of the recording site.

Mapping the spatial distribution of dopamine release in striatum on a micrometer scale is important because local dopamine concentrations measured at disk electrodes are not linearly related to each other, nor to spatially averaged values measured at cylinder electrodes. Arrays of microelectrodes are being developed for this purpose. These arrays consist of four individually-addressable, 1-μm diameter, disk electrodes separated in space by 10-20 μm.

Supported by a grant from the National Institutes of Health (NS31442).

331.4

EFFECT OF HANDLING STRESS ON AMYGDALA DOPAMINE RELEASE: COMPARISON WITH OTHER DOPAMINERGIC REGIONS E. M. Inglis* and B. Moghaddam, Department of Psychiatry, Yale University School of Medicine, VA Medical Center 116A/2, West Haven, CT 06511

Several studies have demonstrated a role for catecholamines in the amygdalar complex in stress, arousal and conditioned behaviours. Using in vivo cerebral microdialysis, TTX-sensitive and calcium-dependent dopamine efflux was assessed in the basolateral nucleus of the amygdala in the rat. The effects of twenty minutes of handling on dopamine release in the basolateral amygdala was examined, and the response compared with three other dopamine-containing regions, the medial prefrontal cortex, the nucleus accumbens, and the caudate nucleus. In agreement with previous reports, dopamine release increased during stress in the prefrontal cortex. Dopamine efflux also increased in the nucleus accumbens, but was unaltered in the caudate nucleus. In the nucleus accumbens and prefrontal cortex, a two-fold increase in dopamine occurred in response to stress: this increase was largest in the twenty minute sample period after the cessation of stress. During this period, rats showed significant behavioural arousal, such as exploratory behaviors and grooming. Dopamine efflux from the basolateral amygdala also increased two-fold during handling stress; in contrast with other areas, this response was maximal during the application of the stress, and returned to baseline more rapidly than in other structures examined. These results provide evidence for differential regulation of dopamine release in these brain regions in response to handling stress.

This work was supported in part by US PHS awards, MH-48404, MH-44866.

331.5

EFFECTS ON *IN VIVO* DOPAMINE RELEASE IN THE STRIATUM AND IN THE SUBSTANTIA NIGRA BY VOLTAGE-SENSITIVE CALCIUM CHANNEL BLOCKERS. F. Bergquist*, J. Jonason and H. Nissbrandt, Department of Pharmacology, Göteborg University, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

Dopamine (DA) is released not only from the terminals of the nigro-striatal neurons but also from their dendrites. The importance of voltage-sensitive calcium channels (VSCC) for terminal DA release in the striatum has hitherto mostly been characterised by *in vitro* studies and overall very few studies on their importance for nigral dendritic DA release has been published.

The aim of the present study was therefore to comprehensively investigate in vivo the relative physiological importance of L-, N- and P/Q/R-type VSCC in the striatum and in the substantia nigra pars reticulata (SNr). The effects of local perfusion of Ca^{2+}-free buffer and specific VSCC blocking drugs on DA release were determined by microdialysis in awake rats.

Perfusion with Ca^{2+}-free buffer decreased DA release by 90% and 70%, respectively, in the striatum and in the SNr. In both brain parts the L-type VSCC blocker, nimodipine (100 μM), decreased DA release modestly (by 10% to 15%) whereas neomycin (0.5 mM), an unselective P/Q/R-type VSCC blocker, decreased the release by 75% to 85%. The N-type VSCC blocker ω-Conotoxin GVIA (1.0 μM) and the P-type VSCC blocker ω-Agatoxin IVA (1.0 μM) inhibited DA release in the striatum by 55% and 80%, respectively, but unexpectedly induced an increase of DA release by 30% and 45%, respectively, in the SNr.

Our observations support a functional coexistence of different VSCC-types on striatal DA terminals with a dominance of the P-type of VSCC. The clear-cut effect of neomycin in the SNr suggests a role for P/Q/R-type VSCC. However, the stimulatory effects of the selective N- and P- type VSCC blockers are obscure. Tentatively, the effects of these drugs may be explained by indirect effects due to decreased release of various transmitters localized in nigral afferents.

Supported by Åhlén, Bergvall, Stohnes, Jeppson and Pfannenstill Foundations.

331.6

IN SITU DETERMINATION OF DOPAMINE RELEASE AND UPTAKE IN FREELY MOVING RATS. P. A. Garris*, J. R. C. Christensen, G. V. Rebec and R. M. Wightman, Department of Biological Sciences, Illinois State University, Normal, IL 61790; Department of Chemistry and Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599; Department of Psychology and Program in Neural Science, Indiana University, Bloomington, IN 47405

A technique was established to monitor dopamine in real time in brain extracellular fluid of freely behaving rats. Parameters describing *in situ* dopamine release and uptake processes were determined from these measurements. Fast-scan cyclic voltammetry at carbon-fiber microelectrodes was employed to monitor dopamine elicited by electrical stimulation of the substantia nigra. Extracellular dopamine in the striatum was frequency and current dependent. When low frequencies and currents were applied, detectable levels of dopamine could be elicited without a perceptible behavioral response. An exciting finding was that evoked concentrations of dopamine were significantly higher in freely moving compared to anesthetized rats. Our findings indicate that this difference is the result of an inhibitory effect of anesthesia on dopamine release. In contrast, dopamine uptake is unaffected. *Conclusions*: (1) fast-scan cyclic voltammetry at carbon-fiber microelectrodes is a viable technique for the measurement of dopamine in brain extracellular fluid of freely behaving rats and (2) transient changes in striatal concentrations of extracellular dopamine elicited by electrical stimulation are affected by anesthesia. This research supported by NSF (IBN 9222349).

331.7

EFFECTS OF HALOPERIDOL AND NOMIFENSINE ON EVOKED DOPAMINE EFFLUX IN AMBULANT RATS. J.R.C. Christensen*[1], P.A. Garris[2], R.M. Wightman[3], and G.V. Rebec[1]. [1]Program in Neural Science and Dept. Psychology, Indiana University, Bloomington, IN 47405; [2]Dept. Biological Sciences, Illinois State University, Normal, IL 61790; [3]Dept. Chemistry and Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599.

We used fast-scan cyclic voltammetry at carbon-fiber microelectrodes to assess the effects of haloperidol, a dopamine (DA) receptor antagonist, and nomifensine, a DA reuptake blocker, on extracellular DA in the striatum of freely moving rats. DA-containing neurons in the substantia nigra compacta were electrically stimulated over a frequency range of 10 - 60 Hz. Haloperidol (0.5 mg/kg, sc) produced an increase in maximal efflux inversely proportional to stimulation frequency with a maximum response of 300% at 10 Hz, but no change at 60 Hz. Nomifensine (5.0 mg/kg, sc), in contrast, produced a constant increase of approximately 200% at each stimulation frequency. Taken together, these results suggest that in awake, unrestrained rats inhibitory autoreceptors modulate DA release only at low stimulation frequencies, while blockade of reuptake continues to be a major factor in the clearance of synaptic DA efflux even at high frequencies.

Supported by NIDA (DA 02451) and NSF (IBN-9222349).

331.8

DOPAMINE RELEASE AND INTRACRANIAL SELF-STIMULATION. *R. M. Wightman* and P. A. Garris.* Department of Chemistry and Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599-3290; Department of Biological Sciences, Illinois State University, Normal, IL *61790-4120*

Animals with stimulating electrodes implanted in select regions of the brain repeatedly press a lever to receive a train of electrical pulses. Considerable evidence suggests that dopamine plays a role in this behavior termed intracranial self-stimulation (ICSS)[1]. In this study, fast-scan cyclic voltammetry was employed to investigate relationships betweeen extracellular dopamine and ICSS with high temporal resolution. Under anesthesia, a stimulating electrode was permanently implanted in the ventral tegmental area at a site that elicits dopamine release in the nucleus accumbens as measured by a carbon-fiber microelectrode. ICSS was observed in all animals tested following recovery from surgery. The stimulus trains (24 pulses at 60 Hz, 1ms pulse widths, 40-60 μA amplitude) that supported ICSS also evoked dopamine release when applied by the experimenter to freely moving rats. However, in only one out of three animals was dopamine clearly observed during ICSS. The preliminary conclusion is that dopamine release can occur during ICSS but is not necessary. This research supported by NSF (IBN 9222349).

[1]Phillips, A.G., Fibiger, H.C., Neuroanatomical bases of intracranial self-stimulation: untangling the Gordian knot, in The Neuropharmacological Basis of Reward, J.M. Liebman, S.J. Cooper, eds., Clarendon Press, Oxford (1989).

331.9

THE EFFECTS OF CHOLECYSTOKININ FRAGMENT 26-33, SULFATED (CCK-8S), ON DOPAMINE (DA) RELEASE FROM OLFACTORY TUBERCLE AND STRIATAL SLICES *IN VITRO*. Mark D. Barsamian, Cheryl Aretha, and Matthew P. Galloway, Cellular & Clinical Neurobiology Program, Dept. of Psychiatry & Behavioral Neurosciences, Wayne State University Sch of Med, Detroit, MI 48202.

CCK-8S is colocalized within DA terminals of both the mesolimbic and mesostriatal systems, and where CCK containing 'dense-core' vesicles co-store DA, CCK-8S is released from DAergic terminals. Microiontophoretic administration of CCK-8S excites the firing rate of identified midbrain DA neurons and locally perfused CCK-8S augments DA release at a number of DA terminal projection sites. Furthermore, clinical studies have implicated CCK-8S as an anxiogenic compound since administration of CCK-8S fragments (CCK-4 or pentagastrin) induced panic attacks. Therefore, to further characterize DA-CCK interactions, we examined the effects of CCK-8S (0.1-100 nM) on DA release *in vitro* using either rat striatal (STR) or olfactory tubercle (OT) slices. Incubation with 100 nM CCK-8S did not significantly alter basal DA release in either the STR (25±3 vs. 37±8 nM/mg) or OT (21±4 vs. 15±1 nM/mg). However, incubation with 0.1 nM CCK-8S significantly enhanced K^+-stimulated (25 mM) DA release in slices from both regions (STR: 73±4 vs. 150±7 nM/mg, $p < 0.001$; OT: 70±7 vs. 126±16 nM/mg, $p < 0.009$). These results are consistant with previous suggestions that CCK-8S may suppress an auto-inhibitory mechanism and that CCK-8S may increase Ca^{++} influx. Support: NIDA-04120 and Joe Young Sr. Research Fund.

331.10

ENDOGENOUS NITRIC OXIDE RELEASES STRIATAL DOPAMINE *IN VIVO* VIA A GLUTAMATE-MEDIATED FACILITATION. *Anthony R. West, Maureen E. Stress, and Matthew P. Galloway, CCN Program, Psychiatry and Behavioral Neurosciences, Wayne State University, Detroit, MI 48202.

We have shown previously that intra-striatal infusion of nitric oxide (NO) donors released vesicular stores of dopamine (DA) in a calcium-dependent manner via an *N*-methyl-D-aspartate (NMDA) receptor-dependent mechanism. As an extension of these studies, we have investigated the influence of the nitric oxide synthase (NOS) substrate, N^G-hydroxy-L-arginine (HO-ARG) on striatal DA and glutamate (GLU) release *in vivo*. Local infusion of HO-ARG (either 100μM or 1 mM for 120 min) increased DA release to 57± 5% and 122 ± 28% above basal levels respectively. Additionally, perfusion with HO-ARG (1mM) increased extracellular (EC) GLU to 71 ± 9% over basal concentrations with a time course similar to that observed for DA. Unexpectedly, HO-ARG (2mM) did not increase EC DA when infused under similar conditions. The DA releasing effect of HO-ARG (1mM) was abolished following pretreatment (80 min) with the constitutive NOS inhibitor 7-nitroindazole (7-NIT). Intra-striatal administration of the NMDA receptor antagonist MK-801 (10μM administered 80 minutes prior to and during HO-ARG delivery), significantly attenuated the HO-ARG (1mM)-induced elevation of EC DA levels to approximately 32 % above baseline. Combined with our previous work (J. Neurochem. 66: 1971-80, 1996), these results demonstrate that both endogenous and exogenous NO releases vesicular stores of striatal DA via GLU-mediated NMDA-receptor activation.

Supported by NIDA-04120 and Joe Young Sr. Research Fund.

331.11

EVIDENCE THAT EXTRACELLULAR DOPAMINE REGULATES DOPAMINE SYNTHESIS *IN VIVO*: A MICRODIALYSIS STUDY. Richard S.J. Nassar and Matthew P. Galloway, Cellular & Clinical Neurobiology Program, Psychiatry & Behavioral Neurosciences, Wayne State U Sch Med, Detroit, MI 48202

To assess striatal tyrosine hydroxylase (TH) activity (dopamine (DA) synthesis) *in situ*, extracellular DOPA levels were monitored after intrastriatal perfusion of the amino acid decarboxylase inhibitor α-difluoromethyl-DOPA (MDL 71,801). Increasing endogenous levels of DA (137 ± 24%) by the intrastriatal perfusion of the DA transporter inhibitor GBR-12909 (10μM) decreased extracellular DOPA levels by 40 ± 8% ($p<0.01$). Blockade of DA autoreceptors with eticlopride (100μM) prevented the GBR-12909 induced decrease of extracellular DOPA. Conversely, the intrastriatal perfusion of either tetrodotoxin (3μM) or Ca^{++}-free artificial cerebral spinal fluid decreased extracellular DA levels (>80% reduction) with a simultaneous increase of extracellular DOPA levels (69 ± 6% and 126 ± 22%, respectively). The increase in DOPA levels was ascribed to the loss of dopaminergic tone at DA synthesis modulating autoreceptors since replacement of the tone with quinpirole (100μM) returned extracellular DOPA levels to basal levels without effecting DA levels. Additionally, intrastriatal perfusion of dibutryl-cAMP (to promote phosphorylation of TH) transiently increased extracellular levels of DOPA by 33 ± 9% ($p<0.01$). The results suggest that *in vivo* microdialysis is a useful technique to dynamically monitor both TH activity and DA autoreceptor function. Moreover, the results provide direct evidence that extracellular striatal DA regulates DA synthesis *in vivo* and support a physiological role for DA autoreceptors. Supported by NIDA-04120 and the Joe Young Sr. Research Fund.

331.12

EFFECTS OF NEUROPEPTIDE Y AND SIGMA RECEPTOR LIGANDS ON NMDA-STIMULATED [^{3}H]DA RELEASE FROM SLICES OF RAT NUCLEUS ACCUMBENS. D.T. Ault*, J.M. Radeff, and L.L. Werling, Neuroscience Program and Dept. of Pharmacology, The George Washington University Medical Center, Washington, DC 20037.

Although the identity of the endogenous ligands for sigma receptors is unknown, various neuropeptides and steroids have been shown to compete for binding to these receptors. Among endogenous sigma receptor ligand candidates is neuropeptide Y (NPY), which mimics electrophysiological actions of sigma ligands in some preparations. We have previously shown that prototypical sigma agonists such as (+)pentazocine and BD737 inhibit stimulated [^{3}H]DA release in various brain regions. Using a superfusion system, we compared the effect of NPY on stimulated [^{3}H]DA release to the effects of these ligands. In contrast to (+)pentazocine- and BD737-mediated inhibition of release, NPY enhanced release at nM concentrations. However, the same sigma antagonists (BD1008, DUP734, haloperidol and DTG) that reverse (+)pentazocine- or BD737-mediated inhibition, as well as a Y receptor antagonist, PYX_1, all reversed the enhancement. To facilitate determination of the receptor through which NPY-mediated enhancement of release occurs, the effects of ligands selective for Y receptor subtypes were compared to that of NPY. In addition, the effect of PYX_1 on BD737-mediated inhibition of release was investigated. Our findings suggest an overlap in populations of receptors currently designated sigma and Y. These findings may be important because regulation of dopamine release in the nucleus accumbens is critical for both the positive symptoms of schizophrenia and for reinforcement caused by drugs of abuse. (Supported by a NIDA grant to LLW.)

331.13

COMPARISON OF THE EFFECTS OF HALOPERIDOL ADMINISTRATION ON AMPHETAMINE-STIMULATED DOPAMINE RELEASE IN THE RAT PREFRONTAL CORTEX AND DORSAL STRIATUM. E.A. Pehek* and M.A. Schaldach, Dept. Psychiatry, Case Western Reserve University and Cleveland VAMC, Brecksville, OH 44141.

Previous studies have shown that haloperidol administration potentiates the neurochemical effects of dopamine (DA) uptake blockers on nigrostriatal DA neurons. Other work has demonstrated that basal DA release in the mesocortical system is relatively insensitive to the effects of D2 antagonists such as haloperidol. The present study thus examined whether a similar interaction between haloperidol and the DA releaser/uptake blocker d-amphetamine (AMPH) would be observed in the medial prefrontal cortex (mPFC) relative to the dorsal striatum. *In vivo* microdialysis was employed in male, Sprague-Dawley rats in order to recover extracellular fluid for subsequent determination of DA levels by HPLC/ED. All drugs were administered i.p. Haloperidol (1.0 mg/kg) was given 30 min prior to d-AMPH (5.0 mg/kg). In the striatum, AMPH increased DA levels from 5.42 pg/20 μl to 151.02 pg at 60 min post-administration. Haloperidol pre-treatment potentiated this increase to 212.38 pg, or 41% greater than AMPH alone. In the mPFC, AMPH increased DA levels from 0.51 pg/20 μl to 3.87 pg at 60 min post-injection. Haloperidol potentiated this increase to 8.23 pg, or 113% greater than AMPH alone. Pre-treatment with 20 mg/kg apomorphine blocked the haloperidol potentiation in the striatum (value = 159.68 pg at 60 min post-AMPH) but not in the mPFC (value = 10.52 pg). A dose of 2.0 mg/kg apomorphine did, however, attenuate the potentiation in the cortex (value = 5.16 pg). These results demonstrate that haloperidol augments transporter-dependent DA release in both the mesocortical and nigrostriatal systems. This potentiation was greater in the mPFC and was at least partially mediated by antagonism of DA D1/D2 receptors.

Supported by NIH grant MH-52220 and the PhRMA.

331.14

RELATIONSHIP BETWEEN PLASMA AND PLATELET CATECHOLAMINES UNDER BASAL AND ACUTE STRESS CONDITIONS. L. Jerabek*, J.P. Boulenger, F.B. Jolicoeur, Psychiatry and Pharmacology Dpt., Sherbrooke University, Quebec, Canada, J1H 5N4.

One of the major difficulties in assessing the role of catecholamines is related to their short half-life in plasma or urine. Recently, it has been proposed that measuring platelet catecholamine concentrations could alleviate this problem. Platelet catecholamine levels have been shown to reflect the overall sympatho-adrenal activity during the 8-day long life-time of the platelet and to be only modestly affected by acute and short-lived elevations in plasma concentrations following psychological and physical stress. In the present study, we examined the relationship between plasma and platelet concentrations of catecholamines under basal and acute stress conditions. For this latter purpose, we used cholecystokinin tetrapeptide (CCK_4), a neuropeptide known to induce physiological and psychological stress in humans. Sixteen healthy subjects were subjected to CCK_4 challenge (25 μg I.V.) and their blood samples were taken before and repeatedly after the administration. Plasma and platelet norepinephrine (NE) and epinephrine (EPI) levels were measured using HPLC-ECD. Results indicate that CCK_4 administration increases both plasma and platelet levels of NE and EPI; however, the time course for each blood compartment is markedly different. On the other hand, no correlation patterns were found between plasma and platelet NE or EPI concentrations, under neither basal nor acute CCK_4-induced stress. Nevertheless, significant positive correlations between basal and post-stress NE levels were observed in plasma but not in platelets. For EPI, positive correlations were found between basal and post-stress levels in both blood fractions. In conclusion, our results reveal that both plasma and platelet catecholamine concentrations are affected by acute stress, albeit with different time course. Furthermore, our results indicate a poor co-relationship between plasma and platelets. Funded by FRSQ and FCAR

331.15

EFFECT OF TOLCAPONE, A CATECHOL O-METHYLTRANSFERASE INHIBITOR, ON BRAIN DOPAMINE METABOLISM IN AMPHETAMINE- OR PARGYLINE-TREATED RATS: A MICRODIALYSIS STUDY.

M. Törnwall, P. Tuomainen, S. Kaakkola[1]* and P.T. Männistö[2]. Dept. of Pharmacol. Toxicol., Inst. Biomedicine, and [1]Dept. of Neurology, University of Helsinki, Finland; [2]Dept. of Pharmacol., University of Uppsala, Sweden.

Inhibitors of catechol O-methyltransferase (COMT) are novel drugs to be used as adjuncts for L-dopa therapy in Parkinson's disease. One of these substances, tolcapone, inhibits COMT both in peripheral tissues and in the brain. In our previous studies, the new COMT inhibitors were found to increase the availability of L-dopa to the brain by preventing its metabolism to 3-O-methyldopa by COMT in the intestinal wall. This salvage is particularly important when the peripheral dopa decarboxylase is inhibited and L-dopa is not converted to dopamine outside the brain. In the present study, we used *in vivo* microdialysis to examine the effects of tolcapone (30 mg/kg) on endogenous dopamine metabolism in anaesthetized rats treated either with amphetamine (5 mg/kg) or pargyline (75 mg/kg). Amphetamine- or pargyline-induced decreases in the levels of extracellular 3,4-dihydroxyphenylacetic acid were counteracted by tolcapone. Tolcapone decreased homovanillic acid effluxes below those caused by amphetamine or pargyline, but dopamine effluxes were not further enhanced. These results show that central metabolism of dopamine can be modulated by COMT inhibition even without exogenous L-dopa. However, extracellular dopamine levels are not easily increased.

Supported by: Sigrid Juselius Foundation

331.16

EFFECT OF S-ADENOSYL-L-METHIONINE ON DOPAMINE METABOLISM IN THE RAT STRIATUM: AN IN-VIVO MICRODIALYSIS STUDY.

T. Bottiglierr and K. Hyland. Kimberly H. Coutwright and Joseph W. Summers Institute of Metabolic Disease at Baylor University Medical Center, and the Dept of Neurology University of Texas Southwestern Medical School, Dallas, TX, USA.

Using in-vivo microdialysis we studied the effect of acute and chronic S-adenosyl-L-methionine (SAMe) administration on dopamine metabolism in the striatum of male Sprague Dawley rats (250-300 g). Microdialysis probes were implanted and animals allowed to recover. The next day probes were perfused with artificial CSF (1µl/min) and samples collected every 20 minutes for analysis of SAMe, dopamine, DOPAC, HVA, & 3-MT. Baseline levels were established over 1 hr then rats were injected with either S-adenosyl-L-methionine butane disulphonate (SAMe-SD4; 200 mg/Kg i.p.) or butane disulphonate (vehicle control) and samples collected for a further 3 hrs. In chronic studies (SAMe-SD4 200 mg/kg/day for 7 days i.p.), dialysis probes were implanted on the 6th day. Rats received the final SAM-SD4 injection the next day and after 30 minutes were perfused with NSD 1015 (100 µM in artificial CSF) and in-vivo tyrosine hydroxylase activity indirectly assessed by the accumulation of L-DOPA.

Dialysate concentrations of SAMe increased after a single i.p. injection of exogenous SAMe-SD4, an effect that was augmented by a repeat injection. Acute injection of SAMe-SD4 had no effect on striatal dialysate concentrations of dopamine or its metabolites. Chronic treatment led to a significantly greater accumulation of L-DOPA in the SAMe-SD4 treated rats as compared to rats receiving vehicle. Our findings indicate that peripherally administered SAMe-SD4 increases brain extracellular levels of SAMe and causes activation of TH. This effect of SAMe on dopamine metabolism may be the mechanism for its antidepressant action.

331.17

A PARAMETRIC ATLAS OF THE METABOLISM OF [^{3}H]DOPA IN BRAIN OF LIVING RAT

P. Deep, H. Kuwabara[1], A. Gjedde[2], J. Reith*[2], and P. Cumming. McConnell Brain Imaging Centre, Montreal Neurological Institute, Montreal, Canada H3A 2B4, [1]West Virginia, [2]Aarhus, Denmark.

We have developed a multi-compartmental model describing the uptake and metabolism of [^{3}H]DOPA in living human brain. Non-linear regression analysis of time-activity curves obtained by PET yield estimates of unidirectional blood-brain clearance (K_1, ml g^{-1} min^{-1}), relative activity of DOPA-decarboxylase (DDC, k_3, min^{-1}) and the rate constant for the net elimination of [^{3}H]dopamine formed within brain (k_{out}, min^{-1}). The net blood-brain clearance (K, ml g^{-1} min^{-1}) is calculated by linear means. In order to validate this PET method, similar approaches were used to calculate the corresponding kinetic parameters in rat brain from a time-series (5-240 min circulation) of autoradiograms obtained from individual rats (n=13) receiving [^{3}H]DOPA (200 µCi, i.v.), all scaled to a common arterial input curve.

	K_1	k_3	k_{out}	K
caudate	0.047±0.001	0.07±0.01	0.018±0.001	0.009±0.001
n. accumbens	0.052±0.002	0.11±0.01	0.023±0.001	0.011±0.001
olf. tubercle	0.057±0.001	0.08±0.01	0.026±0.001	0.010±0.001
amygdala	0.043±0.001	0.05±0.02	0.031±0.002	0.004±0.001
hippocampus	0.044±0.001	0.03±0.01	0.029±0.003	0.003±0.002
hypothalamus	0.059±0.002	0.03±0.01	0.031±0.002	0.005±0.001

We conclude that the autoradiographic method permits estimation of the kinetic parameters in multiple rat brain regions. The unidirectional blood-brain clearance of [^{3}H]DOPA is nearly homogenous, while values for DDC activity vary considerably between rat forebrain regions. [^{3}H]Dopamine formed in brain is eliminated more rapidly from the ventral forebrain and hypothalamus than from the basal ganglia. Net clearance (K) of tracer into brain is the composite of K_1 and k_3 values. This work was supported by the MRC (Canada) SP-30.

331.18

IN VIVO PHARMACOLOGY OF DIHYDROXYPHENYLACETALDEHYDE (DOPAL).

F. Fornai*, M.T. Torracca, L. Bassi, A. Colzi, R. Maggio and G.U. Corsini. Inst. of Pharmacology, Univ. of Pisa, Italy

Using brain dialysis coupled with HPLC with coulometric electrochemical detection we measured dihydroxyphenylacetaldehyde (DOPAL) in the rat trans-striatal dialysate. The peak identified as DOPAL in vivo had the same retention time measured for the enzymatically and chemically synthesized DOPAL which was identified by GLC-mass spectrometry. Based on these findings we carried out a series of experiments to measure, directly in the rat striatal extracellular fluid, DOPAL levels after various pharmacological treatments. MAO-A inhibition suppressed, whereas MAO-B inhibition did not modify DOPAL levels in the dialysate. Administration of non-specific MAO irreversible inhibitors or the co-administration of specific reversible MAO-A together with MAO-B inhibitors led to the same decrease of extracellular DOPAL obtained after administration of single MAO-A inhibitors. Blockade of aldehyde dehydrogenase (ALDH), or microinfusion of DA through the dialysis probe increased DOPAL extracellular levels. Similarly, administration of a DA vesicular releaser produced a slight increase of DOPAL. By contrast, the selective DA uptake inhibitor GBR 12 909 did not significantly modify striatal extracellular DOPAL concentration. Methamphetamine, at different doses, suppressed striatal DOPAL levels. The assay of DOPAL together with DOPAC represents a reliable tool to measure directly, in freely moving animals, DA oxidative metabolism distinguishing between MAO and ALDH activity. Recent studies (Mattamal, 1995) have shown that microinfusions of exogenous DOPAL cause neurotoxicity; in line with this, in vivo measurement of DOPAL could shed new lights on the molecular basis of neurodegenerative processes involving monoaminergic neurons.

331.19

INDIRECT EVIDENCE FOR DOPAMINE METABOLISM BY CYTOCHROME P450 2E1 IN THE SUBSTANTIA NIGRA IN VIVO.

H. Nissbrandt*, F. Bergquist, J. Jonason, A. Elverfors and G. Engberg. Department of Pharmacology, Göteborg University, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

The major dopamine (DA) metabolites in the brain are 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid and 3-methoxytyramine. Other alternative pathways for DA must, however, also be taken into consideration, although no data are available on the quantitative importance of alternative catabolic pathways. Such pathways for DA are conjugation of DA with sulphate or glucuronide, formation of 5-S-cysteinylDA or condensation of DA with aldehydes that will yield isoquinolines.

We have previously presented measurements of DA synthesis and catabolism in the substantia nigra (SN) suggesting that a substantial part of synthesised DA in this brain part is metabolised by unknown non-classical metabolic pathways.

In the SN in rat brain relatively high densities of cytochrome P450 2E1 have been detected. The aim of the present study was therefore to investigate the possibility that this enzyme directly or indirectly is involved in the metabolism of DA in the SN.

Administration of the specific inhibitor of cytochrome P450 2E1, diallyl sulfide (200 mg/kg, an ingredient from garlic) in vivo to rats induced a 30% increase in DOPAC concentrations in the SN but not in the striatum. Diallyl sulphide (200 mg/kg) also increased DA release by around 50% in the SN when measured with microdialysis in awake animals.

These data suggest the possibility that cytochrome P450 2E1 directly or indirectly is involved in the metabolism of DA in the SN. The presence of the enzyme in the SN is especially interesting since the enzyme is known to produce superoxide radicals which can induce cell injury and cell death.

Supported by Swedish Medical Research Council, Gothenburg Medical Society, Åhlén, Bergvall, Stohnes, Jeppson and Pfannenstill Foundations.

331.20

BOTH MAO A AND MAO B METABOLIZE DOPAMINE SYNTHESIZED FROM L-DOPA IN SQUIRREL MONKEYS.

D.A. Di Monte, I. Irwin, L.E. DeLanney, P. Chan, G.M. Petzinger* and J.W. Langston. The Parkinson's Institute, Sunnyvale, CA 94089.

Experimental evidence suggests that, under normal conditions as well as after L-dopa administration, MAO A is primarily responsible for dopamine deamination in rodents. The present study was aimed at determining the effects of MAO inhibitors on the metabolism of dopamine synthesized from exogenous L-dopa in the striatum and substantia nigra of squirrel monkeys. Administration of a single dose of L-dopa (methyl ester, 40 mg/kg, i.p.) caused a significant increase in the levels of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and in the DOPAC/dopamine ratio in the putamen, caudate and substantia nigra. These changes were more pronounced in the substantia nigra than in the striatum. When L-dopa treatment was preceded by the injection of clorgyline or deprenyl at a concentration (1 mg/kg) which selectively inhibited MAO A or MAO B, respectively, striatal dopamine was increased while striatal DOPAC and HVA levels and DOPAC/dopamine ratio were significantly reduced as compared to the values obtained with L-dopa alone. The two MAO inhibitors also counteracted the increase in DOPAC and HVA levels and DOPAC/dopamine ratio induced by L-dopa in the substantia nigra. The extent of reduction of dopamine catabolism (as assessed by the decrease in DOPAC and HVA levels) in the striatum and substantia nigra was similar with clorgyline and deprenyl. These results indicate that in primates both MAO A and MAO B are involved in the metabolism of dopamine when higher levels of this neurotransmitter are generated from exogenous L-dopa. Furthermore, the substantia nigra may be at a greater risk than the striatum for the potential toxic effects of L-dopa-induced increased dopamine turnover.

This work was supported by the Parkinson's Institute and The Mather and Lookout Foundations (GMP).

332.1

D-GLUCOSE -EVOKED INHIBITION OF CRUSTACEAN HYPERGLYCEMIC HORMONE-CONTAINING NEURONS OF THE X-ORGAN OF THE CRAB *CANCER BOREALIS*.

R. Glowik[1,2], J. Golowasch[1], R. Keller[2] & E. Marder[1]*. [1]Volen Center, Brandeis University, Waltham, MA 02254, USA; [2]Institut für Zoophysiologie, Rheinische Friedrich Wilhelms-Universität, D-53115 Bonn, FRG.

The X-Organ, located in the eyestalks of decapod crustaceans, contains a heterogeneous population of neurosecretory cells. These neurons produce several neuropeptides involved in the regulation and homeostasis of physiological processes such as color change and retinal pigmentation, molting, gonad development and carbohydrate metabolism. We studied the process by which glucose concentration in the hemolymph is regulated. We used single electrode voltage and current clamp of cultured X-organ neurons of the crab *Cancer borealis*.

A subpopulation of the cells responded to D-glucose with a hyperpolarization. These cells all showed immunoreactivity to Crustacean Hyperglycemic Hormone (CHH). Glucose-insensitive cells were not CHH-immunoreactive. Glucose-sensitive cells were also inhibited by serotonin and GABA but were not affected by dopamine or Leu-enkephalin. The response was specific for D-glucose, with an EC_{50} of 0.25 mM. No response was seen to either L-glucose, sucrose, galactose, mannose or fructose. The glucose response persisted in the absence of extracellular Na^+ and in low Ca^{2+}/Mn^{2} saline. In voltage clamp, D-glucose evokes a small current with a reversal potential close to that of voltage-dependent K^+ currents.

We conclude that D-glucose activates a K^+ current in CHH^+ cells that, in normal saline induces a hyperpolarization. We propose that this enables glucose to directly regulate the release of CHH into the hemolymph, thus constituting a negative feedback mechanism regulating hemolymph glucose concentration.

Supported by NS17813 (EM), HFSP, Graduiertenförderung NRW, Deutsche Forschungsgesellschaft and DAAD.

332.2

DIFFERENTIAL EFFECTS OF 20-HYDROXYECDYSONE ON THE MORPHOLOGY OF INSECT NEUROSECRETORY CELLS *IN VIVO* AND *IN VITRO*.

HF McGraw, K. Prier, JC Wiley, and NJ Tublitz*. Inst. of Neurosci., U. Oregon, Eugene OR 97403 USA

The tobacco hawkmoth *Manduca sexta* contains 4 pairs of lateral neurosecretory cells (LNCs) in each ganglion. Previous work showed that the LNCs undergo a transmitter switch during metamorphosis, changing from producing the Cardioacceleratory Peptides (CAPs) in larvae to expressing bursicon in adults. This transmitter switch is controlled by the two Vth instar pulses of the steroid hormone 20-hydroxyecdysone (20-HE), the commitment peak (CP) and prepupal pulse (PP). The LNCs also undergo a major morphological transformation during metamorphosis. LNCs from day 2 Vth instar larvae (before the CP) and from pharate adults were dye-filled with cobalt. The extent and complexity of the central arbor was much greater in pharate adult LNCs compared to larval LNCs.

To determine the involvement of 20-HE in this morphological change, single LNCs were cultured in either zero, 0.1 (CP), 0.5 or 1.5 (PP) μg/ml of 20-HE for 10 days or a combination of 0.1 and 1.5 μg/ml for 5 days each. The extent 1°, 2°, and 3° branching was measured daily for each cell. Low concentrations of 20-HE preferentially increased 1° processes whereas the higher 20-HE doses caused a significant increase in the extent and number of 2° and 3° branches. These data suggest that the morphological changes exhibited by the LNCs during metamorphosis are differentially regulated by the CP and PP of 20-HE.

Supported by NSF grant # 9314537

332.3

ALTERNATIVE SPLICING AND PERIPHERAL EXPRESSION OF THE L5-67 GENE mRNA OF *APLYSIA CALIFORNICA*.

A. Angers* and L. DesGroseillers. Département de Biochimie, Université de Montréal, Montréal, Québec, Canada, H3T 3J7.

While screening for the expression of the L5-67 gene in *Aplysia* tissues, using RT-PCR, we amplified two different fragments in kidney mRNA. The longest fragment corresponds to an amplification from the full-lentgh L5-67 transcript while the shorter band contains a deletion of 148 nucleotides when compared to the cDNA sequence. Isolation of genomic clones showed that these 148 nucleotides are included in a single exon and that the shorter transcript arises by alternative splicing and exon skipping. The first 45 amino acids upstream of the splice site are identical to those of the L5-67 peptide, suggesting that the decapeptide LUQIN may be processed from the short transcript. However, a reading frame shift completely disrupts the C-terminal extremity of the precursor. The shorter L5-67 transcript can only be detected in the kidney, whether northern blotting or PCR is used, whereas the full-lenght transcript is amplified in all tissues. *In situ* hybridization experiments on kidney sections shows an abundant L5-67 specific signal in nerve fibers throughout the dorsal wall of this organ, and in the vicinity of the renal pore. By contrast, no cell body was stained in this tissue. Therefore, the L5-67 mRNA present in the kidney seems to be restricted to the axonal network and most likely originates in the LUQ cells. (Supported by the Medical Research Council of Canada).

332.4

CLONING OF THE cDNA ENCODING *HELIX* PEDAL PEPTIDE PRECURSOR.

A.V. Belyavsky, D.A. Poteryaev, I.S. Zakharov and P.M. Balaban*, Engelhardt Inst. of Molec. Biol. Moscow, Russia 117984, and Inst. of Higher Nervous Activity and Neurophysiol., Moscow, Russia 117865

During the searches for the genes preferentially expressed in serotonergic neurons of terrestrial snail CNS, a cDNA encoding multiple copies of a neuropeptide closely related to *Aplysia* pedal peptide (PeP) was cloned. According to the cDNA sequence, *Helix* PeP exists in two forms which differ in one amino-acid residue. *In situ* hybridization studies revealed expression of the PeP gene in all ganglia of the CNS and in the periphery. PeP gene was found to be expressed by (i) some of the identified serotonergic neurons in pedal and cerebral ganglia, (ii) groups of sensory neurons of olfactory analyzer in procerebrum, (iii) some of the peripheral neurons in olfactory bulb, mantle and leg, (iiii) several groups of neurons in pedal ganglia involved presumably in the locomotion control. PeP mRNA is detected in several pioneering neurons early in the course of nervous system formation. The level and pattern of the expression changes during embryogenesis and early post-embryogenesis. The fact that the PeP gene is expressed by functionally different neurons suggests multiple roles for PeP in the nervous system of molluscs.

This work was supported by Russian Foundation for Basic Research (grants Nos. 93-04-7909 and 94-04-12209).

332.5

GABA AS A PROCTOLIN-COTRANSMITTER IN THE CRAB STOMATOGASTRIC GANGLION.

J. Golowasch[1]*, A. Swensen[1], E. Marder[1], M.J. Coleman[2] & M.P. Nusbaum[2]. [1]Volen Center, Brandeis University, Waltham, MA 02254; [2]Univ Penn, Department of Neuroscience, Philadelphia, PA 19104.

GABA-like immunoreactivity is co-localized with the neuropeptide proctolin in identified projection neurons MCN1 and MPN within the stomatogastric nervous system of the crab *Cancer borealis*. To verify that the GABA-like immunoreactivity is authentic GABA, HPLC analysis of ganglion extracts was performed. GABA levels were 25 pmoles/stomatogastric ganglion (STG), 50 pmoles/oesophageal ganglion and 1nmole/commissural ganglion.

We also applied GABA focally to STG neurons. All neurons tested showed both depolarizing and hyperpolarizing responses. LP and Int1 responded with a net depolarization that triggers action potentials. All other neurons in the STG showed either a net depolarization that suppressed action potential generation or a net hyperpolarization. The depolarization was mimicked by muscimol and *trans*-aminocrotonic acid (TACA), blocked by the $GABA_A$ antagonist picrotoxin, and appears to be a mixed cation conductance with a reversal potential around -20mV. The hyperpolarizing component is primarily an increase in K^+ conductance, it is mimicked by baclofen and TACA and is not blocked by picrotoxin. Neither response is sensitive to bicucullin and the $GABA_C$ agonist *cis*-aminocrotonic acid (CACA) has very little effect on either component. We have preliminary evidence that in some but not all STG neurons a third component is present. This response is sensitive to Cl^- injections.

We are currently characterizing in more detail the 3 currents activated by GABA in the different STG neurons and correlating the distribution of these responses with the distribution of proctolin responses on specific target neurons of the STG.

Supported by NS17813 (EM), IBN9309804 (MPN) and HFSP.

332.6

DIRECT NEUROPEPTIDE ASSAY USING MASS SPECTROMETRY OF ISOLATED IDENTIFIED MOLLUSCAN NEURONS AND ITS APPLICATION TO THE VERTEBRATE CNS.

R.W. Garden[1], L.L. Moroz[2], J. Jing[2], T.P. Moroz[1], E.A. Gallman[3]*, M.U. Gillette[3], R. Gillette[2], and J.V. Sweedler[1] [1]Beckman Inst.; [2]Dept. Molec. Integr. Physiol. and [3]Cell and Struct. Biol., Univ. Illinois, IL, 61801.

Matrix assisted laser desorption ionization (MALDI) in combination with time-of-flight mass spectrometry (TOF MS) has been used to determine neuropeptide content in identified neurons from the sea slugs *Pleurobranchaea* and *Aplysia*. Several groups of identified neurons from the CNS of *Pleurobranchaea* have been assayed: (i) dorsal buccal motoneurons (DBM); (ii) feeding buccal interneurons [efference copy cell (ECC) and ventral white cells (VWC)]; (iii) escape swim central pattern generator (CPG) interneurons (A1) and modulator interneurons for the swimming CPG (A2); (iv) egg laying hormone releasing cells (ELH) and some SCP_B-immunopositive cells. Identified neurons of *Aplysia* with previously characterized peptides were used for comparison to verify this method. The individual neurons were isolated without protease treatment in the presence of the MALDI matrix 2,5-dihydroxybenzoic acid (DHB, 1% solution), which acts as a moderate fixative to facilitate the isolation procedure. Consistent neuropeptide mass spectra were obtained in the majority of the cells, suggesting that up to 10-12 individual peptides can be co-localized in individual neurons. Neuropeptides can be reliably detected in smaller peptidergic neurons (30-50 µm). ELH cells of *Pleurobranchaea* have a major peak (5000-5040) associated with the egg laying hormone.Two similar neuropeptides with MW 607 and 1548 were found both in feeding (VWC) and swimming (A1) interneurons. However, SCP_B was not detected in many SCP_B-immunoreactive buccal (including ECC) and pedal neurons from *Pleurobranchaea*, but was detected in *Aplysia* preparations. The developed protocol has been successfully applied to the rat brain and six peaks suggestive of peptides have been observed in rat retina and six in the SCN. Supported by NS31609 to JVS and NIH RO1NS268338 grant to RG.

332.7

NEUROPEPTIDE PROFILES OF SINGLE NEURONS AND CELL CLUSTER RELEASE. Scott A. Shippy, Rebecca W. Garden, Leonid L. Moroz, and Jonathan V. Sweedler*. Beckman Inst./Dept. of Chem., Univ. of Illinois, Urbana, IL 61801.

In order to understand signal transduction between individual neurons more fully, it is necessary to characterize both the neurochemicals and the dynamics of release. We describe a highly sensitive and selective assay for neuropeptides from individual bag cells from *Aplysia californica*. Matrix assisted laser desorption ionization (MALDI) in combination with time-of-flight mass spectrometry (TOF MS) is used to profile the neuropeptide content of single cells. The MALDI matrix 2,5-dihydroxybenzoic acid is used in a stepwise fashion to stabilize cell membranes, remove salts, and deactivate endogenous proteolytic enzymes prior to spectrum acquisition. Mass spectra indicate the presence of neuroactive peptides previously characterized by conventional methods. For example, acidic peptide, egg laying hormone, five bag cell peptides and two C-terminally cleaved α-bag cell peptides are detected by MALDI-TOF MS of a single *Aplysia* bag cell. Furthermore, spectra reveal the possible presence of modified and/or novel peptides. Additionally, we follow the dynamics of neuropeptide release from a bag cell cluster of *Aplysia*. Release is monitored from an electrically stimulated bag cell cluster previously cultured in ^{35}S-methionine containing medium. The inlet of a fused silica capillary (i.d. 50-75 μm) is placed near to a cluster for continuous gravimetric pressure flow. At the outlet end, the sample (100 nL/min.) is absorbed onto a moving membrane strip. Labeled peptides absorbed onto the membrane are detected using a phosphoimaging system. Results demonstrate significant radioactivity only during the afterdischarge. In contrast to stimulated release, data from damaged cell clusters suggests the release of aggregates of neuropeptides. Time and species resolved peptide release is explored by combining the deposition of the sample onto a gel with subsequent separation by electrophoresis. Spatial resolution of release is accomplished by judicious placement of the sampling capillary. The described methods are applicable to neurosecretory cells and clusters in other model systems. Supported by grants NIH NS31609 and NSF CHE925704.

332.8

EFFECTS OF TACHYKININ-RELATED PEPTIDES AND OTHER PUTATIVE NEUROTRANSMITTERS ON DORSAL UNPAIRED MEDIAN NEURONS OF LOCUST THORACIC GANGLIA. C.T. Lundquist* and D.R. Nässel. Dept. Zoology, Stockholm University, S-106 91, Stockholm, Sweden.

Four tachykinin-related peptides, Locustatachykinin 1-4 (LomTK 1-4), are known to be distributed in neurons throughout the central nervous system of the locust *Locusta migratoria*. It has been suggested that these peptides have functions in neural transmission because of their abundant distribution in processes in most neuropils within the CNS of the locust. In search for central actions we have analyzed the physiological response of putative post-synaptic target neurons - the dorsal unpaired median (DUM) neurons of the locust metathoracic ganglion. LomTKs were either bath-applied to an isolated thoracic ganglion or pressure-ejected onto the soma while recording intracellularly the effects on the membrane potential and action potential firing frequency of single DUM neurons. For comparison, the effects of acetylcholine, GABA and glutamic acid were studied. Bath application of LomTK-1 at 0.1μM caused a small and relatively slow, but reversible depolarization (2-5 mV) and a substantial increase in firing frequency of action potentials of the DUM neurons. Bath application of acetylcholine at 0.1mM induced a rapid depolarization and a large increase in firing frequency whereas both GABA and glutamic acid (0.1mM) caused a rapid hyperpolarization, abolishing all spontaneous action potentials. Almost identical results were obtained when LomTK-1 was pressure-ejected (0.1μM, 20-200 msec.) onto the DUM neuron soma. In this case, however, a fast depolarization, followed by a slower and more sustained depolarization was observed. The results indicate a role for LomTK-1 as a transmitter or modulator acting on the thoracic DUM neurons, but the mechanisms remain unknown. We will further investigate the mechanisms that underlie the depolarization and increase in firing frequency by examining which ion channels and/or second messenger systems are involved.

The project is sponsored by the Swedish Natural Science Research Council (NFR).

332.9

SCPb-INDUCED PHOSPHORYLATION OF A 53 kD PROTEIN IN ISOLATED MYOCARDIAL CELLS OF *HELIX ASPERSA*. G. Reich, K.E. Doble and M.J. Greenberg*. Whitney Laboratory, of the University of Florida, St.Augustine, FL32086

The myocardial excitation induced by the molluscan neuropeptide SCPb is accompanied by an increase in cAMP. To study the events that follow this increase in cAMP, we digested heart tissue with trypsin to produce dissociated myocardial cells that were spindle shaped, around 400mm long, and that excluded trypan blue. The effects of SCPb on the levels of cAMP in the isolated myocardial fibers was compared in detail with the cAMP levels of whole ventricles. Levels of cAMP levels rise markedly when dissociated cells are treated with SCPb, and the time course of this response is virtually identical to that of the intact tissue. A set of synthetic analogs - FMRMa, FPRMa, FPRFa, and FMRFa - previously used to characterize the structural specificity of the SCPb effect on whole hearts, had strikingly similar potencies when tested on the cell preparation. Thus the SCPb receptors seem not to have been damaged by dissociation. Next, isolated cells were incubated with inorganic ^{32}P, then treated with SCPb. After homogenization, electrophoresis, and exposure to x-ray film, SCPb was seen to have induced the phosphorylation of a 53 kD protein. The intensity of the band increased in a dose dependent manner; threshold was 10^{-9}-10^{-8} M SCPb. Forskolin and the cAMP analog 8-CPT-cAMP mimicked the SCPb-induced phosphorylation. NIH HL28440

332.10

MODULATION OF POWER OUTPUT FROM THE SWIMMERET SYSTEM BY CRUSTACEAN CARDIOACTIVE PEPTIDE. B. Mulloney*, S. Starsinic, H. Agricola, and W.M. Hall. Neurobiology, Physiology, and Behavior, Univ. Calif., Davis CA 95616-8755, and Inst. f. allg. Zool. u. Tierphysiol., Friedrich-Schiller-Uni., Jena, D-07743 Germany.

The swimmerets of crayfish make rhythmic movements that propel the animal forward in the water column. The force, the period, and the phase of these movements can be altered to the animals' behavioral requirements, but the neural mechanisms that accomplish these alterations are not known. Crustacean Cardioactive Peptide (CCAP) affects visceral and retinal functions in crabs, so we explored its affects on the swimmeret pattern-generating circuits.

Bath-application of CCAP to isolated crayfish abdominal nerve cords increased the intensity of bursts of impulses in power-stroke motor neurons, but did not alter the period, durations, or phases of these bursts. This modulation was manifest both as an increase in spike frequency in individual motor neurons and as an increase in the number of motor neurons firing during each burst.

In each ganglion that innervates swimmerets, three pairs of interneurons labeled strongly with a CCAP-antiserum. The shapes and locations of the cell-bodies of these interneurons were the same in each ganglion. These interneurons send processes into the Lateral Neuropils, the loci of the swimmeret pattern-generating modules.

Thus, these interneurons have properties that suggest to us that they regulate the force produced by the swimmeret system.

Supported by NSF grant IBN-9514889 to BM.

332.11

BIOLOGICAL ACTIVITY OF CEREBRAL PEPTIDE 2 IN THE ABDOMINAL AND BUCCAL GANGLIA OF *APLYSIA*. G. A. Phares* and P. E. Lloyd Committee on Neurobiology, University of Chicago, Chicago, IL. 60637

Cerebral peptide 2 (CP2) is a newly identified 41 amino acid peptide with an amidated carboxyl terminal. This peptide was identified because it was transported from the cerebral ganglion of *Aplysia* to target ganglia including the abdominal and pedal-pleural ganglia. Immunocytology indicates that CP2 was widely distributed and particularly dense in the neuropil of the buccal and abdominal ganglia. Standard electrophysiological techniques were used to record responses of identified neurons in these ganglia to applications of synthetic CP2. Many neurons in the abdominal ganglion were depolarized by CP2 including some in the respiratory pumping circuit (R20, R25/L25, L7) and some with unknown functions (L5, RG neurons). The depolarization appeared direct because it persisted in low Ca^{2+}, high Mg^{2+} ASW. Many of these neurons also received a compound PSP during the application of CP2. In R20, R25/L25, and the RG neurons the compound PSP was inhibitory, but in L7 it was an E/IPSP. The compound PSP appears to arise from other neuron(s) depolarized by CP2 and was abolished in low Ca^{2+}, high Mg^{2+} ASW. Application of CP2 to the buccal ganglion evoked rhythmic activity in a number of ventral cluster motor neurons. The pattern of activity was similar to ingestive buccal motor programs recorded by others. The only neuron in the ventral cluster that appeared to respond directly to CP2 was the interneuron B41. These results suggest that CP2 is likely to be involved in the generation of several behaviors in *Aplysia*. Supported by NSF Grant IBN 9418815.

332.12

LYS-CONOPRESSIN INDUCES TWO SLOWLY-INACTIVATING INWARD CURRENTS IN AN IDENTIFIED NEURON OF *LYMNAEA STAGNALIS*. P. F. van Soest, R. F. Jansen* and K. S. Kits. Graduate School Neurosciences Amsterdam, Research Institute Neurosciences, Vrije Universiteit, Faculty of Biology, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

The oxytocin-like peptide lys-conopressin is expressed in neurons of the right anterior lobe of the central nervous system of the pond snail *Lymnaea stagnalis*. Many of these neurons also express a *Lymnaea* conopressin receptor, suggesting that auto-transmission may be important for regulating their activity. Neurons in the anterior lobe are believed to be involved in the regulation of certain aspects of male copulatory behaviour. Both in the co-localisation of peptide and receptor and their putative function, these neurons seem similar to oxytocinergic magnocellular neurons of the vertebrate hypothalamus.

The effects of lys-conopressin on an identified neuron, isolated from the anterior lobe of *Lymnaea*, were studied using whole-cell voltage clamp. When the cell was clamped at its normal resting membrane potential, conopressin induced a slowly-inactivating, inward current. Voltage ramp protocols were employed to study the voltage dependence of the current. Under control conditions, the steady-state current-voltage (I-V) relation shows a region of negative slope resistance. Application of conopressin shifted the entire I-V relation, ranging from -90 to +10 mV, in the inward direction. This effect was dose-dependent. Vasopressin and oxytocin, as well as lower doses of conopressin, could only activate current at voltages above -40 mV. Apparently, the conopressin-activated current is not homogeneous, but consists of two separate currents. Sodium appears to be the main charge carrier for both currents. In neighbouring neurons, only a current corresponding to one of these components could be activated by conopressin. Desensitisation and recovery experiments further support the notion that the conopressin-activated current is comprised of two separate components. Both currents may contribute to the strong excitation that is observed upon conopressin application under current clamp conditions.

332.13

SINGLE-CHANNEL ANALYSIS OF A PEPTIDE-INDUCED POTASSIUM CURRENT WHICH MODULATES CONTRACTILITY IN *APLYSIA* MUSCLE. C. Erxleben and V. Brezina*. Dept. of Biology, University of Konstanz, Germany, and Mt. Sinai Medical School, Dept. of Physiology and Biophysics, New York, NY.

Contractions of the ARC muscle of *Aplysia* are depressed by neuromodulators that activate a K current in the muscle (Brezina et al 1994: *J Neurosci* 14, 4412). Large K currents are activated by MM_A, a peptide of the myomodulin family, and FMRFamide related peptides (FRFs), and smaller currents by serotonin (5-HT).

We investigated the molecular basis of the modulation at the level of single K channels in dissociated ARC muscle fibers. The bath solution was normal artificial sea water, while the patch pipette was filled with high K^+ solution. Under these conditions, the K^+ equilibrium potential for the patch is shifted more positive than 0 mV, and K channel openings at the resting potential can be seen as inward currents.

While recording from cell-attached patches, application of MM_A, FRF_C or 5-HT to the rest of the fiber resulted in the slow activation of channels which were inactive in the absence of the modulators. The time course of the response was very similar to that of the macroscopic current recorded under voltage clamp. At the resting potential, the channels carried a mean inward current of -7.8 ± 0.2 pA (SEM, n = 12). The open probability of the channels showed no significant voltage dependence. Under these conditions of high K^+ concentration on both sides of the membrane, the single-channel current-voltage relationship was linear with a slope conductance of 100 pS. The channels were identified as K channels based on their ion selectivity and the reversal potential (76 mV positive to rest with high K^+ solution in the pipette) near the K^+ equilibrium potential. Consistent with the pharmacology of the macroscopic current, single-channel gating and amplitude were independent of extracellular Ca^{2+} and insensitive to external Ba^{2+} (10 mM), but the current amplitude was slightly reduced by 10 mM and significantly reduced by 50 mM external tetraethylammonium.

The peptide-induced K channels resemble 'classical' transmitter-gated (ionotropic) ion channels in that they are apparently inactive in the absence of the modulators. The slow time course of activation and the fact that application of the modulators to remote parts of the cell can elicit the response is, however, typical for a second-messenger-activated (metabotropic) ion channel.

Supported by NIMH and the Whitehall Foundation.

332.14

NEURONS SURVIVE AND INDUCE FUNCTIONAL TENTACLES WHEN GANGLIA ARE IMPLANTED INTO HOST SNAILS, AS REVEALED BY IMMUNOHISTOCHEMISTRY AND BEHAVIORAL EVALUATION. J.L.Wilson and S.B.Moffett*, Department of Zoology, Washington State University, Pullman WA 99164.

Implanted ganglia become incorporated into the CNS of the pulmonate snail *Ovatella myosotis* and induce the growth of eyes and tentacles within 1-2 months; these intraspecific implants had a 50% induction rate. A cerebral ganglion from *Melampus bidentatus* implanted into *Ovatella* can also be integrated into the CNS to induce supernumerary structures (showing a 10% success rate). The ganglia were inserted into the lateral head region or between the tentacles, with the latter position being more effective in inducing supernumerary structures. Tactile stimulation of the new tentacle evoked both tentacle and whole-body withdrawal. Sacrifice after 1-6 months allowed microscopic and immunohistochemical observations of the implanted ganglia and innervation of the supernumerary structures. The third tentacle may undergo constriction or diminish and disappear after approximately two months; this is associated with the degeneration of the ganglion.

Research supported by WSU College of Sciences, award to S.M.

332.15

CHARACTERIZATION OF A POTENTIAL PHEROMONAL ATTRACTANT IN *APLYSIA*. S.D. Painter*, B. Clough, X. Fan, and G.T. Nagle. Marine Biomedical Institute and Department of Anatomy & Neurosciences, University of Texas Medical Branch, Galveston, TX 77555-1043.

Water-borne pheromones play an important role in regulating male and female reproductive behavior in the marine opisthobranch mollusc *Aplysia*. They attract normally solitary animals into breeding aggregations during the summer reproductive season, and coordinate mating and egg-laying activity in the aggregations. The pheromones elute from recently deposited egg cordons associated with the aggregations, but relatively little else is known about them. None have, as yet, been chemically characterized. *A. californica* were injected with atrial gland extract to induce egg laying, the cordons collected at 30-min intervals, and eluted in artificial seawater. The eluate was purified on C18 Sep-Pak cartridges, lyophilized and fractionated by C18 reversed-phase HPLC. There were 4 peaks that were attractive in a T-maze assay. The fractions were subjected to amino acid and microsequence analyses. Surprisingly, the sequences were identical in all 4 fractions, except for length. All had two potential glycosylation sites, but it is not known whether the sites are utilized. The longest sequence (48 residues) was compared to the BIONET protein sequence database using their Search and Align program. There is no evidence of homology with any sequence currently in the database. Supported by NIH grant HD28500.

332.16

CHARACTERIZATION OF cDNAs ENCODING A POTENTIAL PHEROMONAL ATTRACTANT AND THE NEUROPEPTIDE APGWamide IN *APLYSIA*. G.T. Nagle*, X. Fan, B. Wu, and S.D. Painter. Marine Biomedical Institute and Department of Anatomy & Neurosciences, University of Texas Medical Branch, Galveston, TX 77555-1043.

A partial amino acid sequence has been obtained for a potential water-borne pheromonal attractant in *Aplysia* (Painter et al., Soc. Neurosci. Abstr. 1996). This information was used to synthesize degenerate oligonucleotide primers to produce RT-PCR and 3'RACE probes in order to isolate cDNAs encoding the complete pheromone sequence. A central nervous system cDNA library was initially screened because: 1) the tissue source of the pheromone was not known; 2) cDNA libraries were available for the CNS, but not for reproductive tract organs that could secrete onto the egg cordon (with the exception of the atrial gland); and 3) other peptides involved in regulating reproduction in *Aplysia* are expressed in both the CNS and the reproductive tract. A CNS 3'RACE product was generated and preliminary sequence analysis established that it encodes multiple copies of the neuropeptide Ala-Pro-Gly-Trp-NH_2 (APGWamide); this was then used to screen a λZAP CNS cDNA library. In addition, an RT-PCR product of the predicted size was generated using atrial gland total RNA, which was consistent with earlier studies showing that extracts of the atrial gland are attractive in T-maze experiments and induce copulatory behavior. This product was used to screen CNS and atrial gland libraries. We are currently characterizing the cDNA clones to determine whether they encode the pheromone. Supported by NSF and a UTMB Small Grant.

332.17

CHARACTERIZATION OF cDNAs ENCODING *APLYSIA* AMIDATION AND CARBOXYPEPTIDASE-RELATED ENZYMES. X. Fan, G.V. Childs*, R. Rodriguez, and G.T. Nagle. Marine Biomedical Institute and Department of Anatomy & Neurosciences, University of Texas Medical Branch, Galveston, TX 77555-1043.

The bag cells of *Aplysia* express the egg-laying hormone (ELH), a neuroendocrine peptide that induces ovulation and its associated behaviors. It is encoded by the ELH gene, which also encodes eight other peptide products. Several prohormone converting enzymes (PCs) have been cloned from the bag cells, and are likely to be responsible for the intravesicular cleavage at basic residue sites. The basic residues are then removed by an enzyme(s) belonging to the carboxypeptidase (CP) family. CPs have been well characterized in many systems but had not been studied in *Aplysia*. We recently cloned a neuronal 561-amino acid preproenzyme that was most closely related to mammalian CPE (Fan & Nagle, DNA Cell Biol., in press). A second neuronal enzyme was detected by RT-PCR. It was most closely related to carboxypeptidase D (Song & Fricker, J. Biol. Chem. 270: 25007-25013, 1995), a novel CPE-like enzyme present in bovine anterior pituitary secretory vesicles. It is known that one of the ELH gene products (ELH) is then amidated. In order to characterize *Aplysia* peptidylglycine α-amidating monooxygenase (PAM), a CNS cDNA library was screened using a PAM-related RT-PCR product generated from bag cell RNA. The partial clone that was isolated encoded a PAM-related enzyme that was most closely related to vertebrate PAM sequences. We are currently attempting to determine the remaining sequence of the *Aplysia* carboxypeptidase D- and PAM-related enzymes by 3'RACE and library screening. Supported by NSF and a UTMB Grant.

332.18

CIRCULATING LEVELS OF ECDYSIS-TRIGGERING HORMONE DURING PRE-ECDYSIS AND ECDYSIS OF MANDUCA SEXTA. J. L. Hermesman[1], T. G. Kingan[3], W. Gray[4], D. Zitnan[5] and M.E. Adams*[2]. Depts. of [1]Biology and [2]Entomology, U. of California, Riverside, CA 92521; [3]Dept. of Biological Sciences, U. of Maryland Baltimore County, Baltimore, MD 21228; [4]Dept. of Biology, U. of Utah, Salt Lake City, UT 84112; [5]Slovak Academy of Sciences, Institute of Zoology, Dubravska cesta 9, 84206 Bratislava, Slovakia.

Growth and development in insects is punctuated by ecdysis, the shedding of old cuticle at the end of each instar. In the tobacco hornworm, *Manduca sexta*, ecdysis-triggering hormone (ETH) released from epitracheal glands elicits pre-ecdysis followed by ecdysis in all life stages through a direct action on the central nervous system (Zitnan *et al., Science* **271**: 88-91; 1996). Here we report for the first time the detection of ETH in pharate 5th instar hemolymph by quantitative ELISA. An antiserum specific for ETH was produced in rabbits following immunization with a multiple-antigen peptide (MAP) version of ETH. Hemolymph was taken from pharate 5th instar larva just prior to pre-ecdysis, and at time points during the course of the behavioral sequence through ecdysis. Hemolymph samples were heated to 90°C, centrifuged, and assayed directly by ELISA. Several blood samples also were fractionated using HPLC and all fractions were assayed. Only the fractions that co-eluted with ETH showed immunoreactivity. Detectable levels of ETH appear immediately prior to pre-ecdysis, reaching levels of 50-100 nM at the onset of this behavior. Hemolymph concentrations of ETH remain elevated during pre-ecdysis and ecdysis. Our results confirm the hormonal nature of ETH and indicate that physiological levels of ETH during the performance of natural pre-ecdysis and ecdysis are in the range previously shown to elicit these behaviors in the isolated central nervous system.

Partially supported by the UCR Agricultural Experiment Station and USDA grant #93-37302-8968

332.19

ECLOSION HORMONE-EVOKED RELEASE OF ECDYSIS TRIGGERING HORMONE FROM EPITRACHEAL GLANDS. T. G. Kingan*[1], J. L. Hermesman[2], W. Gray[3], D. Zitnan[4], M. E. Adams[5]. [1]Dept. of Biological Sciences, U. of Maryland Baltimore County, Baltimore, MD 21228; Depts. of [2]Biology and [5]Entomology, U. of California, Riverside, CA 92521; [3]Dept. of Biology, U. of Utah, Salt Lake City, UT 84112; [4]Slovak Academy of Sciences, Institute of Zoology, Dubravska cesta 9, 84206 Bratislava, Slovakia.

In the tobacco hornworm, *Manduca sexta*, ecdysis-triggering hormone (ETH) released from epitracheal glands elicits pre-ecdysis followed by ecdysis in all life stages through a direct action on the central nervous system (Zitnan *et al.*, *Science* **271**: 88-91; 1996). The neuropeptide eclosion hormone (EH) also evokes ecdysis behavior *in vivo*, but its *in vitro* action on the CNS requires the presence of the tracheal system, to which the epitracheal glands are attached. Together, these observations raise the possibility of an endocrine interaction between EH and ETH. We now report that eclosion hormone (EH) evokes release of ETH *in vivo*, and *in vitro* from individual epitracheal glands. EH prepared by solid-phase chemical synthesis showed chemical and biological identity with the native peptide isolated from extracts of adult corpora cardiaca. Release of ETH was detected by immunohistochemistry and quantitative ELISA. Injection of EH (1-2 pmol) into the hemocoel of pharate larvae and pupae resulted in the loss of immunohistochemical staining in Inka cells of the epitracheal glands and appearance of ETH in the hemolymph. To detect ETH release *in vitro*, epitracheal glands were removed 3-4 hr prior to natural ecdysis ("anterior shrink" in pharate pupae and "air-filled head capsule" in pharate 5th instars). Individual glands were placed in a drop of physiological saline, to which EH was added. Up to 1.7 pmol of ETH appeared in the medium bathing pharate 5th instar glands following exposure to 0.3-1 nM EH. No release was detected after exposure to CCAP or a myotropic *Manduca* FLRFamide. These findings support the hypothesis that EH initiates the ecdysis sequence through a signaling pathway in the epitracheal glands, resulting in the release of ETH.

Partially supported by USDA grant 93-37302-8968 and the UCR Ag Experiment Station.

TRANSMITTERS IN INVERTEBRATES: NEUROPEPTIDES II

333.1

MECHANISM OF ACTION OF THE CARDIOINHIBITORY PEPTIDE, *DROSOPHILA* MYOSUPPRESSIN (DMS)

Nichols, R.[12]*, Dickerson, M.[2], McCormick, J.[2] and Paisley, K.[2]
Depts of BiolChem[1] and Biol[2], University of Michigan, Ann Arbor, MI 48109-1048

Myosuppressin peptides, isolated from several organisms, have a high degree of structure identity; only the N-terminal amino acid residue differs. Similar to the myoinhibitory actions of <EDVDHVFLRFamide or leucomyosupressin, LMS (Nachman and Holman) and PDVDHVFLRFamide or SchistoFLRFamide (Robb and Evans), *Drosophila* myosuppressin, TDVDHVFLRFamide or DMS (Nichols), inhibits heart rate in a dose dependent manner (Dickerson and Nichols).

Amino acid residues critical for the cardioinhibitory action of DMS have been determined by an alanine scan. The alanyl-substituted analogs revealed the following effects on cardioinhibition: T1 (N-terminal threoninyl residue) and D2 accentuate, L8 reduces, and F7, F10, and the C-terminal amide abolish activity. Substitution of R9 has no effect, an observation that is in contrast to the essential arginyl residue of LMS and SchistoFLRFamide inhibitory actions of gut and oviduct motility, respectively. These data suggest that the structural requirements for cardioinhibition are different than those for inhibiting gut and oviduct motility. Supported by NSF and AHA/MI.

333.2

flp-1 DELETION MUTANTS SUGGEST FMRFAMIDE-LIKE PEPTIDES ARE INVOLVED IN MULTIPLE SENSORY AND MOTOR FUNCTIONS IN *CAENORHABDITIS ELEGANS*.

L. S. Nelson* and C. Li. Department of Biology, Boston University, Boston, MA 02215

FMRFamide-related peptides (FaRPs) have been found throughout the animal kingdom, and have been implicated in many functions, including cardiovascular control and neuromodulation. Recently, mammalian FaRPs have been shown to have anti-opioid activity. In the nematode *C. elegans*, FMRFamide-like immunoreactivity has been localized to about 10% of the neurons and at least five genes encoding these peptides have been identified. These genes, *flp-1* through *flp-5*, appear to be expressed in the animal, and are now being further characterized in our lab.

The *flp-1* gene of *C. elegans* encodes multiple copies of FLRFamide-containing peptides. Animals carrying *lacZ* reporter constructs containing 1.5 kbp of the *flp-1* promoter region have shown that *flp-1* is expressed in certain interneurons in the head region of the animal. Further upstream regions have now been isolated and will be used to determine whether additional cells express the *flp-1* gene.

A *flp-1* deletion mutant, or "knockout", has now been isolated from population screens of *flp-1*::Tc1 animals (kindly provided by Ron Plasterk). The deletion was 1.4 kbp, and included almost 4 of the 6 exons as well as the start site of transcription. Multiple phenotypes have been correlated with the *flp-1* deletion. These include uncoordinated movement, nose-touch insensitivity, and defects in osmolarity detection. Many of the phenotypes are also seen in mutants missing the α subunit of the neuronal G protein. Rescue of these phenotypes with the wild type copy of the gene is currently underway. Further characterization of phenotypes in *flp-1* deletion animals as well as non-complementation screens for additional *flp-1* alleles are ongoing.

Supported by grants from NIH and NSF.

333.3

GENETIC ANALYSIS OF NEUROPEPTIDE SIGNALING: IDENTIFICATION OF A P ELEMENT INSERTION IN *DROSOPHILA PHM*.

P.H. Taghert*, N. Jiang, M.S. Roberts, Q. Wang, A.S. Kolhekar. Anat. & Neurobiology, Washington University Sch. Med., St. Louis MO 63110, and Neurosci., Johns Hopkins University Sch. Med., Baltimore MD 21205.

Peptide alpha-amidation is widespread and often essential for the synthesis of biologically active peptides. In vertebrates, a single gene (*PAM* - peptidylglycine-alpha amidating monooxygenase) encodes a large protein with two separable enzymatic domains. The first domain is a monooxygenase (PHM) and the second is a lyase (PAL); together these enzymes convert peptides with C terminal glycines to the alpha amide. To further the genetic analysis of *PAM*, we have begun to study this aspect of neuropeptide biosynthesis in *Drosophila*.

Drosophila contains both enzymatic activities. We identified a *Drosophila* gene encoding PHM but lacking PAL. We suspect *PAL* is a separate gene. *dPHM* is expressed in a variety of tissues, including nervous system, endocrine glands and gut. Within each tissue, PHM-like immunoreactivity is present in a heterogeneous subset of cells. In embryos, PHM staining first appears around Stage 16 in salivary glands.

dPHM is located on the right arm of the second chromosome at 60A12-16. We screened available mutations in this interval and identified a candidate P element insertion within the *PHM* transcription unit by PCR methods. We then used blotting, restriction and sequence analysis to confirm the position of the insertion within the signal sequence of the *dPHM* open reading frame. Two lines of evidence suggest that PHM protein expression may be decreased due to the insertion. Animals heterozygous for the insertion display a weaker band of PHM immunoreactivity on Western blots and produce a lower ratio of PHM to PAL enzyme activity. The stock displays homozygous lethality that appears to be due to the insertion. Preliminary analysis of homozygous insertion embryos indicates that some of these animals progress to at least Stage 17 (~75% of embryonic development).

Supported by the NIH (#DK-32949 to Betty A. Eipper and #NS-21749 to PHT) and by the McDonnell Center for Cellular and Molecular Neurobiology (PHT)

333.4

ANALYSIS OF A CNS-PERITRACHEAL NEUROPEPTIDE SYSTEM IN INSECTS.

M.A. O'Brien* and P. H. Taghert, Anat. and Neurobiol. Dept., Washington Univ. Med. School, St. Louis, MO 63110.

We have been studying a *Drosophila* "enhancer trap" line that has reporter gene expression co-localized with expression of a myomodulin-like peptide. There is co-expression in prominent neurosecretory cells in the CNS, and in one of two segmental peritracheal cells that have strong reporter gene expression. The myomodulin-positive peritracheal cell is also immunoreactive with an antibody against the *Drosophila* PHM protein, an enzyme in the peptide amidation pathway (see abstract by Taghert *et al.*). Since many neuropeptides are amidated, this is a good marker for neuropeptide-expressing cells.

This peritracheal neuropeptide system appears to be widespread in insects. We have found myomodulin-like expression in peritracheal cells in at least two other insect orders. In *Manduca sexta*, a peritracheal, myomodulin antibody-positive cell has been identified; this cell is either identical to, or a neighbor to, the inka cell, which produces the ecdysis-triggering hormone (Zitnan *et al.*, 1996). The *Drosophila* cell may be the homolog to the inka cell, though the cell morphology is quite distinct. In a grasshopper, there is staining in cells with similar morphology to the *Drosophila* cell, but they are widely distributed along the tracheae. Whether this peritracheal neuropeptide system has a general, endocrine function or a more local function is an important question. Assessing peptide expression in a variety of insects with different physiological demands, as well as monitoring expression throughout development should provide insight into the possible functions of this conserved peritracheal neuropeptide system.

In order to study this system at a molecular genetic level, we have cloned the region surrounding the transposon in *Drosophila*, with the goal of identifying the gene responsible for the expression pattern and mutating it. We have isolated several cDNAs in the vicinity of the transposon and are in the process of screening them by *in situ* hybridization to determine if their expression coincides with the reporter gene expression.

Supported by NIH Grant NS-21749 (P.H.T)

333.5

MODULATION OF THE STOMATOGASTRIC GANGLION OF THE LOBSTER, *HOMARUS AMERICANUS*. K. S. Richards*, V. Kilman, J. Golowasch and E. Marder. VOLEN CENTER, BRANDEIS UNIVERSITY, WALTHAM, MA 02254.

An extensive literature on the modulation of the stomatogastric nervous system (STNS) of several decapod species has been gathered. The STNS of *Homarus americanus* has been relatively neglected, however, partially because it has been less reliable in *in vitro* preparations than some of the other crustacean species. Because of its potential use to study STNS development, we decided to study the presence and action of putative neuromodulatory substances in the *H. americanus* STNS, and to study the effects of these on motor patterns produced by the stomatogastric ganglion (STG).

Anatomical studies of the adult *H. americanus* showed the presence of immunoreactivity in the STNS after staining with antisera generated to the following transmitter and modulator substances: GABA, glutamate, octopamine, FMRFamide proctolin, buccalin, histamine, red pigment concentrating hormone (RPCH), and cholecystokinin (CCK). In addition, we showed the presence of FMRFamide -like staining in the STG of stage 1 larvae. We are currently extending these studies to other larval stages.

Electrophysiological studies on adults using bath application showed that proctolin, SDRNFLRFamide and crustacean cardioactive peptide (CCAP) enhanced pyloric and/or gastric mill activity. Allatostatin (AST) was inhibitory, as were GABA and histamine. Dopamine evoked rapid tonic firing but not bursting. Surprisingly, the muscarinic agonists pilocarpine and oxotremorine, which strongly activate the STG of other decapod species were not effective.

In summary, as in other crustacean STNS, the *H. americanus* STNS contains and is modulated by a large number of substances.

Supported by NS17813 and the HFSP.

333.6

DEFINITION OF A CELL TYPE-SPECIFIC ENHANCER OF NEUROPEPTIDE TRANSCRIPTION IN *DROSOPHILA*. R.J. Benveniste* and P.H. Taghert, Dept. of Anat. and Neurobiol., Washington Univ. Sch. of Med., St. Louis, MO 63110

The *Drosophila FMRFamide* neuropeptide gene is transcribed in about 50 neurons in the CNS, representing at least 17 different cell types. The most prominent are the Tv neurons, six neuroendocrine cells in the ventral ganglion. Previous work suggested that the *FMRFamide* promoter is large and mosaic with respect to regulation in different cell types. A 300 bp enhancer dedicated to one of the 17 cell types (OL2) was previously defined. We now define a distinct enhancer specific for the Tv neurons.

The Tv regulatory region was previously mapped by deletion analysis to a 446 bp sequence between 476 and 922 bp upstream of *FMRFamide*. We tested whether sequences from this region could drive expression by a heterologous promoter (hs43). The 446 bp sequence drove expression of a reporter gene in the Tv neurons of transgenic larvae, as well as in four cells that do not express FMRFamide. While Tv FMRFamide expression continues through adulthood, Tv lacZ expression waned during metamorphosis.

Reduction of the 446-bp sequence defined 169 bp that are necessary for reporter expression in larval Tv neurons. Preliminary results suggest that 59 bp within the 169-bp sequence are necessary for reporter expression in larval Tv neurons. This 59-bp sequence includes a homeodomain consensus-binding site and a CRE-like element, both of which are conserved between the *FMRFamide* promoters of *D. melanogaster* and *D. virilis*. We are currently searching for transcription factors that interact with these sequence elements.

Supported by NIH grants NS-21749 to PHT and NS-07071 to RJB

333.7

REGULATION OF *DROSOPHILA* HEART RATE

Dickerson, M.[1]*, McCormick, J.[1], Paisley, K.[1], and Nichols, R.[1,2] Depts of Biol.[1] & Biol. Chem.[2], University of Michigan, Ann Arbor, MI

Although *Drosophila melanogaster* has served as a versatile experimental organism for developmental neurobiology little is known about the action of neuropeptides due to the lack of structure data, antisera specific to individual neuropeptides, and the development of bioassays. We have isolated peptides, generated sequence-specific antisera, and established *Drosophila* heart rate assays. By application of neuropeptides, classical transmitters, and pharmacological reagents, and the utilization of mutants, we have begun to analyze the regulation of *Drosophila* heart rate.

Drosophila FMRFamide peptides, DPKQDFMRFamide, TPAEDFMRFamide, and SDNFMRFamide, increase heart rate; the molluscan cardioexcitatory peptide FMRFamide is inactive. In contrast, *Drosophila* myosuppressin, TDVDHVFLRFamide, inhibits heart rate. These data indicate that the variant N-terminal sequences of -FXRFamide peptides are important in affecting heart rate.

We have determined that serotonin is cardioexcitatory, while dopamine, GABA, glutamate, proctolin, and octopamine inhibit heart rate. A mixture of serotonin and DMS has an additive effect suggesting that the two substances act through different mechanisms. The action of DMS is suppressed in potassium channels mutants and in the presence of potassium channel blockers, supporting the conclusion that the regulation of heart rate involves potassium channels. Supported by NSF and AHA/MI.

333.8

THE EFFECT OF FMRFamide RELATED PEPTIDES (FaRPs) ON THE PHARYNGEAL AND SOMATIC MUSCULATURE OF THE NEMATODE ROUNDWORM *ASCARIS SUUM*. David J Brownlee*, Lindy Holden-Dye and Robert J Walker. Department of Physiology and Pharmacology, Bassett Crescent East, University of Southampton, Southampton, SO16 7PX, England, UK.

The nervous system of the parasitic nematode, *Ascaris suum* exhibits widespread immunoreactivity to the FMRFamide-like peptides. More recently a number FMRFamide related peptides, (FaRPs) have been isolated from both free-living and parasitic forms. An *in vitro* pharmacological approach was used to investigate the functional role of endogenous nematode FaRPs in the control of *Ascaris* pharyngeal and somatic muscle. Intracellular recordings from *Ascaris* somatic muscle cells were also performed. The peptides tested on the two muscle preparations were: AF1 (KNEFIRFamide), AF2 (KHEYLRFamide), AF8 (KSAYMRFamide) and PF4 (KPNFIRFamide).

	AF1	AF2	AF8	PF4
Pharyngeal muscle	-	+	- -/+	0
Somatic muscle	+	+/-	++	- -

0 no effect; + excitatory; - inhibitory (1nM - 10 μM; n=8)

AF8 inhibited pharyngeal pumping behaviour in a dose-dependent manner (threshold 0.1 nM). The inhibition was characterized by the pharynx remaining in a hyper-contracted state, with no pumping movements. AF8 also caused contraction of the somatic musculature. The peptides tested have potent effects on both the pharyngeal and somatic musculature of *Ascaris*. However, the peptides cause diverse effects depending on the nematode muscle type under study (see table). This indicates that some, if not all of the FaRPs may have an important functional role in the control of nematode feeding and motility, and the possible coordination of these two behaviours.

This work was supported by the Medical Research Council and the BBSRC.

333.9

IDENTIFICATION OF LEPIDOSTATIN, AN ALLATOSTATIN-LIKE PEPTIDE, AND MAPPING AND FUNCTIONS OF LEPIDOSTATIN-IMMUNOREACTIVE NEURONS IN *MANDUCA SEXTA*. N.T. Davis[1]*, J.A. Veenstra[2], R. Feyereisen[2], and J.G. Hildebrand[1,2]. [1]ARL Division of Neurobiology and [2]Department of Entomology, University of Arizona, Tucson, AZ 85721.

In the sphinx moth *Manduca sexta*, we have identified a neuropeptide, Lepidostatin (LS), whose primary structure is AKSYNGLamide. Its C-terminal structure indicates that LS is related to cockroach allatostatins. We used an antiserum that recognizes LS to map LS-immunoreactive (LS-ir) neurons in *M. sexta* larvae. Many interneurons are LS-ir; thus, LS may have neuromodulatory functions. Thoracic motor neurons are LS-ir, but immunoreactivity is lost at metamorphosis. LS-immunoreactivity of these motor neurons may indicate myotrophic or myomodulatory functions. LS and diuretic hormone immunoreactivities are colocalized in neurosecretory cells of the brain and abdominal ganglia; thus LS, released as a neurohormone, may have functions related to diuresis. LS-ir cells of the terminal ganglion innervate the hindgut; bioassays indicate that LS inhibits hindgut contractions. LS does not appear to have an allatostatic function.

[Supported in part by NIH grant AI-23253 to JGH.]

333.10

FRFamide FAMILY OF NEUROPEPTIDES IN *APLYSIA*: MOLECULAR, BIOCHEMICAL, AND PHYSIOLOGICAL INVESTIGATIONS. J. Rybak*[1], V. Alexeeva[1], V. Brezina[1], E. C. Cropper[1], I. Kupfermann[2], I. Orekhova[1], D. A. Price[2], F. S. Vilim[1], and K. R. Weiss[2]. [1]Mt. Sinai Med. Schl., NY, NY 10029; [2]Columbia Univ., NY, NY 10032; [3]C.V. Whitney Lab, St. Augustine, FL 32086.

Buccal muscles of *Aplysia* are controlled by primary transmitters and modulatory neuropeptides that interact with each other to shape the characteristics of muscle contractions. Thus, to understand the normal functioning of the buccal mass it is desirable to characterize the full complement of neuropeptides that modulate this organ. Often modulatory neuropeptides exist as members of multi-peptide families. Some peptides were originally characterized using biochemical techniques, others were identified using molecular biology.

Based on cloning studies we now report that three previously identified FRF peptides (a, b, and c; Cropper et al., J. Neurophysiol. 72:2181, 1994) are members of a larger family. The FRF precursor encodes two additional peptides: FRFd and FRFe. Sequential IEX-HPLC and RP-HPLC have shown that all five FRFs are expressed in buccal neurons. Like FRFa-c, FRFd and FRFe are bioactive and depress the size of contractions of the ARC muscle (FRFe is less potent than FRFa-d in this system). Both B15- and B16-elicited contractions are depressed, but the latter more effectively. This differential effect suggests a presynaptic site of action of the FRFs. However, all the FRFs also act directly on the muscle where they activate a voltage-dependent K current, thus depressing contractions. These actions are likely to be physiologically relevant since we find that the FRFs are released in a calcium dependent manner in response to nerve stimulation.

Supported by NIMH, DFG, and the Whitehall Foundation.

333.11

CLONING AND SEQUENCE OF A FULL LENGTH FMRFAMIDE CODING cDNA FROM THE BRAIN OF THE CUTTLEFISH *Sepia officinalis*. P.K. Loi* & N.J. Tublitz. Inst. of Neuroscience, University of Oregon, Eugene OR 97403

The overall aim of our work is to determine the neurotransmitters involved in the regulation of chromatophore function in cephalopods. Our previous work (Loi *et al*, *J. of exp. Biol.*,1996) showed that FMRFamide Related Peptides (FaRPs) play an important regulatory role in this system, causing contraction of the chromatophore muscles in the cuttlefish *Sepia officinalis* when applied to an isolated chromatophore bioassay. Immunological and biochemical data in that study also showed that there is likely more than one FaRP involved in chromatophore function. The purpose of this study was to identify the number and structure of FaRPs in *Sepia* using molecular techniques. As a first step in this process, PCR was performed on a *Sepia* cDNA brain library (gift of Stratagene Corp.) using primers whose sequences were taken from a FMRFamide coding transcript isolated by Dr.David Price from the squid *Loligo opalescens*. The PCR generated a 500 base pairs product which was subcloned and sequenced. This partial sequence was used as a template for generating probes to screen the cDNA library for full length transcripts that coded for FaRPs. Sequence analysis of one positive clone revealed a full length transcript of about 1900 bases (b) long. It has about 280 b at the 5' end prior to start codon, 1200 b between the start and the stop codon, and about 400 b from the end codon to the poly A tail. This transcript encodes for one copy of FIRFamide, one copy of a FaRP decapeptide (ALSGDAFLRFamide), one copy of FLRFamide, and 11 copies of FMRFamide. Each FaRP is, flanked at the 5' end with 1or 2 basic residues and an arg at the 3' end. All end with a glycine, which is necessary for the C-terminus amide. In summary, we have demonstrated that the cuttlefish expresses at least four FaRPs, one or more of which may play an important role in regulating chromatophore function.

(supported by grants from NSF and medical research foundation of Oregon)

333.12

FMRF-AMIDE ACTIVATES AN UNUSUAL RESTING NA CURRENT IN LEECH RETZIUS CELLS. J. A. Strong*, M. D. Hilborn, and C. L. Sahley. Dept. Biol. Sci., Purdue Univ., West Lafayette IN 47907

The Leech Retzius (R) cell is a large, serotonergic neuron which regulates several behaviors. The cell's usual activity pattern is sporadic or regular firing of single action potentials. FMRF-amide related peptides profoundly alter this activity pattern, causing a small depolarization and increased activity, followed within seconds by induction of endogenous, long-lasting, bursting[1]. We have used single- and two-microelectrode voltage clamp (SEVC and TEVC) in R cells from isolated, desheathed ganglia to examine possible ionic bases for these effects. SEVC experiments showed that *Helix* FMRF related peptide (FMRF; 20-50 μM) rapidly activated a small inward current (0.5 to 2 nA) and increased conductance at subthreshold potentials near rest (-60 to -50 mV), closely coinciding with the onset of increased activity. The current increased with depolarization over the range studied (-100 to -45 mV). The voltage dependence is very shallow compared to that of the Na currents underlying the action potential, and no time dependence was seen. The current persisted in nominally Ca-free solutions, but required extracellular Na. It bears some similarity to a "persistent" Na current[2], and was not blocked by Cs or saxitoxin. Modeling of the R cell indicates that the current may mediate the initial depolarization caused by FMRF. A more complete characterization of its voltage dependence is needed to determine whether it could also account for bursting. TEVC experiments showed that FMRF had no effect on the voltage-activated Ca current, but did modestly reduce voltage activated K currents (10-25%). This may account for the previously reported FMRF-induced broadening of action potentials.

[1]*Sahley et al. Neurosci Lett 164:37* [2]*Opdyke , Calabrese, J. Comp. Physol A 175:781*

MH 44789

333.13

CELLULAR SPECIFIC DISTRIBUTION AND PHYSIOLOGICAL ACTIONS OF MasFLRFamide PEPTIDES. Y.S. Miao*, E.M Waters, K. Heinzen and J.L. Witten. Department of Biological Sciences, U. Wisconsin-Milwaukee, Milwaukee, WI 53201.

A family of at least three FLRFamide peptides is present in the nervous and neuroendocrine systems of the tobacco hornworm, *Manduca sexta* (Kingan et al., 1996). Furthermore, these peptides are developmentally regulated (Witten and Truman, 1996; Kingan et al., 1996). Here we present our preliminary data demonstrating 1) a cellular specific distribution for the peptides in motoneurons (MasF7G) and neurosecretory cells (varying levels of all MasFLRFamides), 2) developmental plasticity in the expression of the peptides in the thoracic neurosecretory cells that parallels previously reported plasticity in the motoneurons (Witten and Truman, 1996) and 3) differential physiological actions of the peptides on skeletal muscle. We analyzed the actions of the MasFLRFamide peptides on the skeletal muscle target of an identified motoneuron that contains MasF7G (abdominal ISMs). As expected, MasF7G was the most potent and significantly enhanced stimulus evoked muscle tension 5 fold. This peptide-specific enhancement of muscle tension is consistent with our hypothesis for different functional roles for the MasFLRF-NH2 peptides: neurotransmitter, local neuromodulator and circulating hormone.

This research was supported by NS34117-01 to J.L.W.

333.14

FUNCTIONAL REDUNDANCY OF *DROSOPHILA* FMRF-NH_2-RELATED NEUROPEPTIDES AT THE LARVAL NEUROMUSCULAR JUNCTION. R. S. Hewes*, E. Snowdeal III, and P. H. Taghert. Dept. of Anat. and Neurobiol., Wash. U. Med. School, St. Louis, MO 63110.

Neuropeptide genes commonly encode large propeptides, from which multiple peptides are derived through enzymatic processing. The functional significance of this organization is not understood. The *Drosophila FMRFamide* gene encodes at least seven distinct neuropeptides. These peptides share a C-terminal FMRF-NH_2-like consensus sequence, while their N-terminal sequences are divergent. We have examined the effects of six of these peptides on neuromuscular transmission in *Drosophila* larvae at concentrations ranging from 1 pM to 10 μM. Five peptides enhanced abdominal muscle contractions at a threshold of 10 nM; one peptide, SAPQDFVRS-NH_2, had no effect on muscle contraction. At 10 nM the response latencies, the times to peak, and the times to full recovery for each peptide were identical. Thus, at low concentrations, these peptides have equivalent effects on nerve-stimulated muscle contraction.

Three sequence-dependent differences in peptide action were apparent at higher concentrations. At 10 μM, the dose-response curves for 2 peptides had a positive slope. For the other three peptides the dose-response curves peaked or reached a plateau at 0.1-1 μM. At these higher concentrations, the responses to the various peptides also displayed significantly different latencies and times to peak. Thus, (1) these peptides appear to be functionally redundant at low concentrations, and (2) they elicit different responses at higher concentrations. The latter may reflect sequence-dependent differences in kinetics for receptor occupancy and/or the existence of multiple receptor subtypes. We are examining these differences at a cellular level.

Supported by NIH Grant NS-21749 (P.H.T.) and American Cancer Society Postdoctoral Fellowship PF-4212 (R.S.H.).

333.15

MODULATORY EFFECTS OF FMRFamide-like PEPTIDES AND DISTRIBUTION OF FMRFamide-LIKE IMMUNOREACTIVITY IN THE CRAYFISH STOMATOGASTRIC NERVOUS SYSTEM. A.J. Tierney*, J.K. Blanck and A.J. Mercier. Dept. of Psychology, Colgate University, Hamilton, NY 13346 and Dept. Biol. Sci., Brock Univ., St. Catherines, Ontario, Canada.

FMRFamide-like peptides have been shown to modulate neural network activity in the stomatogastric ganglion (STG) of marine crustacea. We found that several FMRFamide-like peptides also alter network activity in the STG of the crayfish *Procambarus clarkii.* Peptides were bath-applied to isolated, quiescent STGs and activity was recorded via electrodes placed on dvn, lvn and mvn nerves. Relatively high concentrations (5×10^{-7} M) of NF1 (NRNFLRFamide), DF2 (DRNFLRFamide), and F1 (TNRNFLRFamide) elicited robust rhythmic activity in most preparations. We are presently investigating the effects of these and additional FMRFamide-like peptides on bursting activity of individual STG neurons. As in other crustaceans, FMRFamide-like immunoreactivity was detected in somata located in ganglia and nerves anterior to the STG. Commercially-available primary (bovine thyroglobulin conjugated anti-FMRFamide generated in rabbit) and secondary (FITC-labelled goat anti-rabbit IgG) antibodies revealed stained somata in the paired commissural ganglia (12-20 stained somata per ganglion) and the esophageal ganglion (1-3 stained somata per ganglion). An additional pair of stained somata were located in the stomatogastric nerve where the two superior esophageal nerves join this nerve. The stomatogastric ganglion contained no stained somata, but the neuropil was brightly stained. Also, 2-4 stained axons projected laterally in small nerves directly from each STG, possibly indicating direct modulation of stomatogastric muscles by a FMRFamide-like peptide.

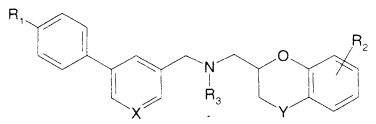
Supported by NSF-ILI Grant DUE-9550846 (AJT) & NSERC Canada (AJM).

334.1

SAR FOR NOVEL CHROMANES: ATYPICAL NEUROLEPTICS WITH 5HT1A AGONISTIC AND D2 ANTAGONISTIC ACTIVITY

H. Böttcher[1], G.D. Bartoszyk[1], J.J. Berthelon[2], M. Brunet[2], R. Devant*, H.E. Greiner[1], R. Gottschlich*[1], J. März[1], C.A. Seyfried[1], and J.J. Zeiller[2]. [1]Merck KGaA Pharm. Res., CNS-Research, 64271 Darmstadt, Germany, and [2]Lipha Centre Recherche Lacassagne, 69003 Lyon, France.

Conventional D2 neuroleptics provoke catalepsy in rats corresponding to undesired extrapyramidal side effects in man. Because 5HT1A agonists are known to antagonize haloperidol-induced catalepsy, the combination of D2 antagonistic with 5HT1A agonistic properties may have therapeutic advantages, *i.e.* may not induce catalepsy. Comparing binding (D2; 5HT1A) and behavioral data (apo climbing mouse; catalepsy rat; ultrasonic vocalization rat, USV) it turned out: unsubstituted R1



led to loss of DA activity, un- or 8-substituted R2 is essential for both DA and 5HT1A activity, H for R3 required for both DA and 5HT1A. For 5HT1A, X = N and Y = O led to the most active compound 2-[5-(4-fluorphenyl)-3-pyridylmethylaminomethyl]-chromane (IC50 values [nM]: D2 9; 5HT1A 2; ED50 values [mg/kg sc] USV 1.2, climbing 3.4, catalepsy >100).

334.2

EFFECTS OF APOMORPHINE ON THE SINGLE-UNIT ACTIVITY OF SEROTONERGIC DORSAL RAPHE NEURONS IN BEHAVING CATS.

C.A. Fornal *, C.W. Metzler, F.J. Martín and B.L. Jacobs. Program in Neuroscience, Princeton University, Princeton, NJ 08544.

Although it is well established that brain serotonin can alter dopaminergic function, relatively little is known about the possible influence of brain dopamine on serotonergic function. Recent neurochemical evidence suggest that increased dopaminergic transmission inhibits the activity of serotonergic neurons in the dorsal raphe nucleus (DRN) through increased release of somatodendritic serotonin and subsequent autoreceptor stimulation (Ferré et al., J. Neurosci. 14: 4839-4846, 1994). The present study tested this hypothesis by examining the effects of the dopamine receptor agonist apomorphine on the spontaneous activity of serotonergic DRN neurons in behaving cats. Serotonergic neurons were recorded and identified as described previously (Fornal et al., JPET 270: 1345-1358, 1994). Systemic administration of apomorphine (0.5 mg/kg, i.v.) increased the firing rate of the majority of cells studied, in contrast to its predicted action. The stimulatory action was evident within 30 sec of injection and lasted for approximately 10 to 30 min. The maximal increase in neuronal activity produced by apomorphine ranged from about 10% to 150% above baseline levels. None of the cells tested were inhibited by apomorphine in these trials. In additional experiments, apomorphine (0.5 mg/kg, i.v.) was found to potentiate the increase in serotonergic DRN neuronal activity produced by a maximally-effective dose (0.1 mg/kg, i.v.) of the 5-HT_{1A} autoreceptor antagonist WAY-100635. Overall, these results suggest that increased dopaminergic transmission exerts a stimulatory, rather than inhibitory, influence on the activity of serotonergic DRN neurons in awake cats. Supported by grants from the AFOSR (F 49620-94-1-0128) and the NIMH (MH 23433).

334.3

SPROUTING OF CATECHOLAMINERGIC FIBERS IN THE CORTEX OF RATS WITH NEONATAL LESIONS OF THE RAPHE NUCLEI.

J.B. Taylor*, M. Cunningham and F.M. Benes. Lab for Structural Neuroscience, McLean Hospital, Belmont, MA; Dept. of Psychiatry, Harvard Medical School, Boston, MA.

To explore whether trophic interactions occur between catecholaminergic and serotonergic projections to rat medial prefrontal cortex (mPFCx), a series of experiments were conducted in which lesions of the raphe nuclei were induced during the neonatal period. On postnatal days 3 and 6, male CD rat pups were given an intraventricular unilateral injection of either vehicle or 5,7-dihydroxytryptamine (10μg/μl) together with an IP injection of nomifensin (25 mg/kg). Lesioned rats showed nearly complete ablation of 5HT-IR staining in the nucleus raphe dorsalis, the nucleus raphe medianis and mPFCx. An immunoperoxidase localization of a monoclonal antibody against tyrosine hydroxylase (TH) revealed a rich plexus of varicose fibers throughout the cortical mantle of both vehicle and lesioned rats, with the greatest density being observed in layers V and VI of both groups. In lesioned rats, there was a two-fold increase in density of TH-IR varicosities on cell bodies ($t = 5.35$; $p = 0.0007$) and in neuropil ($t = 4.08$; $p = 0.0035$) of layer V; a significant increase was also observed in the neuropil of layer VI ($t = 2.63$; $p = 0.03$). When the distribution of TH-IR varicosities was compared for different neuronal subtypes in layer V, the density was increased by 100% on pyramidal neurons ($p = 0.002$), but only 60% on nonpyramidal cells ($p = 0.02$) in the lesioned group. Overall, these data are consistent with the idea that an early disturbance in the development of cortical 5HT projections may be associated with a preferential sprouting of catecholaminergic fibers in layer V, and to a lesser extent, layer VI of rat mPFCx and suggests that trophic interactions between these two monoaminergic systems may occur postnatally. Supported by MH31154 and the Stanley Foundation.

334.4

THE EFFECT OF UNILATERAL LESIONS TO THE SEROTONIN INNERVATION ON THE UPTAKE OF [^{3}H]DOPA IN RAT BRAIN.

P. Cumming*, V. Ljubic-Thibal, C. Laliberté, and M. Diksic. McConnell Brain Imaging Centre, Montreal Neurological Institute, Montreal, Canada H3A 2B4.

To test the contribution of the forebrain serotonin innervation, which contains the enzyme DOPA-decarboxylase (DDC), to the metabolism of [^{3}H]DOPA, unilateral lesions were produced by infusion of 5,7-DHT (2 μg) to the MFB of Wistar rats, some pretreated with desmethylimipramine (DMI, 10 mg/kg). One week later, rats were cannulated in the femoral artery and vein, and received [^{3}H]DOPA (200 μCi, i.v.), which circulated for 90 minutes. In vivo [^{3}H]dopamine synthesis in caudate was measured by quantitative autoradiography. Brain sections were incubated with [^{3}H]citalopram (2 nM) to label serotonin terminals, or [^{125}I]RTI (50 nM) to label catecholamine terminals.

		Ipsilateral	Contralateral
sham	[^{3}H]Citalopram	50±25 fmol/mg	25±5 fmol/mg
(n=3)	[^{125}I]RTI	45±4 fmol/mg	46±5 fmol/mg
	[^{3}H]dopamine	0.37±0.02 nCi/mg	0.45±0.02 nCi/mg
5,7-DHT	[^{3}H]Citalopram	7±2 fmol/mg	23±6 fmol/mg
(n=3)	[^{125}I]RTI	19±13 fmol/mg	40±5 fmol/mg
	[^{3}H]dopamine	0.18±0.05 nCi/mg	0.49±0.03 nCi/mg
5,7-DHT + DMI	[^{3}H]Citalopram	6±3 fmol/mg	24±1 fmol/mg
(n=3)	[^{125}I]RTI	44±10 fmol/mg	46±5 fmol/mg
	[^{3}H]dopamine	0.10±0.04 nC/mg	0.26±0.01 nCi/mg

5,7-DHT infusion extensively damaged serotonin and dopamine innervations, but DMI protected the catecholamine fibres. The decline in striatal [^{3}H]dopamine synthesis in vivo, correlating with reduced [^{125}I]RTI binding in vitro, also occurred in the DMI-treated group. This suggests that the forebrain serotonin innervation plays an important role in the synthesis of [^{3}H]dopamine from [^{3}H]DOPA in vivo, but not the storage of [^{3}H]dopamine within the caudate. This research was supported by MRC (Canada) SP-30.

334.5

EFFECT OF UPREGULATION OF $5HT_{2A}$ RECEPTORS ON DOPAMINE-MEDIATED BEHAVIORS.

J.G. Hensler*, J.M. Scalzitti and K.A. Truett. Dept. of Pharmacology, Univ. of Texas Health Sci. Center, San Antonio, TX. 78284.

Intracerebroventricular (i.c.v.) infusion of antisense oligodeoxynucleotide (AS oligo), directed against the translation initiation site of the serotonin2A ($5HT_{2A}$) receptor, for 8 days results in a 50% increase ($p \leq 0.05$) in the density of cortical $5HT_{2A}$ receptors as compared to control oligo or vehicle (see Scalzitti et al., Soc. Neurosci. Abst. in press, 1996). We have assessed the effect of these treatments on locomotor behavior and grooming behavior mediated by D2 and D1 dopamine receptors, respectively. On the 7th day of infusion, there was no significant difference in the number of line crossings induced by the D2 receptor agonist quinpirole (0.5 mg/kg, s.c.) in rats receiving AS oligo, control oligo or vehicle infusions. Following 8 days of infusion, grooming behavior (time spent grooming in a 30 min period) induced by the D1 receptor agonist SKF 38393 (2 mg/kg, s.c.) was significantly reduced (Vehicle: 447 ± 61 sec; Sense oligo: 503 ± 42 sec; AS oligo: 262 ± 13 sec, n=5 per group, $p \leq 0.01$). This decrease in D1 receptor-mediated grooming behavior in AS oligo infused rats was accompanied by a marked increase in oral dyskinesias (e.g. tongue protrusions) and directed oral stereotopies (e.g. gnawing at the cage bottom). There was no change in the binding of ^{3}H-SCH23390 (0.1 - 8nM) to D1 receptors in striatal homogenates. These data suggest that an upregulation of $5HT_{2A}$ receptors in brain, as a result of 8-day AS oligo infusion i.c.v., increases the functional sensitivity of D1 receptors. USPHS grant MH52369 and research funds from NARSAD.

334.6

MESOSTRIATAL DOPAMINERGIC ACTIVITY IS POTENTIATED BY STRIATAL 5-HT_{2A} RECEPTORS.

V. L. Taylor, G. M. Fadayel and C. J. Schmidt*. Hoechst Marion Roussel, Inc. 2110 E. Galbraith Rd. Cincinnati, OH 45215.

Although postmortem investigations have provided evidence of elevated serotonergic activity in the basal ganglia of schizophrenic patients, the consequences of such increased activity remain unknown. Evidence from rodent studies indicates that 5-HT_{2A} receptors play a permissive role in some states of dopaminergic activation and that 5-HT_{2A} receptor antagonists can attenuate such activation. In the current experiments, the *in vivo* stimulation of dopamine (DA) synthesis produced by administration of the amphetamine analogue, 3,4-methylenedioxymethamphetamine (MDMA) was potentiated by the mixed 5-$HT_{2A/C}$ agonist, 3,5-dimethoxy-4-iodophenylisopropylamine (DOI). Similar results were observed in the nucleus accumbens. The pro-synthesis effect of DOI was blocked by the selective 5-HT_{2A} receptor antagonist, MDL 100,907. The anatomical site of this interaction between 5-HT_{2A} receptors and the striatal DA system was examined using *in vivo* microdialysis in awake, freely moving rats. Systemic administration of MDMA (10 mg/kg, s.c.) produced a significant elevation of striatal DA efflux which was significantly potentiated by the infusion of DOI (50 uM) into the striatum via the microdialysis probe. DOI infusion alone had no effect on extracellular concentrations of striatal DA. The results suggest that a hyper-responsive dopaminergic system is one potential consequence of increased serotonergic activity in the basal ganglia. Although baseline dopaminergic function would be unaffected under such conditions, any physiological or pharmacological stimuli producing enhanced dopaminergic drive could precipitate the abnormal increases in dopamine release believed to underlie the initiation of a psychotic episode.

334.7

PHARMACOLOGICAL CHARACTERIZATION OF THE RECEPTORS INVOLVED IN THE MODULATORY EFFECTS OF SEROTONIN ON [^{3}H]-DOPAMINE RELEASE FROM RAT STRIATAL SLICES. B. J. van Vliet, R.R. Terpstra, E. Ronken* & M.A.W. van der Neut. Solvay Duphar BV, Department of Pharmacology, PO Box 900, 1380 DA Weesp, The Netherlands.

Serotonin (5-HT) and dopamine have been proposed as mediators of several neuropsychiatric diseases, including depression, schizophrenia and obsessive compulsive disorder. Most research about 5-HT and dopamine has focused on these neurotransmitters individually, with less attention for the pharmacology of central interactions among serotonergic and dopaminergic systems. Therefore, in the present study, we investigated the receptors involved in the modulatory effects of serotonin on [^{3}H]-dopamine release from rat striatal slices. 5-HT (0.1-10 μM) caused a dose dependent increase in spontaneous [^{3}H]-dopamine release. The selective 5-HT receptor agonists 8-OH-DPAT, 5-carboxyamido-tryptamine, DOB, DOI and 2-methyl-5-HT tested at the same concentration range did not affect [^{3}H]-dopamine release. Moreover, the selective 5-HT$_4$ receptor antagonist GR 113808 did not affect the stimulatory effect of 5-HT on dopamine release. Interestingly, we observed that the dopamine-reuptake inhibitors GBR13069 and nomifensine, but not the selective noradrenaline-reuptake inhibitor desmethylimipramine, or the highly selective 5-HT-reuptake inhibitor fluvoxamine, fully inhibited the effect of 5-HT. Taken together, our data indicate that 5-HT-evoked dopamine release is not mediated by either the 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_2$, 5-HT$_3$ or 5-HT$_4$ receptor, but rather due to the uptake of serotonin into dopaminergic terminals via *"selective"* dopamine transporters and subsequent displacement of [^{3}H]-dopamine.
Solvay Duphar B.V.

334.8

MODUALTION OF GABA BY SEROTONIN MAY INFLUENCE STRIATAL DOPAMINE RELEASE: MICRODIALYSIS STUDIES IN VIVO. Camille S. Norton* and Matthew P. Galloway, CCN Program, Dept. of Psych and Behav Neurosciences, Wayne State Univ Sch of Med, Detroit, MI 48202.

Recent studies suggest that intrastriatal application of serotonin (5-HT) facilitates dopamine (DA) release and multiple 5-HT receptor subtypes have been implicated in this phenomenon. The neuronal localization of these subtypes may elucidate whether this facilitation is mediated directly on the DA nerve terminal, indirectly through a striatal neuron or a combination of both indirect and direct influences. We and others have suggested previously that the 5-HT4 receptor subtype may regulate striatal DA release *in vivo*. Although 5-HT4 receptors are consistently found in high densities of DA projection fields, evidence suggests that they are on γ-aminobutyric acid (GABA) neurons. To investigate further the 5-HT/GABA/DA interactions, we examined the ability of 5-HT to influence extracellular GABA levels *in vivo* and the ability of selective GABA antagonists to influence the 5-HT/DA interaction in the rat striatum. Local perfusion with 5-HT (10uM) significantly increased both GABA levels (approximately 50% over baseline 24.75 fmol/ul, n=6) and DA levels nearly 7-fold over basal levels (0.96 ± 0.13 fmol/ul, n=6). In the presence of the GABA A antagonist, biccuculine (100 uM), the effect of 5-HT on DA levels was significantly attenuated to approximately 3.5-fold above baseline (1.4 ± 0.19 fmol/ul, n=6). In the presence of the selective GABA B antagonist, 2-hydroxysaclofen (100uM), the 5-HT enhancement of DA levels over baseline (2.26 ± 0.16 fmol/ul, n=6) did not differ from controls. These results support a role for GABA (possibly via a striatonigral pathway) in the 5-HT facilitation of striatal DA release.
Supported by NIDA 04120 and the Joe Young Sr. Research Fund.

334.9

ENHANCEMENT OF ANTIPSYCHOTIC-LIKE PROPERTIES OF RACLOPRIDE IN RATS USING THE 5-HT$_{2A}$ RECEPTOR ANTAGONIST MDL 100,907. M-L.Wadenberg*, P. B. Hicks, T. J. Richter and J. L. Browning. Dept. of Psychiatry, Scott & White Clinic, Temple, Texas.

High 5-HT$_{2A}$ receptor affinity in the presence of low DA D$_2$ receptor affinity is implicated in the favorable clinical profile of clozapine. Recently, MDL 100,907, a potent 5-HT$_{2A}$ receptor antagonist with a 300 fold selectivity for the 5-HT$_{2A}$ over the DA D$_2$ receptor site, was proposed as potentially antipsychotic. Using the conditioned avoidance response (CAR) behavior (an animal model traditionally presumed to reflect antipsychotic rather than extrapyramidal side effect, EPS, liability) as a tentative index for antipsychotic efficacy, MDL 100,907 was evaluated as a potential antipsychotic, and also tested as an adjunctive with the specific DA D$_2$ receptor antagonist raclopride. Adult male Sprague-Dawley rats were used. Rats were trained and tested in a computer-assisted traditional shuttlebox paradigm. Behavioral variables recorded: avoidance, escape, escape failures, and intertrial crosses.

MDL 100,907 (0.1, 0.5 or 1.0 mg kg^{-1}, s.c.) produced a slight suppression of CAR only at 60 minutes after administration. Raclopride (0.14 mg kg^{-1}, s.c.; ED$_{50}$) alone suppressed CAR only at 30 min after administration. Pretreatment with MDL 100,907 (0.5 - 1.5 mg kg^{-1}, s.c.) enhanced and prolonged the raclopride-induced suppression of CAR. Raclopride (50 μg kg^{-1}, s.c.) alone had no effect on CAR. Co-treatment with MDL 100,907 (1 mg kg^{-1}, s.c.) and raclopride (50 μg kg^{-1}, s.c.), produced a long lasting suppression of CAR both compared to vehicle treated and raclopride treated animals. **Conclusions:** The selective 5-HT$_{2A}$ antagonist alone does not display an antipsychotic profile. However, a selective 5-HT$_{2A}$ receptor antagonist as an adjunctive therapy to a weak DA D$_2$ receptor blocking antipsychotic might be helpful in improving the treatment of schizophrenia, and at the same time minimize EPS.

334.10

DIFFERENTIAL EFFECTS OF CHRONIC FLUOXETINE (FLU), CLOMIPRAMINE (CIM) AND IMIPRAMINE (IMP) ON BASAL AND AMPHETAMINE (AMP)-INDUCED DOPAMINE (DA) RELEASE. J. Ichikawa*, T. Kuroki, M.T. Kitchen and H.Y. Meltzer. Dept. Psychiatry, Case Western Reserve Univ. Sch. Med., Cleveland, OH 44106

We reported that the tricyclic antidepressant drugs (ADD) CIM (10 mg/kg) and IMP (10 mg/kg) increased extracellular DA levels in the striatum (STR) but not the nucleus accumbens (NAC) whereas the selective serotonin reuptake inhibitor FLU (10 mg/kg) decreased extracellular DA levels in both regions (Ichikawa and Meltzer, 1995), suggesting that DA transmission may not be related to the therapeutic action of ADD. However, the antidepressant effects of ADD develop over several days to weeks after starting medication. Therefore, we examined the effect of chronic FLU, CIM, IMP and the vehicle control (VEH) (10 mg/kg/day for 24-28 days in drinking water) on basal and AMP-induced DA release in rat STR and NAC after 3-5 days withdrawal, using in vivo microdialysis (N=6-8). Basal striatal DA levels were not altered by chronic FLU (11.5±1.1 fmol/30 min), CIM (12.7±2.0) and IMP (11.0±2.1), as compared to VEH (13.1±1.1) whereas chronic FLU (6.5±0.6) and CIM (5.1±0.6) decreased, but IMP (12.7±1.5) increased basal DA levels in the NAC (VEH, 9.1±0.7). AMP (0.5 mg/kg, SC)-induced DA release was potentiated by chronic IMP (189±25% of VEH net-AUC: 178±29 fmol/180 min) but was attenuated by chronic CIM (55±18%) and not significantly altered by FLU (140±21%) in the NAC whereas chronic FLU (54±8%), CIM (63±11) and IMP (39±4) attenuated AMP-induced DA release in the STR (VEH, 319±35 fmol/180 min). These results suggest that chronic ADD may attenuate AMP-induced activation of striatal DA transmission while sparing basal transmission. Potentiation of basal and AMP-induced DA transmission in the NAC by chronic IMP, but not FLU or CIM, could relate to the potential to produce mania in patients with major depression. Supported by NARSAD.

334.11

SEROTONIN-DOPAMINE INTERACTIONS AFTER CHRONIC ANTIDEPRESSANT TREATMENT S. Koch* and M. P. Galloway, CCN program, Dept. of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI 48202

Repeated administration of antidepressant agents such as the selective serotonin reuptake inhibitor (SSRI) fluvoxamine (FLV) is generally thought to lead to enhanced serotonergic neurotransmission. Since we, and others have shown previously that intracerebral application of serotonin (5HT) facilitates dopamine (DA) release in vivo, we used microdialysis to determine the effect of repeated FLV treatment (10 mg/kg bid x14 d, 1 or 2 day washout) on extracellular (EC) striatal DA levels after a challenge with either FLV, MDMA, or 5HT. In naive rats, acute FLV (10 or 20 mg/kg; ip) increased EC-DA 9-15% whereas in animals pretreated with FLV, EC-DA levels were increased by 33±11% (n=7) and 73±23% (n=12), respectively. Administration of the 5HT-releasing hallucinogen (+)-MDMA (1 mg/kg iv) increased EC-DA 503±101% (n=10) in naive subjects, an effect that was not significantly different in animals treated chronically with FLV (451±103%, n=10). Intrastriatal perfusion of 5HT (10 μM, 20 min) increased EC-DA to 521±56% (n=5) in untreated rats whereas the ability of 5HT to facilitate DA release was significantly attenuated to 305±16% (n=8) in subjects pretreated with chronic FLV. The results support the contention that repeated treatment with an SSRI modifies the interaction between 5HT and DA and suggest that 5HT regulation of DA release may be relevant to the therapeutic action of antidepressants. Supported by NIDA-04120 and the Joe Young Sr Research Fund.

334.12

MODULATION OF THE FIRING ACTIVITY OF LOCUS COERULEUS NEURONS IN THE RAT BY THE 5-HT SYSTEM. P. Blier, N. Haddjeri and C. de Montigny* Neurobiological Psychiatry Unit, McGill University, Montréal, Canada, H3A 1A1.

The aim of the present study was to investigate a putative modulation of locus coeruleus (LC) norepinephrine (NE) neurons by the serotonin (5-HT) system using *in vivo* extracellular unitary recordings and microiontophoresis in anesthetized male Sprague-Dawley rats. To this end, the potent 5-HT$_{1A}$ receptor antagonist WAY 100635 was used. WAY 100635 and 5-HT failed to modify the spontaneous firing activity of LC NE neurons when applied by microiontophoresis. However, the injection of WAY 100635 (100 μg/kg, i.v.) suppressed the spontaneous firing activity of LC NE neurons. Lesioning 5-HT neurons increased the firing activity of LC NE neurons and abolished the effect of WAY 100635 on these neurons. The selective 5-HT$_3$ receptor antagonist BRL 46470A (10 and 100 μg/kg, i.v.), the 5-HT$_{1D}$ receptor antagonist GR 127935 (100 μg/kg, i.v.) and the 5-HT$_{1A/1B}$ receptor antagonist (-)pindolol (15 mg/kg, i. p.) failed to prevent the suppressant effect of WAY 100635 on the firing activity of LC NE neurons. However, it was prevented by the 5-HT$_{1A}$ receptor antagonist BMY 7378 (1 mg/kg, i. v.), the non-selective 5-HT receptor antagonists spiperone (1 mg/kg, i. v.) and metergoline (1 mg/kg, i. v.), and the 5-HT$_2$ receptor antagonist ritanserin (0.5 mg/kg, i. v.). In conclusion, these results indicate that 5-HT neurons exert a tonic inhibitory effect on NE neurons which is potentiated by WAY 100635. 5-HT$_{1A}$ receptors are not likely involved in this effect of WAY 100635 because it is not prevented by the 5-HT$_{1A}$ receptors antagonist (-)pindolol. Given that WAY 100635 has significant 5-HT$_7$ affinity and that all drugs that prevented its suppressant effect on NE neurons are endowed with 5-HT$_7$ affinity (except BMY 7378 which shuts off 5-HT neuron firing activity), it is concluded that the latter effect of WAY 100635 is mediated by presynaptic 5-HT$_7$ receptors.

334.13

Concentrations of Free Dopamine, Serotonin, Norepinephrine and Their Metabolites in Ventricular CSF of Parkinson's Disease Patients H. Zheng, M.C. Schiess*, H.J. Nauta, J.G. Bonnen Departments of Neurology and Neurosurgery, Univ. of Texas Medical Branch, Galveston, TX 77555-0539.

In order to correlate neurotransmitter concentrations with relative severity of various Parkinsonian symptoms this study determined the concentrations of dopamine (DA), serotonin (5-HT),norepinephrine (NE), and their metabolites in ventricular CSF (V-CSF) of Parkinson's Disease (PD) patients compared to non-PD controls. V-CSF was obtained intra-operatively from eight hyperkinetic PD patients, 4F & 4M ages 43 - 71, undergoing stereotactic posteroventral pallidotomy and four age-matched non-PD controls. CSF which had been immediately stored at -70 °C was analyzed for DA, 5-HT, NE, and their metabolites including DOPAC, HVA, 5-HIAA, and precursor 5-HTP using high performance liquid chromatography with electrochemical detector and quantified by the internal standard method with DHBA and a lower limit of detection of 20 pg/ml. The mean concentration of 5-HT in controls of 0.388 $\pm$0.036 ng/ml was significantly lower than PD of 3.134 $\pm$1.707 ng/ml; 5-HTP, HVA, NE were lower and 5-HIAA higher in controls. Free DA in controls was not detected but had a mean of 8.37 $\pm$2.58 ng/ml in PD. Our preliminary results suggest that free 5-HT is elevated in the V- CSF of hyperkinetic PD patients relative to controls and that free DA concentrations are elevated which may be due to treatment with levodopa.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION II

335.1

PCRH-REB MEDIATES TRANSCRIPTIONAL REGULATION OF POMC GENE UPON CRH TREATMENT OF AtT20 CELLS Q. Han and J. L. Roberts* Fishberg Center for Neurobiology, Box 1065, Mount Sinai Medical School, New York, NY 10029

CRH stimulates POMC transcription through several promoter elements, including PCRH-RE(POMC CRH-responsive element) shown to bind PCRH-REB, which was cloned and shown to be indentical to the largest subunit of Replication Factor C. PCRH-REB is a nuclear protein and has multiple PKA sites. In order to determine the mechanism of PCRH-REB activation of POMC transcription we characterized changes in synthesis or post-translational modification of this transcription factor upon CRH treatment of AtT20 cells. We performed Western blot analyses using a polyclonal antibody raised against recombinant PCRH-REB and a "T7-Tag" monoclonal antibody. While CRH did not appear to change the levels of PCRH-REB, the factor appeared to undergo proteolytic processing. The full length protein,140kD, exists only in the initial AtT20 protein extract; whereas the primary immunoreactive proteolytic product, about 70kD, is predominant in the isolated nuclear extract. Interestingly, although there was only a single predominant species of PCRH-REB from nuclear extract upon CRH treatment of AtT20 when analyzed on SDS-PAGE, multiple species came out on a non-denaturing gel. One species was replaced by another one with different mobility characteristic and highest level of expression at 60min when CRH stimulation of POMC transcription reaches maximum, a pattern change that is consistent with the time course of CRH stimulatory effects on POMC transcriptional activity. (supported by NIH-DK27484)

335.2

PCRH-REB APPEARS TO PARTICIPATE IN THE BAY K STIMULATION OF POMC TRANSCRIPTION IN AtT20 CELLS. Jacques Jospitre Jr.* & James L. Roberts, Fishberg Center for Neurobiology, Box 1065, Mount Sinai Medical School, New York, NY 10029

Previous studies have shown that the L-type calcium channel agonist BayK can stimulate POMC transcription in AtT20 cells similarly to CRH, yet BayK does not induce the large increase in cAMP levels that CRH does. Our group has recently characterized a putative transcription factor, PCRH-REB (POMC Corticotropin Releasing Hormone-Regulatory Element Binding protein) which is induced by CRH, presumably through the activation of the cAMP/PKA system. Since BayK can apparently activate POMC transcription while bypassing the PKA system, we wanted to determine if BayK affected the PCRH-REB system. Cultures of AtT20 cells were treated with BayK (100nM) or CRH (10nM) for 60 min and nuclear extracts were prepared according to the Dignam method. Gel shift analysis was performed with a labeled PCRH-RE (-178/-155 in rat POMC promoter). BayK treated extracts gave a 2-3 fold induced gel shift relative to the control AtT20 cells, that was apparently identical to that observed with CRH. To further characterize the composition of the BayK induced gel shift, an antisera raised against PCRH-REB was used for supershift analysis. A quantitative shift of the BayK induced shift was observed, which was similar to the CRH shift, implying that PCRH-REB was responsible for both shifts. Further, it is known that CRH induced POMC transcription is inhibited by divalent cations, such as cadmium or cobalt, independent of their effects on calcium channels, and they have been shown to inhibit the CRH induced PCRH-REB gel shift. Current work is focused on determining the dose response of the divalent cation shift inhibitions in both the Bay K treated and the CRH treated cells. Preliminary data suggest that the nuclear extracts from the BayK or CRH treated cells show different sensitivities to the different cations. Thus, although BayK is able to produce an identical shift to CRH, the different sensitivities to the cations and cAMP suggest that they may be acting through different pathways that eventually converge on PCRH-REB or an associated protein.(Supported by NIH-DK27484)

335.3

CLONING AND CHARACTERIZATION OF AN OVINE PITUITARY CORTICOTROPIN RELEASING HORMONE (CRH) RECEPTOR COMPLEMENTARY DEOXYRIBONUCLEIC ACID. D.A. Myers* and T.R. Myers. Dept. Physiology, Univ. of Oklahoma HSC, Okla. City, OK 73190.

In sheep, controversy exists regarding the role of CRH in regulating the hypothalamo-pituitary-adrenal axis (HPA). While numerous reports indicate that AVP is the major "CRH" in sheep, other studies support CRH as the primary ovine corticotropin releasing hormone. To aid in elucidating the role of CRH in activating the ovine HPA we cloned two ovine pituitary CRH receptor (CRHR) cDNAs. Degenerate primers based on pituitary CRHR sequences from human, mouse and rat were used to PCR a 380bp cDNA from ovine anterior pituitary mRNA. The PCR cDNA exhibited >90% nucleic acid homology to rodent and human pituitary CRHR and was used to screen an ovine pituitary cDNA library. We identified two cDNA clones (oCRa and oCRb). The nucleotide sequence of oCRa predicts a truncated CRHR (158 amino acids) exhibiting >90% nucleic and amino acid homology to human, rat and mouse CRHR1 subtype containing the N-terminal extracellular domain and the first transmembrane domain (TM1). oCRa diverges from rat, human, mouse pituitary CRHR and oCRb immediately 3' of the TM1 domain after a putative signal for an exon splice junction (AG/GT), resulting in an in-frame stop codon and thus a premature C-terminus. The sequence of oCRb is identical to oCRa up to the putative splice site in oCRa. The sequence of oCRb encodes a protein with >90% nucleic and amino acid homology to rat, human and mouse pituitary CRHR through TM7. oCRb differs from human, mouse and rat pituitary CRHR in that 132bp have been deleted within the C-terminal intracellular domain. The deletion may result from alternate splicing, or may represent divergence of ovine CRHR from other CRHRs. We are currently expressing oCRa and oCRb in COS-7 cells and examining their binding and signal transduction properties. NIH HD33147

335.4

AtT20 CELLS TRANSFECTED WITH THE RAT PREPRO-TRH cDNA EXHIBIT INCREASED SENSITIVITY TO ACTH SUPPRESSION BY DEXAMETHASONE: PROPOSED ROLE OF CORTICOTROPIN RELEASE INHIBITING FACTOR (CRIF). S. Revskoy, F. Aird, P. Whybrow, E. Redei* Lab. of Neuroendocrinology, Dept. of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

The objective of this study was to elucidate a possible link between the effects of the preproTRH intervening peptide (178-199) and glucocorticoids on the inhibition of ACTH secretion from cortiocotrophs. We studied the effect of dexamethasone (DEX) on AtT20 cells transfected with rat preproTRH cDNA containing deletions of either a region of preproTRH cDNA encoding 178-199 (i.e. lacking CRIF) or a fragment located down-stream from the latter region. In addition, the influence of different cell culture conditions, including high and low cell culture density and the effect of steroid-free fetal calf serum, on ACTH suppression by DEX was also investigated in these cell lines. CRIF containing transfected cells demonstrated higher sensitivity to DEX, reaching more than 50% suppression of ACTH secretion at 1-10 nM DEX, while ACTH secretion by both wild type (*wt*) and transfected cells AtT20 lacking CRIF was suppressed to the same extent by 10-100 nM DEX. Therefore, we suggest that the expression of preproTRH in corticotrophs lowers the concentration of DEX required for inhibition of ACTH compared to those cells lacking CRIF. Significant modifying effect of the cell culture conditions on DEX-induced ACTH suppression was also demonstrated. In particular, unlike high density cultured *wt* AtT20 cells, CRIF containing transfected cells did not respond to steroid deprivation by increasing ACTH secretion, similarly to *wt* AtT20 cells cultured at low density. Furthermore, the steroid depleted media did not promote cell proliferation, measured by ^{3}H-TdR incorporation, in CRIF containing transfected cells cultured at high cell density. Hence, CRIF may exert its effect, partially independent of glucocorticoid actions, perhaps through tissue specific growth modifying factors. *Supported by Berman Foundation.*

335.5

IMMUNOTARGETED LESIONS OF PARAVENTRICULAR (PVN) CRF AND AVP NEURONS IN DEVELOPING RATS REVEAL THE PATTERN OF MATURATION OF THESE SYSTEMS AND THEIR FUNCTIONAL IMPORTANCE. C.-D. Walker*[1], P. Tankosic[2], F.J.Tilders and A. Burlet[2]. Douglas Hospital Research Center[1], Dept of Psychiatry, McGill University, Montreal, PQ H4H 1R3, Canada and INSERM U308[2], University of Nancy, Nancy, France.

During the first weeks of life in rats, the control of ACTH secretion is critical in maintaining low circulating levels of corticosteroids. In neonatal rats, the specific role of both CRF and AVP from the hypothalamic PVN in controlling basal and stimulated ACTH release has not yet been defined. We performed specific PVN lesions of CRF or AVP neurons in 4- and 14 day (d)-old pups by injecting ricin A (Tx) associated with monoclonal antibodies directed towards CRF (CRF/Tx) or AVP (AVP/Tx) or with nonspecific IgGs (IgG/Tx) as controls. Pups received either sham surgery or adrenalectomy (ADX) at the time of lesion and were sacrificed 5 days later for determination of plasma ACTH levels, brain CRF and AVP peptide and mRNA levels. Although the specificity of the lesion was greater in 19d than in 9d-old pups, CRF and AVP lesions did not alter basal ACTH secretion. In 9d pups, CRF mRNA levels were greatly reduced in the CRF/Tx and AVP/Tx groups compared to IgG/Tx group, while in 19d pups, only the CRF/Tx group had reduced CRF mRNA levels. At both ages, AVP mRNA levels were decreased only in the AVP/Tx group. After ADX, AVP and CRF mRNA levels increased in 19d-, but not 9d-old pups and lesions reduced the ADX-induced increases. Plasma ACTH levels after ADX were not affected significantly by the lesions at both ages. These results indicate that 1) neonatal PVN neurons are still phenotypically immature in the 2nd week of life, 2) the control of ACTH secretion by corticosterone is exerted at different levels as a function of age and 3) lesions of either CRF or AVP neurons are not sufficient to significantly alter ACTH secretion during development. (Supported by NSERC Canada).

335.6

LOCALIZATION OF CORTICOTROPIN RELEASING HORMONE RECEPTOR (CRHR) MESSENGER RIBONUCLEIC ACID IN THE FETAL PITUITARY DURING DEVELOPMENT. T.R. Myers* and D.A. Myers. Dept. Physiology, Univ. Oklahoma HSC, Oklahoma City, OK 73190.

In sheep, development of the fetal hypothalamo-pituitary-adrenocortical (HPA) stress axis in late gestation results in a preterm rise in fetal plasma cortisol. Fetal cortisol provides the signal for birth in sheep, and matures organs necessary for neonatal survival in most species. In fetal sheep, the paraventricular nucleus is fundamental for HPA maturation. However, little is known about neuroendocrine signals controlling maturation of the anterior pituitary (AP) corticotrope. We have cloned a cDNA for the ovine CRHR; in this study we used double-labelling *in situ* hybridization to localize mRNA for CRHR in POMC mRNA expressing cells of the pituitary during development. Fetal pituitaries were collected at 105; 118; 128 and 144 days of gestation (DG;term=148DG;-n=5/group) and subjected to simultaneous *in situ* hybridization utilizing a digoxigenin labelled 450 base ovine POMC cRNA probe and a ^{33}P labelled 380 base ovine CRHR cRNA probe. At all ages, CRHR mRNA co-localized with corticotropes in the anterior pituitary. Of interest, lower POMC expressing corticotropes were typically observed with greater CRHR signals at all ages. We did not observe CRHR hybridization over POMC labelled cells of the neurointermediate lobe melanotropes at any age. CRHR mRNA levels in corticotropes were higher in 105DG fetal AP than at other ages. 144DG fetuses exhibited the lowest hybridization signal; CRHR mRNA in 118 and 128DG groups were intermediate. We have previously shown that CRH expression in the PVN is low at 105DG and peaks at 128DG (Endocrinol.132:2109). Our results indicate that CRHR mRNA levels decline during the period of development that the fetal HPA matures possibly indicating a down regulation of CRHR by CRH or negative feedback regulation by increasing levels of fetal cortisol. NIH HD33147

335.7

DEVELOPMENTAL PROFILE OF THE CORTICOTROPIN RELEASING FACTOR RECEPTOR (CRF_2) IN THE RAT. M. Eghbal-Ahmadi, C. Hatalski and T.Z. Baram* Pediatrics and Anatomy & Neurobiology, University of California, Irvine, CA 92717.

Rationale: CRF_2, the second member of the CRF receptor family, has a distinct distribution in the adult brain, suggesting that it may mediate different functions than those of CRF_1. The goal of this study was to determine the age-dependent distribution of CRF_2 messenger RNA (CRF_2 -mRNA) in the rat.

Methods: Pups aged 3, 5 ,7, 9 and 12 days (2-3/age point), as well as adults, were sacrificed under stress-free conditions and brains were frozen and sectioned coronally. In situ hybridization histochemistry (ISH) using a riboprobe (courtesy Drs. Lovenberg & Chalmers) was followed by quantitation using the MCID system.

Results: In the adult, maximal optical density (OD) occurred over the ventromedial hypothalamic nucleus (VMH), followed by the hippocampus and the cortical and medial amygdala nuclei (ACO, AM). Lower signal intensity was observed over the lateral septum. Within the hippocampus, OD rank order was CA3 > dentate gyrus granule cell layer > CA1. CRF_2 was first detectable in the VMH, hippocampus, septum and amygdala complex on postnatal day 3. At that age, OD over the hippocampus was lower than the OD over VMH and the lateral septum. By day 7, CRF_2-mRNA levels in the hippocampus equaled those in the VMH. By day 9, CRF_2-mRNA was clearly localized in the AM, and VMH levels were significantly higher than hippocampal levels, a pattern maintained to adulthood. By day 12, CRF_2-mRNA was evident in the ventral thalamus.

Conclusion: Using ISH, the onset of CRF_2 gene expression is region specific. The rates of increase in CRF_2-mRNA abundance varies among the regions studied, consistent with differential maturation of the functions mediated by this receptor. Supported by NIH NS 28912 (TZB).

335.8

HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL (HPA) AXIS CHANGES IN TRANSGENIC MICE WITH IMPAIRED GLUCOCORTICOID RECEPTOR FUNCTION. J.M.H.M. Reul*, A.C.E. Linthorst, F.J.H. Tilders[1], I. Dijkstra[1], N. Barden[2], F. Holsboer and S. Karanth. Max Planck Inst. of Psychiatry, Clinical Inst., Munich, Germany; [1]Research Inst. Neurosciences, Free Univ., Amsterdam, The Netherlands; [2]Molecular Psychogenetics Lab., Laval Univ., Quebec, Canada.

Recently, a transgenic mouse with impaired glucocorticoid receptor (GR) function was created to serve as an animal model for the study of HPA axis changes as occuring in major depression. Adult male transgenic mice showed no changes in baseline plasma ACTH and corticosterone (CORT) levels. Novel environment stress produced an enhanced ACTH response in transgenic mice, whereas CORT responses were not different. In view of this disparity in pituitary and adrenocortical responses, we directed our studies toward the *in vitro* regulation of ACTH secretion in the intact pituitary-hypothalamic complex (PHc) and the pituitary (PI) alone. Basal ACTH release by PHc and PI from normal and transgenic mice was similar, while the release by PI was lower than that by PHc. Stimulation of tissues with either high K^+ (56 mM) or CRH produced an enhanced ACTH release from both PHc and PI, while the response in PI was larger than that in PHC. The responses to these stimuli were markedly enhanced in tissues from transgenic mice. CORT feedback on basal and CRH-stimulated ACTH release was more effective in PHc than in PI. The feedback capacity of CORT to restrain basal as well as CRH-stimulated ACTH release was highly impaired in tissues of transgenic mice, whereas the feedback appeared to be more affected in the PHc than that in the PI of these animals. In conclusion, the *in vitro* data revealed intrahypothalamic mechanisms operating to fine-tune stimulus-evoked ACTH responses and to facilitate feedback action of glucocorticoids. In the transgenic mice we found impaired negative feedback combined with "normal" (i.e., noncompensated) *in vivo* plasma corticosterone responses. Therefore, this transgenic animal may be regarded as a valuable model to study functional glucocorticoid insufficiency at the central nervous system level. *Supported by VW-Foundation (I/70 543), Dutch Found Med Health Res (900-564-034) and EC (CI1*-CT93-0092)*

335.9

CORTICOSTERONE MODULATES CORTICOTROPIN-RELEASING HORMONE GENE EXPRESSION ASSOCIATED WITH HYPOVOLEMIA IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS S. M. Tanimura*, G. Sanchez-Watts & A. G. Watts NIBS program and Dept. of Biological Sciences, USC, Los Angeles, CA 90089

Results from our lab have shown that polyethylene glycol (PEG)-induced iso-osmotic hypovolemia leads to increased levels of corticotropin-releasing hormone (CRH), proenkephalin (pENK), and neurotensin/neuromedin N (NT/N) mRNAs in the dorsal medial subdivision of the paraventricular nucleus of the hypothalamus (PVHmpd). Increases in CRH mRNA levels are seen despite the rising plasma concentrations of corticosterone (CORT). To investigate the potential role of CORT in mediating changes in CRH mRNA in this model, we have used *in situ* hybridization to determine CRH mRNA levels associated with hypovolemia in the presence of constant plasma concentrations of CORT. Male SD rats were bilaterally (or sham) adrenalectomized (ADX) under halothane anesthesia and implanted with either placebo, 25mg, 50mg, or 100mg CORT replacement pellets (Innovative Research of America). Seven days later, animals were briefly anesthetized and injected subcutaneously with 5mls of 40% PEG (w/v). 5 hours after injection, rats were anesthetized, blood samples drawn and perfusion-fixed. Frozen 15 µm coronal sections were cut through PVH and hybridized using cRNA probes for CRH and pENK. In the intact and all CORT treatment groups, there was a significant increase in the level of CRH mRNA hybridization measured in the PVHmpd of PEG-treated rats as compared to vehicle injected controls. However, in the absence of CORT, the accumulation of CRH mRNA signal was significantly decreased. An increase in pENK mRNA was measured in both intact and placebo-treated ADX'd groups administered PEG, suggesting that the PVHmpd neurons were still able to respond to PEG in the absence of CORT. At least two mechanisms may explain the decreased CRH mRNA levels measured in the PEG-treated placebo group (1) CORT acts (with other factors) to direct transcriptional regulation; in the absence of CORT, CRH gene expression is not activated. (2) In the absence of CORT, mRNA stability is compromised and thus, mRNA degradation occurs more rapidly. Experiments are currently in progress to investigate these two possibilities. (Supported by NS29728).

335.10

Regulation of Corticotropin Releasing Factor (CRF) mRNA in Primary Amygdalar Cultures as Determined by *In Situ* Hybridization. JW Kasckow*[1,2], A Regmi[1], PS Gill[1], MK Norman[1], NM Richtand[1,2], SJ Fudala[1], TD Geracioti[1,2]. [1]U. Cincinnati College of Med., Dept. Psychiatry, Cincinnati, OH. 45267. [2] Cincinnati VAMC, Psychiatry Service, Cincinnati, OH 45220.

The CRF containing amygdalar neurons have been postulated to play a role in the stress response. Little is known as to which stimuli regulate CRF messenger RNA in the amygdala. The effects of dexamethasone (DEX) and the cytokines - interleukin 1 (IL1) and interleukin 6 (IL6) on CRF mRNA levels in primary rat amygdalar cultures derived from E19 pups were evaluated. The amygdala was dissected bilaterally and dissociated with 0.25% trypsin. Cells were plated in DMEM:F12, 10% fetal bovine serum, 14 mM glucose, 15 mM $NaHCO_3$, 5 mM Hepes and pen/strep on Nunc tissue culture slides. At 17 days, triplicate sets of cells were treated 12 hours with either DEX, IL1 or IL6 in β-Pit Julip. Following fixation, cells were hybridized with an ^{35}S-labelled rat CRF probe (provided by Dr. A Seasholtz; U Michigan, Ann Arbor, MI). Following this, slides were dipped in NTB-2 emulsion and subsequently developed. Silver grains in cells were counted per treatment and the effects of various treatments were determined based on significance at the $P<0.05$ level. 100 nM DEX produced very slight but non-significant increases in CRF mRNA relative to control (+18%). Similar non-significant increases were seen with 100 pM IL1 treatment (+14%). 100 pM IL6 treatment produced a significant 2 fold increase in CRF mRNA relative to control. Further characterization of these amygdalar cultures is under way in order to investigate their responsiveness to other potential stimulators and inhibitors of CRF involved in the stress response. These preliminary data suggest that the role of glucocorticoids in regulating amygdalar CRF differs from that in the hypothalamus and that some cytokines do appear to regulate amygdalar CRF. Dr. Kasckow is a Pfizer awardee and a recipient of a NARSAD Young Investigators Award.

335.11

Regulation of Corticotropin Releasing Factor (CRF) mRNA in Clonal Cell Lines as Determined by *In Situ* Hybridization. A Regmi[1], JW Kasckow[1,2], PS Gill[1], AB Norman[1], NM Richtand*[1,2], TD Geracioti[1,2]. [1]U. Cincinnati College of Med., Dept. Psychiatry, Cincinnati, OH. 45267. [2] Cincinnati VAMC, Psychiatry Service, Cincinati, OH 45220.

The NPLC-KC human hepatoma and the BE(2)-M17 human neuroblatoma cell lines have been shown to synthesize and secrete the CRF peptide as well as express CRF messenger RNA. Here, we have analyzed the effects of various second messengers on CRF mRNA utilizing *in situ* hybridization. Cells were plated in DMEM:F12, 8% fetal bovine serum, 2 mM L-glutamine, non-essential amino acids and 10 U/ml pen/strep on Nunc tissue culture slides. BE(2)-M17 cells had been pretreated with 5 μM retinoic acid for 3 days prior to treatment with various stimuli. Both clonal cell lines were exposed to various stimuli for 12 hours in β-Pit Julip. Following fixation, cells were hybridized with an ^{35}S-labelled human CRF probe (provided by Dr. A Seasholtz; U Michigan, Ann Arbor, MI). Following this, slides were dipped in NTB-2 emulsion and subseqeûently developed. Silver grains in cells were counted per treatment and the effects of various treatments were determined based on a significance level of $P<0.05$ level. In the BE(2)-M17 cells, 100 nM dexamethasone produced slight but significant increases in CRF mRNA relative to control (+64%). Significant increases in CRF mRNA in these cells were also noted with 20 nM TPA and 30 μM forskolin (3 fold elevation relative to controls in both). With the NPLC-KC cells, 100 pM interleukin-1, 20 nM TPA and 30 μM forskolin produced significant elevations relative to control (respectively +45%, +71% and +64%). The above findings make these cells potentially useful models for studying gene expression of CNS CRF neurons. Dr. Kasckow is a Pfizer awardee and a recipient of a NARSAD Young Investigators Award.

335.12

NEUROENDOCRINE DYSFUNCTION IN CORTICOTROPIN-RELEASING FACTOR RECEPTOR 1 (CRFR1)-DEFICIENT MICE K.-F. Lee*, J.-M. Aubry, W.T. Berggren and W.W. Vale. Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037

Signaling pathways dependent on the members of the CRF gene family, including CRF and urocortin, are involved in a wide-range of neural and endocrine functions. Two CRF receptors, CRFR1 and CRFR2, have been identified. To understand the developmental and physiological roles of CRF-dependent pathways, mice carrying a CRFR1 null mutation have been generated. Mice heterozygous for the CRFR1 mutation have no overt phenotype. Homozygote mice derived from intercross of heterozyote mice develop to term and become sexually mature. The levels of circulating corticosterone in both sexes of mutant mice are significantly lower than those of control mice; histological analysis revealed that zona fasiculata of the adrenal gland is substantially diminished. The levels of ACTH were in the normal range, although these are considered low given the low levels of corticosterone. Female mutant mice have higher levels of corticosterone compared to male mutant mice, a finding that correlates with higher mortality rate in male mutant mice. Study of the expression of other components of the CRF-signaling pathway has revealed that the expression of CRF in the paraventricular nucleus was up-regulated. The expression of the other potent ACTH secretagog, vasopressin, was unchanged. In contrast to the offspring of heterozygote intercross, the offspring of homozygote intercross die within 24 hr after birth, likely due to abnormal lung development. These result suggest that transplacental corticosterone of heterozygote females may be sufficient to promote normal in utero lung development of homozygotes. To test this possibility, corticosterone was added to the drinking water of pregnant homozygous females beginning at E12 for two weeks. The offspring survived to adulthood. Explorations of the endocrine, autonomic and behavioral roles of CRFR1 are in progress.

Supported by NIH DK-26741 and the Fdn. for Medical Research, Inc.

335.13

CRF AND GR GENE EXPRESSION IN THE PARAVENTRICULAR NUCLEUS OF IMMUNE-CHALLENGED TRANSGENIC MICE EXPRESSING TYPE II GLUCOCORTICOID RECEPTOR ANTISENSE RIBONUCLEIC ACID. N. Laflamme, N. Barden* and S.Rivest, Mol. Endocrinol. Lab., CHUL Research Center and Laval University, Québec, Canada, G1V 4G2.

The purpose of this study was to investigate the effect of the immune activator lipopolysaccharide (LPS) on the expression of corticotropin releasing factor (CRF) and glucocorticoid receptor (GR) mRNA in the PVN of transgenic mice with impaired GR function caused by endogenous expression of GR antisense RNA. At 3 and 8 weeks of age, control and transgenic mice were sacrificed 4.5 h after a single i.p. administration of LPS (100 μg/100 g of body weight) or vehicle. Frozen brains were mounted on a microtome and cut in 30 μm sections. mRNAs encoding rat CRF and GR were assayed by *in situ* hybridization histochemistry using a ^{35}S-labeled riboprobes and localization of Fos-immunoreactive (Fos-ir) nuclei was determined by immunocytochemistry. Basal expression of CRF mRNA in the PVN, central nucleus of the amygdala (CeA) and geniculate complex was similar in the control and transgenic mice. LPS induced a comparable neuronal activation in the PVN of control and transgenic mice as revealed by the number of Fos-ir neurons. Moreover, the endotoxin caused a significant increase in the CRF mRNA levels within the PVN and CeA, an effect observed in both animal models. The endotoxin did not notably modulate CRF expression in other regions. Although GR mRNA was expressed in the PVN of control mice under basal conditions, this transcript was not detected in this hypothalamic structure in LPS-treated and transgenic animals. This indicates that endogenous type II GR mRNA is decreased in the PVN of mice expressing type II GR antisense RNA and that gene is down-regulated by LPS. Hybridization signal for CRF and GR transcripts was not notably altered by the age of mice. These results provide evidence that the basal expression of CRF and the increase of neuroendocrine CRF transcription in response to immunogenic challenges are not significantly affected by impairment of the type II GR function.

Supported by the MRC of Canada.

335.14

C-*FOS* mRNA PATTERN AND CRF NEURONAL ACTIVITY THROUGHOUT THE BRAIN OF RATS INJECTED CENTRALLY WITH A PROSTAGLANDIN OF E2 TYPE. S.Lacroix, L.Vallières, R. Rivest* and S.Rivest, Mol. Endocrinol. Lab., CHUL Research Center and Laval University, Québec, Canada, G1V 4G2.

The present study investigated the effect of central administration of the prostaglandin of E2 type (PGE_2) on the distribution of the immediate *early* gene (IEG) c-*fos* mRNA and the transcriptional activity of corticotropin-releasing factor (CRF) and its type 1 receptor in the rat brain. mRNAs encoding the IEG c-*fos* and CRF_1 receptor were assayed by *in situ* hybridization histochemistry using ^{35}S-labeled exonic riboprobes whereas the primary transcript {heteronuclear (hn)RNA} for CRF was detected using intronic probe technology as an index of CRF transcriptional activity. Thirty min after PGE_2 injection into the lateral ventricle, a moderate to strong positive signal for c-*fos* mRNA was detected in multiple structures of the brain such as the medial preoptic area/organum vasculosum of the lamina terminalis, supraoptic nucleus (SON), paraventricular nucleus (PVN) of the hypothalamus, central nucleus of the amygdala, nucleus of the solitary tract, dorsal motor nucleus of the vagus, area postrema, ambiguus nucleus, and throughout the choroid plexus and leptomeninges. In the parvocellular nucleus of the PVN, c-*fos* was expressed in CRF-immunoreactive (ir) and oxytocin (OT)-ir neurons, whereas in the magnocellular part of that nucleus and in the SON, this transcript was essentially colocalized in OT-ir neurons. Activation of CRF neuroendocrine cells was also associated with an increase in CRF transcription as revealed by the selective presence of CRF hnRNA in the PVN. Central administration of PGE_2 also induced expression of the CRF type 1 receptor in the parvocellular PVN. Taken together, these results provide clear anatomical evidence that central PGE_2 injection causes specific and selective expression of c-*fos* in several brain structures recognized to be activated in the brains of acutely immune-challenged rats. It is therefore possible that PG of E2 type plays a crucial role within the CNS in the interface between the immune system and brain functions responsible to modulate neuroendocrine circuitry involved to prevent exaggeration of the immunogenic processes.

Supported by the MRC of Canada.

335.15

INFLUENCE OF INTERLEUKIN-6 ON NEURAL ACTIVITY AND TRANSCRIPTION OF THE GENE ENCODING CORTICOTROPIN-RELEASING FACTOR IN THE RAT BRAIN. L. Vallières, S. Lacroix & S. Rivest*. Lab. of Molecular Endocrinology, CHUL Research Center, Ste-Foy, Québec, Canada, G1V 4G2.

Interleukin-6 (IL-6) is a pleiotropic cytokine produced by various lymphoid and neural cells. In addition to its classic role during immune and inflammatory responses, IL-6 acts on the central nervous system to elicit changes, such as activation of the hypothalamic-pituitary-adrenal (HPA) axis. This study investigated the effects of systemic and central injection of IL-6 on neural activity and transcription of the corticotropin-releasing factor (CRF) gene in the brain of conscious rats. The animals were sacrificed 1 and 3 h after a single infusion of IL-6 into the right jugular vein (0.83 or 3.0 μg) or the right lateral ventricle (0.2 μg) and their brains cut from the olfactory bulb to the end of the medulla in 30-μm coronal sections. Messenger RNA encoding the protein Fos, a marker of neural activity, and the neuropeptide CRF were localized by *in situ* hybridization histochemistry using ^{35}S-labeled riboprobes. The results show that systemic injection of IL-6 induced specific transcription of c-*fos* gene in most of the sensorial circumventricular organs (CVOs), including the organum vasculosum lamina terminalis, subfornical organ, median eminence and area postrema, as well as in the central nucleus of the amygdala and bed nucleus of the stria terminalis. On the other hand, central injection of IL-6 increased cellular level of c-*fos* mRNA in the ependymal layer and the walls of the ventricles, meninges, nucleus of the solitary tract and CVOs. These effects seem to be rapid and transient since the signal for c-*fos* mRNA was detected 1 h after both treatments and vanished 3 h afterwards. Moreover, CRF gene was not activated by either systemic or central administration of IL-6 in the paraventricular nucleus of the hypothalamus. Taken together, these results suggest that CVOs may play a crucial role in the effects of systemic IL-6 on neural activity whereas centrally-injected IL-6 can strongly activate cells of the ventricular system and the surrounding structures. Although this differential circuitry may explain distinct origin-dependent functions of IL-6, this cytokine seems to be insufficient by itself to induce transcription of the gene encoding neuroendocrine CRF, the neuropeptide responsible for the control of the HPA axis.

Supported by the MRC of Canada.

335.16

OBESITY AND FOOD DEPRIVATION REDUCE THE EXPRESSION OF THE CRF TYPE 2α RECEPTOR IN THE VMH OF RATS. E. Timofeeva, D. Richard*, Q. Huang. Department of Physiology, Faculty of Medicine, Laval University, Québec, Canada, G1K 7P4.

The mechanisms underlying the effects of corticotropin-releasing factor (CRF) and its receptors in the regulation of energy balance have yet to be determined. In this respect, the study was carried out to investigate the main and interactive effects of food deprivation (48 hours) and obesity on the transcriptional activity of CRF, and CRF type 1 and 2α receptors. The experiments were performed in lean (Fa/?) and obese (fa/fa) Zucker rats. The mRNA levels of CRF and its receptors were determined using *in situ* hybridization histochemistry. In Fa/? rats, the mRNA levels of CRF in the paraventricular nucleus of the hypothalamus (PVH) were lower in food-deprived rats compared to *ad libitum*-fed animals. There was no difference between Fa/? and fa/fa in the levels of CRF_1 receptor mRNA, notwithstanding the brain structures examined. The expression of the $CRF_{2\alpha}$ receptor transcript in the ventromedial nucleus of the hypothalamus (VMH) was lower in the obese rats compared to the lean rats fed *ad libitum*. In addition, food deprivation caused a significant decrease in the expression of the $CRF_{2\alpha}$ receptor transcript in the VMH of the lean rats. The reduction in the biosynthesis of the $CRF_{2\alpha}$ receptor within the VMH in response to obesity and food deprivation is consistent with a role for CRF in the control of food intake and energy expenditure.

Supported by the Medical Research Council of Canada.

335.17

THE EFFECTS OF MULTIPLE SCLEROSIS AND CORTICOSTEROIDS ON HYPOTHALAMIC CRH AND AVP NEURONS. Z. A. Erkut and D. F. Swaab (SPON: European Neuroscience Association), Netherlands Institute for Brain Research, Meibergdreef 33 1105 AZ Amsterdam, The Netherlands.

The hypothalamo-pituitary-adrenal axis (HPAA) is involved in the susceptibility to and recovery from immune-mediated diseases like multiple sclerosis (MS). A higher HPAA activity decreases the susceptibility to the disease, and once the disease is established, helps to recover from the symptoms. Increased activity of HPAA at the adrenal, pituitary and hypothalamic level in MS was concluded from increased blood levels of cortisol, urinary free cortisol and blood levels of adrenocorticotropic hormone (ACTH). Our group found an increased activity of hypothalamic corticotropin-releasing hormone (CRH) neurons in MS patients, as indicated by increased number of CRH neurons and increased proportion of vasopressin (AVP) co-localization in CRH neurons in the paraventricular nucleus.

However, in MS patients, the increase in endogenous HPAA activity seems often not to be sufficient to suppress the ongoing immune reaction, and exogenous corticosteroid administration is required to quell the symptoms of the disease. We studied the effects of corticosteroids on human hypothalamic CRH and AVP neurons by a double labelling immunocytochemical method on postmortem brain material of patients who were exposed to corticosteroids before death. The findings show for the first time that both CRH and AVP cell populations express less peptide, during corticosteroid therapy. The number of CRH expressing neurons was only 6 % of the controls ($p < 0.003$) and the number and staining intensity of AVP cells were quantitatively clearly less than the controls, and hardly detectable in m ost cases. The results also revealed that this suppression lasts at least 2 weeks after the corticosteroid treatment is stopped. Suppression of CRH and AVP neurons may not only cause endocrine effects, but may also be related to the commonly seen cognitive and mood changes of patients under corticosteroid therapy.

(Brain material was obtained from Netherlands Brain Bank, and this study was supported by Foundation Friends MS Research, The Netherlands (grant nr. 94-188) and by TUBITAK, Turkey).

335.18

INDIVIDUAL DIFFERENCES IN THE NORADRENERGIC REGULATION OF HIPPOCAMPAL CORTICOSTEROID RECEPTORS. M. Kabbaj, PV. Piazza, M. Le Moal and S. Maccari*. INSERM U.259 - Rue C.S.Saëns, 33077 Bordeaux, France.

Two types of hippocampal corticosteroid receptors (CR) play an important role in regulating the secretion of corticosterone. Type I regulates both basal and stress induced release of corticosterone whereas type II seems to be involved only in the stress response. This study was designed to compare the noradrenergic (NA) regulation of these CR in rats separated, on the basis of their locomotor reactivity in response to novelty in two groups: high responder rats (HR) having a higher locomotor reactivity and low responder rats (LR). NA regulation of CR was assessed in these rats by measuring binding capacity of CR and stress induced corticosterone secretion after chronic treatment with the α1 NA antagonist prazosin (0.5 mg/Kg, i.p.). Before the chronic treatment, HR and LR rats were either left with intact adrenal glands or adrenalectomized, their plasma levels of corticosterone being maintained in the physiological range by implanted corticosterone pellets. In this way, direct drug-induced changes in NA transmission, which may be confounded by drug-induced changes in corticosterone secretion occuring in the intact rats were effectively isolated in the adrenalectomized group, allowing the assessment of direct NA regulation of CR. The results demonstrate that in LR rats, the prazosin treatment decreased the number of type I CR in both adrenalectomized and adrenal-intact rats whereas type II CR were reduced only in adrenal-intact rats. In the adrenal-intact LR rats, prazosin induced hypersecretion of corticosterone after stress which may account for the reduction of type II CR noted in this group. No changes were seen in hippocampal CR nor corticosterone secretion in HR rats after prazosin treatment. These results suggest greater NA regulation of type I CR and a greater hormonal control of type II CR. This differential regulation depends on the individual differences, as it occurs only in LR rats. *Supported by INSERM*

335.19

ROLE OF THE MINERALOCORTICOID RECEPTOR IN REGULATION OF HIPPOCAMPAL ADRENOCORTICOSTEROID RECEPTOR EXPRESSION. J.P. Herman*, G.L. Bowers and C.M. Dolgas. Department of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536-0084.

Mechanisms underlying regulation of hippocampal adrenocorticosteroid receptors are presently ill-defined. This study evaluated the hypothesis that hippocampal glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) biosynthesis is controlled through the neuronal MR. Experiments examined GR and MR mRNA and GR protein expression following 1) blockade of the neuronal MR by systemic administration of spironolactone (SPIRO) and 2) saturation of MR and GR receptors with high doses of corticosterone (CORT). Male rats received subcutaneous injections of SPIRO (50 mg/kg),CORT (5 mg/kg) or vehicle twice a day for three days. Animals were killed 4 hours following the last injection and tissue processed for in situ hybridization and immunoautoradiography. Plasma corticosterone was markedly elevated in the CORT group (591±159 ng/ml), whereas both OIL and SPIRO groups showed normal AM levels (9.1±1.6, 9.6±1.9 ng/ml, respectively). Corticotropin releasing hormone mRNA expression in the paraventricular nucleus was increased two-fold in SPIRO rats relative to controls, consistent with MR-mediated regulation of hypothalamo-pituitary-adrenocortical tone. In hippocampus, GR mRNA was increased by 65% in CA1 and 46% in dentate gyrus of SPIRO treated rats. Parallel increases in GR protein were observed both regions (26% and 19%, respectively). MR mRNA was slightly increased in CA1 (13%) and CA3 (11%). High-dose CORT treatment did not affect GR or MR mRNA expression or GR protein levels in any region analyzed. The results are consistent with previous adrenalectomy studies, and indicate that baseline expression of GR and perhaps MR is maintained by low doses of steroids acting through the neuronal MR. However, high-dose CORT treatment does not appear to down-regulate neuronal GR or MR. The latter data suggest that adrenocorticosteroid receptor down-regulation likely requires neuronal input. Supported by MH49698 and AG 10836.

335.20

THE EFFECTS OF CHRONIC INGESTION OF NATURAL OR SYNTHETIC GLUCOCORTICOIDS ON LEARNING AND CHOLINE ACETYLTRANSFERASE ACTIVITY IN YOUNG SPRAGUE-DAWLEY RATS. A.P. Pinney and L.A. Meserve*, Dept. Biological Sciences, Bowling Green State Univ., Bowling Green, OH 43403-0212

Chronic elevation of circulating glucocorticoids has been shown to impair cognitive function, usually by causing neuron death. However, cognitive deficits resulting from glucocorticoid exposure have been demonstrated in the absence of neuron loss. The purpose of the present study was to compare the effects of a synthetic glucocorticoid (prednisone) with a natural one (corticosterone) on learning of the Morris water maze and on Choline Acetyltransferase (ChAT) activity. ChAT is responsible for the production of acetylcholine, a primary neurotransmitter for learning pathways in the basal forebrain and the hippocampus. Thirty day old Sprague-Dawley rats were given either prednisone or corticosterone in their drinking water for 30 days. At 60 days of age the animals were administered 10 trials in the water maze (twice a day for 5 days). At 65 days of age, the animals were rapidly decapitated and ChAT activity was analyzed in the hippocampus and basal forebrain. All results were analyzed by ANOVA. The animals given corticosterone spent significantly more time in the water maze than controls on days three, four and five, although ChAT activity was not altered. These animals gained significantly less weight than controls. ChAT activity was significantly depressed in the animals receiving prednisone, but these animals id not demonstrate learning deficits. Thus, this study was unable to correlate between depression of ChAT activity and deficits in cognitive function.

Supported by OBOR-BGSU Research Challenge.

335.21

REDUCED SENSITIVITY OF MONOSODIUM GLUTAMATE(MSG)-LESIONED RATS TO EXOGENOUS LEPTIN. R. Dawson, Jr.*, W.J. Millard, S.C. Liu, B. Eppler and M.A. Pelleymounter. Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, FL 32610 and Dept. Neurobiology, Amgen, Inc., Thousand Oaks, CA 91320.

Leptin (OB protein) is a protein secreted by adipocytes that may act as a feedback signal in the regulation of body composition, appetite and metabolism. The exact target for leptin's feedback signal is unknown but could be the arcuate nucleus of the hypothalamus (ANH) since the ANH plays a pivotal role in neuroendocrine and metabolic regulation. Neonatal administration of MSG to rodents destroys the ANH and produces obesity characterized by extreme metabolic efficiency, hypophagia, hypothermia and growth stunting. Therefore, the current study was designed to test if MSG-induced ANH damage would attenuate the actions of exogenous leptin on food intake and body weight.

Male and female rats received s.c. injections of either MSG (4 mg/g) or saline on postnatal days 2 and 4. Leptin was administered by osmotic minipumps at an approximate dose of 1 mg/kg/day to MSG-treated (n=7) or saline-treated rats (n=6) starting on postnatal day 60 (males) or 61 (females). The control groups were MSG-treated (n=7) or saline-treated rats (n=6) implanted with osmotic minipumps filled with phosphate buffered saline.

Leptin significantly ($p<0.05$) suppressed night time food intake in saline-treated males on treatment days 2-5 and in saline-treated females on treatment days 3-5. Male and female MSG-treated rats given leptin had modest but nonsignificant reductions in food intake. Male and female saline-treated rats given leptin failed to gain weight, whereas the control groups continued to gain weight. Leptin had no effect on the weight gain in MSG-treated rats of either sex.

The results of the present study suggest that MSG-induced damage to the ANH can blunt the actions of exogenous leptin. Funded by a grant from Amgen, Inc.

335.22

GOLDTHIOGLUCOSE LESIONS PARTIALLY BLOCK LEPTIN'S EFFECTS IN OB/OB MICE. M. Baker, M Cullen, W. Karbon* and M. Pelleymounter. Amgen, Thousand Oaks, CA 91320.

Several variants of the leptin receptor have been described, with localization in kidney, testes, white fat, choroid plexus and hypothalamus. The only form that is mutated in the db/db mouse is expressed in the hypothalamus, supporting the theory that leptin regulates energy utilization and intake through a central mechanism. If the receptor that mediates leptin's effects is localized in the hypothalamus, then destruction of areas of the hypothalamus associated with regulation of appetite and energy utilization should reduce or abolish leptin's effects on body weight. Large or small hypothalamic lesions were induced with two doses of goldthioglucose (GTG). The low dose (.3mg/g) destroyed cells forming the ventrolateral border of the ventromedial hypothalamus (VMH). The high dose (1mg/g) caused a more widespread destruction, involving most of the VMH and smaller percentages of the paraventricular and arcuate nuclei. The small GTG lesion did not reduce leptin's efficacy in ob/ob mice, in that leptin's effects on body weight and food intake were essentially the same in mice with sham or GTG lesions. The larger lesion, however, did reduce leptin's efficacy on body weight and food intake by approximately 60% in comparison to ob/ob mice with sham lesions. These data suggest that the leptin receptor may be primarily localized in the VMH, with extension into the arcuate and paraventricular nuclei. The fact that this fairly extensive lesion of the ventromedial hypothalamus did not completely block leptin's effects suggests either that 1) the receptor is more diffusely localized in the hypothalamus than previously believed, or 2) the peripheral forms of the receptor play a role in leptin's effects on body weight regulation.

336.1

PERIPHERAL CYTOKINE INDUCTION OF INTERLEUKIN-1ß GENE EXPRESSION IN SPECIFIC BRAIN SITES. J. R. He*, Y. Gao, and L. P. Kapcala. Endocr. Div., Dept. of Med. Univ. of Maryland Sch. of Med., and Baltimore VA Med. Ctr., Baltimore, MD 21201

Although many studies show lipopolysaccharide(LPS) stimulates interleukin(IL)-1 or its mRNA in brain, precise localization of IL-1 production is not clear. Neither is it known whether IL-1 stimulates its own production in brain as in the periphery and to what extent LPS stimulation occurs via generation of IL-1. We compared effects of IL-1ß(20µg/kg) and LPS(4,10mg/kg) on IL-1ß mRNA(riboprobe in situ hybridization) in various brain sites at 2.5 or 3 hrs after their peripheral(ip) administration to adult male SD rats. Control(saline) rats did not exhibit IL-1ß mRNA in brain. LPS potently induced IL-1ß mRNA in circumventricular organs(CVOs: OVLT,ME,SFO,AP) choroid plexus, meninges, some blood vessels and in some parenchymal sites including hypothalamic paraventricular and arcuate/periarcuate nuclei, and vagal nuclei. IL-1ß weakly induced IL-1ß mRNA(number of positive cells and signal intensity/cell were much less vs LPS). CONCLUSIONS : 1. Peripheral LPS potently induced IL-1ß gene expression in CVOs, choroid plexus, meninges, blood vessels, and in specific parenchymal brain sites. 2. Although the pattern of brain IL-1ß mRNA induced by peripheral IL-1ß and LPS was similar, weak stimulation by IL-1ß implicated the importance of other cytokines in the potent stimulation by LPS.

Supported by VA Merit Review Research Grant

336.2

ALCOHOL STIMULATES INTERLEUKIN-1 ß GENE EXPRESSION IN HYPOTHALAMIC NEURONS AND GLIA. L. P. Kapcala*, Y. Gao, and J.R. He. Endocrine Div., Dept. of Med. Univ. of Maryland Sch. of Med., and Baltimore VA Med. Ctr., Baltimore, MD 21201

IL-1 exerts various potent central effects including activation of the hypothalamic-pituitary-adrenal(HPA) axis. Alcohol(ETOH) is also a potent stimulator of the HPA axis and produces pseudo-Cushing's syndrome in some alcoholics. Consequently, we questioned whether ETOH might activate the HPA axis via induction of brain IL-1. We tested the hypothesis that ETOH stimulates hypothalamic IL-1 gene expression. Effects of prolonged(~52 hrs)ETOH (25 mM=115 mg/dl) exposure on IL-1ß mRNA(RNase protection assay) were studied in enriched hypothalamic neuronal and glial(predominantly microglia and astrocytes) cultures of day 17 fetal rats. We also studied ETOH effects on IL-1ß and lipopolysaccharide (LPS) stimulation of brain IL-1ß mRNA. ETOH exposure stimulated IL-1ß mRNA in neuronal(~3 fold) and glial(~4.5 fold) cultures without toxic effects on cell viability. In neuronal and glial cultures ETOH selectively abolished stimulation of IL-1ß mRNA by IL-1ß but did not alter stimulation by LPS. CONCLUSIONS : 1. ETOH stimulated IL-1ß gene expression in hypothalamic neuronal and glial cultures. 2. Presumed induction of hypothalamic IL-1ß by ETOH could mediate some central effects of ETOH especially HPA axis activation. 3. ETOH selectively altered stimulation of IL-1ß gene expression by specific immune stimuli in neuronal and glial cultures.

Supported by VA Merit Review Research Grant

336.3

INTERLEUKIN-1ß AND LIPOPOLYSACCHARIDE REGULATION OF GENE EXPRESSION OF INTERLEUKIN-1ß AND INTERLEUKIN-1 TYPE 1 RECEPTOR IN HYPOTHALAMIC NEURONS AND GLIA. Y. Gao, J.R. He, M.P. Lilly* and L.P. Kapcala. Endocr. Div., Dept. of Med. Univ. of Maryland Sch. of Med. & Baltimore VA Med. Ctr., Baltimore, MD 21201

Different pathological or pharmacological stimuli induce brain IL-1 which can exert various central effects including activation of the hypothalamic-pituitary-adrenal axis. However, little is known about specific factors regulating brain IL-1. We tested the hypothesis that IL-1 and lipopolysaccharide(LPS), a potent generator of IL-1 and other cytokines, stimulates hypothalamic gene expression of IL-1 and its type 1 receptor(IL-1R1). We studied effects(4 hrs) of IL-1ß or LPS on IL-1 ß and IL-1R1m RNAs(RNase protection assays) in enriched hypothalamic neuronal and glial(predominantly microglia and astrocytes) cultures of day 17 gestation fetal rats. IL-1ß(10^{-11}-10^{-7}M) dose-dependently stimulated (~2 fold maximally) IL-1ß and IL-1R1 mRNAs in neuronal and glial cultures. Although LPS potently dose-dependently(1-10^4 ng/ml) stimulated(6-330 fold) IL-1ß mRNA in neuronal and glial cultures, it did not increase IL-1R1 mRNA. CONCLUSIONS : 1. IL-1ß modestly stimulated gene expression of IL-1ß and IL-1R1 in hypothalamic neuronal and glial cultures suggesting a positive autofeedback regulation. 2. LPS differentially and potently stimulated IL-1ß mRNA but not IL-1R1 mRNA in hypothalamic neuronal and glial cultures, presumably because of effects of cytokines induced by LPS in addition to IL-1ß.

Supported by VA Merit Review Research Grant

336.4

ADRENOCORTICOSTEROID FEEDBACK IS NOT NECESSARY FOR ADRENOCORTICOTROPIN STIMULATION BY PERIPHERAL INTERLEUKIN-1ß. M.K. Selmanoff*, L.P. Kapcala, J.R. He, Y. Gao, D.N. Darlington, and D.E. Carlson. Endocr. Div., Dept. Med., & Res. Div., Dept. Surg., Univ. of Maryland S.O.M. and Baltimore VA Med. Ctr., Baltimore, MD 21201

The observation that adrenalectomy(ADX) abolishes plasma adrenocorticotropin(ACTH) stimulation by central(icv) interleukin (IL)-1ß has been interpreted as evidence supporting the necessity of adrenocorticosteroid feedback for IL-1 stimulation of ACTH secretion. Different mechanisms appear to mediate IL-1 activation of the hypothalamic-pituitary-adrenal axis when IL-1 originates in different body compartments(e.g. different routes of administration). Thus, we tested the hypothesis that loss of adrenocorticosteroid feedback after ADX diminishes plasma ACTH stimulation by peripheral(iv or ip) IL-1ß. Adult male SD rats were studied 7 days after ADX or sham ADX for plasma ACTH responses to iv(2µg/kg) and ip(6µg/kg) IL-1ß or iv/ip saline(control). Insulin-induced hypoglycemia was a positive control to show ACTH stimulation after ADX. ADX did not diminish ACTH stimulation by iv IL-1ß. Stimulation of ACTH secretion by iv IL-1ß in ADX rats ranged between normal to exaggerated responses. Neither did ADX diminish ACTH stimulation by ip IL-1ß.

CONCLUSION : Adrenocorticosteroid feedback is not necessary for facilitating stimulation of ACTH secretion by IL-1ß originating in peripheral body compartments(e.g. circulatory-iv, or abdominal-ip).

Supported by VA Merit Review Research Grant

336.5

COMPARATIVE EFFECT OF ACUTE CENTRAL CYTOKINE ADMINISTRATION ON BEHAVIORAL, NEUROCHEMICAL, ENDOCRINE & IMMUNE PARAMETERS IN THE R AT.

C. Song*[3], T.J. Connor [1], H. Anisman [3], A.V. Ravindran [4] and Z. Merali [2]. [1]Dept. of Pharmacol., UCG, Ireland. [2]Dept. of Pharmacol. & Psychol., Univ. of Ottawa. [3]Dept. of Psychol., Carleton Univ.,[4]Dept. of Psychiat. Royal Ottawa Hospital. Ont. Canada.

In addition to the immune system, cytokines and their receptors have recently been located in many other tissues including the peripheral and central nervous systems (CNS). The pharmacological role and/or physiological function of the various cytokines within the CNS is not fully understood. The present study compared the effects of central (i.c.v.) administration of IL-1β, IL-2, IL-6 (20 ng) and TNF-α (40 ng) on elevated plus maze behavior, monoamine levels in the hypothalamus, plasma corticosterone and catecholamine concentrations and Con A induced spleenic lymphocyte proliferation in the rat, 45 min post administration. IL-1β and TNF-α reduced the number of entries and time spent on the open arms of the elevated plus maze, whereas, IL-2 and IL-6 did not. Cytokine specific alterations in monoamine levels were evident at the hypothalamus. Both IL-1β and TNF-α increased 3-MT and decreased 5-HIAA and DA concentrations, while IL-2 and IL-6 increased HVA levels. In addition, IL-6 increased 3-MT level. Plasma corticosterone was elevated by IL-1β but not by any of the other cytokines, furthermore IL-1β and IL-6 reduced plasma DA levels. IL-1β reduced and TNF-α increased Con A-induced lymphocyte proliferation. These results demonstrate that in some but not all respects, IL-1β administration may produce stress-like effects on behavior, the HPA axis and immune function, while the other cytokines produce less consistent effects on these parameters.

Supported by Medical Research Council of Canada.

336.6

EFFECTS OF INTERFERON β-1B AND TRIPTOLIDE ON CONCANAVALIN A-INDUCED PERIPHERAL BLOOD MONONUCLEAR CELL PROLIFERATIVE RESPONSES AND NON-SPECIFIC SUPPRESSOR FUNCTION IN MULTIPLE SCLEROSIS. Z Qu, M. A. Jensen and B. G. W. Arnason*. Dept. of Neurology, University of Chicago, Chicago. IL 60637

Multiple Sclerosis (MS) is an immune mediated demyelinating disease. Non-specific suppressor function, as measured in the Con A suppressor assay, is decreased in MS during active disease. Interferon β-1B (IFNβ-1B) is an approved treatment for relapsing-remitting MS. IFNβ-1B therapy augments non-specific suppressor function in MS patients as measured ex vivo. We investigated potential additive effects of IFNβ-1B and Triptolide on Con A-induced lymphocyte proliferation and non-specific suppressor function of PBMC *in vitro*. Triptolide is a drug derived from the roots of *Triperygium Wifordii f.* The results indicate that (1) Both IFNβ-1B and Triptolide inhibit Con A-induced proliferative responses of PBMC in a dose dependent manner. Drug concentrations that yielded 50% inhibition were 53.3 ± 8.3 U/ml (range 37.01 to 72.35) of IFNβ-1B and 1.99 ± 0.13 ng/ml (range 1.73 to 2.30) of Triptolide; (2) Isobologram analysis of dose response curves revealed that IFNβ-1B and Triptolide suppress PBMC proliferative responses in an additive manner; (3) IFNβ-1B augments non-specific suppressor function in a dose dependent manner; the concentration that reduced suppressor function to 50% of optimum was 97.2 + 7.8 U/ml; (4) Triptolide augments Con A induced non-specific suppressor function (for Con A vs Con A plus Triptolide; Control = 13.8 ± 1% vs 22 ± 3% and for MS 11.6 ± 1% vs 17.5 ± 2%; $p < 0.03$ for both); (5) IFNβ-1B and Triptolide together fail to synergistically augment non-specific suppressor function. Study supported by PHS-PO-1-NINDS-NS 24575; a gift from the Butz Foundation; a grant from Berlex, Inc. (all to B.G.W.A.).

336.7

MOLECULAR REGULATION OF THE IL-1beta SYSTEM IN RAT BRAIN REGIONS IN RESPONSE TO THE CHRONIC ICV MICROINFUSION OF rhIL-1beta AT DOSES THAT YIELD PATHOPHYSIOLOGICAL CONCENTRATIONS IN THE CSF. S.E. Ilyin*, G. Sonti, D. Gayle and C.R. Plata-Salamán. Sch. Life Hlth. Sci., Univ. Delaware, Newark, DE 19716-2590, USA.

RhIL-1beta (RHI-1) was chronically infused (via osmotic minipumps) into the 3rd ventricle of rats, at doses that yield estimated pathophysiological concentrations in the CSF. RHI-1 induced significant anorexia. The nighttime food intake at 60-72 h after initiating the chronic ICV infusion was 21.1±0.8 g (n=7) for heat-inactivated, 15.7±0.6 g (n=8) for 2.0 ng, and 11.5±1.0 g (n=7) for 8.0 ng RHI-1/24 h ($p<0.0001$). At 72 h, brain regions were isolated from all rats. The IL-1beta system [IL-1 receptor type I (IL-1RI), IL-1 receptor antagonist (IL-1Ra) and IL-1beta] mRNAs levels were determined in brain regions by RNase protection assays. The chronic ICV infusion of RHI-1 increased ($p<0.005$) the IL-1Ra and IL-1beta mRNA levels in the cerebral cortex, hippocampus, hypothalamus, cerebellum and midbrain, and increased ($p<0.03$) the IL-1RI mRNA level in the cerebellum. The profiles of mRNAs induced were highly intercorrelated within a brain region. Inactive RHI-1 had no effect. The data suggest the operation of a feedback system (IL-1beta/IL-1Ra/IL-1RI). Dysregulation of the CNS IL-1beta feedback system (IL-1beta/IL-1Ra ratio) may be reflected in the pathogenicity and severity of neurological diseases associated with increased CSF concentrations of IL-1beta such as CNS infections.

The present novel integrative approach can be used to study cellular and molecular mechanisms involved in cytokine-induced anorexia (and other neurological manifestations) including cytokine, neurotransmitter, and neuropeptide subsystem (ligands, receptors, transducing molecules, endogenous inhibitors) profiles. Supported by Univ. Del. Res. Grants.

336.8

LOCALIZED INFLAMMATION AND THE RELEASE OF BETA-ENDORPHIN FROM IMMUNE CELLS. P.J. Cabot*, L. Carter, C. Gaiddon[§], J. P. Loeffler[§] and C. Stein. Dep. of Anesthesiology and Critical Care Medicine, Johns Hopkins Univ., Baltimore, MD, 21287-8711, USA and [§]Univ. of Strasbourg, Strasbourg, France.

Recent findings have suggested a significant involvement of the immune system in the control of pain. Immune cells contain opioid peptides which are released within inflamed tissue and act at opioid receptors on peripheral sensory neurons. These immune cells have cell surface receptors for various cytokines and corticotropin-releasing factor (CRF) which, when activated, can evoke the release of opioid peptides. In these studies we sought to characterise the release of the prominent opioid peptide, β-endorphin, in distinct populations of lymphocytes from blood, non-inflamed lymph nodes (LN) and inflamed popliteal LN to assess the role of cell trafficking and the cellular mechanisms of opioid peptide release from immune cells. Male wistar rats were treated with freund's complete adjuvant (150 µl) in the right hind paw which induced an inflammatory response, maximal at four days. The in-vitro release of β-endorphin from immune cells was evoked by interleukin-1β (IL-1β) and CRF and was demonstrated to be dose-dependent from both inflamed and non-inflamed LN. The release of β-endorphin by IL-1β and CRF was reversed by specific receptor antagonsist IL-1ra and alpha-helical-CRF, respectively, and the release was shown to be calcium dependent, indicating the vesicular containment of β-endorphin within the immune cells. Circulating lymphocytes of either treated (1.98 ± 0.08 ng/10^6 cells) or untreated animals (0.83 ± 0.04 ng/10^6 cells) contained significantly lower levels of β-endorphin than those within normal (6.88 ± 0.77 ng/10^6 cells, $p<0.05$) or non-inflamed LN (5.54 ± 0.98 ng/10^6 cells) and were not sensitive to IL-1β or CRF. Similarly, levels of β-endorphin were lower in inflamed LN (3.36 ± 0.21 ng/10^6 cells) than in non-inflamed LN ($p<0.05$). Conversely, POMC mRNA levels were highest in inflamed LN. These findings suggest that circulating lymphocytes travel to sites of imflammation where they are activated and enhance the production of β-endorphin. Stimulated via cellular receptors for cytokines or CRF, the β-endorphin is released into the inflamed tissue to act at peripheral sensory neurons to reduce pain and neurogenic inflammation. Immune cells depleted of β-endorphin then travel through afferent ducts to the inflamed popliteal lymph node.

This study was funded by NIH/NINDS grant R01NS32466.

336.9

ACTIVATION OF THE ANTERIOR HYPOTHALAMIC SEROTONINERGIC SYSTEM BY INTERLEUKIN-6 AND INTERLEUKIN-1ß: IN VIVO MICRODIALYSIS STUDIES IN AWAKE F344 RATS. E. Khan Shaghaghi[1], Yue Wu[1], M. Pallardy[2], H. Lebrec[2], C. Jacquot[1*] and A.M. Gardier[1]. [1]Lab. Neuropharmacol. JE DRED 92-372, [2]Lab. Toxicol. CJF INSERM 9301, Fac. Pharmacie Univ. Paris-Sud, F92296 Chatenay-Malabry, France.

Recently, we demonstrated that a primary immune response to T-cell dependent antigen (sheep red blood cell, SRBC, or keyhole limpet hemocyanin, KLH) decreases hypothalamic and cortical serotonin ((5-hydroxytryptamine, 5-HT) levels in F344 rats while increasing extracellular 5-HT levels in vivo {Gardier et al., *Brain Res.*, 645 (1994) 150-156}. Pretreatment with an immunosuppressive drug, cyclosporine A prevented these effects suggesting that a T-lymphocyte product, but not a macrophage one, may be involved in the central serotoninergic activation induced by one of this antigen. Thus, interleukin-6 (IL-6) might be a good candidate for being a link between the immune system and the central nervous system. To further characterize this hypothesis, we used a microdialysis probe equiped with a microinjection tube as designed by Shintani et al. {*J. Neurosci.* **13** (1993) 3574-3581} to compare the effects of interleukin-6 (IL-6) and interleukin-1ß (IL-1ß) administered directly into the anterior hypothalamus, on 5-HT release in the anterior hypothalamus of conscious, freely moving rats. At 10 and 50 ng doses, IL-6 induced rapid and transient increases in extracellular 5-HT levels: the maximal increases were to 157% and 309% of the respective basal values (100 %). Extracellular 5-HT levels were back to baseline values 1 hour after local IL-6 perfusion. Furthermore, 5-hydroxyindolacetic acid (5-HIAA) efflux remained unchanged. However, at a lower dose (1 ng), central IL-6 did not alter extracellular 5-HT and 5-HIAA levels compared to the control group. Central IL-1ß (1 ng) induced a dramatic, rapid and transient increase in extracellular 5-HT levels: the maximal increase was to 1127% of the basal value (100%) as well as those of 5HIAA. The present findings provide the first reported *in vivo* evidence that IL-6, although much less effective than IL-1ß, is able to directly increase hypothalamic 5-HT release.

336.10

THE INTRACEREBROVENTRICULAR INJECTION OF CORTICOTROPIN-RELEASING FACTOR OR INTERLEUKIN-1β INCREASES mRNA LEVELS OF CONSTITUTIVE NITRIC OXIDE SYNTHASE IN THE RAT HYPOTHALAMUS. S. Lee* and C. Rivier. Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037

Nitric oxide (NO) synthase (NOS), the enzyme responsible for NO formation, is found in hypothalamic neurons that are important for the activity of the hypothalamic-pituitary-adrenal (HPA) axis. We reported earlier that systemic (iv) endotoxin injection significantly upregulated mRNA levels of constitutive (c) NO synthase (NOS, the enzyme responsible for NO formation) in the paraventricular nucleus (PVN) of the hypothalamus, while other investigators found that physical stresses induced similar changes. However, the mechanisms leading to increased brain NOS values remain unclear. The purpose of the present work was to determine whether neuromodulators known to be increased following stress or endotoxin treatment, were able to stimulate NOS mRNA in the paraventricular nucleus (PVN) of the hypothalamus. Corticotropin-releasing factor (CRF) is the hypothalamic peptide that represents the primary mediator of the activity of the HPA axis; its levels are significantly elevated in the PVN of rats exposed to stress or endotoxin. Interleukin-1β (IL-1β) is a cytokine whose concentrations increase in the brain of rats administered endotoxin systemically. The intracerebroventricular (icv) injection of CRF (1 µg) or IL-1β (100 ng) was therefore used as pharmacological means to mimic changes in the hypothalamic levels of these secretagogues during exposure to physical stress or endotoxin. Both icv treatments significantly increased hypothalamic cNOS mRNA levels measured 2 h later. These results suggest that CRF and/or IL-1β may be specifically responsible for increased NO formation in rats exposed to physical stress or injected with endotoxin.

Supported by NIMH R01-MH51774.

336.11

THE CENTRAL INJECTION OF INTERLEUKIN-1β INHIBITS TESTOSTERONE SECRETION INDEPENDENTLY OF CHANGES IN PLASMA LUTEINIZING HORMONE LEVELS. C. Rivier* and A.V. Turnbull. Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037

It is well known that central (intracerebroventricular, icv) injection of interleukin-1ß (IL-ß) significantly lowers plasma luteinizing hormone (LH) concentrations, which in turn reduces testosterone (T) secretion in adult male rats. We have recently observed that in addition, icv IL-1ß (30-80 ng) also inhibits the surge in T secretion due to the intravenous (iv) injection of human chorionic gonadotropin (hCG). This diminished testicular responsiveness to gonadotropin appears to be independent of reduced plasma LH concentrations, since blockade of LH alone (using gonadotropin-releasing hormone antagonists) does not acutely impair the T response to hCG. Icv IL-1ß increases the plasma concentrations of corticosteroids and IL-6, both of which directly inhibit gonadal steroidogenesis. However, neither adrenalectomy nor CRF antibodies affect the diminished T response to hCG in animals icv injected with IL-1ß, indicating that elevated plasma corticosterone is not responsible for the blunted testicular response. Similarly, elevated plasma IL-6 levels do not seem to be the primary cause of icv IL-1ß-induced suppression of the T response to hCG. Indeed the peak values of IL-6 over the time course of our experimental paradigm while differing temporally, are quantitatively similar after both iv and icv IL-1ß, yet the icv IL-1ß far more effectively blunts the T response to hCG. Collectively, these data indicate that IL-1ß within the central nervous system can inhibit testicular activity independently of plasma LH or corticosterone levels, and that this phenomenon is probably not directly correlated with elevated plasma IL-6 concentrations. We postulate that neural influences on testicular function, similar to those described for the adrenals and the ovaries, may mediate this action of IL-1ß on T secretion. In view of the wide range of pathologies associated with increased brain IL-1 levels, this mechanism may be important during stress-induced inhibition of reproductive function in the male.

Supported by NIH HD-13527.

336.12

EVIDENCE FOR THE INVOLVEMENT OF THE CENTRAL NORADRENERGIC SYSTEM IN SYSTEMIC INTERLEUKIN-6 INDUCTION BY PERIPHERALLY ADMINISTERED INTERLEUKIN-1ß. Q.-H. Huang, A. Takaki, A. Kastin*[1] and A. Arimura. US-Japan Biomed. Res. Labs, Tulane Univ. Hebert Ctr., Belle Chasse, LA 70037, USA, [1]VA Med. Ctr., New Orleans, LA 70146, USA.

The role of the central noradrenergic system in systemic interleukin-6 (IL-6) induction by peripherally administered interleukin-1ß (IL-1ß) was examined in rats. The results demonstrated: (1) intravenous (iv) injection of rhIL-1ß (2µg/kg body wt) caused a notable rise in plasma IL-6 levels, and this action was markedly attenuated by intracerebroventricular (icv) pretreatment with either 6-hydroxydopamine (6-OHDA, 100 or 200 µg/rat) or a1-adrenergic receptor antagonist prazoxin (20 µg/rat), suggesting that the central a1-noradrenergic system is involved in the iv applied IL-1-induced systemic IL-6 induction; (2) the levels of IL-6 in the brain (hypothalamus, medulla oblangata, and cortex) did not rise after iv IL-1ß, suggesting that the brain noradrenergic system probably acts on the peripheral IL-6-producing cells, but not those in the brain, to contribute to the elevation of systemic IL-6; (3) chemical sympathectomy with intraperitoneal (ip) injection of 6-OHDA, but not adrenalectomy, resulted in dramatic reduction of the plasma IL-6 response to iv IL-1ß, suggesting that the autonomic sympathetic nervous pathway, but not the hypothalamus-pituitary-adrenal (HPA) axis, conveys the neural signals to the periphery to facilitate IL-6 induction; (4) norepinephrine (NE) *in vitro* at dose range 10^{-6}-10^{-4}M was able to stimulate IL-6 production, and at dose range 10^{-9}-10^{-7}M synergized with IL-1ß in inducing IL-6 production from splenocytes. Taken together, the findings support the proposal that the high levels of systemic IL-6 induced by iv IL-1ß is partially mediated through the activation of the a1-noradrenergic autonomic system in the brain and the consequent increase in the sympathetic outflow to the periphery, and NE released from sympathetic terminals may function as a modulator to interact with IL-1ß in inducing IL-6 production from sympathetic nerve innervated peripheral organs. Sponsored in part by Off. of Naval Res. grant N00014-93-1-0829.

336.13

NEURONAL SIGNALING BY INTERLEUKIN-1.

Y. Bai, E. Kouranova, G.M. Jonakait, E. Bonder and R.P. Hart*,
Dept. Biol. Sci., Rutgers Univ., Newark NJ 07102.

The effects of cytokines on neuronal function have been largely ascribed to mediation by non-neuronal cells. In order to determine if neurons may be capable of directly responding to inflammatory cytokines, we have examined IL-1 signaling in cultured superior cervical (sympathetic) neurons. Enriched neuronal cultures (<5% non-neuronal cells by visual inspection) contain both Type I IL-1 receptor mRNA and IL-1 receptor accessory protein (IL-1Rap) mRNA. The relative level of these two mRNAs is similar to that found in a Schwann cell line, arguing that these mRNAs are found in neurons in the enriched neuronal cultures and are not due to the presence of contaminating cells. When enriched neuronal cultures are treated with 10 ng/ml hrIL-1β for 2 hr, RNA differential display detects several bands either up- or down-regulated indicating a gene expression response. Some of these bands are different from those regulated in a Schwann cell line in a parallel experiment, indicating that they are specific to neurons. Treatment of enriched neuronal cultures with IL-1 also leads to nuclear localization of the responding transcription factor NF-κB by immunocytochemistry with an anti-p65 antibody. NF-κB translocation is observed in both neurons and Schwann cells in the culture within 1 hr of IL-1 treatment. These results demonstrate that sympathetic neurons likely express IL-1 receptor components, that they respond to IL-1 treatment by activating a transcription factor and that they regulate responding genes. Supported by NIMH.

336.14

INDUCTION OF IL-1b EXPRESSION AND CELL DEATH BY HEXACHLOROBENZENE IN DIFFERENTIATED N1E-115 NEUROBLASTOMA. A. J. Malin, C. F. Ide, A. Arimura* U.S. Japan Biomedical Research Laboratory, and Center for Bioenvironmental Research, Tulane University, New Orleans, LA 70115.

IL-1β has been shown to play a critical role in repair of nervous tissue and has been implicated in both cell death and cell survival. IL-1β is synthesized by both neurons and glial cells. IL-1β protein has been localized to neurons in the suprachiasmatic nucleus, cortex, dentate gyrus, median eminence, lateral hypothalamus, and granular layer of the cerebellum in rat. Hexachlorobenzene (HCB) is a environmental contaminant shown to cause an array of health effects in mammalian nervous, endocrine, and immune systems. In this study, the effects of HCB on IL-1β expression and cell death were investigated using differentiated murine N1E-115 neuroblastoma cells as a neuronal model. Cell death was measured using the trypan-blue exclusion assay after treatment of differentiated N1E-115 cultures with 50 ppb and 150 ppb HCB at 6, 12, 24, 36, 48 and 72 hours. Levels of IL-1β were measured by ELISA for each dose and time point. IL-1β-like immunoreactivity was confirmed in the differentiated N1E-115 cells and found to be localized to the nucleus. No nuclear stain was present with pre-absorbed antibody. Results indicate that cell-associated IL-1β levels and cell death both increase in a time and dose dependent manner. These results suggest that hexachlorobenzene induces a neural immune response which may be regulating cell death in a neuronal model. Supported by Office of Naval Research grant number N00014-3-1-0829.

336.15

EXPRESSION OF A FUNCTIONAL INTERLEUKIN-15 RECEPTOR COMPLEX BY MICROGLIAL CELLS IN CULTURE. U.-K. Hanisch*, S. Lyons, F. Kirchhoff, C. Nolte, and H. Kettenmann. Department of Cellular Neurosciences, Max Delbrück Center (MDC) for Molecular Medicine Berlin, 13122 Berlin-Buch, Germany.

The study demonstrates the presence of mRNAs for interleukin-15 (IL-15) and all the components of an IL-15 receptor (IL-15R) system in cultured mouse microglia. RT-PCR results, supported by partial sequencing, show that mouse microglia synthesize the novel cytokine, IL-15, and express the cytokine receptor subunits, IL-15Rα, IL-2Rß and IL-2Rγ, that build up a heterotrimeric high-affinity IL-15R complex. IL-15 supported the growth of microglia *in vitro* at doses of 0.1 to 10 ng/ml, indicating that the microglial IL-15R could transmit signals. The IL-15 effect was demonstrated with complementary experimental protocols estimating cell death and survival (LDH and MTT-3 assays), respectively.

The findings suggest that microglial cells produce IL-15 as an autocrine growth factor. Microglia may serve as an IL-15 source for other neural cell populations throughout the brain. Furthermore, *in vivo* IL-15 could likely be responsible for several CNS effects currently ascribed to the T cell growth factor, IL-2, as IL-15 and IL-2 have overlapping activities. Being related by their immune functions (but not by sequence), IL-15 and IL-2 both share IL-2Rß and IL-2Rγ for signaling, while IL-15Rα and the homologous, non-signaling IL-2Rα may serve as specific binding subunits. Under pathophysiological conditions, microglial IL-15 could act as a chemoattractant and stimulator for invading T cells. In turn, (T cell- or astroglia-derived) IL-2 could affect (activated) microglia. Together the data point to a possible role of the IL-15/IL-15R system in the regulation of microglial physiology and communication. Supported by the BMBF and DFG of Germany.

336.16

INTERLEUKIN-15 (IL-15) INDUCES TOXICITY IN MESENCEPHALIC, SEPTAL AND HIPPOCAMPAL NEURONAL CELL CULTURES, F. Mennicken*, S. Bastianetto and R. Quirion. Douglas Hospital Research Centre, McGill University, Department of Psychiatry, 6875 Blvd. LaSalle, Verdun, Quebec, H4H 1R3, Canada.

We have previously shown that IL-15 decreased choline acetyltransferase (ChAT) activity in septal cell cultures grown under serum-free medium after 5 days *in vitro* (DIV) (Soc. Neurosci., San Diego, 1995). The IL-15-induced decrease in ChAT activity (ED_{50}≈4 ng/ml) is due to marked neuronal toxicity. We have now investigated if IL-15 is similarly neurotoxic to different types of primary neuronal cultured cells assessing viability by the MTT method (Manthorpe et al., Dev Brain res 25: 191, 1986).

Mesencephalic, septal and hippocampal cultures were prepared from 15, 17 and 19-day old rat embryos respectively. Following enzymatic and mechanical dissociation, cells were allowed to grow in a serum medium for 24 h. This medium was then change to serum-free conditions (N_2 supplement) with IL-15 being added at various concentrations (0.1-50 ng/ml). After 6 DIV, IL-15 induced significant decreases in cell viability (up to 80% at 50 ng/ml) in these 3 types of cultures. IL-15 was highly toxic in septal cell cultures with a significant cell loss at 5 ng/ml whereas 10-30 ng/ml were required for hippocampal and mesencephalic cell cultures. We also observed that IL-15-induced toxicity decreased proportionaly with cell density increments (1.5-7.5 x10^5 cells/well). The addition of IL-15 after 4 DIV was also found to be neurotoxic. Hence, IL-15 toxicity is not only related to initial phase of *in vitro* neuronal survival and growth.

Taken together, these results demonstrate the potent neurotoxic effect of IL-15 on neuronal survival. Supported by FRSQ and MRCC.

336.17

UP-REGULATION OF THE C-C CHEMOKINE RECEPTOR-LIKE GENE (RBS11) IN THE RAT FACIAL NUCLEUS AFTER MOTONEURON AXOTOMY. Y. Jiang, R.K. McNamara, M.N. Salafranca, N.A. Pennell, W.J. Streit, and J.K. Harrison*. Departments of Pharmacology and Therapeutics, Psychiatry, and Neuroscience, University of Florida, Gainesville, FL 32610-0267.

We have previously reported on the molecular cloning of a novel G-protein coupled receptor whose conceptualized amino acid sequence is most similar to the subfamily of C-C chemoattractant cytokine (chemokine) receptors (Harrison, J.K. *et al., Neurosci. Lett.*, 169:85-89, 1994). This receptor gene (RBS11) is widely expressed in several tissues of the rat with prominent expression in both brain and spinal cord. In order to determine the cellular localization of this receptor in the central nervous system we initially examined for the presence of RBS11 mRNA in extracts of primary cultured neurons, astrocytes, and microglia. Only cultured microglia expressed detectable levels of RBS11 mRNA. The effect of nerve transection on the level of expression of RBS11 mRNA in the rat facial nucleus 4 days after motoneuron axotomy was also assessed using the techniques of northern blot and *in situ* hybridization analysis. Both methods revealed a significant elevation of RBS11 mRNA in the facial nucleus on the axotomized side 4 days after motoneuron transection. Since microglial proliferation and activation are found in the axotomized facial nucleus, it is our hypothesis that RBS11 is expressed by microglia *in vivo*. These data implicate chemokine-dependent mechanisms in the cellular response of the facial nucleus to nerve transection. *(Supported by grants from the American Heart Association - Florida Affiliate and the National Multiple Sclerosis Society)*

336.18

IMMOBILIZATION STRESS MODULATES THE EXPRESSION OF INTERFERON-α mRNA IN THE MOUSE. S.Take[1*], Y.Kanemitsu[1], T.Yasaka[1], T.Katafuchi[1], T.Hori[1] and F.Eckenstein[2] [1]Dept. of Physiology, Kyushu Univ. Faculty of Med., , Fukuoka 812-82, Japan and [2]Dept. of Cell Biology, Oregon Health Science Univ., Portland, OR 97201.

The brain and the immune system communicate with each other using the common ligand-receptor systems. Interferon-α (IFN-α) is one of such ligans that is produced in the brain during neurotropic viral infections, brain injury, etc. and induces a variety of central symptoms including immunomodulation. In the present study, we examined whether or not immobilization stress, one of the "psycological" stress, might modulate the biosynthesis of IFN-α in the mouse.

Male BALB/c mice were immobilized for 15 to 180 min in a prone position by fasting their extremities to a board by adhesive tapes. At the end of immobilization, the mice were decapitated and their organs were rapidly removed to procede to Northern blot analysis of IFN-α mRNA. IFN-α mRNA was constitutively expressed in the brain and immobilization stress for 30 min markedly increased its expression especially in the cerebral cortex. Among the peripheral organs, expression of IFN-α mRNA was markedly enhanced in the liver and spleen by 90 min immobilization stress. Immobilization stress for 90 min also suppressed the cytotoxic activity of splenic natural killer cells measured by a standard chromium release assay using YAC-1 cells as a target.

We have previously reported that intracerebroventricular injection of IFN-α suppressed the cytotoxic activity of natural killer cells which was mediated by the brain opioid-CRF-splenic sympathetic pathway. Taken all things together, we may speculate that immobilization stress-induced IFN-α in the brain might be involved in the brain modulation of the immunity, especially in stress-induced immunosuppression. The further study is going to examin this hypothesis.

This work was supported by Grants-in-Aid for Scientific Research 07770047 from the Ministry of Education, Science, and Culture of Japan.

336.19

DEFICIENT INTRACEREBRAL IFN-γ PRODUCTION IN HSV-2-INFECTED MICE: T CELL RECRUITEMENT, INOS PRODUCTION, AND APOPTOSIS OF HSV-INFECTED NEURONS

Gail Lewandowski*, Dept. of Neuropharmacology, The Scripps Research Inst., La Jolla, CA 92037.

In mice infected with virulent herpes simplex virus type 2 (HSV-2), intracerebral IFN-γ production was observed to be significantly less than that in mice infected with avirulent strains of HSV-1. Consequently, the following questions were investigated, (i) is the decreased IFN-γ production due to decreased T cell recruitment in the brains of HSV-2-infected mice? (ii) does the decreased IFN-γ production prevent production of nitric oxide (NO), a putative anti-HSV agent?, (iii) does the decreased IFN-γ production affect survival of infected neurons, by either promotion of apoptosis or disruption of synthesis of anti-apoptotic proteins?

Following intravitreal inoculation, numerous $CD4^+$ and $CD8^+$ T cells were immunohistochemically detected in brain regions positive for HSV-1 antigens. By contrast, very few $CD4^+$ and $CD8^+$ T cells were detected in the same brain regions in HSV-2-infected mice. Preliminarily, abundant inducible nitric oxide synthase (iNOS) was immunohistochemically detected in HSV-1-postive brain regions, but only minimally detected in HSV-2-postive brain regions. Considerable production of the Bcl-2, an anti-apoptotic protein, was immunohistochemically detected in HSV-positive brain regions in both HSV-1 and HSV-2-infected mice. No significant difference in the number of apoptotic neurons in HSV-2-infected brains, as compare to HSV-1-infected brains, was observed using the TUNNEL method.

Conclusions: (i) insufficient recruitment of CD4+ and CD8+ T cells in the brains of HSV-2 infected mice is the likely basis for decreased IFN-γ production, (ii) decreased IFN-γ production may stem the induction of iNOS, preventing production of NO, (iii) the presence or absence of IFN-γ does not appear to affect apoptosis of HSV-infected neurons.

Supported by NIMH Grant #R29MH51926

336.20

CYTOKINE RESPONSES TO ADENOVIRUS VECTORS IN THE BRAIN.

M.B. Lee, A.P. Byrnes, J. Lang, H.M.Charlton and M.J.A. Wood. Dept. of Human Anatomy, University of Oxford, Oxford, OX1 3QX, United Kingdom.*

Adenovirus vectors can deliver a wide range of genes to post-mitotic cells and are therefore a valuable research tool and potential therapy for neurological disorders. Although E1-deleted adenovirus vectors are non-replicating, they nonetheless can induce a T cell-mediated immune response in the brain and other organs. In the liver and lung, vector transgene expression is short-lived unless this immune response is suppressed. Although transgene expression in the mouse and rat brain persists for much longer (at least two months), subsequent peripheral exposure to the vector can result in local demyelination and an elimination in marker protein expression. Therefore, adenovirus vectors in the brain can remain as a potential target for the immune system and this limits the therapeutic potential of these vectors. A better understanding of the factors that control the immune response to adenovirus vectors in the brain is needed and an effective immunosuppressive strategy developed if these vectors are to be used in neural gene therapy.

We are currently examining murine cytokine mRNA production in order to characterise the immune response to adenovirus vectors in the brain. Adenovirus vectors were stereotaxically injected into the striatum of C3H mice. RNA from both the brain and cervical lymph nodes were examined. Analysis of IL-4 and IFN-γ message and protein allows different components of T cell immunity (Th1 and Th2 responses) to be differentiated. A semi-quantitative study of cytokine message using the reverse transcriptase polymerase chain reaction (RT-PCR) was used to compare the relative amounts of IL-4 and IFN-γ message over a time course. We show here that this is a reliable method to test our hypothesis that the T cell response in the brain is different from the T cell response in the periphery. The results presented here are important for evaluating our efforts to maintain a potentially less deleterious Th2 type response by expressing specific cytokines from the adenovirus vectors. (Supported by the MRC, U.K.).

CARDIOVASCULAR REGULATION: VENTRAL MEDULLA I

337.1

CHOLINERGIC CONTROL OF C1 ADRENERGIC AND OTHER VASOMOTOR NEURONS OF THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) P. G. Guyenet*, A. M. Schreihofer and D. Huangfu. Dept. of Pharmacology, University of Virginia, Charlottesville, VA 22908.

In chloralose-anesthetized rats iontophoresis of carbachol (carb) excited all barosensitive neurons of the RVLM (+8.2 ± 1.2 spikes / s, n=29).The excitation was reduced 85% by iontophoretic methylatropine (n=7) and was blocked by i.v. scopolamine (1mg/kg). Nicotine or hexamethonium (iontophoresis) produced no effect. Whole-cell recordings of bulbospinal RVLM neurons (n=54) were made in slices of neonate rat brain (3-10 day-old) using previously described techniques (Li et al., Am. J. Physiol. 269: R1356, 1995). Twenty four of 41 histologically recovered neurons were C1 cells (bulbospinal and immunoreactive for tyrosine-hydroxylase). Carb (1-30 μM) depolarized the cells, increased PSP frequency and decreased input resistance. In voltage clamp (approx. -50 mV, 1μM TTX present), both carb and muscarine produced inward current (12.6 ± 2 pA with 30 μM carb, n=16; EC_{50} for carb: 10 ± 1 μM; 2.6 ± 0.6 pA with 30 μM muscarine). The carb-induced current was reduced 46% by 5 μM methylatropine (n= 15) and 84% by 200 μM hexamethonium (n=9). It was linearly related to the holding potential (-120 to -40 mV) and reversed at -22 ± 2 mV, (n=18; linear extrapolation).

In conclusion, carb exerts pre- and post-synaptic effects on C1 and other vasomotor neurons of RVLM. "In vitro", the postsynaptic effect of carb has a mixed nicotinic and muscarinic pharmacology . "In vivo", the predominant response of these cells to iontophoretically applied carb is muscarinic. The dual cholinergic pharmacology of C1 cells is reminiscent of that of the noradrenergic neurons of the locus coeruleus. (Supported by the NIH: RO1 HL39841 and NIH training grant 5T32HL07284-19).

337.2

PROPERTIES OF IRREGULAR FIRING NEURONS IN THE RAT ROSTRAL VENTROLATERAL MEDULLA *IN VITRO* AND POSSIBLE INVOLVEMENT IN SYMPATHOEXCITATORY FUNCTION. A. Hayar*, P. Piguet and R. Schlichter. Lab. Physiologie Générale, Univ. Louis Pasteur, 21 rue R. Descartes, 67084 Strasbourg, France.

The rostral ventrolateral medulla (RVL) is thought to be the major central nervous system site where substances with α2-adrenoceptors agonist properties exert their sympatholytic effects. The purpose of this work was to examine in particular a type of RVL neurons designated as irregularly firing (IF). These neurons were shown to have relatively larger spike duration and amplitude, and were slowly discharging (1- 2 Hz). They were less frequently encountered (25% of the total RVL neurons sampled) and were interspersed between regularly firing pacemaker-like and silent neurons. Using intracellular recordings in rat medullary slices, we looked for properties that could distinguish these neurons (n=50) as a unique group. These neurons were found to have a predominant GABAergic postsynaptic activity that was essentially irregular. This contrasted with the finding that other neurons have a rather glycinergic regular synaptic activity. Noradrenaline (10-100 μM) hyperpolarized about 80 % of IF neurons. This response was associated with a decrease in membrane input resistance and was mimicked by the α2-adrenoceptor agonist clonidine (3-10 μM). Most of these neurons have an inward rectifier current and their input resistance was significantly reduced at hyperpolarized potentials. Preliminary results using intracellular labeling with Lucifer Yellow indicated that neurons of this group appeared to have a distinctive morphology. Taken together, our data indicate that IF neurons recorded *in vitro* could correspond to a similar group of slowly and irregular firing neurons recorded *in vivo* and found to be sensitive to α2-adrenoceptors agonists, suggesting that they could assume a sympathoexcitatory function.

A. Hayar is supported by a predoctoral scholarship (CIES, Univ. Louis Pasteur).

337.3

PONTINE RETICULAR FORMATION CONTRIBUTES TO THE TONIC CONTROL OF BLOOD PRESSURE. S. Ito and A.F. Sved*. Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Neurons in the pontine reticular formation (PRF) appear to contribute to the tonic control of arterial pressure (AP), by providing tonic excitation of bulbospinal neurons in the rostral ventrolateral medulla (RVLM) (Hayes et al. Am. J. Physiol. 266:R237, 1994). However, inhibition of neuronal activity in the PRF elicits only a transient decrease in BP. The present study sought to confirm this observation, and to examine the factors that may contribute to the transient nature of the response. Injection of muscimol (100 pmol in 100 nl CSF) unilaterally into the PRF (1.8 mm lateral from the midline, 8.0 mm below the dura, and 5.0-5.5 mm rostral to the obex with the pipette angled 10° caudally) of chloralose-anesthetized, paralyzed, ventilated male rats decreased AP 22±5 mm Hg and heart rate 22±4 bpm (n=3). These responses were transient, returning to control levels in 37±4 sec. Similar responses were obtained with injections of GABA (10 nmol) or glycine (65 nmol) and responses produced by bilateral injections were not significantly different from those produced by unilateral injections. The size of the depressor response was not altered by either acute or chronic baroreceptor denervation, although responses were slightly prolonged (approx. 1 min). In contrast, in rats in which the inhibitory input from the caudal ventrolateral medulla (CVLM) to the RVLM was inhibited by bilateral injection of muscimol into the CVLM, injections of muscimol into the PRF elicited large and sustained decreases in AP. Muscimol injection into the CVLM increased AP from 102±4 mm Hg to 164±4 mm Hg and subsequent injection of muscimol into the PRF decreased AP to 67±5 mm Hg, and this response was maintained for longer than 15 min (n=5). In 3 additional rats, AP fell to below 40 mm Hg and these rats then died. These results suggest that the PRF provides a prominent tonic excitatory input to RVLM vasomotor neurons, and this tonic excitatory input is offset by an inhibitory input from CVLM to RVLM. (Supported by NIH grant HL-38786.)

337.4

Efferents from both the Nucleus of the Solitary Tract and the Caudal Ventrolateral Medulla Synapse on C1 Neurons in the Rostral Ventrolateral Medulla. S.A. Aicher*, I. Jeske, S. Cravo, S. F. Morrison, D. J. Reis and T.A. Milner. Dept. of Neurol. & Neurosci., Cornell Univ. Med. College, NY, NY 10021.

The rostral ventrolateral medulla (RVL) contains reticulospinal adrenergic (C1) neurons that are thought to be sympathoexcitatory and form the medullary efferent limb of the baroreceptor reflex pathway. The RVL receives direct projections from two important autonomic regions, the caudal ventrolateral medulla (CVL) and the nucleus tractus solitarius (NTS). The present study used anterograde tracing from the CVL or NTS combined with immunocytochemical identification of C1 adrenergic neurons in the RVL to compare the morphology of afferent input from these two autonomic regions into RVL. NTS (N=203) and CVL (N=380) efferent terminals had similar morphology and vesicular content, but CVL efferent terminals were slightly larger than NTS efferent terminals. Overall, efferent terminals from either region were equally likely to contact adrenergic neurons in the RVL (21% for NTS; 25% for CVL). While efferents from both regions formed symmetric synapses, NTS efferent terminals were more likely to form asymmetric synapses than CVL efferent terminals. CVL efferent terminals were more likely to contact adrenergic somata than were NTS efferents, which usually contacted dendrites. These findings (1) support the hypothesis that a portion of NTS efferents to the RVL may be involved in sympathoexcitatory, e.g. chemoreceptor, reflexes (via asymmetric synapses) while those from CVL mediate sympathoinhibition (via symmetric synapses) exclusively; and (2) provide an anatomical substrate for differential postsynaptic modulation of C1 neurons by projections from the NTS and CVL. With their more frequent somatic localization, CVL inhibitory inputs may be more influential than excitatory NTS inputs in determining the discharge of RVL neurons. (Support: NIH #HL18974; Amer. Heart Assoc. Grant-in-Aid to SAA).

337.5

ADENOSINE INHIBITS RETICULOSPINAL VASOMOTOR NEURONS OF ROSTRAL VENTROLATERAL MEDULLA AND DOES NOT MEDIATE THEIR EXCITATION BY HYPOXIA *IN VIVO*. M.-K. Sun* & D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Reticulospinal vasomotor neurons of the rostroventrolateral reticular nucleus (RVL) are excited by acute hypoxia even after peripheral chemodenervation (Sun, *Prog. Neurobiol.* **47:** 157-233, 1995). Their membrane response *in vitro* is not abolished by blocking synaptic transmission with tetrodotoxin (Sun & Reis, *J. Physiol. Lond.* **476:** 101-116, 1994), suggesting their role as central oxygen-detectors. Synaptic blockade, however, may not affect release of excitatory amino acids (EAAs) and/or adenosine through non-synaptic mechanisms. Blocking EAAergic inputs onto RVL-spinal vasomotor neurons has been shown to have no effect on the hypoxic sympathoexcitatory pressor responses *in vivo*. Whether adenosine may mediate sympathoexcitatory pressor responses was investigated in chemodenervated rats *in vivo*. Microiontophoresis of adenosine onto RVL-spinal vasomotor neurons inhibited the neurons and microinjection of adenosine into RVL produced a depressor response. Both were abolished by an i.c. injection of adenosine deaminase. The i.c. adenosine deaminase, however, did not attenuate the excitatory responses of RVL-spinal vasomotor and sympathetic neurons and the associated pressor response, consistent with our *in vitro* observations. We conclude that (*a*) excitatory responses of RVL-spinal vasomotor neurons to acute hypoxia are not mediated by adenosine and (*b*) RVL-spinal vasomotor neurons are central oxygen detectors responsible for the initiation of the excitatory pressor responses of the sympathetic nervous system to acute hypoxia. (Supported by an NHLBI grant HL-18974)

337.6

IMMUNOLABELLING OF IONOTROPIC AND METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES IN RAT DORSOMEDIAL AND VENTROLATERAL MEDULLA. E.K.A. Corbett, T.F.C. Batten, S. Saha, and P.N. M^{c}William*. Institute for Cardiovascular Research, Leeds University, Leeds LS2 9JT, U.K.

Central cardiovascular regulation at brain stem sites including the nucleus tractus solitarii (NTS), nucleus ambiguus (NA) and ventrolateral medulla (VLM), involves the release of the excitatory amino acid (EAA) glutamate, and its action at both ionotropic and metabotropic EAA receptor subtypes (FJ Gordon, 1995, Clin Exp Hypertens 17: 81). We have investigated the presence of immunoreactivity (IR) for different GluR subtypes or subunits on neurons in the NTS/ dorsal motor vagal (DMVN) complex, the NA, and VLM catecholamine cell areas, using antibodies specific for AMPA subunits (GluR1, 2/3 & 4), kainate subunits (GluR5,6/7 & KA2), NMDA subunits (NMDAR1 & 2A/B), and for the metabotropic subtypes (mGluR1α, 2/3 & 5). Vibratome sections of rat brains perfused with paraformaldehyde/glutaraldehyde mixtures were immunolabelled for receptor subunits using ABC-peroxidase techniques. Sections were dual labelled with antibodies to choline acetyltransferase (ChAT), tyrosine hydroxylase (TH), or nitric oxide synthase (NOS), using alkaline phosphatase conjugated IgGs to label known cell groups with a blue product. Subsets of cell bodies in NTS, NA and VLM labelled strongly for GluRs 1-4, moderately for NMDAR1, but only weakly for NMDAR2A/B. Kainate subunit-IR was markedly stronger in NA and VLM neurons than in NTS neurons. Antibodies to mGluRs also gave characteristic labelling patterns, with the comparative labelling intensity of fibre terminals in NTS and surrounding NA and VLM neurons being mGluR2/3 > mGluR5 > mGluR1α. Through this study we have begun a mapping of the complex distributions and combinations of GluR molecules on specific neuronal populations in the medulla concerned with cardiovascular and other autonomic control mechanisms.

Supported by a British Heart Foundation project grant.

337.7

A5 NEURONS IN NEONATAL RATS: AUTOACTIVITY AND SENSITIVITY TO α_2-ADRENERGIC RECEPTOR AGONISTS. D. Huangfu*, and P. G. Guyenet. Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908.

Tyrosine-hydroxylase-immunoreactive (TH-ir) cells of the ventrolateral pons (A5 cells) control autonomic functions and nociception. The electrophysiological properties of spinally-projecting A5 cells were analyzed in brain slices of neonatal rats (age 3-10 days) with whole-cell patch-clamp techniques (electrodes containing Lucifer yellow). Cells were identified in the living slice by the presence of retrogradely transported fluorescent microbeads injected in the thoracic spinal cord 1 or 2 days after birth and their catecholaminergic phenotype was confirmed by immunohistochemistry posthoc (presence of TH-ir and Lucifer yellow). 69% of the spinally-projecting cells recorded (n=36) were TH-ir. 83% of A5 cells were spontaneously active (0.1- 6 spikes/s), their average interspike membrane potential was -63.1±1.6 mV and their input resistance 0.76±0.11 GΩ. Most cells displayed a regular pacemaker-like pattern at rest. Perfusion with a mixture of blockers of ionotropic receptors (kynurenic acid, bicuculline and strychnine) eliminated PSPs but did not reduce the spontaneous activity of the cells significantly (n=5). Slow membrane oscillations were observed when the cells were slightly hyperpolarized. These oscillations persisted when PSPs were eliminated with the mixture of postsynaptic blockers or with 1 μM TTX (n=5). Under voltage clamp (-55 to -60 mV; 1μM TTX) 1 μM clonidine, 10 μM α-methylnorepinephrine or 10 μM UK-14304 produced outward current (5-25 pA) in most A5 cells (14/17). Conclusion: A5 neurons have intrinsic pacemaker properties and possess autoinhibitory α_2-adrenergic receptors.

(Supported by NIH: RO1HL28785-15).

337.8

CARDIOVASCULAR & RESPIRATORY RESPONSES TO ACTIVATION OF A5 PONTINE NEURONS OF THE ANAESTHETIZED RAT. J.P.Lara, M.S. Dawid-Milner, P. López de Miguel, J.A. Aguirre*, K.M. Spyer & S. González-Barón. Dept. of Physiology. School of Medicine. 29080 Málaga (SPAIN).

The role of A5 neurons in cardiovascular regulation is controversial, but very little is known of its respiratory role. In this study both electrical stimulation (20 μA, 0.4 ms pulses, 50 Hz for 5 s) and glutamate microinjections (20 nl, 100 mM, pH 7.4 ± 0.1) were delivered to the A5 region using multibarrel electrodes to characterize its cardiorespiratory function. Experiments were performed in anaesthetized rats (Pentobarbitone, 60 mg kg^{-1}, i.p.). Phrenic nerve activity and blood pressure were recorded. In all cases, electrical stimulation elicited an increase in both blood pressure ($p<0.001$) and heart rate ($p<0.05$). The response to glutamate was variable. In 2 i animals, glutamate evoked increases of blood pressure ($p<0.05$) and heart rate ($p<0.05$). In 6 animals, the response was biphasic with a transient pressor response ($p<0.05$) followed by a small depressor response ($p<0.05$). The depressor component of the biphasic response was not elicited by baroreflex activation as the increase in heart rate ($p<0.05$) was maintained during both phases. In 1 animal, glutamate evoked a depressor response. No topographical organization of these responses was evident. This diversity of cardiovascular responses may reflect the diversity of neurons located within the limits of the A5 region although, a predominant sympatoexcitatory role was demonstrated. These stimuli always produced an expiratory facilitatory response: a decrease in respiratory rate ($p<0.01$) due to an increase of expiratory time ($p<0.01$). The respiratory response to electrical and chemical stimulation persisted after blocking the cardiovascular changes with guanethidine (10 mg kg^{-1}, i.v.). These results add new data regarding the cardiorespiratory role of the A5 pontine region.

This work has been supported by the Spanish DIGICYT and the BHF.

337.9

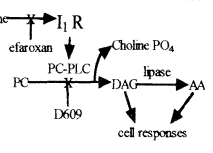
DIFFERENTIAL INDUCTION OF FOS-IMMUNOREACTIVITY IN HINDBRAIN CATECHOLAMINE (CA) CELL GROUPS BY SYSTEMIC 2-DEOXY-D-GLUCOSE (2DG)-INDUCED GLUCOPRIVATION. T.T. Dinh* and S. Ritter. Department of VCAPP, Washington State University, Pullman, WA 99164.

Decreased intracellular glucose utilization stimulates a number of physiological responses including increased food intake and adrenal medullary secretion. Central CA neurons appear to be involved in these responses: glucoprivation increases turnover of norepinephrine in the hypothalamus; hypothalamic norepinephrine stimulates feeding; hindbrain catecholamine neurons of the A1/C1 and A5 cell groups directly innervate sympathetic preganglionic neurons that project to the adrenal medulla and are activated by glucoprivation. In this experiment, we examined the effect of systemic glucoprivation on Fos-immunoreactivity (-ir) in brainstem CA neurons. The non-metabolizable glucose analogue, 2DG (200 mg/kg), was used to block glucose utilization. Rats were killed 2 hrs after 2DG or saline injection and prepared for immunohistochemistry. Brain sections were double labeled to reveal tyrosine hydroxylase (TH)- and Fos-ir. Neurons in the caudal part of the A1/C1 cell group, some in A6 and a few in C3 along the floor of the 4th ventricle contained both labels. However, rostral C1, A2/C2, A5 and A7 TH-ir neurons did not express Fos in response to 2DG. Saline control injections did not induce c-fos. Results indicate differential involvement of hindbrain CA neurons in responses to glucoprivation. Supported by PHS grant 40498.

337.10

IMIDAZOLINE RECEPTORS COUPLE TO PHOSPHATIDYLCHOLINE-SELECTIVE PHOSPHOLIPASE C TO GENERATE DIACYLGLYCERIDE AND TO ELICIT VASODEPRESSION IN ROSTRAL VENTROLATERAL MEDULLA. P Ernsberger, D Separovic, M Kester & MA Haxhiu. Case Western Reserve Univ, Cleveland OH 44106

Imidazoline receptors of the I_1-subtype (I_1R) selectively bind the agonist moxonidine and the antagonist efaroxan, and are expressed in the rostral ventrolateral medulla (RVLM) where they mediate vasodepression. In pheochromocytoma PC12 cells, I_1R are expressed in the plasma membrane in the absence of α_2-adrenergic receptors, which also bind moxonidine and efaroxan. Knowing I_1R are not coupled to cAMP, cGMP or inositol(PO_4) synthesis, we sought to define I_1R's signaling pathway. PC12 cells were treated with moxonidine (1μM) for 15s to 10 min, and diacylglyceride (DAG) was measured in two ways: formation of [^{3}H]DAG from [^{3}H]myristate-labeled phosphatidyl-choline (PC), and cellular mass of DAG (DAG kinase method). Phospholipase D (PLD) was assayed as [^{3}H]phosphatidylethanol accumulation. PC-selective phospholipase C (PC-PLC) was assayed by release of [^{3}H]choline-PO_4. Moxonidine increased [^{3}H]DAG by 39±13% at 1min. Total cellular DAG increased 32±10% at 15s. Differentiation with nerve growth factor (50 ng/ml for 36h) potentiated the DAG mass increase at 15s (to 69±12%) and prolonged it >10min. DAG accumulation was dose-dependent (EC_{50}=0.27±0.09μM) and efaroxan (1μM) was a competitive antagonist (apparent EC_{50}=2.4±0.5μM). The α_2-antagonist SK&F86466 (10μM) had no effect. The selective PC-PLC inhibitor D609 (100μM) abolished the rise in DAG mass. Moxonidine elicited a 41% rise in [^{3}H]choline-PO_4 release, which also was blocked by D609. Moxonidine neither released [^{3}H]choline nor activated PLD. Microinjection of D609 (10 nmol) into the RVLM of SHR rats abolished the vasodepressor response to iv moxonidine (40μg/ /kg). All data support the model shown, implicating PC-PLC in the cellular and organismic response to I_1R stimulation. *Support:*R29HL44514



337.11

VENTRAL MEDULLARY ADMINISTRATION OF AN OPIOID AGONIST MODULATES THE CARDIOVASCULAR RESPONSES DURING STATIC MUSCLE CONTRACTION. D.C. Caringi, D.J. Mokler, D. Koester, and A. Ally*. Departments of Pharmacology, Biochemistry & Anatomy, University of New England, College of Medicine, Biddeford, ME 04005

Static muscle contraction evokes increases in mean arterial pressure (MAP) and heart rate (HR) in anesthetized animals. It has been reported that activation of opioid receptors in the dorsal horn of the spinal cord attenuates these cardiovascular responses during muscle contraction. The present study determined the effects of administering the μ-opioid agonist [D-Ala^2]-methionine enkephalinamide (DAME, 100 μM) into the ventrolateral medulla (VLM) on the cardiovascular responses elicited during static contraction of the triceps surae muscle using chloral hydrate-anesthetized Sprague Dawley rats. Two microdialysis probes were inserted into the VLM at co-ordinates AP:-2.2; L: +1.9; H: -2.6 with respect to the calamus scriptorius using a stereotaxic guide. Static muscle contraction, evoked by stimulation of the tibial nerve at 3 X motor threshold, 0.1 msec duration, and 40 Hz frequency for 30 sec, increased mean arterial pressure (MAP) and heart rate by 22 ± 1 mmHg and 24 ± 1 bpm, respectively (n = 5). The developed tension during the contraction was 384 ± 50 g. Microdialysis of DAME for 40 min attenuated the contraction-evoked increase in MAP and HR (7 ± 3 mmHg and 10 ± 3 bpm). Developed tension did not change during contraction before and after microdialyzing DAME (372 ± 58 g). Prior administration of an equimolar concentration of naloxone, an opioid antagonist, blocked the attenuating effects of subsequent microdialysis of DAME. These results suggest that activation of opioid receptors within the VLM appear to play a role in modulating the cardiovascular responses during static muscle contraction.

337.12

CARDIOVASCULAR EFFECTS OF NITRIC OXIDE AND ADENOSINE IN RAT BRAINSTEM NUCLEI. C. J. Tseng*, W. J. Lo, H. C. Lin and C. S. Tung. Department of Medical Education & Research, Venterans General Hospital-Kaohsiung; Departments of Pharmacology & Physiology, National Defense Medical Center, Taipei, Taiwan, R.O.C.

Nitric oxide (NO) functions as a second messenger and neurotransmitter in the central and peripheral nervous systems. We reported recently that microinjection of L-arginine (L-Arg) into the Nucleus Tractus Solitarii (NTS) or rostral ventrolateral medulla produced inhibition of blood pressure, heart rate and sympathetic nerve activity. The purpose of the present study was to investigate the possible interaction of NO and adenosine in the NTS. In normotensive Sprague-Dawley rats, intra-NTS microinjection of L-Arg (33 nmol/60 nl) decreased blood pressure and heart rate. These effects were attenuated by prior administration of the specific adenosine receptor antagonist 1,3-dipropyl-8-p-sulfophenyl-xanthine (0.92 nmol). Similarly, prior administration of NO synthase inhibitor N^G-methyl-L-arginine or N^G-nitro-L-arginine methyl ester significantly attenuated the depressor and bradycardic effect of adenosine (2.3 nmol/60 nl). These results demonstrate a reciprocal attenuation of adenosine receptor antagonist and NO synthase inhibitor on L-Arg and adenosine responses respectively in the brainstem nuclei, and suggest that there is an interaction between NO and adenosine in central cardiovascular regulation.

This study was supported by grant NSC 85-2331-B075B-008, Taiwan, R.O.C.

337.13

EXPRESSION OF C-FOS PROTEIN IMMUNOREACTIVITY FOLLOWING PARTIAL BRAIN ISCHEMIA. Sexcius, L.M.*[1], Trouth, C.O.[1], Douglas, R.M.[1], James, S.D.[1], Valladares, E.R.[1], Khansari, P.[1], Peters, J.W.[1] and McKenzie, J.C.[2]. Departments of 1. Physiology and Biophysics and 2. Anatomy, Howard University College of Medicine, Washington, D.C. 20059.

Cerebral ischemia produces a powerful vasomotor response. In this experiment, the expression of c-Fos was examined by immunocytochemistry following permanent occlusion of the right common carotid artery in rats. Histological examination demonstrated the localization of a few c-fos positive cells, along the caudal and rostral VMS, in the A1 and C1 areas, in the NTS, in the locus coeruleus and in the hypothalamus, regions that have been implicated in cardiorespiratory control. These results indicate that centrally induced hypoxia stimulates cells in brainstem cardiorespiratory control sites as well chemosensitive sites at the ventral surface.

(Supported by ONR Grant # N00014-94-1-0523)

337.14

IMAGING OF VENTRAL MEDULLARY SURFACE CHANGES IN THE FREELY BEHAVING CAT. D.M. Rector*, M. Oguri and R.M. Harper. Dept. Neurobiology, UCLA, Los Angeles, CA 90095; Toho University, Tokyo, Japan.

We examined ventral medullary surface (VMS) activity during sleep-waking states and pressor and depressor challenges with imaging procedures. Under sterile surgery, cats were instrumented with electrodes to monitor sleep physiology and with a miniaturized video camera coupled to a coherent fiber probe and a narrow bandwidth illuminator with two wavelengths: red light (700nm), optimized for cell volume changes, and green light (565nm) which is preferentially responsive to perfusion changes. The probe was placed over a 7 mm^2 intermediate VMS area, and a 96 by 82 pixel image was acquired (12 bits, 100Hz) with alternating wavelengths. Cardiac and respiratory sources could be detected (0.01% peak to peak), as well as pronounced baseline state effects. Large slow oscillations, 0.05% in magnitude, emerged for both red and green light illumination during rapid eye movement sleep (REMS), and not during quiet sleep (QS). Noise-evoked arousal from QS caused a 0.3% drop in reflectance for both wavelengths. A 20µg/kg phenylephrine pressor challenge caused a sustained increase in green light reflectance and a biphasic response in red light reflectance. Depressor challenges with 25µg/kg nitroprusside caused a sustained reflectance decrease in both wavelengths in all states. Light reflectance changes from the VMS during sleep and pressor challenges are both wavelength- and behavioral state-dependent. *(Supported by HL-22418 and NIDR DE-07212)*

337.15

FOS EXPRESSION DEFINES NEURAL SUBSTRATES MEDIATING AUTONOMIC RESPONSES TO AIR PUFF STARTLE. A. A. Palmer, M. P. Printz *, Dept. of Pharm., UC, San Diego, La Jolla, CA, 92093-0636

SHR and WKY rats show a differential heart rate and blood pressure response when subjected to air puff startle. Prior studies in our lab indicate that this difference is mediated by the CNS. To determine which brain regions account for the air puff startle response, and the strain differences in the response, we used immunohistochemical labeling for fos protein in rat brains in both strains after either startle or chamber restraint, but no startle. A subset of animals from all groups were habituated to the test chamber environment prior to the day of testing. Our results show an elevation of fos positive neurons following startle in the following brain regions: RVLM, DCo, VCo, LC, KF, DLL, LDTg, DCG, MGN, PRC, PVA, LH, VMH, DM, PVN, MPA, and LPO ($p < 0.05$). Additionally, we found that there was a strain dependent difference in the fos response in NTS, RVLM, LC, LH, VMH, and DM with SHR showing a greater response than WKY ($p < 0.05$). Habituation showed an effect only in the RVLM ($p < 0.05$). Interestingly, heart rate, blood pressure, and motor responses were unchanged by habituation. These data identify a diverse group of brain regions activated following the delivery of the air puff startle stimulus, and indicate some brain stem and hypothalamic regions are differentially activated in the two strains. This work supported by NIH HL 35018.

337.16

INPUTS FROM THE CAUDAL VENTROLATERAL MEDULLA TO THE LATERAL TEGMENTAL FIELD OF THE CAT: A POSSIBLE BRAINSTEM CIRCUIT FOR VESTIBULOSYMPATHETIC REFLEXES. B.C. Steinbacher Jr.*, S.D. Stocker, C.D. Balaban and B.J. Yates. Dept. of Otolaryngology, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15213.

Previous studies have demonstrated that cardioregulatory areas of the brainstem receive input from vestibular afferents. Activation of the otolith organs in the "nose-up" direction has been shown to drive an increase in sympathetic nerve activity and a concomitant increase in blood pressure. From electrophysiological studies it has been shown that cells in the caudal ventrolateral medulla (CVLM) respond to activation of vestibular afferents with a short latency response. Neurons in the CVLM have a distinct inhibitory projection to the rostral ventrolateral medulla (RVLM). Since the RVLM projects to sympathetic preganglionic neurons in the thoracic spinal cord, it was initially hypothesized that vestibular information which drives an increase in sympathetic activity is relayed to cells in the RVLM through the same barosensitive cells located in the CVLM. However, two populations of cells exist in an overlapping region in the CVLM: 1) A group of cells which respond to vestibular stimulation and have no blood pressure related activity; 2) A group of cells which have a firing pattern correlated to blood pressure (i.e., barosensitive cells), but do not respond to vestibular activation. None of the cells that responded to vestibular afferent activation could be antidromically activated from the RVLM. In order to identify what other areas of the brainstem the CVLM might send projections, the anterograde tracer *Phaseolus vulgaris leucoagglutinin* and the anterograde/retrograde tracer dextramethylrhodamine were microionophoretically injected into discrete areas of the CVLM. Results of this labeling pattern confirm that there is dense projection to the sub-retrofacial area, as well as a diffuse, but prominent projection into the LTF. Serial sections of tissue were additionally stained with an antibody to tyrosine hydroxylase to determine if PHA-L labeling overlapped central catecholaminergic neurons. In fact, PHA-L positive fibers were found in catecholaminergic containing areas of the brainstem corresponding to the A1, A5, A2, C1 regions and the locus ceruleus. These results provide a possible pathway for which cells in the CVLM could relay vestibular related information to the RVLM. A good candidate is the area of the LTF, since it has already been shown that cells in the LTF respond to vestibular afferent activation and make direct connections with cells in the RVLM.

Supported by NIH grant RO1 DC-00693

337.17

CHANGES IN GENE EXPRESSION OF NEURONAL NITRIC OXIDE SYNTHASE (nNOS) IN HYPOTHALAMUS AND BRAINSTEM OF ADULT RATS WITH SPONTANEOUS HYPERTENSION. T.L. Krukoff* and D. Plochocka-Zulinska. Dept. of Anatomy & Cell Biology, Faculty of Medicine, Univ. of Alberta, Edmonton, Canada T6G 2H7.

Nitric oxide (NO) has been implicated in a variety of neuronal functions including regulation of sympathetic outflow and cardiovascular activity. We have previously shown that gene expression of nNOS, the synthetic enzyme of NO in neurons, is altered in discrete brain regions of renal hypertensive rats. To determine if the brain NO system is also altered in association with genetic hypertension, we measured levels of nNOS gene expression in discrete areas of the brain from spontaneously hypertensive rats (SHRs) at pre-hypertensive (4 week) and hypertensive (14 week) stages. Levels of nNOS mRNA in hypothalamus (HYP), dorsal pons (DP), dorsal medulla (DM), rostral ventrolateral medulla (RVLM), and caudal ventrolateral (CVLM) were measured with semi-quantitative polymerase chain reaction and compared to levels in control Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats. In 4 week rats, no significant changes in nNOS gene expression were found in any brain region among the groups. In 14 week rats, significant increases in nNOS mRNA levels were found in the following areas from SHR brains: HYP (56% increase compared to WKY, 104% increase compared to SD), DM (27%, WKY; 45%, SD), and CVLM (23%, WKY; 27%, SD). No significant differences were found in the DP or RVLM. *In situ* hybridization revealed that, in the HYP, increases in nNOS mRNA levels occurred primarily in the paraventricular (parvo- and magnocellular divisions) and supraoptic nuclei. The results suggest that NO production is increased in response to elevated arterial pressure in hypothalamus and brainstem of adult SHRs and support the hypothesis that NO is involved in regulating sympathetic output to the periphery.

Supported by the MRC and Heart and Stroke Foundation of Canada.

337.18

BRAINSTEM TUMORS IMPAIR CARDIAC SYMPATHOVAGAL BALANCE. C. Cogliati*, S. Guzzetti, S. Marzorati, R. Magatelli, N. Montano, A. Malliani. Medicina Interna II, Ospedale L.Sacco University of Milan & Div. di Neurianimazione, Ospedale Maggiore, Milano. Italy.

Brainstem represents the major site of integration of neural regulation of cardiovascular function. To evaluate the contribution of some of its structures to the sympatho-vagal balance, we evaluated heart rate variability (HRV) and its oscillatory components in 13 hospitalized subjects with tumoral brainstem lesions before neurosurgery intervention and in 10 control subjects (C) of similar age. All patients were studied with TC scan or NMR for a precise description of the localization of the lesion. ECG was recorded for 30 min at rest. Patients enrolled in the study were not in therapy with medications interfering with autonomic function. Three patients (Group A) presented a lesion in the ponto-cerebellar region, on the dorsal surface of the brainstem, while the remaining (n = 10; Group B) had a more diffuse localization within the ventral part. Group B displayed a significantly lower RR interval compared to C (684 ± 36 vs. 990 ± 28 msec; $p < 0.05$). Moreover both groups A and B had a significant reduction in total HRV (respectively, 598 ± 254 and 450 ± 232 msec^2) compared to C (2399 ± 423 msec^2; $p < 0.01$). A clear difference was found in the ratio between LF (Low Frequency, around 0.1 Hz) and HF (High Frequency, synchronous with respiration) spectral components, considered an index of the sympathovagal balance (Group A: 0.45 ± 0.15, B: 5.98 ± 1.5 vs. C: 1.55 ± 0.25; $p < 0.01$). In conclusion, while a sympathetic predominance seems to accompany diffuse ventral brainstem lesions, a vagal predominance characterizes patients in whom expansive lesions were likely to involve structures regulating vagal modulation.

337.19

Neurons in the fastigial pressor area project fibers to the lower medulla in the common tree shrews. (Tupaia glis). W.Singhaniyom[1], M.Phoglin., P.Kanluan., R.Sudsuang, P.Boonsinsuk and S.Casalotti*; [1]Faculty of Medicine, Srinakharinwirot University, Bangkok and *Neuro-behavioral centre, Mahidol University, Nakornpathom, Thailand.

Previous reports have shown the influence of the fastigial nucleus on arterial blood pressure(ABP) and heart rate(HR) in various animals. However, no information reveals on the precise area of the nucleus responsible for the activities including mechanism underlying the actions. Electrical simulations of the nucleus in the rostro-caudal direction reveal the highest ABP and HR response in the rostral area (430 umposterior to the rostral pole) while the lesser response was obtained in the middle area (430-640 um) and no response in the caudal area (640-1,200 um). Simultaneous electrical stimulatios and injections of biocytin(30 picolitre) through glass micropipette into the three areas with 12-16 hours survival time. Then, the brainstem were vibratome sectioned(100 um) and processed for biocytin localization. The labelled fibres were found to project bilaterally from the nucleus only after injection into the rostral and middle areas. They are observed just dorsal to the brachium conjunctivum and curved dorso ventrally between the spinal tract of the trigeminal nerve(TSV) and brachium pontis toward the lateral boundary of the superior olivary nucleus(OS). At the caudal level of OS the fibers are splitted into the upper and lower groups. The upper group are observed between the superior vestibular nucleus and the inferior cerebellar peduncle(ICP), and then in ICP caudally . The lower one project caudally occupied the area near the edge of the section between the OS and pyramidal tract. Caudally, the fibres in the ICP move ventrally and along the lateral boundary of the TSV and join the lower group. The joined tract projects caudally toward the medulla and spinal cord. The results suggest the nucleus may exert the cardiovascular effects partly through this tract. (Supported by Srinakharinwirot University)

337.20

AUTORADIOGRAPHIC QUANTIFICATION OF CHOLECYSTOKININ RECEPTORS IN CARDIOMYOPATHIC AND SYRIAN HAMSTER BRAIN. S. Merani[1], T.V. Dam[2], R.M.Palmour[1]*, F.R.Ervin[1] and J.Gutkowska[2]. [1]Depts. Biology and Psychiatry, McGill Univ., H3A 1A1; [2]Cardiovascular Biochemistry, Hotel-Dieu Hospital, H2W 1T8, Montreal, Canada.

Quantitative *in vitro* autoradiography was used to examine cholecystokinin (CCK) receptor density in the cardiomyopathic and Syrian hamster brain. The cardiomyopathic hamster, an inbred strain of the Syrian hamster, is a paradigm for the cardiac necrosis that occurs in certain human myocardial diseases. Although the exact neuronal mechanisms in cardiomyopathy are not established, recent findings indicate increased catecholaminergic and serotonergic immunoreactivity in this model. CCK is a gastrointestinal and neuropeptide that has been implicated in both physiological and behavioral processes and is one of the most abundant peptides in the central nervous system. Previous studies indicate that neuronal CCK release is mediated by the 5-HT system. Furthermore, CCK is implicated in the etiology of panic disorder, a psychiatric illness that has been associated with idiopathic cardiomyopathy and which may be accompanied by cardiovascular abnormalities such as reduced heart rate variability, decreased plasma noradrenalin, increased cardiac left ventricular mass and mitral valve prolapse. In the present study, the distribution and density of neuronal CCK receptors in cardiomyopathy were investigated.

^{125}I-CCK-8 binding was examined in coronal and saggital brain sections of age- and sex-matched cardiomyopathic and Syrian hamsters, using unlabelled CCK-4 and CCK-8 as competitors. Binding profiles revealed high density labeling in the cortex and olfactory bulb, while moderate binding was observed in the hypothalamus, thalamus, amygdala, striatum and hippocampus. CCK receptor density was comparable in 30-day old cardiomyopathic and control animals. Increased CCK binding in the frontal cortex was observed in 120-day old cardiomyopathic hamsters as compared to age-matched controls. The results indicate that an altered CCK receptor density is present in the cardiomyopathic hamster brain and that this alteration is concomitant with the time-dependent progression of the disease.

The Medical Research Council of Canada, Group Grant.

CARDIOVASCULAR REGULATION: BLOOD PRESSURE REGULATION

338.1

Alterations in Stress-Responsiveness in the mRen2(27) Rat. M. Morris*, A.B. Lucion, P. Li, C. Barrett, D. Ganten, C.M. Ferrario and M.F. Callahan. Department of Physiology/Pharmacology and Hypertension Center, Wake Forest Univ., Winston Salem, NC, and Max Delbruck Center, Berlin-Buchs, FRG.

The mRen (27) transgenic model was used to study the influence of the renin angiotensin system on stress responsiveness. The effect of shaker stress on cardiovascular and endocrine parameters was evaluated in male Tg+ and Tg- rats either water replete or salt loaded (2 days of 2% NaCl). The rats were prepared with chronic arterial catheters. After recovery, they were exposed to the stress paradigm, 5 mins of shaking at 150 rpm, with measurement of mean arterial pressure (MAP), heart rate (HR) and plasma oxytocin (OT). The hypertensive Tg+ rats showed an exaggerated cardiovascular response to stress. MAP was increased 26.6±2.3mmHg as compared to 15.8±3.9mmHg (Tg+ vs Tg-). Salt consumption did not alter the magnitude of the pressure response. The tachycardia was also greater in the Tg+ than in the Tg- (74.4±10.3 vs 38.2±9.6 bpm) with a decreased response seen in the Tg+ rats consuming NaCl (48.7±11.1 vs 48.7±10.6 bpm). Stress produced an increase in plasma OT with no difference noted between the groups or with salt loading. The exaggerated MAP and HR stress responses in the Tg+ may be mediated by a greater sympathetic activation previously demonstrated in this model.

(Supported by NIH HL51952 and AHA NC93-GS-15)

338.2

Blunted Baroreflex Control of Heart Rate in mRen2(27) Rats is Unaffected by NaCl Loading. M.F. Callahan*, D. Ganten, C.M. Ferrario and M. Morris. Department of Physiology and Pharmacology and The Hypertension Center, Wake Forest Univ., Winston Salem, NC, and Max Delbruck Center for Molecular Medicine, Berlin-Buchs, FRG.

The mRen2(27) transgenic rat (Tg+) shows increased arterial pressure and circulating angiotensin. We have shown that salt loading rapidly increases arterial pressure in these rats. We examined the effects of two days of salt loading (2%NaCl in drinking water) on pressor responsiveness and baroreflex function in Tg+ and Hanover Sprague Dawley controls. An aorta catheter was used to measure mean pressure (MAP) and a jugular vein catheter was used to give phenylephrine (0.25-1.25μg). The regression of the change in pulse interval on the pressor effect was determined.

At baseline, MAP was significantly elevated in Tg+ rats(130±13 v. 89±3 mmHg). Tg+ rats showed elevated phenylephrine pressor response (62±10 v. 41±9) and blunting of the slope of the reflex curve (0.6±0.2 v. 2.7±0.3). Salt loading increased MAP in the Tg+ rats (147±13 mmHg) but had no effect on pressure in the controls (76±3). Salt loading had no significant effect on the slope of baroreflex curve but tended to diminish the pressor responsiveness. The results indicate that acute salt loading is not associated with a worsening of baroreflex function in mRen2 transgenic rats.(Supported by NIH NL51952 and AHA NC93-GS-15)

338.3

BLOOD PRESSURE OF MREN-2 RATS IS A FUNCTION OF SALT INTAKE. S. Sesoko, D.B. Averill, D. Ganten, D.I. Diz*, and C.M. Ferrario. Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27257.

Replacement of drinking water with 2% NaCl exacerbates the hypertension of mRen-2 transgenic (TG) rats. The objective of the current study was to determine whether salt restriction of mRen-2 TG rats caused a commensurate change in blood pressure. Adult (12 weeks) male mRen-2 TG rats (n=4) were instrumented for the measurement of blood pressure and heart rate by telemetry. Rats were placed in metabolic cages and fed a diet containing 0.13% NaCl (low salt) for twelve days. The diet was then changed to one containing 0.5% NaCl (normal salt) and rats kept on this diet for 6 days. Rats had access to water ad libitum. Blood pressure and heart rate for the last three days of low salt averaged 176 ± 3 (SEM) mm Hg and 350 ± 9 beats/min, respectively, whereas by the last three days of normal salt intake blood pressure and heart rate were 193 ± 3 mm Hg and 355 ± 8 beats/min, respectively. This represented a significant ($p<0.01$) increase in blood pressure. The change to a normal salt diet was also associated with a significant increase in water intake (52 ± 1 ml/day vs 69 ± 1 ml/day, $p=0.0001$), but body weight was unchanged. The results of this study demonstrate that reduction of salt intake and an associated decrease in water intake ameliorates the hypertension of mRen-2 TG rats. In conclusion, these data demonstrate that mRen-2 TG are salt sensitive on the basis of blood pressure changes commensurate with salt intake. Supported in part by NIH HL51952

338.4

MREN-2 TRANSGENIC RATS HAVE REDUCED SYMPATHETIC VASCULAR RESPONSE. D.B. Averill*, M. Brady, E. Eyler, D. Ganten, and C.M. Ferrario. Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27257.

Previous work from this laboratory has shown that enhanced sympathetic outflow may contribute to the hypertension of mRen-2 transgenic (TG) rats. The objective of the current study was to assess the vascular responsiveness of TG rats. Experiments were performed in TG (10-12 weeks old) and age matched Sprague-Dawley (SD) rats. Rats were anesthetized with halothane; catheters were inserted in a femoral artery and vein for measurement of blood pressure (BP) and injection of drugs, respectively. Rats were pithed with a stainless steel rod inserted along the length of the spinal cord. Immediately after pithing, rats were paralyzed, artificially ventilated, and removed from halothane. A second rod was inserted subcutaneously to serve as a ground return for electrical stimulation. A minimum of 1 hour was permitted for BP to stabilize (TG: 61 ± 13 (SEM) mm Hg and SD: 56 ± 2 mm Hg). Sympathetic responsiveness was assessed by measuring the BP response to graded electrical stimulation (40 V, 1 msec; 0.25, 0.5, 1, 2 and 4 Hz) of the spinal cord. Both TG and SD exhibited a frequency dependent increase in BP during spinal cord stimulation. However, the increase in BP produced by spinal cord stimulation was approximately 50% ($p<0.001$) of that obtained in SD. Vascular responsiveness was assessed by measuring the BP response to intravenous injection of phenylephrine (0.1, 0.2, 0.4, and 1.0 μg). In contrast to the diminished blood BP produced by spinal cord stimulation, the pressor response to each dose of phenylephrine was approximately 30% ($p<0.001$) larger in TG than in SD rats. These data suggest a defect exists in the sympathetic vasoconstrictor neuroeffector pathway that may be compensated by upregulation of α1 adrenergic receptors or enhanced signal transduction downstream from α1 adrenergic activation. Supported in part by NIH HL51952.

338.5

EVIDENCE THAT 5-$HT_{1D\alpha}$ AND 5-HT_{1A} RECEPTORS PLAY A SIMILAR ROLE IN CENTRAL CARDIOVASCULAR REGULATION IN ANAESTHETIZED RATS. M. Gallacher and A.G. Ramage.* Department of Pharmacology, Royal Free Hospital School of Medicine, London, NW3 2PF., U.K.

The regional haemodynamic effects of the 5-HT_1 receptor agonists DP-5-CT (1A; 3 nmol kg^{-1}) and sumatriptan (1B/1D; 10 nmol kg^{-1}) given i.c.v. (5μl over 20s) were compared in anaesthetized Sprague-Dawley (250-350g) rats. This comparison was carried out in the presence of the following 5-HT_1 receptor antagonists, 100 or 200 nmol kg^{-1} i.c.v. of WAY-100635 (1A) and/or 600 nmol kg^{-1} (i.v.) of GR127935 (1B/1D), mianserin or ketanserin (1Dα). The rats were anaesthetized with α-chloralose (80 mg kg^{-1}) after induction with halothane and artificially ventilated following neuromuscular blockade with decamethonium. BP and HR were measured from the carotid artery. Doppler flow probes were placed on the mesenteric (M) and renal (R) arteries and the abdominal aorta (hindquarters, H, flow) from which changes (%) in vacular conductance (C) were calculated.

DP-5-CT (n=6) and sumatriptan (n=5) caused immediate and significant ($p < 0.05$; ANOVA) increases in BP of 10±2 and 14±1 mmHg and HR of 124±17 & 56± 24 bpm and decreases in MC of 10±1 & 14±1 %, respectively. GR127935 blocked the effects of sumatriptan but not those of DP-5-CT. In the presence of WAY (100 nmol kg^{-1}) DP-5-CT and sumatriptan caused delayed falls in BP and only the DP-5-CT tachycardia was reduced to 43±4 bpm. The high dose of WAY abolished the effect of DP-5-CT on BP, however the depressor effect of sumatriptan was unaffected. Both drugs still caused a similar tachycardia. The sumatriptan depressor effect could be blocked by the addition of GR127935, mianserin or ketanserin. Only mianserin blocked the sumatriptan induced tachycardia reversing it to bradycardia. These results indicated that activation of central 5-$HT_{1D\alpha}$ receptors has a similar effect on blood pressure as 5-HT_{1A} receptors, in the forebrain causing a rise while in the hindbrain a fall in BP. (Supported by the Wellcome Trust)

338.6

INTRAVENOUS INFUSION OF MIVAZEROL, A NOVEL SELECTIVE ALPHA 2-ADRENOCEPTOR AGONIST, ATTENUATES THE TACHYCARDIC RESPONSE TO INTRATHECAL INJECTON OF NMDA, S.Y. Kim[1], K. Raikoff[1], E. Wülfert[2] and J. Hanin[1]*. [1]Dept. Pharmacol., Loyola University Chicago Stritch School of Medicine, Maywood, IL 60153, and [2]UCB Pharmaceutical Sector, Braine-L'Alleud, Belgium.

Our previous studies have shown that intravenous (i.v.) infusion of Mivazerol (MIV) produces a dose-related decrease in heart rate (HR) but not in blood pressure, suggesting that MIV has some specific effects different from those of "classical" hypotensive α_2-adrenoceptor agonists. The present study was performed to investigate the mechanism of bradycardic action of MIV, focussing on the possibility of a functional interaction between spinal α_2-adrenoceptors and *N*-methyl-D-aspartic acid (NMDA) receptors, in the control of HR. Experiments were carried out in pentobarbital-anesthetized and respiratory assisted male Sprague-Dawley rats in which i.t. cannulae were implanted at the T_2 level 5 days earlier. The i.v. infusion of MIV (15.3 μg/kg/hr), which was maintained from 30 min earlier until the end of experiment, significantly attenuated the tachycardic response to intrathecal (i.t.) injection of NMDA (15 nmol/kg). When an α_2-adrenoceptor antagonist yohimbine (150 nmol/kg, i.t) was administered 10 min before the i.v. infusion of MIV (15.3 μg/kg/hr), the tachycardic response to i.t. injection of NMDA (15 nmol/kg) was significantly greater than that observed in the control group. In addition, co-administration of MIV (15.3 μg/kg/hr, i.v.) with the NMDA antagonist 2-amino-5-phosphonovaleric acid (150 nmol/kg, i.t), in doses that gave significant bradycardic responses, had no additive effect on the decrease in HR. These results suggest that: (1) the bradycardic effect due to i.v infusion of MIV is mediated, at least in part, by its inhibition of the postsynaptic response to NMDA in upper thoracic spinal segments; and (2) this effect of MIV is mediated by spinal α_2-adrenoceptors. (Supported by UCB s.a. Pharm. Co.).

338.7

BRADYCARDIC EFFECT OF IMIDAZOLINE α_2 AGONISTS IS MEDIATED BY PERIPHERAL, PRE-SYNAPTIC α_2-ADRENOCEPTORS IN RATS, J.P. Lillibridge & K.A. Skau*. The Procter & Gamble Co. and U. of Cincinnati College of Pharmacy, Cincinnati, OH.

Using *in vitro* and *in situ* models we investigated the bradycardic effect of several imidazoline drugs. Clonidine, oxymetazoline and UK-14304 had no effect on the rate of isolated, spontaneously beating right atria at concentrations up to 10 μM, indicating that these drugs have no direct effect on cardiac rate. The peripherally selective benzimidazole PGE-6201204, which is a potent α_2-agonist, caused bradycardia in pentobarbitol anesthetized rats; this bradycardia was not reduced by acute bilateral vagotomy. Thus, cholinergic mechanisms appeared not to be involved in the bradycardia. Bradycardia by PGE-6201204 was attenuated by treatment with the β-antagonist atenolol and by chemical sympathectomy (iv 6-hydroxydopamine). Idazoxan antagonized the PGE-6201204 bradycardia at doses that inhibit α_2-adrenoceptors but not at doses that inhibit imidazoline receptors. Likewise, RX-821002 blocked the bradycardia at doses that block α_2-adrenoceptors. These results suggest that the bradycardic effect of PGE-6201204 and other imidazolines, is mediated by peripheral α_2-adrenoceptors possibly by reduction in norepinephrine secretion from cardioaccelerator nerves. (Supported by the Procter and Gamble Co.)

338.8

CENTRAL PRESSOR AND TACHYCARDIC EFFECTS OF γ-MELANOCYTE SIMULATING HORMONE (γ-MSH) ARE MEDIATED BY AN AS YET UNIDENTIFIED MELANOCORTIN RECEPTOR (MC-R). S-J. Li, K. Varga, P. Archer, V.J. Hruby, S.D. Sharma, R.A. Kesterson, R.D. Cone and G. Kunos*. Dept. Pharmacol. & Toxicol., Med. Coll. of VA, Richmond, VA 23298

γ-MSH is known to have potent pressor and tachycardic effects when injected intravenously into conscious rats, but the underlying mechanism is unknown (Hypertension 7 (Suppl I): I145-I149,1985). The present experiments were conducted to identify the nature of the MC-R involved in the cardiovascular effects of γ-MSH. In urethane-anesthetized rats, intracarotid (ic.) injection of γ_2-MSH elicited dose-dependent (0.1-12.5 nmol) pressor and tachycardic effects. Ic. injections of 1.25-12.5 nmol ACTH(4-10), a γ-MSH analog with minimal affinity for MC3-R or MC4-R, caused similar but smaller effects, whereas ic. injections of similar doses of α-MSH were without effect. The effects of γ_2-MSH were much more pronounced after ic. than after iv. injection, and were completely blocked by pretreatment with the ganglionic blocker mecamylamine (2 mg/kg, i.v.). Of the five MC-R cloned to date, MC3-R and MC4-R are expressed in brain, including regions involved in cardiovascular regulation. The novel, selective neural MC-R antagonist SHU9119 inhibits melanotropin effects at the cloned MC-3R and MC-4R at antagonist/agonist dose ratios of ≤1. SHU9119, 1.25-12.5 nmol ic., failed to alter the pressor and tachycardic effects of γ-MSH, 1.25 nmol ic. These findings indicate that γ_2-MSH increases BP and HR by increasing sympathetic outflow at a site(s) in the brain, and that the receptors mediating these effects are distinct from MC3-R or MC4-R.

Supported by HL-49938 (GK), DK-17420 (VJH), HD-30236 (RDC).

338.9

CENTRAL NERVOUS SYSTEM ACTIONS OF UROCORTIN AND CORTICOTROPIN-RELEASING FACTOR ON CARDIOVASCULAR FUNCTION IN CONSCIOUS RATS. M.K. Borsody and L.A. Fisher*. Dept. of Physiology, The Ohio State Univ. Col. of Med., Columbus, OH 43210

Owing to its central nervous system (CNS) distribution and actions, coticotropin-releasing factor (CRF) is hypothesized to be utilized as a transmitter in neural pathways that mediate the endocrine, autonomic and behavioral responses to stressful stimuli. Urocortin (UCN) is a recently characterized mammalian neuropeptide comprised of 40 amino acids and sharing 45% sequence identity with CRF. While the neural distribution of UCN appears to be more restricted than that of CRF, UCN exhibits greater affinity than CRF for both CRF receptor subtypes *in vitro*. Therefore, the objective of the present study was to compare the CNS actions of CRF and UCN on autonomic nervous activity and cardiovascular function. All experiments were performed in conscious unrestrained male Sprague-Dawley rats (230-250 g) previously instrumented with intracerebroventricular (icv) cannulas for injection of vehicle (artificial CSF, 10 μl) and test compounds (45, 150 and 450 pmol CRF or UCN) and with iliac arterial catheters for direct measurement of pulsatile arterial pressure (AP) and heart rate (HR). As expected, icv administration of CRF elicited dose-related elevations of AP and HR as well as behavioral changes consisting of intermittent episodes of grooming, chewing and tunneling under the cage bedding. Surprisingly, at all doses tested, icv administration of UCN produced considerably attenuated cardiovascular and behavioral changes compared to those evoked by CRF injection. In addition, icv administration of the highest dose of UCN (450 pmol) produced delayed reductions of AP, an effect not observed after injection of CRF.

Supported by MH53720 and The Roessler Foundation.

338.10

Influence of NO synthetase inhibitor on the cardiovascular responses to dopamine and apomorphine in anesthetized rats. Nobufumi Ono*, Yuichi Ono, Hidenori Noguchi, Shuuji Hara, Misa Fukuzawa and Takeshi Kuroda. Medicinal Informatics and Research Units, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, 814-80, Japan.

We examined the influence of NO synthetase inhibitor on the cardiovascular responses including in the regional blood flow (rBF) to dopamine agonists in urethane-anesthetized rats. The systemic blood pressure was measured from right femoral artery, heart rate from the pressure pulse, and rBF by laser doppler flowmetry under inhalation of O_2. Nω-nitro-L-arginine-methylester (L-NAME, 10 mg/kg) administered intravenously increased blood pressure, although decreased heart rate, the rBF of right biceps femoral muscle and right auricle skin. Nitropurusside elicited the depressor effect. Dopamine produced the depressor action at a dose of 5 μg/kg, but the pressor actions at the range of 20-100 μg/kg. The depressor action of dopamine was abolished, and reversed to pressor action by L-NAME. The pressor actions of dopamine at high doses were potentiated by L-NAME. The dose-dependent depressor actions of apomorphine (0.1-1 mg/kg) were markedly inhibited by L-NAME. The duration of pressor response to norepinephrine and epinephrine, moreover, was potentiated by L-NAME, although the maximal response was not altered. These results suggest that L-NAME modifies the cardiovascular response to dopamine and NO may participate in the modulation of sympathetic nerve transmission.

338.11

BIPHASIC PRESSOR RESPONSE TO CENTRAL BLOCKADE OF NITRIC OXIDE SYNTHASE WITH L-NAME IS INDEPENDENT OF DRINKING BEHAVIOR AND THE ADRENAL GLANDS. H. Liu, M.L. Terrell, J.Y. Summy-Long[§] and M. Kadekaro*. Div. of Neurosurgery, UTMB, Galveston, TX and [§]Dept. of Pharmacology, Hershey Medical Ctr., Penn. State Univ., Hershey, PA.

We have shown that intracerebroventricular (icv) blockade of nitric oxide (NO) synthase with N^G-nitro-L-arginine (L-NAME) attenuates drinking in dehydrated rats and increases arterial blood pressure (BP) in a biphasic mode in water sated and water deprived rats. The objective of this study was to investigate whether the drinking behavior or the absence of the adrenal glands modifies either of the pressor responses after central inhibition of NO production. Two studies were performed: 1) In the first (n=35), two groups of rats were dehydrated for 24 h then injected icv with either L-NAME (250 μg/5 μl) or the vehicle, artificial cerebrospinal fluid (aCSF; 5 μl). Water was made available immediately after the icv injections in only half of the animals. 2) In the second (n=13), two groups of acutely adrenal-ectomized and water sated rats treated with dexamethasone (0.25 mg/kg, i.m.) received an icv injection of either L-NAME or aCSF. BP was recorded before and continuously for 120 min after injection. In dehydrated rats, L-NAME increased BP in a biphasic mode, regardless of whether the animals drank or not. The first peak occurred within 5 min and decayed in 15 min; the second began at 45 min, with a continual rise until the end of the experiment. In dehydrated rats receiving aCSF there was a difference in BP responses associated with drinking. In contrast to the stable levels in animals that did not drink, there was an initial transient rise in BP, as expected in response to drinking, followed by a continuous fall to levels below basal values by the end of the experiment. Adrenalectomy did not modify either pressor response to L-NAME. These results indicate that the biphasic pressor response to icv L-NAME is independent of drinking behavior and release of catecholamines from the adrenal glands. Funded by 2R01-NS23055 (MK).

338.12

VASOPRESSIN MICROINJECTION INTO THE RAT SUBFORNICAL ORGAN CAUSES DECREASES IN BLOOD PRESSURE. P.M. Smith, K. Sun*, and A.V. Ferguson. Dept. of Physiology, Queen's University, Kingston Ontario, Canada, K7L 3N6

Vasopressin (VP), in addition to its direct vasoconstrictor actions, has been shown to be involved in the central control of blood pressure (BP). This peptide has been implicated in the development of experimental and clinical hypertension as well as increasing the sensitivity of the baroreflex. The subfornical organ (SFO) is one of the seven circumventricular organs and, as is characteristic of these specialized central nervous system (CNS) structures, lacks the normal blood brain barrier, has an extensive vascular supply, and has a dense aggregation of a variety of peptidergic receptors, including VP. It is thus ideally suited to its primary role as a receptor site for blood borne information that would otherwise not have access to the CNS. SFO neurons influence neuroendocrine and cardiovascular systems through extensive efferent neural projections to hypothalamic autonomic nuclei (such as the PVN and SON) which secrete oxytocin and VP.

The present study was conducted to evaluate the cardiovascular effects of VP microinjection directly into the SFO. Urethane anaesthetized (1.4g\kg) male Sprague Dawley rats were fitted with femoral arterial catheters for BP measurement. The animals were placed in a stereotaxic frame and a cannula electrode was advanced into the region of SFO. The effect on BP of 5 pmol (in 500nl) of VP microinjected into the region was assessed. The BP response for each animal was calculated as the mean area under the curve (area between the baseline and each BP response) for 165 sec following microinjection. Post mortem histological verification of electrode placement was performed and animals were grouped accordingly. Microinjection of 5 pmol of VP into the SFO resulted in a mean BP decrease of -626.8 ± 161.4mmHg*sec* ($p < .05$, Student's t test). VP microinjection into Non-SFO sites or into the ventricle resulted in mean increases in BP of 322.9±218.1 mmHg*sec and 1417.1±448.6mmHg*sec** ($p < .01$, Student's t test), respectively. These results suggest that the SFO may be an essential structure in the feedback control loop through which VP influences hypoythalamic neurosecretory centres.

Supported by Medical Research Council (Canada) and Heart & Stroke Foundation Ontario

338.13

ACUTE AND SUBACUTE ADRENALECTOMIES ALTER THE PRESSOR EFFECT OF INTRATHECAL VASOPRESSIN IN THE RAT. D.-P. Tan*# and K. Tsou. Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China. #present address: Lab. of Biochemical Genetics, NHLBI, NIH, 9000 Rockville Pike, Bethesda, MD 20892, USA.

Previous reports from our group and others have shown that intrathecal injection (IT) of arginine vasopressin (AVP) increase the blood pressure of the rat. This pressor effect is mediated by the spinal V1 receptors and through the sympathetic nervous system. To study the role of the adrenal gland in the pressor effect of IT AVP, we used rats that were sham-operated, 2h or 48h adrenalectomized (ADX) and 48 h ADX with dexamethasone replacement (40 μg/kg/day). Two hour ADX did not change the basal blood pressure (2h ADX 93 ±5 mmHg vs sham-operated 85 ± 5 mmHg; $p>0.05$). When IT AVP 25 ng was given to 2h ADX rats, the pressor effect was reduced significantly (ΔMBP 27 ± 3 vs 15 ± 1.1 mmHg; $p <0.01$). This suggests the involvement of adrenal gland in the pressor effect of IT AVP. When IT AVP 25 ng was given to 48 h ADX rats, the pressor effect was not reduced but increased significantly (ΔMBP 27±3 vs 48±6 mmHg; $p<0.05$). Dexamethasone replacement reduced the increased pressor effect of IT AVP in 48h ADX rats (ΔMBP 28±5 vs 48±6 mmHg; $p<0.05$). There was no significant difference between the 2h and 48 h ADX rats when the pressor effect of the intravenous injection of nonadrenaline 1.5 μg were compared (ΔMBP 33±5 vs 29±2 mmHg; $p>0.05$). These data suggest that 1) the reduction of the effect of IT AVP in acute ADX rats is due to the elimination of adrenaline; 2) the increased pressor effect of IT AVP in 48h ADX rats is due to the hypersensitivity induced by the lack of glucocortical hormones; 3) blood vessels do not play a major part in the increased pressor effect of IT AVP in 48 h ADX rats.

338.14

CENTRAL CARDIOVASCULAR CONTROL THROUGHOUT REPRODUCTION.

Y. Takahashi and Q. J. Pittman*. Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

In response to altered body water content during pregnancy and the demands of lactation, cardiovascular control mechanisms have to adapt to changing states. In addition, there are alterations in circulating hormone levels and in both the density and in the distribution of central neurotransmitter receptors involved in autonomic control mechanisms. We have measured cardiovascular variables in unanesthetized female rats at various times before and throughout the gestational and lactational periods. Rats were instrumented with chronic indwelling vascular canulae attached to radio telemetry devices permitting chronic telemetric recording of blood pressure in unstressed, freely moving rats. In addition, rats were prepared with indwelling cannulae in the lateral ventricle to permit later administration of arginine vasopressin (AVP), 10 pmol/5 μl ICV. After baseline recordings of blood pressure, cardiovascular and behavioural responses to AVP, (assessed on a 0-6 point scale), rats were bred; (detection of a vaginal plug). At a minimum of one week intervals thereafter, basal blood pressure recordings were taken and cardiovascular and behavioural responses were again assessed after ICV AVP. Virgin female rats displayed mean arterial pressure of 93 ± 3.84 mm Hg (mean ± SEM, N=9); blood pressures were statistically unchanged throughout gestation and lactation, except during the last trimester, at which time mean arterial pressure was 80 ± 3.8 mm Hg ($P < 0.05$). Pressor responses to 10 pmol AVP averaged 16-22 mm Hg throughout the various gestational, lactational and virgin states; no significant difference in pressor or motor behaviour responses were seen over the various times. We conclude that, although basal blood pressure changes throughout gestation, no significant changes are seen in responses to central AVP.

Supported by Heart and Stroke Foundation of Canada.

338.15

THE GALANIN ANTAGONIST M40 BLOCKS THE CARDIOVASCULAR EFFECTS ELICITED BY GALANIN N-TERMINAL FRAGMENT (1-15), BUT NOT BY GALANIN (1-29).

J.A. Narváez*, Z. Díaz, P.B. Hedlund§, J.A. Aguirre, R. Coveñas# S. González-Barón and K. Fuxe§. Dept. de Fisiología. Universidad de Málaga. Spain. §Dept of Neuroscience. Karolinska Institute. Stockholm. Sweden. # Dept. de Biología Celular. Universidad de Salamanca. Spain

Central administration of Galanin N-terminal fragment (1-15) [GAL(1-15)] elicits hypertension whereas Galanin (1-29) [GAL(1-29)] produces hypotension; both molecules have a tachycardic action. Furthermore, GAL(1-15) decreases baroreceptor sensitivity but GAL(1-29) has no effect, and both molecules modulates differentially the cardiovascular responses of Neuropeptide Y. The aim of this work is to investigate if these molecules could exert their action through different receptor subtypes. Doses of GAL(1-15) (3.0 nmol/rat) and GAL(1-29) (3.0 nmol/rat) were injected intracisternally alone or in presence of subthreshold doses of the Galanin antagonist M40 (0.1 nmol/rat). Mean arterial pressure (MAP) and Heart rate (HR) were recorded during 60 minutes after injections. GAL(1-29) elicited hypotension and tachycardia which remain unchanged in the presence of M40. However, M40 abolished the hypertensive and tachycardic action elicited by GAL(1-15) ($p<0.001$). In these animals a hypotensive response, similar to that induced by GAL(1-29) alone, was observed. These results demonstrate that M40 blocks the central cardiovascular effects elicited by GAL(1-15) but not by GAL(1-29). Thus, at least regarding the cardiovascular role of Galanin, M40 might distinguish the putative N-terminal recognizing Galanin receptor subtype from the GAL(1-29) receptor. This work was supported by Spanish CICYT (PB93-0992).

338.16

Influence of $GABA_A$ agonist, muscimol, on cardiovascular changes induced by carbachol in conscious rats. F. Onat*, T. Tellioğlu, Z. Gören, Ş. Oktay, K. Berkman. Dept. of Pharmacology, Marmara Univ. Sch. of Med., Haydarpaşa, 81326, İstanbul, Turkey.

Previous experimental studies have shown that intracerebroventricular (icv) injection of $GABA_A$ agonist, muscimol, resulted in marked decreases in blood pressure and heart rate due to a decrease in sympathetic nervous system activity. Likewise, it is well recognized that central cholinergic system is also involved in the regulation of central cardiovascular control. In this study, we examined the effect of muscimol on the cardiovascular responses induced by the stimulation of central cholinergic system. All experiments were performed in conscious, Sprague-Dawley rats instrumented with icv guide cannula for drug injection and iliac arterial catheters for direct measurement of mean arterial pressure (MAP) and heart rate (HR). The administration of cholinergic agonist, carbachol (CCh; 0.8 nmol, icv) produced an increase in MAP (22.8 ± 3.1 mm Hg) and a decrease in HR (-97.1 ± 13.1 beats/min). On the other hand, 5 nmol of icv muscimol decreased MAP (-14.7 ± 2.9 mm Hg) and HR (-35.7 ± 17.8 beats/min). All cardiovascular changes in response to both CCh and muscimol reached their max at 10 min after the drug injection. Pretreatment with muscimol 10 min before CCh administration completely abolished maximum MAP and HR changes induced by the icv injection of CCh. Additionally, displacement of (^{3}H)-QNB by muscimol (10^{-9} -10^{-4} M) at cerebrocortical and hypothalamic homogenates was investigated. Muscimol failed to displace the muscarinic radioligand from its binding sites suggesting that it does not exert any direct antagonistic activity at muscarinic receptors. All these results suggest that GABAergic system has a inhibitory influence on cholinergic neurons. (Supported by TÜBİTAK, SBAG-AYD-13)

338.17

ACTIVATION OF NEURONAL POPULATIONS BY HIGH SODIUM INTAKE IN WKY RATS F. Vahid-Ansari* and Frans H.H. Leenen Hypertension Unit, University of Ottawa Heart Institute, Ottawa, ONT., Canada, KIY 4E9.

High sodium intake affects BP and osmoregulation in part via central effects. In order to localize the central neuronal populations involved, immunohistochemical detection of Fra-like immunoreactivity (Fra-LI) was used to identify those neurons activated by high sodium diet. Groups of WKY rats (4 weeks old) received either high sodium (8% NaCl) or regular sodium (0.5% NaCl) diet for 3, 7 or 14 days. After perfusion, immunohistochemistry was processed on brain sections, 30 μm thick. Fra-LI expression was quantified within osmosensitive nuclei of lamina terminalis: SFO and MnPo and within BP-regulating areas like PVN, NTS and CG. On regular sodium diet, expression of Fra-LI was low and did not significantly change over the time course in the SFO and MnPo. However, high sodium diet increased Fra-LI in the SFO (> 3 times) after 3 days and maximal expression was observed in the SFO and MnPo after 7 days. In the BP-regulating areas, Fra-LI expressed differently in two divisions of the PVN. After 3-14 days of regular sodium, expression of Fra-LI in the magnocellular and parvocellular portions of the PVN was low and similar. In contrast, high sodium increased Fra-LI in the parvocellular part after 3 days diet (> 2 times) and and the highest expression (> 3 times) after 7 days. Maximal neuronal activation (> 10 times) of the magnocellular part of PVN was observed after 14 days of high sodium diet. High sodium increased Fra-LI in the NTS after 3 days and the highest expression after 7 days (> 2 times). In the CG, high sodium diet increased Fra-LI after 3 days and maximal activation (> 2 times) was observed after 14 days. The present results indicate that immunohistochemical detection of Fra-LI can be used to identify neurons chronically activated by high sodium diet. Since these neuronal populations may differently mediate the BP and osmoregulation by high sodium diet in salt-sensitive hypertension, determining the connectional and neurochemical character of the neurons that display Fra-LI after chronic high sodium diet may yield insight into its neuronal basis.

Supported by a grant from MRC.

338.18

BRAIN NATRIURETIC PEPTIDE MEDIATED CARDIOVASCULAR CHANGES IN DIFFERENT RAT STRAINS . B. R. Dev*, L. Philip and S. J. John. Dept. of Physiology, Faculty of Medicine, Kuwait Univ., P. O. Box 24923, Safat, Kuwait 13110.

Cardiovascular functional changes in response to local administration of brain natriuretic peptide (BNP) into the nucleus tractus solitarius (NTS) were assessed in age-matched, urethane anesthetized spontaneously hypertensive (SHR), Wistar-Kyoto normotensive (WKY) and Sprague-Dawley rats. Catheterization of the femoral artery provided the continuous measure of both blood pressure and heart rate. Microinjection of the vehicle (phosphate buffered saline) alone into the NTS of all the three rat strains did not elicit any significant change in blood pressure or heart rate. A significant reduction in the mean arterial blood pressure and heart rate was observed only in SHRs, whereas in the other two strains, namely, the WKY and Sprague-Dawley rats, there was no appreciable change in their mean arterial blood pressure and heart rate in response to local injection of different doses of BNP into the caudal NTS. These results suggest that the SHRs might be much more susceptible to exogenous BNP than the rats of normotensive strains. Supported by Kuwait University (MY 025).

338.19

MOTHER/PUP INTERACTIONS DURING PREWEANLING DEVELOPMENT IN SPONTANEOUSLY HYPERTENSIVE RATS, E. Perdomo, A.K. Johnson, and R.F. Kirby*. Departments of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, Iowa 52242-1407

The expression of hypertension in the spontaneously hypertensive rat (SHR) is dependent on both genetic and environmental factors present in early development (Cierpial, Shasby, & McCarty, 1987). In our first study, we examined the mother/pup interactions of the SHR and their normotensive control strain, the Wistar-Kyoto (WKY). Animals were videotaped across 24 hour periods at 3 ages during preweanling development; postnatal day (PD) 6, PD 12, and PD 18. In both the SHR and WKY, contact varied over the 24 hour light/dark cycle, with greater contact during the light period. Strain-related differences were also noted on PD 6. During the dark period, SHR displayed much shorter contact bouts than WKY, but at an increased frequency. SHR also had relatively greater amounts of contact during the day than WKY. Early preweanling manipulations, such as cross-fostering, can reduce the elevated blood pressure of adult SHR (Cierpial & McCarty, 1987). Therefore, our second study examined cross-fostering influences on mother/pup interactions. Previous patterns of SHR and WKY differences were maintained. These results suggest that mother/pup interactions during early preweanling development are determined by the mother rather than the pups. Supported by NHLBI HL 14388 and NASA NAGW-4358.

338.20

HEART RATE DYNAMICS IN APNEA OF INFANCY. R.K. Harper*, V.L. Schechtman, M.Y. Lee, J.A. Henslee, G.J. Luna and R.M. Harper. *Brain Research Inst. and Dept. of Neurobiology, UCLA School of Med., Los Angeles, CA 90095; and Southwest SIDS Research Inst., Lake Jackson, TX 77566.*

Heart rate variability is significantly enhanced in infants with apnea of infancy (AOI); moreover, apneic episodes are associated with marked changes in heart rate. Several conditions associated with altered heart rate variability result in aberrant beat-to-beat dynamics that can provide easy and inexpensive screening tools for the conditions. We studied the dynamics of beat-to-beat heart rate change in infants with persistent apnea and control infants. Polysomnographic recordings were obtained from fifteen 4-7-mo-old infants with persistent AOI. From these recordings and 17 recordings of control infants, R-R intervals (the intervals between successive R waves of the ECG) were determined with 1ms accuracy. For a 6-hr, evening period of each recording, each R-R interval was plotted as a function of the previous interval, and dispersion of subsequent intervals was quantified at slow, fast, and intermediate heart rates. Apneic periods were accompanied by substantial R-R interval patterning changes; however rapid (beat-to-beat) changes occurred only at the onsets of these episodes, and no significant difference in heart rate dynamics emerged between apneic and control infants across the 6-hr periods. We conclude that beat-to-beat dynamics across extended periods cannot be used to screen for AOI, but that the distinctive patterns of heart rate dynamics during apneic events may shed light on the physiology of persistent central apnea. *This research was supported by HD 22695.*

338.21

HEART RATE AND VARIABILITY IN FULL-TERM INFANTS WITH APNEA OF INFANCY. V.L. Schechtman,* J.A. Henslee and R.M. Harper. *Brain Research Inst. and Dept. of Neurobiology, UCLA School of Med., Los Angeles, CA 90095; and Southwest SIDS Research Inst., Lake Jackson, TX 77566.*

During periods of regular breathing, heart rate is slower and heart rate variability enhanced in selected prematurely-born infants with persistent apnea of prematurity (those with no history of perinatal respiratory distress), relative to full-term infants of comparable post-conceptional ages. Evidence suggests that this difference may be linked to the persistent apnea rather than premature birth. Thus, we assessed heart rate and variability in full-term infants with persistent apnea of infancy and control infants. Heart rate and variability (standard deviation of R-R intervals) were compared during periods of regular breathing in 48 infants with persistent apnea and 72 age-matched control infants. Heart rates did not differ significantly between the two groups of infants at any age; however, after 3 mo prenatal age, heart rate variability was significantly enhanced in infants with persistent apnea of infancy, relative to control infants. The findings indicate tonic changes in cardiac-variation in 4-6-mo-old infants who continue to suffer from apnea of infancy, and demonstrate a physiologic similarity in full-term infants with persistent apnea and prematurely-born infants with persistent apnea but no history of respiratory distress. It is not clear if this autonomic aberration is a consequence of persistent apnea or a pre-existing difference between normal infants and those who continue to show apnea of infancy.

This research was supported by HD 22695.

338.22

CENTRALLY ADMINISTRATED THA RESTORES HYPOTENSION IN HEMORRHAGIC SHOCK IN CONSCIOUS RATS. V. Savcı*, I. H. Ulus, S. Gürün, S. Çavun and B. K. Kıran. Uludag University Medical School, Department of Pharmacology, Görükle, 16059, Bursa-Turkey.

Right common carotid artery and left cerebral ventricles of male Wistar rats were cannulated under light ether anaesthesia to monitor blood pressure and drug injections, respectively. Hemorrhagic shock were produced by bleeding 2.1-2.2 ml of blood per 100 g of body weight within 5-10 min Intraventricular (i.c.v.) injection of tetrahydroaminoacridine (THA; 10, 25, 50 μg) increased blood pressure in a dose-and time-dependent manner in both normotensive and hemorrhaged rats. Atropine (10 μg; i.c.v.) greatly attenuated the blood pressure response to i.c.v. THA (25 μg) in both conditions. However, mecamylamine (50 μg; i.c.v.) caused slight blockade in hemorrhaged rats, while it failed to change the response in normotensive conditions. The increase in blood pressure to i.c.v. THA was associated several fold increase in plasma levels of vasopressin and catecholamines but not of renin. Pretreatment of rats with prazosine,an α1-adrenergic receptor antagonist (0.5 mg kg -1; i.p.) or vasopressin V1 receptor antagonist [β-mercapto-β,β-cyclopenta-methylenepropionyl^1,O-Me-Tyr^2-Arg^8]-vasopressin (10 μg kg-1; i.a.). was attenuated the pressor response to THA in both conditions. These data show that THA can restore hypotension in hemorrhaged rats through the activation of both central muscarinic and nicotinic cholinergic receptors, however the only central muscarinic cholinergic receptors are involved in pressor effect of THA in normotensive animals. Moreover, the increase in plasma vasopressin and catecholamine levels, both, participate the blood pressure effects of THA in both conditions.

338.23

CENTRAL ADMINISTRATION OF CHOLINE INCREASES PLASMA VASOPRESSİN LEVELS IN CONSCIOUS RAT. İ. H. Ulus*, V. Savcı, and B. K. Kıran. Uludag Univ. Medical Sch., Department of Pharmacology, 16059, Bursa, Turkey.

In the present study, we examined the effects intracerebroventricularly (i.c.v.) injected choline on both basal and stimulated release of vasopressin (AVP) in conscious rats. Choline (50-150 μg) caused time- and dose-dependent increases in plasma AVP levels. The i.c.v. choline-induced increase in plasma AVP was greatly attenuated by the pretreatment of rats with mecamylamine, a nicotinic receptor antagonist. Atropine, a muscarinic receptor antagonist, failed to alter choline's effect. Osmotic stimuli induced by either oral administration of 1ml of hypertonic saline (3 M NaCl) following 20-h deprivation of drinking water of rats, or an i.c.v. injection of hypertonic saline (1M NaCl) increased in plasma AVP several fold. Under these conditions, i.c.v. injection of 50 or 150 μg of choline produced a greater increase in plasma AVP than the increase observed in the basal condition. Choline (50 or 150 μg, i.c.v.) also enhanced the increase in plasma AVP in response to graded blood loss and acute hemorrhage. The effects of choline on plasma AVP levels in normal rats or in the rats subjected to graded blood loos were reduced by the pretreatment with hemicholinium-3, a high affinity choline uptake inhibitor. Stimulation of central nicotinic cholinergic receptors by presynaptic mechanisms is apparently involved in effects of choline on plasma AVP.

SENSORY SYSTEMS: SPINAL CORD I

339.1

PARTICAL SCIATIC NERVE LIGATION LEADS TO A MICROGLIA REACTION WHICH CORRELATES WITH ALLODYNIC BEHAVIOR

D.E. Coyle* and C. S. Sehlhorst Department. of Anesthesia, University of Cincinnati, Cincinnati, OH 45267-0531

A majority of studies on neuropathic pain have focused on the alterations that occur within neurons following peripheral nerve injury. Since glia have a profound influence on the CNS neural environment and are capable of generating an inflammatory response, this study investigated the response of microglia and astroglia to partial sciatic nerve ligation (PSNL). Forty female Harlan Sprague-Dawley rats were randomized into 10 groups containing 3 PSNL and 1 sham animal per group. Rats were evaluated for withdrawal from touch using Von Frey filaments (evaluator blinded). Spinal cords were evaluated for immunoreactivity (IR) to complement receptor iC3b (OX42), major histocompatibility complex II (OX6) and glia fibrillary acidic protein (GFAP) on post-injury days 1, 2, 4, and weeks 1, 2, 4, 6, 8, 10, and 12. The intensity of staining of the ipsilateral verses contralateral gray matter was determined by measuring average pixel intensity. OX42 and GFAP IR was confined to the ipsilateral cord (L3-L5) sections. The main areas of staining were confined to Lamina (Lam) I-III and part of Lam IV of the Dorsal Horn (DH) and Lam IX of the Ventral Horn (VH). A linear correlation was found to exist between gram force (withdrawal) verses percent change in intensity from the contralateral cord yielding a correlation coefficient of 0.8 for OX42 and 0.5 for GFAP. Cells staining for OX6 were rare and occurred in both the DH and VH. This study presents data that support the role of microglia in the development of neuropathic pain. The mechanism by which microglia may potentiate neuropathic pain is unknown but may be mediated through increased hypersensitivity of the DH as well as neural damage due to microglia release of excitatory amino acids, free radicals, and/or cytokines. This study was supported in part by an ASRA Carl Koller Award.

339.2

PLASTIC CHANGES IN SYNAPTIC TRANSMISSION OF RAT DORSAL HORN NEURONS FOLLOWING PERIPHERAL NERVE TRANSECTION.

M. Yoshimura*, M. Okamoto[1], H. Baba[1], K. Shimoji[1] and H. Higashi,

Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830, Japan, [1]Dept. Anesthesiol., Niigata Univ. Sch. Med., Niigata 951, Japan.

Following peripheral nerve injury, pathological state such as allodynia and/or hyperalgesia has been reported to develop in subpopulation of patients. Persistence of the pain after healing of the damaged tissue suggests that plastic changes in CNS, including the spinal cord, may play a important role in processing the pathological pain transmission. To investigate plastic changes in synaptic transmission at the spinal level, whole cell recordings were made from substantia gelatinosa (SG) and laminae IV/V neurons in the spinal cord slice with attached dorsal root from adult rats with or without the sciatic nerve transection (SNT). No significantly changes in passive and active membrane characteristics, including resting membrane potential, input resistance and configuration of action potential and spike after potentials, were detected between normal and SNT rats. In the normal rats, primary afferent stimulation with intensity sufficient to activate Aδ afferents elicited a monosynaptic fast EPSC in the majority of SG neurons. However, in the SNT rats, a polysynaptic EPSC with a long latency was evoked by activation of afferent fibers. The stimulus intensity for eliciting the polysynaptic EPSCs was much lower than that for activation of Aδ afferents, suggesting that Aβ afferents are likely responsible for generation of the responses. In addition, the conduction velocity and threshold for each afferents were not significantly altered. These observations suggest that synaptic plasticity occurred in a subset of deep dorsal horn neurons tends to transmit tactile sensory information to SG and this plastic change may account for underling mechanism in the pathological pain such as allodynia.

339.3

LONG-TERM CHANGES IN AFFERENT-EVOKED DORSAL HORN FIELD POTENTIALS ACCOMPANY SCIATIC LIGATION IN RATS. V. Miletic* & G. Miletic. Dept. Comp. Biosci., Univ. Wisconsin, Madison, WI 53706

Following insertion of a glass microelectrode to a depth of about 100 μm, we recorded sciatic-evoked field potentials (FPs) in the spinal dorsal horn of urethane-anesthetized control and ligated rats. The sciatic was stimulated with 0.5 ms long pulses at 0.5 Hz, and the FPs remained essentially invariable for about 30 min of control recordings. We then applied tetanic stimulation to the nerve (a 400 ms train of 0.1 ms pulses at 50 Hz), and recorded the evoked FPs again up to 4 hrs post-tetanus. In control animals, tetanic stimulation produced increases in FPs which peaked at 30 min (175% of pre-tetanus amplitudes), and returned to baseline values at 2 hrs post-tetanus. Interestingly, after 2 hrs the evoked FPs began to decrease, and attained only 50% of their pre-tetanus values at 4 hrs. In ligated rats the increase in FPs also peaked at 30 min (175%), but in contrast to control animals, was seen to persist, and at 4 hrs post-tetanus was still 155% of baseline values.

We also recorded sciatic-evoked FPs in another group of control and ligated rats but this time following low-frequency stimulation (a 400 ms train of 0.1 ms pulses at 5 Hz). We again noted different responses in control vs. ligated rats. In controls, a long-term depression in evoked potentials was seen, with the FPs reaching only 40% of their baseline values at 4 hrs. On the other hand, in ligated animals the depression was less pronounced (80%), was maximal at 2 hrs, and by 4 hrs the FPs had essentially returned to baseline.

These data suggest that sciatic ligation modifies afferent-evoked processing in the spinal dorsal horn to invoke plastic changes in excitability that resemble long-term potentiation and long-term depression. These long-lasting plastic changes in neuronal excitability may underlie the development of neuropathic pain in ligated animals. [Supported in part by NIH NS 21278.]

339.4

CALCIUM CHANNEL BLOCKER REVERSES PROSTAGLANDIN $F_{2\alpha}$ -INDUCED INCREASE OF SPINAL DORSAL HORN NEURONAL RESPONSES IN RATS.
Y.Yamamori, Y. Saito, T. Yanagidani*, H. Ishii, M. Shinzawa, Y. Kosaka
Dept. of Anesth., Shimane Med. Univ., Izumo, 693 Japan

Increase in the response to low intensity stimulation caused by prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) may contribute to the prostaglandins (PGs) induced long lasting hypersensitive state (Neurosci. Abst., 20, 307 1994). The purpose of this study was to examine the effects of calcium channel blocker, diltiazem on $PGF_{2\alpha}$ -induced increases of spinal dorsal horn neuronal responses.

Male Sprague-Dawley rats weighing 350-450g were used. Extracellular activity of single spinal dorsal horn neurons were recorded in decerebrated rats. The size of low-threshold RF and responses to brushing were determined. Following determination of neuronal responses after intrathecal(i.t.) administration of $PGF_{2\alpha}$(100ng/10 μl), evaluation was repeated after i.t. administration of diltiazem (500 μg/ 10 μl). To date eight neurons (7 LT neurons and 1 WDR neuron) have been studied. Four neurons out of five showed increase of RF, and one neuron showed no change after $PGF_{2\alpha}$, while intrathecally administered saline induced no change of RF in three neurons. The mean RF size was increased by 33%, 57% and 66% from the baseline level at 10, 30 and 60 minutes after $PGF_{2\alpha}$, respectively. The responses to brushing was increased in the neurons with the increased RF after $PGF_{2\alpha}$. The $PGF_{2\alpha}$-induced increase of the RF size was reduced by 91%, 83% and 53% at 10, 20 and 30 minutes after the administration of diltiazem. The responses to brushing were also reduced. These results suggest that calcium channel blocker may reverse PGs induced hypersensitive state by changing spinal transduction of non-noxious information.

339.5

EFFECTS OF CALCIUM CHANNEL BLOCKER ON PROSTAGLANDIN $F_{2\alpha}$-INDUCED LONG LASTING ALLODYNIA .
K.Hara, Y.Saito, Y.Kirihara, N.Imamachi, Y.Yamada, Y.Yamamori* and Y.Kosaka.
Dept. of Anesthesiol., Shimane Med.Univ., Izumo, Shimane 693 Japan

We have reported that intrathecal (i.t.) prostaglandins (PGs) induce long lasting allodynia (Neurosci. Abst., 1995; Pain, 1996). The present study examined the effect of calcium channel blocker, diltiazem on the $PGF_{2\alpha}$-induced long lasting allodynia.

Male Sprague-Dawley rats weighing 270-350 g were used. The response of the animals to non-noxious mechanical stimulus produced by Semmes-Weinstein monofilaments (SWM) was graded from 0 (=no response) to 3 (=vigorous escape and frequent vocalization). Each SWM was applied at 3 different sites (neck, flank and hip) on the right and left side of the body. Agitation score (AS) was calculated as sum of the scores in six sites. Following the determination of baseline score, diltiazem 300 - 1000 μg or normal saline was intrathecally administered 20 min before (pretreatment) or 30 min after (posttreatment) i.t. injection of 100 ng $PGF_{2\alpha}$. Measurements were repeated for 7 days.

Posttreatment and pretreatment with saline had no effect on the increase of ASs induced by $PGF_{2\alpha}$. Posttreatment with diltiazem 1000 μg temporarily reduced ASs by 36% for 10 min and then ASs increased to the same level of posttreatment with saline. From 1 to 7 days after injection, ASs of posttreatment with diltiazem was lower than that of saline. Intrathecal administration of $PGF_{2\alpha}$ following pretreatment with diltiazem 1000 μg did not produce any increases of ASs for 7 days. These results suggest that L-type calcium channel may participate in the initiation and sustaining of $PGF_{2\alpha}$-induced allodynia in the spinal cord.

339.6

INTRATHECAL PROSTAGLANDIN $F_{2\alpha}$ EVOKES FOS EXPRESSION IN THE RAT SPINAL DORSAL HORN .
Y.Saito*[1], Y.Kirihara[1], K.Hara[1], T.Gonda[2], H.Miyamoto[1] and Y.Kosaka[1]. 1)Dept. of Anesthesiol., 2)Inst. of Exp. Animals, Shimane Med.Univ., Izumo, Shimane 693 Japan

Intrathecal (i.t.) prostaglandins (PGs) initiate long lasting allodynic state (Neurosci. Abst., 1995; Pain, 1996). To investigate the neuroanatomical aspects of this phenomenon, we examined expression of c-Fos-like immunoreactivity (FLI) in the spinal neuron.

Seven days after implantation of i.t. catheter, male Sprague-Dawley rats were i.t. injected with 100 ng of $PGF_{2\alpha}$ (n=5) or saline (n=4). Agitation scores (ASs) to non-noxious mechanical stimuli evaluated for 3hrs, and then rats were anesthetized and perfused with fixative. Transverse 40 μm frozen sections of the lumbar spinal cord processed for FLI standard immunohistochemical method.

I.t. administration of $PGF_{2\alpha}$ significantly increased ASs comparing the baseline values, while i.t. saline injection did not produce any increases of ASs. The FLI was significantly elevated after i.t. $PGF_{2\alpha}$ injection, although little expression of FLI was shown after i.t. saline injection. The mean number of FLI expressed neurons following $PGF_{2\alpha}$ and saline were 22.3 ± 9.7 (SD) and 7.4 ± 3.2 in lamina I-II, 10.4 ± 6.5 and 2.6 ± 0.7 in III-IV, and 11.3 ± 4.3 and 3.8 ± 1.9 in V-VI, respectively. The number in lamina VII-X following $PGF_{2\alpha}$ injection did not increase.

These results indicate that spinal dorsal horn neuron is capable of expressing FLI following $PGF_{2\alpha}$ treatment, suggesting the alteration of the ability in response to a non-noxious stimulus.

339.7

PLASTICITY IN THE DORSAL HORN FOLLOWING NEUROPATHIC INJURY AND INFLAMMATION. *Jonathan Goff** and *Luc Jasmin*. Neurosurgery, Georgetown University, Washington DC.

There is evidence to suggest that the development of chronic pain is related to plasticity in the central nervous system. We report time-related neural changes in the lumbar spinal cord of the rat in three models of chronic pain and a close correlation with the hyperalgesic behavior quantified in these animals. The neural markers studied were the NK-1 and μ-opioid receptors and constitutional Nitric Oxide Synthase (cNOS) labeled with immunocytochemistry. The Chronic Constriction Injury (CCI) was used as a model of neuropathic pain and a plantar injection of 200 μl of Complete Freund's Adjuvant (CFA) (n=12) was used as a model of inflammatory pain. The animals were divided into four survival times: 4, 7, 14, and 28 days. Behavior was monitored using the thermal withdrawal test for hyperalgesia and von Frey hairs for mechanical allodynia, at 4, 7, 14, and 28 days. Immunolabeling was quantified using NIH "Image" software. The results show that CCI leads to an hyperalgesic hindpaw, which is paralleled by a marked ipsilateral increase in NK1 receptor density, in L1 to L6, that peaks at 4 days and persists at 28 days. A moderate increase in ipsilateral μ-opioid receptor density was found in this model at short survival at L4-L5 while a large decrease in ipsilateral cNOS labeled neurons was observed at L4-L5 throughout the 28 day survival. The CFA model also leads to local hyperalgesia which is observed at 4 and 7 days but not at 14 days and beyond. NK1 receptor density increases were most pronounced at early survival times. μ-opioid receptor labeling also showed an increase at L4-L5 at early survival times. cNOS reactivity did not change significantly throughout the survival time of the CFA model. We suggest that there is a correlation between the development of chronic pain and neuronal plasticity in the central nervous system. Furthermore, the type of peripheral injury will determine the central changes. While NK1 and μ-opioid receptor density levels increase in both neuropathic and inflammatory pain models, cNOS decreases after nerve injury and is unaffected by peripheral inflammation. NIH RO1 DK47523-01; MRC (Canada).

339.8

INHIBITORY SYNAPTIC TRANSMISSION IN THE SUBSTANTIA GELATINOSA AND ITS MODULATION BY μ-OPIOID AND $GABA_B$ RECEPTOR ACTIVATION. T. J. Grudt* and G. Henderson. Department of Pharmacology, University of Bristol, Bristol BS8 1TD, UK.

The substantia gelatinosa (SG) is the primary site of termination of sensory neurons activated by painful stimuli. Most of the neurons in the SG are interneurons thought to be involved in the processing and modulation of the afferent signal and many contain the inhibitory transmitters GABA or glycine. In order to examine inhibitory transmission in the SG, whole-cell recordings were made from SG neurons in horizontal slices (275 μm) of the spinal trigeminal nucleus at 30 °C. In the presence of the excitatory amino acid receptor antagonists CNQX (10 μM) and D-APV (30 μM) and the $GABA_A$ receptor antagonist bicuculline (10 μM), stimulation within the SG evoked IPSCs sensitive to the glycine receptor antagonist strychnine (1 μM) in 20 of 20 neurons. In CNQX, D-APV and strychnine, IPSCs sensitive to bicuculline were evoked in 30 of 32 neurons. In some neurons, spontaneous glycine or $GABA_A$ IPSCs (sIPSCs) were present in TTX (0.5 μM). The decay of the glycine sIPSCs and some of the $GABA_A$ sIPSCs were well-fit by one exponential whereas the decay of other $GABA_A$ sIPSCs required two exponentials. Application of the μ-opioid agonist DAMGO (1 μM) caused a reduction in the amplitude of evoked glycine IPSCs in 8 of 8 neurons to 51±10% of control but reduced the amplitude of evoked $GABA_A$ IPSCs in only 4 of 8 neurons, to 55±4.8% of control in the neurons affected. By comparison, the $GABA_B$ receptor agonist baclofen (30 μM) reduced the amplitude of the IPSC in all neurons tested, to 5.0±1.4% of control for glycine (n=5) and 11±2.6% of control for $GABA_A$ (n=6). Inhibition of the evoked IPSCs was accompanied by an increase in failures of transmission, indicating that at least part of the inhibition was presynaptic. These results indicate that inhibitory synaptic transmission mediated by glycine and GABA acting at $GABA_A$ receptors is present in most if not all SG neurons. This work was supported by NIH grant DA05534-0182.

339.9

DIRECT APPOSITION OF SUBSTANCE P AND NK-1 RECEPTOR IMMUNOREACTIVITIES IN THE RAT SPINAL DORSAL HORN: AN ULTRASTRUCTURAL DOUBLE-LABELLING STUDY. A.L. McLeod*[1], V.V. Karpitskiy[2], J.E. Krause[2] and A. Ribeiro-da-Silva[1]. [1]Dept. of Pharmacology & Therapeutics, McGill University, Montréal, Québec, Canada H3G 1Y6, and [2]Dept of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 633110.

Previous studies showed a conspicuous absence of NK-1 immunoreactivity in lamina II, although this area is known to receive numerous substance P (SP)-containing sensory fibres. The clarification of this apparent mismatch between the distribution of SP and its receptor prompted the present investigation. Adult male Wistar rats were anaesthetized and fixed by vascular perfusion with an aldehyde mixture. Segments C3-C5 of the spinal cord were collected and processed for immunocytochemistry at the light and ultrastructural levels. A polyclonal antibody generated against the rat NK-1 receptor was applied and developed using an ABC protocol. SP immunoreactivity was demonstrated using a bi-specific anti-SP/anti-HRP antibody (for light microscopy), and an anti-SP monoclonal antibody applied to ultrathin sections in a post-embedding immunogold protocol (for electron microscopy). NK-1 receptor immunoreactivity was intense in both lamina I and outer lamina II, and occurred in dendrites and cell bodies. In inner lamina II, NK-1 receptor immunoreactivity was still considerable, although the number of immunoreactive (IR) structures was less. In contrast, SP immunoreactivity was intense in the outer two thirds of lamina II. SP-IR boutons were frequently presynaptic to NK-1-IR dendrites and perikarya. In the middle third of lamina II, NK-1-IR dendrites were frequently post-synaptic to the central boutons of synaptic glomeruli, as well as to SP-IR boutons. In contrast with some other studies, the above results indicate a good correlation between SP-IR boutons and NK-1-IR postsynaptic targets, and give support to the concept that SP acts at a very distance from its release site.

(Research funded by the Canadian MRC and the NIH)

339.10

PHARMACOLOGY OF METABOTROPIC GLUTAMATE RECEPTOR -MEDIATED MODULATION OF MEMBRANE PROPERTIES IN RAT DEEP DORSAL HORN NEURONS. V. Morisset, J.-C. Gauffre, M. Allard and F. Nagy*. INSERM U378, Univ. Bordeaux II, 33076 Bordeaux Cedex France.

Activation of mGluRs with 1S, 3R - ACPD induces or enhances voltage-dependent plateau potentials in about 26% of lamina V dorsal horn neurons (DHNs), in the cervical region of the rat spinal cord (Morisset and Nagy, Neurosci. Abstr., **21**, 377, 1995). This promotes expression of long-lasting after-discharge, bistability, and windup of action potentials when neurons are stimulated by intracellular injections of current. We have investigated the pharmacology of the mGluRs involved in this modulation in a transverse spinal cord slice preparation (cervical region) of 18- to 22-day old rats.

The plateau potential modulated by 1S, 3R - ACPD was maintained in the presence of TTX, and blocked by nifedipine-containing medium, indicating that L-type calcium channels were involved. When ionotropic glutamate receptors (AMPA, NMDA) were blocked by a mixture of CNQX and AP-5, quisqualate was 5 to 10 times more potent than 1S, 3R - ACPD in promoting plateau properties of lamina V DHNs. The effects of both agonists were reversed by the mGluRs antagonist MCPG. They were not reproduced by L-CCG-1 and L-AP4, the more specific agonists at group II and group III mGluRs, respectively, indicating the implication of the group I receptors mGluR1 or mGluR5. Because these receptors are known to be coupled to phospholipase C, we measured the effect of 1S, 3R - ACPD on phosphoinositide hydrolysis in a crude synaptosome preparation from 20-day-old rat spinal cord. Increasing concentrations of 1S, 3R - ACPD (1 μM to 1 mM) stimulated IP formation in a dose-dependant manner yielding to an EC_{50} value of about 300 μM. This stimulating effect was inhibited by 100 μM MCPG. Taken altogether, the results suggest the implication of mGluR1 or mGluR5 in the modulation of plateau properties in deep DHNs.

Supported by grants from: DRET *ERS 94-1055*, and Conseil Régional d'Aquitaine *940301212*.

339.11

WDR NEURONAL POPULATION BEHAVIOUR DURING NOXIOUS AND NON-NOXIOUS STIMULI. AN INFORMATION WEIGHING AND NETWORK CODING STUDY WITH MULTIPLE SIMULTANEOUS RECORDINGS. G.E.Biella*°§, P.Gurzi°, F.Gianinetti° and M.L.Sotgiu° °Istituto di Neuroscienze e Bioimmagini, CNR; § Cattedra VI Neurologia, Universita' di Milano; Via Mario Bianco, 9; 20131 Milan, ITALY.

Noxious and non-noxious signals are carried by different diameter peripheral fibers and specifically noxious signals are diversely carried to the central structures by distinct spectra of slow conducting fibers. Differences in conduction and laminar arrangement lead to different design of postsynaptic activation in the spinal cord. We investigated if this structural configuration subserves a variable recruitment of cell populations depending on the incoming stimuli and induces diverse connectivity properties relying on the stimulus features.

Multiple simultaneous recordings from Wide Dynamic Range (WDR) neurons have been performed in the deep dorsal laminae of the spinal cord of anesthetized and paralysed rats. The responses to mechanical, thermal and electrical noxious and non-noxious stimuli have been selected. The data have been analysed by cross-correlographic techniques and by information theory starting from Karhunen-Loève tranformations with probability density function estimates (Parzen method). Finally information amount processing by stochastic integration techniques has been performed.

The overall information weighing has shown differences both in the correlographic profiles and in the information estimate in the responses to different stimulations. Noxious stimuli induce a differently timed connectivity among WDR and diverse information weighing. These results demonstrate some discriminative properties of local spinal circuits depending on stimulus modalities. *Funds by National Research Council..*

339.12

ALTERATION OF FIRING PATTERNS AND ACTIVATION OF SLOW POTENTIALS IN DEEP DORSAL HORN INTERNEURONS BY DIRECT STIMULATION. S.P. Schneider*. Department of Anatomy, Michigan State University, E. Lansing, MI 48824-1316.

To gain insight into dynamics of local integrative function in the deep dorsal horn (laminae III-VI), activity-dependence of neuronal firing patterns was examined using blind whole-cell recordings from hamster spinal slices.

Injection of suprathreshold depolarizing current pulses through the recording pipette (20-30 pulses, 500-700ms, 0.2-0.5Hz) induced changes in spike firing, accompanied by shifts in V_m, that were dependent on stimulus rate and intensity (39/56; 70% of cells). During repeated activation resting V_m hyperpolarized (2-5mV), spike firing decreased and/or frequency adaptation increased (16/39; 41%). These effects reversed within 30-120s after stimulation was terminated. The hyperpolarization and altered spike firing were associated with decreased R_N and were not blocked by application of Cd^{2+}. Returning V_m to control levels by passing depolarizing current also did not reverse decreased spike firing. In other cells (23/39; 59%) repetitive activation or single 5s injections of suprathreshold current induced depolarizing shifts in V_m (2-6mV), increased R_N, and increased spike firing to depolarizing test pulses. These were prolonged effects, lasting up to 15 min after cessation of stimuli, and were not associated with changes in synaptic activity. Increased firing could be reversed by hyperpolarizing V_m to control levels but was not blocked by application of D-AP5 or CNQX. Intracellular staining revealed that neurons exhibiting firing pattern plasticity had longitudinally oriented axons with dense terminations in laminae III-V.

The findings demonstrate that firing patterns of many laminae III-V interneurons are capable of considerable plasticity, which may be determined by expression of multiple intrinsic (non-synaptic) mechanisms. Changes in interneuron excitability could alter integrative properties of local networks, contributing to sensory habituation and secondary hyperalgesia. *Supported by NINDS grant NS25771.*

SENSORY SYSTEMS: SPINAL CORD II

340.1

FUNCTIONAL ORGANIZATION OF NEURONAL ENSEMBLES ASSOCIATED WITH SPONTANEOUS CORD DORSUM POTENTIALS IN THE CAT SPINAL CORD. E. Manjarrez, I. Jiménez and P. Rudomin* Dept. of Physiol., Biophys. and Neurosc. CINVESTAV, IPN and IF-BUAP, MEXICO.

We have examined the interaction between the spontaneous cord dorsum potentials (SCDP's) and selected groups of interneurons responding to synaptic inputs from cutaneous and/or muscle afferents. The experiments were performed in 11 pentobarbital anesthetized and unparalyzed cats. SCDP's, dorsal root potentials (DRP's), and spinal extracellular field potentials (EFP's) were recorded by means of conventional methods. Laminar and segmental analysis performed in 6 experiments indicates that the neuronal ensembles involved in the generation of the SCDP's are located in the dorsal regions of the spinal L6-S1 segments, where the cutaneous afferents also terminate (1000 to 1900µm depth). The DRP's as well as the EFP's evoked in the dorsal horn by stimulation of low threshold cutaneous afferents (1.2 to 1.7xT) were facilitated when preceded by the largest SCDP's (selected using a window discriminator). The time course of this facilitation resembled that of the averaged SCDP's and lasted about 50 ms. The DRP's and the EFP's recorded in the intermediate nucleus by stimulation of group Ib fibers (unlike those elicited by group Ia fibers) were also facilitated when conditioned with SCDP's. Single dorsal horn interneurons responding monosinaptically to low threshold cutaneous fibers could be also synchronously activated during the occurrence of SCDP's, suggesting that they contribute, at least in part, to the generation of the SCDP's. Fractal analysis (N=3) of SCDP's has further indicated that the complexity of the organization of the elements associated with the generation of these potentials is increased after spinalization (D=1.5±0.18 to D=2.2±0.16) and is dynamically modulated (but not generated) by descending influences. The experimental paradigm used in this study may provide a novel approach to disclose the functional organization of distributed spinal neuronal ensembles. *Supported by NIH 09196, CONACyT 031N9107 and SNI, México.*

340.2

RATE OF RISE OF CUMULATIVE DEPOLARIZATION REPRESENTS NMDA RECEPTOR INDEPENDENT SUMMATION OF SLOW SYNAPTIC POTENTIALS. G.Baranauskas and A.Nistri, Biophys. Lab, Int Sch Adv Studies (SISSA), 34013 Trieste, Italy.

Action potential windup (an increase in number of spikes after each constant stimulus applied at 0.5-2 Hz frequency) is one of the manifestations of pain induced short term plasticity in the spinal cord. Intracellular recording from neonatal rat spinal neurones in vitro demonstrated that only the rate of rise (RR) of cumulative depolarization (CD) during the train can be correlated with of action potential windup. Several mechanisms for CD were suggested: relief of NMDA channels from Mg block; activation of voltage dependent slow Ca channels; summation of slow tachykinin mediated synaptic potentials. In the present study these issues were examined with intracellular recordings from neonatal rat spinal cord motoneurones in vitro. 19 motoneurones had -71±2 mV E(rest) and RR ranging from 0.1 mV/s to 0.67 mV/s (in 10/19 cells action potential windup could be evoked). RR voltage dependence as well as its amplitude persisted in the presence of NMDA receptor antagonists (25 µM of APV or 20 µM of CPP) even though the peak of CD was reduced by 37 ± 5% (n=6). In 3/6 cells the action potential windup still could be observed in the presence of NMDA receptor antagonists. No correlation was found between RR and CD or single EPSP area indicating that intrinsic voltage dependent conductances cannot explain these phenomena. A late component (at 500-1000 ms after the stimulsu) of the slow EPSP in the presence of NMDA receptor antagonists had the same voltage dependence as RR and was linearly related with the size of RR (r**2=0.75, n=11). The tachykinin NK1 receptor antagonist SR140333 reduced RR by a constant fraction at various membrane potentials. All this data are consistent with the hypothesis of summation of slow EPSP partly mediated by NK1 receptor s. Supported by INFM.

340.3

PROPRIOSPINAL AFFERENT AND EFFERENT CONNECTIONS OF THE LATERAL AND MEDIAL SUBDIVISIONS OF THE DORSAL HORN (LAMINAE I-IV) IN THE RAT LUMBAR SPINAL CORD M. Antal* and M Petkó. Dept. of Anat., Histol. and Embryol., Univ. Med. School, Debrecen, Hungary, H-4012.

It has been noted by several authors that the medial and lateral subdivisions of the superficial dorsal horn of the spinal cord show many distinct features concerning primary afferent inputs, synaptic and neurochemical properties of interneurons as well as numbers of neurons with axons projecting to supraspinal brain centres. Here we demonstrate that, in addition to these disparities, the medial and lateral subdivisions of laminae I-IV of the spinal dorsal horn appear to be different also on the basis of their propriospinal afferent and efferent connections. After injecting *Phaseolus vulgaris* leucoagglutinin and biotinylated dextran into various areas of the dorsal horn (laminae I-IV) at the level of the lumbar spinal cord, we found that a number of propriospinal fibres travel one-two segments rostrally or caudally and terminate in areas of the dorsal horn that are identical to that in which their cells of origin are located, forming rostro-caudally oriented cellular laminae in which the neurons are highly interconnected. These cellular laminae both in the medial and lateral subdivisions of the superficial dorsal horn receive strong inputs from laminae V-VI. It has also been revealed that the medial subdivision of laminae I-IV send an abundant projection to the lateral subdivision, but terminals were only occasionally found in the medial dorsal horn after injecting the tracers into the lateral part of laminae I-IV. We have revealed a strong reciprocal commissural projection between the lateral subdivisions of laminae I-IV on the two sides of the spinal cord. The lateral part of laminae I-IV gave rise also to many fibres that terminated in the ventral horn at the segmental level or ascended in the lateral white matter. Injecting the tracers into the medial dorsal horn, however, no commissural fibres were revealed, and terminals in the ventral horn and ascending fibres in the white matter were found only in limited numbers. The findings indicate that the medial and lateral subdivisions of the superficial spinal dorsal horn of rats may play different roles in sensory information processing.

(Supported by the Howard Hughes Med. Inst. and the Hungarian Natl. Sci. Fund)

340.4

Presynaptic inhibition along the disynaptic reciprocal Ia inhibitory pathway in the cat. M. Enríquez*, J. Nielsen, H. Morita, N. Petersen, and H. Hultborn. Department of Medical Physiology, The Panum Institute. DK-2200, Denmark; and Department of Physiology, Christian-Albrechts-University, Kiel, D-24098, Germany.

Eccles et al (J.Neurophysiol. 26:506. 1963) reported that disynaptic Ia-IPSPs are depressed for about 100 ms following conditioning stimulation of group I afferents from flexor nerves. This long lasting depression was explained as a result of presynaptic inhibitory actions on the terminals of Ia afferents on the Ia-inhibitory interneurones, although the possibility of a presynaptic action onto the terminals of these interneurones was not excluded. Actually, evidence in favor of presynaptic inhibitory actions at the terminals of spinal cord interneurones has been presented (Alford et al, Eur.J. Neuroscience. 3:107. 1991; Aggelopoulos et al, J.Physiol. 487.P:71P. 1995). The present study was undertaken to investigate this possibility in the well studied Ia-inhibitory pathway (Jankowska & Roberts, J.Physiol. 222:597. 1972). In five cats deeply anaesthetized with α-chloralose and pentobarbital, we studied the reduction of the monosynaptic Ia-EPSPs during intracellular recording from Ia-inhibitory interneurones. In two units, conditioning stimulation of deep Peroneal, Sartorius or Quadriceps nerves (1.6-2 T) depressed the Ia-EPSP. In support of the notion that this depression was presynaptic in origin, we found that monosynaptic EPSP from descending fibers in the ventral funiculus was *not* depressed. These data suggest that the tested pathways projecting to these interneurones are subjected to similar patterns of presynaptic inhibition as shown for the terminals projecting to α-motoneurones. We also analyzed the excitability changes of the terminals of individual Ia-inhibitory interneurones following activation of presumed presynaptic inhibitory pathways. Conditioning stimulation of flexor and extensor nerves (1.3-10 T) increased the excitability of the terminals in 7 out of 8 interneurones, that lasted around 200 ms. Finally conditioning activation of cutaneous nerves (1.6-5 T) induced an increase in excitability in 6 out of 8 units. It shall be noted that this pattern of origin is different to that described for Ia afferent fibers. Because we had not observed long lasting postsynaptic actions during intracellular recordings from Ia-inhibitory interneurones, the observed increase in the excitability of their terminals may thus be caused by a depolarization in the terminals themselves, rather than by a somatic depolarization spreading electrotonically to the terminals. If the observed increase in excitability is indeed related to presynaptic inhibition, the depression in transmission of Ia inhibition is explained by both presynaptic inhibition in the terminals of Ia fibers *and* in the terminals of the interneurones at their target motor nuclei. Funded by Danish Medical Research Council.

340.5

INDUCIBLE NITRIC OXIDE SYNTHASE mRNA EXPRESSION IN RAT DORSAL ROOT GANGLIA IS INCREASED BY INTRATHECAL BUT NOT INTRAPLANTAR INJECTION OF SALINE. Y. Cai*, R. A. Velazquez and A. A. Larson. Dept. of Veterinary PathoBiology, University of Minnesota, St. Paul, MN 55108.

Reverse transcription-polymerase chain reaction (RT-PCR) was used to measure inducible nitric oxide synthase (iNOS) mRNA expression in dorsal root ganglia (DRG) of adult male Sprague-Dawley rats. DRG were sampled separately from thoracic and lumbar regions at 6 or 24 hr after intraperitoneal (i.p.), intraplantar (i.pl.), intrathecal (i.t.) or no injection of saline. iNOS mRNA was not expressed in the DRG of non-injected control rats (n=4), in rats injected with 50 µl of saline i.pl. (n=4) or sham injected i.pl. (needle prick in one foot only, n=12). However, an i.p. injection (400 µl) of 10 mg/kg of lipopolysaccharide (LPS) greatly increased the expression of iNOS in the DRG, spinal cord, brain, liver, lung and spleen. When animals were acclimated to the laboratory for only 2 days (n=5), rather than 25.5 ± 3.5 days for those studies described above (n=20), expression of iNOS was strongly induced in DRG but not spinal tissue even in the absence of LPS. Rats also exhibited strong expression of iNOS mRNA in DRG, but not in spinal tissue, following i.t. injections of 10-20 µL of saline. No effects were found on expression of constitutive NOS mRNA in DRG or spinal cord following any treatment. Changes in iNOS were identical in thoracic and lumbar DRG. These results indicate that an i.t. injection or insufficient acclimatization is sufficient to induce iNOS mRNA in DRG. These data suggest a possible mechanism by which stress may affect sensory input. [USPHS grant NIDA04090 to A.A.L.]

340.6

ADENOVIRAL TRANSFER OF *LacZ* TO SPINAL CORD NEURONS. A.J. Mannes*, R.M. Caudle,B. O'Connell, D.J. Kim, M.J. Iadarola. NAB/NIDR/NIH Bethesda, MD 20892.

The spinal cord is an attractive and accessible target for gene therapy. The present study examines the ability of replication deficient adenovirus to transfer a test gene containing the cytomegalovirus promoter driving the expression of the *LacZ* reporter gene (Ad.CMVβgal). Sprague Dawley rats were anesthetized, the spinal cord exposed at C1 and a microinjection of Ad.CMVβgal (5 µl of 1 $X10^{10}$ pfu/ml infused at 1 µl/min.) was made stereotaxically 0.5 mm lateral to the midline and 3 mm below the pial surface. Rats (3/time point) were sacrificed at 1, 3, 7, and 14 days post- infusion and extracts of spinal cord (divided into 1 cm sections from the injection site), pons-medulla, midbrain, hypothalamus and thalamus were assayed for β-gal activity fluorometrically (4-Methylumbelliferyl β-D-Galactoside). Significant β-gal activity was detected at 1 day (2699 ± 739) which progressively increased to 7844 ± 529 by day 7 and remained elevated (6718 ± 3334) at 14 days. Activity at day 7 was elevated 50 fold over non-injected control tissue (137 ± 5). The spatial distribution of activity also increased: on day 7, activity was confined to the 1 cm section containing the injection site but by day 14, activity was detected up to 2 cm caudal to this section. No substantial activity was detected in the 4 brain regions rostral to the injection site. The tip of the cannula was located in the anterior horn. Histochemical staining with x-gal revealed reaction product in the motor neurons and neurons in lamina 6 and 7 bilaterally; no staining was seen in the control tissue. Parallel results were obtained using β-gal immunocytochemistry.

This study demonstrates Ad.CMVβgal injected into the ventral spinal cord results in localized, high levels of β-gal activity which reached a plateau by 7 days and was sustained at 14 days. We are currently investigating microinjections into the spinal cord dorsal horn to target sensory neurons. Funded by: IRP/NIDR/NIH

340.7

DEAFFERENTATION-INDUCED ALTERATIONS OF RECEPTOR DENSITY IN THE DORAL HORN C.C. LaMotte[1]*, K.E. Arsenault [1] M. A. Wolfe [1], M. E. Helgren[2], and S.E. Kapadia[1]. [1]Section of Neurological Surgery, Yale University School of Medicine, New Haven, CT. 06520; [2]Dept. of Physical Therapy, Quinnipiac College, Hamden CT. 06518

Past studies in this laboratory have demonstrated the selective destruction of sciatic primary afferents in the dorsal horn following injection of the rat sciatic nerve with pronase enzymes; subsequently, saphenous afferents sprout into the deafferented territory. Accompanying these changes are a decrease of Substance P (SP) and Calcitonin-gene related peptide (CGRP) immunoreactivity in the dorsal horn and a later partial return toward normal levels. In the present study, we examined changes in density of receptor binding or receptors over a similar time course. SP and CGRP receptor binding was determined with receptor autoradiography and non-NMDA receptor (Glu R1 and Glu R2/3) densities were determined with immunocytochemistry (Chemicon). Computer image analysis of density in the superficial dorsal horn was compared between the pronase treated side and the contralateral, non-treated side. Ten days following pronase injection, there was an increase in SP, CGRP, and GluR2/3 receptors, and a decrease in GluR1 receptors. By 3 months, all receptors were approaching normal. (NIH grants NS10174 and NS13335).

340.8

EFFECTS OF SPINAL CORD TRANSECTION ON NEUROPEPTIDE FF AND OPIOID RECEPTORS: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. Ch. Gouradères, C. Advokat*, and J-M Zajac. Institut de Parmacologie et de Biologie Structurale, CNRS, 205 Route de Narbonne, 31077 Toulouse-Cédex, France.*Dept of Psychology, 236 Audubon Hall, Louisiana State University, Baton Rouge, LA 70803, USA.

Neuropeptide FF (NPFF) acts as a modulator of opioid functions. In particular, spinal administration of NPFF in the rat produces i) antinociception decreased by opioid antagonists; ii) enhancement and prolongation of spinal opioid analgesia. NPFF binds with high affinity to specific receptors distinct from opioid receptors a part of which are carried on primary afferent fibers. In order to reveal the presence of NPFF and opioid receptors on descending fibers, we measured their densities in rats submitted 14 days before to a complete mid-thoracic (T6) spinal transection. The binding was estimated in each side of the injury site in the superficial layers of the dorsal horn by quantitative autoradiography using highly selective iodinated ligands, [D.Tyr^1,(NMe)PHe^3]NPFF, FK33824 and [D.Ala^2] deltorphin-I to label NPFF, µ and δ sites, respectively. Analysis of the comparative distribution of NPFF and opioid binding sites in the sham operated and transectioned rats showed that the lesion did not induce significant changes in labelling except a 25% decrease in the density of δ sites, caudal-proximal to the injury. These results suggest that NPFF and opioid receptors not associated with primary afferent fibers are not located on descending fibers, but present on spinal neurons. Supported by PHS 02845.

340.9

EFFECT OF BICUCULLINE ON HALOTHANE-INDUCED REDUCTIONS OF RAT DORSAL HORN LOW-THRESHOLD RECEPTIVE FIELDS.

H. Iwakura*, T. Tsukamoto, S. G. Shimada, J.G. Collins. Department of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510

We have identified a series of effects of anesthetics on spinal dorsal horn neurons, including reduction of low-threshold receptive field (RF) size and are now using those effects as tools to examine the pharmacology responsible for such changes. We hypothesize that, rather than acting by a unitary mechanism, anesthetics, in an agent-specific way, modulate sensory transmission by influencing the actions of neurotransmitter systems. This is part of a series of ongoing studies designed to examine the role that the inhibitory neurotransmitter GABA may play in anesthetic-induced reduction of low-threshold receptive field RF size.

Extracellular activity of single spinal dorsal horn neurons was recorded in halothane-anesthetized, spinally-transected rats. After mapping the low-threshold RF in the presence of 1.1% (1 MAC) and 1.6% (1.5 MAC) halothane, animals received two injections of 0.5 mg/kg bicuculline intravenously to a cumulative dose of 1.0 mg/kg. Following each bicuculline injection, we reevaluated RF size.

To date, 7 neurons have been examined. Increasing the depth of halothane anesthesia produced a significant reduction in RF size. The administration of bicuculline induced a partial but not complete, significant reversal of the halothane-induced reduction of the low-threshold RF size.

As we have seen with other inhalation anesthetics and the GABA antagonist picrotoxin, GABA appears to play only a partial role in halothane reduction of RF size. These data support site- and drug-specific actions, rather than a unitary mechanism of action for general anesthetics. Supported, in part, by NIH GM 44954

340.10

RAT THORACIC SPINAL CORD CONTAINS M2, M3, AND M4, BUT NOT M1, MUSCARINIC RECEPTOR (mAChR) SUBTYPES. A. U. Höglund* and H. A. Baghdoyan. Department of Anesthesia, The Pennsylvania State University, College of Medicine, Hershey, PA 17033.

Intrathecal administration of cholinomimetics produces antinociception *(Anesthesiol. 80:1338, 1994)* and increased blood pressure *(Brain Res. Bull. 27:47, 1991)*. These responses have been proposed to be mediated, in part, by M2 *(J. Pharmacol. Exp. Ther. 270:1301, 1994)* and by M1 and/or M2 mAChRs *(J. Pharmacol. Exp. Ther. 266:329, 1993)*. These functional studies led us to test the hypothesis that spinal cord contains multiple mAChR subtypes. *In vitro* autoradiography performed on 5 thoracic spinal cords revealed: no M1 receptors; M2 receptors in Rexed laminae I-III (20-30 fmol/mg), IV, VI, VII, IX, and area X; M3 receptors in laminae I-III, with the highest densities in laminae I/II (20-25 fmol/mg). Saturation binding assays using [^{3}H]-pirenzepine (PZ) in spinal cord homogenates confirmed the absence of M1 mAChRs. Competition of [^{3}H]-N-methyl scopolamine binding with PZ yielded a competition curve with a Hill coefficient (n_H) of 0.95±0.01 and a K_i=164.5±30.5 nM, indicating the presence of M3 mAChRs. Competition with methoctramine (METH) resulted in a curve (n_H= 0.99±0.02) with a K_i=24.5±1.5nM, indicating the presence of M2 mAChRs. AF-DX 116 and METH + atropine showed shallow competition curves with n_H=0.76±0.05 and n_H=0.61±0.08, respectively. The fitted K_i constants indicated that AF-DX 116 recognized M2 and M4 mAChRs, and that METH + atropine recognized M3 and M4 receptors. Localization of M2 and M3 mAChRs to laminae I-III is consistent with the possibility that these mAChR subtypes may modulate antinociception. The laminar distribution of M4 mAChRs remains to be identified.
Support: MH-45321 (HAB), HL-47749 (RL), B96-99Z-11159-02 (AUH), Departments of Anesthesia and Comparative Medicine.

PAIN PATHWAYS: SPINAL CORD AND BRAINSTEM

341.1

IN VIVO, WHOLE-CELL RECORDINGS FROM SPINAL SUBSTANTIA GELATINOSA (LAMINA II) NEURONS OF ADULT RATS. A. R. Light*, and H. H. Willcockson. Department of Physiology, University of North Carolina, Chapel Hill, NC 27599-7545

We developed a procedure for *in vivo*, whole-cell recording in adult rats to compare to *in vitro* techniques (Schneider and Light, Soc. Neurosci. Abstr. 20: 1730, 1994). Rats weighing ~250 g were anesthetized with sodium pentobarbital and chloral hydrate, respired, paralyzed with pancuronium bromide and suspended in a spinal frame. Following stabilization and lumbar laminectomy, recordings were made with patch-clamp electrodes (5-8 MΩ, K-gluconate internal solution with ATP, GTP, and biocytin), using an Axo-Clamp in the bridge balance mode. Standard cell attached recordings with initial impedances >1 GΩ, were reduced with gentle suction and rupturing of the membrane to 200-500 MΩ. Thirty neurons were recorded (receptive fields: lateral leg, and hip): 12 slow brush, 11 nociceptive, 2 cooling, and 5 without receptive fields. These cell types are similar to those found in previous conventional recordings in cats and monkeys. Resting membrane potentials were -45 to -65 mV. Unitary postsynaptic potentials were observed with amplitudes <10 mV. Summed evoked EPSP's were often as large as 30 mV leading to 30-80 mV action potentials. Labeling three neurons with biocytin confirmed recording locations in the marginal zone (lamina I) and substantia gelatinosa. *Supported by NINDS grants NS16433, NIDA DA04420 (A.R.L.) and P01NS14899 (Edward R. Perl, PI).*

341.2

PHOSPHORYLATION OF CREB IN THE RAT SPINAL CORD FOLLOWING FORMALIN STIMULATION. R. R. Ji, D. Ginty and F. Rupp*. Dept. of Neurosci., Sch. of Med., Johns Hopkins Univ., Baltimore, MD 21205

Phosphorylation of the transcriptional factor CREB at serine 133 following tissue injury induced-inflammation and hyperalgesia has been examined. Unphosphorylated CREB was present in most nuclei of spinal cord nurons and 10% DRG neurons. However phosphoCREB (pCREB) was only found in few nuclei in the normal spinal cord. Formalin injection into the left hindpaw induced a bilateral phosphorylation of CREB. Numerous pCREB-positive nuclei were found in laminae I, II, V and X of the spinal cord. The phosphorylation was very rapid and reached to the peak level in several min. The induction of pCREB was mainly postsynaptic, since only 5% DRG neurons with small size on both sides were labeled. In contrast c-Fos induction, appearing as early as 30 min after inflammation, was ipsilateral. The formalin evoked-pCREB and c-Fos in the spinal cord was distinctly suppressed by pretreatment of NMDA receptor antagonist MK-801 (i.p, 3.5 mg/kg) or by a brief halothane anesthesia. Our results suggest a) CREB phosphorylation, partially through an activation of NMDA recepor, may be involved in a long-term facilitation of the spinal cord neurons following tissue injury and hyperalgesia, b) CREB phosphorylation may be necessary but not sufficient for c-fos expression.

341.3

C-FIBER RESPONSES OF DORSAL HORN NEURONS ARE FACILITATED FOLLOWING STIMULATION OUTSIDE THEIR RECEPTIVE FIELD. J. Li*[1], D.A. Simone[1] and A.A. Larson[2]. Dept. of Psychiatry[1] and Vet. Pathobiology[2], Univ. of Minnesota, Minneapolis, MN 55455.

Hyperalgesia is mediated, in part, by enhanced excitability of dorsal horn neurons. "Wind-up" of dorsal horn neurons refers to the progressive potentiation of C-fiber response during trains of electrical stimulation and "wind-up" has been used as a model of synaptic plasticity. Increase in the number of responses of dorsal horn neurons have been demonstrated following injury to or inflammation of the receptive field. We investigated whether stimulation outside the receptive field facilitates responses to C-fiber input, as evident by facilitation of wind-up. Recordings were made from wide dynamic range neurons in the dorsal horn of anesthetized rats. Responses evoked by 12 trials of electrical stimuli at low frequency (0.5Hz) and constant intensity to the cutanous receptive field were summed. Responses of dorsal horn neurons were evaluated by monitoring C-fiber input, "wind-up", and the total number of spikes evoked by C-fiber activity in response to the 12 stimuli. We found that electrical stimulation outside the receptive field increased the total number of C-fiber spikes (146%) and caused an increase in C-fiber input (166%) evoked by electrical stimulation inside the receptive field. These data indicate that stimulation outside the receptive field may increase C-fiber responses to stimulation applied within the receptive field. Supported by NIH grants NS31223 and DA04090.

341.4

INTRADERMAL INJECTION OF CAPSAICIN (CAP) EVOKES SPINAL FOS-LABELING AND EXCITES DORSAL HORN NEURONS. E. R. Stevens, J. Li, D. A. Simone, and K. C. Kajander*. Depts. of Psychiatry, Oral Science, and Cell Biology & Neuroanatomy, and Grad. Prog. in Neuroscience, University of Minnesota, Minneapolis, MN 55455

In the spinal cord, immunocytochemical labeling for Fos-protein and electrophysiological responses increase after noxious stimulation, inflammation, or peripheral nerve injuries. We evaluated spinal Fos-labeling and electrophysiological responses of spinal neurons in rats after intradermal injection of CAP (1, 10, or 30 µg--Fos experiments; 10 µg--electrophysiological experiments). In the Fos experiments, one hour after injection into the plantar surface of the hindpaw, rats were deeply anesthetized and perfused transcardially with saline followed by 4% paraformaldehyde. Spinal segments L4-L5 were removed, post-fixed, and cryoprotected. Spinal sections were processed using a Fos-protein antibody (Cambridge Research Biochemicals). Sections were evaluated and drawn using light microscopy, and labeled nuclei were counted off the drawings. As compared to the contralateral side, greater Fos-labeling occurred in the spinal gray matter ipsilateral to the injections (ANOVA, $p<.01$). Also, labeling increased as CAP dose increased ($p<.01$). In electrophysiological experiments, CAP was injected into the receptive field of dorsal horn neurons characterized as responding to both innocuous and noxious stimulation (wide dynamic range neurons). Responses of these neurons upon CAP injection was vigorous and prolonged, usually lasting more than five minutes. These experiments demonstrate that intradermal injection of CAP excites rat dorsal horn neurons and increases spinal Fos-labeling. (Research supported by NIH grants NS29567 & NS31223)

341.5

REDISTRIBUTION OF CAPSAICIN FROM THE INJECTION SITE AND RESULTING EXCITATORY ACTIONS ON REMOTE SPINAL NEURONS, W.J. Roberts,* and R.C. Kramis. R.S. Dow Neurol. Sciences Institute, Legacy Health Systems, Portland OR 97209

Local injections of capsaicin (100 μg) are commonly used to excite primary afferent nociceptors and to change the excitability of nociceptors innervating the injection area. Studies with very large systemic doses of capsaicin (e.g 125 mg/kg) have demonstrated widespread depletion of neuropeptides in many afferent systems (Gamsel et al, 1981). In the present study we have found that injections of small amounts of capsaicin into the rat hindpaw (0.3-1.0 mg) result in widespread activation of dorsal horn neurons as evidenced by expression of c-*fos* in spinal segments anatomically distant from the injection site (e.g. cervical and contralateral lumbar segments). Transection of the nerve innervating the injection area reduces c-Fos immunoreactivity in ipsilateral lumbar segments but not that in cervical segments. These results suggest that capsaicin is redistributed via the circulatory system and that it subsequently induces transmitter release from nociceptors systemically, probably through actions on the central terminals or cell bodies of primary nociceptors. Redistribution of capsaicin beyond the injection site is likely to occur at all doses; the sensory consequences of the redistribution will depend on the resulting peripheral and central concentrations of the algogen.

Supported by NIH (NS32316).

341.6

OPIOID INHIBITION OF TRACT OF LISSAUER, BUT NOT DORSAL ROOT, STIMULATION-EVOKED FIELD POTENTIALS IN THE RAT SPINAL CORD SLICE PREPARATION. G.W. Terman*, and C. Chavkin^, Departments of Anesthesiology* and Pharmacology^, University of Washington, Seattle, WA. 98195.

In vitro slice electrophysiology methods are powerful tools in the study of pharmacological mechanisms in many CNS regions. Such methods allow administration of known drug doses to the studied synapses and facilitate mechanistic studies of physiological circuitry (including studies of pre vs post-synaptic effects, the importance of inhibitory interneurons and the specific ion channels activated). Slice studies in the spinal cord, particularly those involving presumed pain circuitry, have suffered from the inability to preferentially stimulate small fibers (i.e., those fibers most likely to carry nociceptive information) with spinal dorsal root electrical stimulation (DR). The present studies compare the properties of field potentials evoked by DR and Lissauer's tract stimulation (LT). Lissauer's tract is known to contain primary afferents likely to transmit nociception.

Transverse slices were made from the lumbar spinal cord of 14-21 day old rats. The slices were placed in a chamber and perfused with O_2-bubbled artificial CSF also containing bicuculline (10 μM) and strychnine (1μM). The dorsal root remnant and the tract of Lissauer were sequentially stimulated once every minute (at the S1/2 current intensity) and the resultant field potentials recorded in ipsilateral Lamina V with a 3M NaCl filled glass micropipette. DR produced a field potential with a minimum conduction velocity of 5m/sec, whereas the LT evoked field potentials had a 0.3m/sec minimum conduction velocity. Both stimulation sites produced field potentials greatly diminished by $MgCl_2$ (10mM)(n=4) or CNQX (10μM)(n=3). In contrast, only the LT evoked field potentials were significantly inhibited by the mu opioid receptor agonist DAMGO (1μM)(n=6). This inhibition was significantly reversed by naloxone (1 μM)(n=5). Supported by DA00266.

341.7

ULTRASTRUCTURAL COLOCALIZATION AND SYNAPTIC ASSOCIATION OF μ-OPIOID RECEPTORS AND SUBSTANCE P IN THE SPINAL DORSAL HORN. PY Cheng*[1], SA Aicher[1], S Sharma[1], LY Liu-Chen[2], VM Pickel[1]. [1]Dept of Neurology and Neuroscience, Cornell Univ Medical College, New York, NY, 10021. [2]Dept. of Pharmacology, Temple Univ School of Medicine, Philadelphia, PA, 19140.

The spinal analgesic effects of opiates acting at the mu opioid receptor (MOR) are thought to involve presynaptic inhibition of substance P (SP) release from primary afferents in the superficial laminae of the dorsal horn of the spinal cord. To determine if MOR has a cellular distribution that underlies this presynaptic action of opiates, we examined dual immunogold and immunoperoxidase labeling of SP and MOR in this region of the rat cervical spinal cord. By light microscopy, both MOR-like immunoreactivity (MOR-LI) and substance P-like immunoreactivity (SP-LI) were detected in varicose processes in lamina I and II of the spinal dorsal horn. Electron microscopic examination of these laminae showed that MOR-LI was localized mainly to the cytoplasmic surfaces of axons and terminals, as well as numerous dendrites. In contrast, SP-LI was mainly seen in dense core vesicles within unmyelinated axons and terminals. Dendrites more rarely contained detectable SP-LI. The MOR-LI was detected presynaptically in axon terminals containing SP and in dendrites receiving synaptic input from terminals containing the neuropeptide. These results suggest that the analgesic effects of opiates acting at MOR are attributable to both (1) decreased release of SP from dense core vesicles in primary afferents and (2) diminished postsynaptic responses to SP or coexisting neurotransmitters.

(Supported in part by an Aaron Diamond Foundation Postdoctoral Fellowship to PYC, NIDA DA04600 and HL18974 to VMP, and DA04745 to LYLC).

341.8

Substance P Containing Terminals Contact NMDA-R1 Immunoreactive Dendrites in the Dorsal Horn of the Rat Spinal Cord. V. M Pickel*, S. Sharma, P. Y. Cheng and S.A. Aicher. Dept. of Neurol. & Neurosci., Cornell Univ. Med. College, NY, NY 10021.

Substance P-containing terminals in the dorsal horn of the spinal cord predominantly belong to primary afferent fibers arising from the periphery and may play a role in the transmission of noxious stimuli to second-order neurons. These terminals are thought to also release glutamate as a neurotransmitter, and post-synaptic responses to glutamate release may be mediated through NMDA receptors. We combined immunocytochemical localization of Substance P (SP) and the NMDA-R1 receptor subunit, to determine if there may be direct pre- or post-synaptic interactions between these neurotransmitter systems. Regions of laminae I and II of the dorsal horn in the rat cervical spinal cord containing both labels were examined at the ultrastructural level. SP-like immunoreactivity was located primarily in unmyelinated axons and axon terminals. NMDA-R1-like immunoreactivity (NMDA-LI) was seen mainly in dendrites, but also in perikarya, axons, axon terminals, and glial processes. SP was most often seen in large dense core vesicles in axon terminals forming asymmetric synapses with dendrites both with and without detectable NMDA-LI. SP-labeled terminals less frequently either (1) converged on a common target with another terminal containing NMDA-LI or (2) also contained immunolabeling for the NMDA receptor. These results support the conclusion that both the post-synaptic responses and pre-synaptic release of transmitter from SP-containing terminals are modulated by glutamate acting at NMDA receptors in the superficial laminae of the rat spinal cord. (Support: NIH #HL18974; Amer. Heart Assoc. Grant-in-Aid to SAA).

341.9

MICROINJECTION OF GLUTAMATE IN TRIGEMINAL SUBNUCLEUS CAUDALIS (Vc) PRODUCES EXCITATORY EFFECTS IN RAT JAW MUSCLES. C.-M. Tsai*, B.J. Sessle, and J.W. Hu. Dept. of Oral Physiology, Fac. of Dentistry, Univ. of Toronto, Toronto, Canada. M5G 1G6

We have previously shown in rats that lesions of Vc, an important brainstem relay of craniofacial nociceptive information, or preadministration of MK-801, a glutamate subtype (NMDA) antagonist, can reduce the increases of electromyographic (EMG) activity in digastric (DIG) and masseter (MASS) muscles reflexly evoked by injection of mustard oil into the temporomandibular joint region. We have also shown that electrical microstimulation in Vc produces excitatory responses in these jaw muscles and that the loci associated with the lowest response threshold and shortest latency are in the caudal Vc. To determine whether these responses are due to activation of neurones as opposed to fibres of passage in Vc, sodium glutamate (GLU), an excitatory amino acid and cell excitant, was microinjected into Vc. Twenty-four male Sprague-Dawley rats anaesthetized with halothane/N_2O/O_2 were equally divided into four groups. GLU (0.5 M in saline; 40 nL) was injected via a micropipette into the exposed left caudal brainstem at a different site (rostral Vc, caudal Vc, C2 segment, or the adjacent reticular formation) in each group of rats. EMG activity was recorded bilaterally from DIG and MASS to determine the incidence, latency, amplitude and duration of any evoked jaw muscle activity. GLU injection into the caudal Vc evoked an increase in EMG activity of the ipsilateral, but not the contralateral, DIG (mean increase $\pm$S.D., 560$\pm$410 %) and MASS (530$\pm$430 %). The increased EMG activity had a latency of 4.2$\pm$2.8 s, peaked by the first min and lasted for 8.5$\pm$5.9 min. In contrast, GLU injection into the other three loci evoked little or no increase in EMG activity of any of the four muscles. Furthermore, the EMG activity evoked by GLU injection into the caudal Vc was significantly greater and lasted significantly longer ($p<0.05$) than that evoked by GLU injection into the other loci. These findings indicate that the effectiveness of Vc lesions and preadministration of MK-801 in reducing the mustard oil-evoked EMG responses may be explained by their interference with glutamate-mediated excitatory effects on Vc interneurones involved in craniofacial nociceptive reflex pathways. Supported by Canadian MRC MT12286.

341.10

C-FOS EXPRESSION IN TRIGEMINAL NUCLEUS FOLLOWING EITHER SKIN INCISION OR MUSTARD OIL APPLICATION TO MAXILLARY SKIN. S. Chaisin, K.J. Heyeraas, A.R. Light and W. Maixner* Dental Research Center, School of Dentistry and Dept. of Physiology, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7455.

C-fos-like immunoreactivity (Fos-LI) has been shown to be a marker for cells which respond to nociceptive stimuli. We studied the somatotopic distribution of immunoreactive c-fos protein resulting from either a 12 mm incision of the maxillary skin or topical application of C-fiber stimulant mustard oil to the maxillary skin. Fos-LI distribution 2 hours after application of the noxious insult was determined. Both skin incision and mustard oil induced Fos-LI in the trigeminal nucleus caudalis and trigeminal nucleus interpolaris. Fos-LI ipsilateral to the site of skin incision or mustard oil treatment was greater ($P<0.05$) than on the contralateral side in discrete regions of trigeminal nucleus caudalis and trigeminal nucleus interpolaris. In contrast to these findings, the number of immunoreactive cells in the nucleus tract solitaris and ventrolateral medulla of the brain stem were not significantly different for the two treatment groups. Comparison of brain stem Fos-LI ipsilateral to the site of skin incision and mustard oil application revealed qualitative and quantitative differences. Mustard oil evoked greater Fos-LI compared to skin incision in trigeminal nucleus caudalis ($P<0.05$). Also, the somatotopic organization of Fos-LI in the superficial regions of the trigeminal nucleus caudalis was more discretely localized in response to skin incision compared to mustard oil. These findings suggest that the nociceptive responses to skin incision and mustard oil are likely mediated by neurons in trigeminal nucleus caudalis and trigeminal nucleus interpolaris. Further studies will investigate if skin and mustard oil stimulation activate different nociceptive pathways within the trigeminal nucleus. Supported by DE11661 (W.M.) and NS16433 (A.L.).

341.11

ACTIVATION OF NEURONS IN RAT DORSOMEDIAL TRIGEMINAL N. CAUDALIS (Vc) BY APPLICATION OF A SPECTRUM OF IRRITANT CHEMICALS TO THE TONGUE E. Carstens*, N. Künzler and H.O. Handwerker. Inst. Physiology & Exp. Pathophysiology., Univ. Erlangen (FRG) and Sect. Neurobiology, Physiology & Behavior, Univ. Calif., Davis, CA 95616.

To study trigeminal mechanisms of oral irritation, we recorded responses of neurons in brainstem Vc to application of irritant chemicals to the tongue in thiopental-anesthetized rats. Units in superficial layers of dorsomedial Vc responding to noxious heating of the ipsilateral tongue were tested with the following chemicals delivered epilingually: histamine (his), nicotine (nic), capsaicin (cap), piperine (pip), mustard oil (mo), serotonin (5HT), acid (phosphate buffer pH 4 to 1 = pH), NaCl and ethanol (EtOH). Dose-response relationship, and tachyphylaxis to repeated application at one concentration, were tested for each chemical.

31/32 units responded to at least one chemical, and 33% responded to all 9. The unit population responded to 133/173 (77%) of tested chemicals (incidence: his 88%, nic 85%, 5HT 65%, NaCl 74%, pH 94%, EtOH 85%, cap 74%, pip 56%, mo 50%). The incidence of responses was independent of the order of chemicals tested, except that cap reduced subsequent responses (desensitization). Onset and duration of averaged responses were longest for cap, pip and mo. Responses significantly increased in a dose-related manner for his ($9x10^{-1}$-10^{-4} M), nic ($6x10^{-1}$-10^{-4} M), NaCl (0.5-5M), EtOH (15-50%), and pH. Successive responses to repeated application decreased significantly only for nic, NaCl, 5HT, cap and pip. Spontaneous firing increased significantly 5-10 min after cap. Responses to his and nic were significantly reduced by ceterizine (H1 antagonist) and mecamylamine, respectively.

These data support the idea of a common chemical sense ("chemesthesis"), whereby different irritant chemicals activate a common population of sensory neurons in Vc, presumably via independent peripheral transduction mechanisms.

Supported by a Fulbright Award (to EC), Calif. Tobacco-Related Disease Research Program, and the Deutsche Forschungsgemeinschaft, SFB 353.

341.12

RESPONSES OF NEURONS IN RAT VENTROLATERAL TRIGEMINAL N. CAUDALIS (Vc) TO APPLICATION OF A SPECTRUM OF IRRITANT CHEMICALS TO CORNEA AND PERIORBITAL TISSUE. N. Künzler, E. Carstens, R.L. Kitchell*, & H.O. Handwerker. Inst. Physiology Exp. Pathophysiol., Univ. Erlangen (FRG) and Neurobiology, Physiology & Behavior, UC Davis, CA 95616.

To study trigeminal mechanisms of corneal irritation, we recorded responses of single units in ventrolateral Vc to application of irritant chemicals to cornea/periorbital skin in thiopental-anesthetized rats. Units responsive to noxious corneal heating were tested with: histamine (his), nicotine (nic), capsaicin (cap), piperine (pip), mustard oil (mo), serotonin (5HT), acid (phosphate buffer pH 4 to 1 = pH), NaCl and ethanol (EtOH). Dose-response relationship, and tachyphylaxis to repeated application at one concentration, were tested for each chemical.

14/30 units had mechanoreceptive fields restricted to cornea and eyelids, and the rest had larger facial fields encompassing the cornea. 28/30 responded to >1 chemical delivered topically to the cornea. The population responded to 115/177 (65%) of tested chemicals (EtOH 85%, nic 83%, cap 82%, pH 73%, NaCl 67%, pip 62%, mo 58%, his 46%, 5HT 25%). Responsiveness appeared to be independent of the order of chemical application (cap, pip and mo were tested last to avoid desensitization). Averaged responses to NaCl and mo peaked more slowly; duration of responses to pH and cap were longest. Responses significantly increased in a dose-related manner to his ($9x10^{-1}$-10^{-4} M), nic ($6x10^{-1}$-10^{-4} M), NaCl (0.5-5M), pH, cap ($3x10^{-3}$-10^{-5} M) and mo (8-50%). Successive responses to repeated application decreased significantly for nic, EtOH, and mo, tended to decrease for cap, pip and 5HT, and remained constant for the others. Responses to nic were significantly attenuated by prior corneal application of mecamylamine.

Activation of individual trigeminal neurons by many irritant chemicals delivered to cornea and periorbital tissue provides further support for a "common chemical sense".

Supported by a Fulbright Award (to EC), Calif. Tobacco-Related Disease Research Program, and the Deutsche Forschungsgemeinschaft, SFB 353.

341.13

THE EFFECT OF DORSAL COLUMN LESIONS UPON BONE PAIN A. Houghton*, E. James-Hewitt and K. N. Westlund. Marine Biomedical institute. University of Texas Medical Branch, Galveston, TX 77555-1069

Recent studies have shown that a lesion to the midline dorsal column of the spinal cord in rats can block visceral pain induced by colon distension. The aim of the present study was to determine if dorsal column lesions can also reduce bone pain. At present the only treatment for bone cancer pain is radiation and morphine.

Experiments were performed on 14 male rats (240-350 g). In all rats bone pain was induced by making a hole (1mm diameter) through the tibia of the left leg with a hand drill under anaesthesia. In seven of the rats a lesion of approximately 1 mm in depth was made in the midline dorsal column of upper thoracic level of the spinal cord. The lesion was made after a small laminectomy under anaesthesia two days prior to the induction of the bone pain. No neurological defects were observed after the lesion was made. Hyperalgesia was quantified by measuring the paw withdrawal threshold to a mechanical stimulus. Four strengths of von Frey hair were used (8, 10, 50, 100 mN), and were presented to the anterior plantar surface of both hindlimbs ten times. Mechanical threshold was tested before the hole was drilled and at 30 min intervals for 4 hr afterwards. An increased percentage of paw withdrawals to the same strength hair was considered hyperalgesia, whereas response to a strength of hair which previously evoked no response was considered allodynia.

After the hole was drilled, both hyperalgesia and allodynia were observed. The mean response to the 50 and 10 mN hair strengths was increased from 23 ± 9 % to 71 ± 12 % (n = 7) and 0 % to 50 ± 15 % respectively, 2 hr post injury. In contrast, in the lesioned rats no hyperalgesia or allodynia was observed after the hole was drilled.

These studies suggest that information about deep pain is carried through the midline dorsal column and includes bone pain in addition to visceral pain. Thus, dorsal column lesions may prove to be an effective treatment for patients suffering from bone cancer pain. Supported by NIH grants RO1 NS28064 and NS11255.

341.14

CARDIOPULMONARY SYMPATHETIC AFFERENT INPUT EXCITES CUNEATE-THALAMIC NEURONS IN MONKEYS. M.J. Chandler*, J. Zhang and R.D. Foreman. Dept. Physiology, Univ. of Okla. HSC, Okla. City, OK 73190.

Stimulation of cardiopulmonary sympathetic afferent fibers (CPSA) excites thoracic and cervical spinothalamic tract neurons which respond primarily to noxious somatic inputs. Neurons in dorsal column (DC) nuclei respond primarily to innocuous somatic inputs. Recent evidence in rats shows that neurons in gracilis nucleus are excited or inhibited by noxious stimulation of pelvic organs. The hypothesis of this study is that CPSA input excites relay neurons in cuneate nucleus. Extracellular action potentials of 11 neurons antidromically activated from ventroposterolateral (VPL) thalamus were recorded in cuneate nucleus of 4 anesthetized male monkeys. Electrical stimulation of the ipsilateral stellate ganglion to activate CPSA excited 7 cells and did not affect activity of 4 cells. Transection of DC + ipsilateral dorsal lateral pathways (DLF) at C4 eliminated CPSA excitation in 2 of 2 cells. Of 10 cells examined for somatic field characteristics, 6 were low-threshold (LT), 2 were wide dynamic range, 1 was high-threshold, and no somatic field was found for 1 cell; receptive fields were located on small areas of upper body regions. Of 7 cells excited by CPSA, 3 were excited by noxious somatic input, 3 were LT, and 1 cell was not tested for somatic field. These results showed that CPSA input travels in DC and/or DLF to activate cuneate nucleus neurons which project to contralateral VPL thalamus. Thus, these neurons might participate in integration of nociceptive information from the heart. (NIH grant HL22732)

341.15

RETROGRADE IDENTIFICATION OF LAMINA I NEURONS THAT PROJECT TO THE CAUDAL VENTROLATERAL MEDULLA (CVLM) IN THE CAT. K. Krout* and A.D. (Bud) Craig. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, Arizona 85013.

The CVLM, where A1 medullo-hypothalamic neurons are located, is involved in a variety of homeostatic responses to small-diameter somatic afferent input. Recent PHA-L results demonstrating bilateral input to the CVLM (and A1 neurons) of cats and monkeys from lamina I indicate an ascending pathway for such inputs. Because different morphological types of lamina I cells correspond to distinct physiological classes responsive to nociceptive and thermoreceptive inputs (SN 20:547, 1994), we have sought to determine which types of lamina I neurons project to the CVLM with retrograde labeling.

Recordings made in the CVLM in isoflurane-anesthetized cats identified single units responsive to natural noxious (pinch, heat) and thermal (cold) stimuli over large receptive fields (eg hemi-body). Single iontophoretic injections of CTb (2μA, 20 min; to avoid uptake by fibers of passage) were made at such sites and, 1-2 wks later, spinal segments were processed with ABC/DAB. At all spinal levels, lamina I neurons were labeled bilaterally, and all three recognized types were included: fusiform, pyramidal and multipolar.

Our physiological and anatomical findings indicate that the CVLM receives activity from lamina I related to pain, temperature, and probably all aspects of the physiological condition of the body, consistent with a broad role for lamina I projections in homeostasis. Such inputs may be integrated in CVLM projections to the hypothalamus and the RVLM, providing a basis for control of autonomic responses to environmental challenges. (Supported by the Barrow Neurological Foundation)

341.16

RESPONSE PROPERTIES OF INTRACRANIAL MENINGEAL AFFERENTS IN RAT SUGGEST THESE FIBERS CONTRIBUTE TO HEADACHE PAIN. A.M. Strassman*, S.A. Raymond and R. Burstein. Dept. Anesthes., Beth Israel Hosp. and Harvard Med. Sch., Boston, MA 02215.

Theories of migraine headache have focused on the possible activation of intracranial meningeal sensory afferents by mechanical or chemical stimuli, or both. However, there is little direct data on the responses of meningeal afferents, and none on their possible chemosensitivity. We have recorded the activity of rat trigeminal ganglion cells that innervate the dural venous sinuses during mechanical and chemical stimulation of their dural receptive fields. Such cells were identified by their response to electrical stimulation of the dura overlying the ipsilateral transverse sinus.The majority of neurons (22/32) that were activated by dural shock exhibited mechanosensitive receptive fields on the dura from which they could be activated by punctate probing with von Frey hairs or by stroking with a blunt rod. A subset of the neurons (17/23) exhibited chemosensitivity in response to topical application of algesic or inflammatory agents to the dura, including solutions of hypertonic sodium chloride, potassium chloride, capsaicin, high or low osmolarity, and a soup of inflammatory agents (histamine, serotonin, bradykinin, and prostaglandin E_2, pH 5). Sensitization was investigated by determining von Frey thresholds before and after topical application to the receptive field of pH 5.0 buffer or the inflammatory soup. Lowered mechanical thresholds were found in 7 of 9 neurons tested. These properties of meningeal afferents (chemosensitivity and sensitization to mechanical stimuli) could account for the intracranial mechanical hypersensitivity that is characteristic of many types of clinically occurring headaches. We suggest that non-painful cardiac pulsation or head movement may become painful during migraine because of chemically induced mechanical sensitization of meningeal afferents. (Supported by the National Headache Foundation, NIH, and BIH Dept. Anesthes.)

341.17

CENTRAL MECHANISM OF VASCULAR HEAD PAIN - SENSITIZATION OF TRIGEMINOTHALAMIC, TRIGEMINOHYPOTHALAMIC AND TRIGEMINO PARABRACHIAL NEURONS INNERVATING THE INTRACRANIAL SINUSES. R. Burstein , A. Malick and A. Strassman, Department of Anesthesia, Beth Israel Hospital, Department of Neurobiology, Harvard Medical School, 220 Longwood Ave. Boston MA, 02115

Although different theories have been proposed about migraine, most are based on the notion that the headache results from the sensitization and/or excitation of trigeminal sensory neurons that innervate cranial blood vessels. However, direct evidence for the development of sensitization in trigeminovascular neurons is almost entirely lacking. In the present study we have determined the effects of potentially-sensitizing chemical stimuli on the ongoing activity and responsiveness of central trigeminal neurons innervating the intracranial dural sinuses and determined to which brain areas they project.

Trigeminal neurons responding to stimulation of intracranial dural sinuses were identified in the brainstem. The size of their visceral and cutaneous receptive fields and thresholds of mechanical and thermal stimuli were determined before and after exposing the sinus to chemical irritants. Antidromic stimulation technique was then used to map their axons.

Fifteen trigeminovascular neurons recorded in C1, Vc and Vi exhibited dural and cutaneous receptive fields. Following application of chemical irritants to the dural sinus, several changes developed in the studied neurons: their thresholds to mechanical stimulation of the sinus and to mechanical, heat and cold stimulation of the facial skin decreased; the size of the dural receptive field increased; and in some cases they developed ongoing activity. Some neurons were antidromically activated from the parabrachial nucleus, ventroposterior medial thalamus, or lateral hypothalamus.

These findings suggest that irritation of cranial blood vessels can sensitize central trigeminal neurons that project to brain areas involved in somatosensory and affective responses to head pain.

NIH-NIDR 1-R29-DE10904,NINDS-NS32534

341.18

SPINODIENCEPHALIC TRACT (SDT) NEURONS IN THE THORACIC SPINAL CORD OF RATS: RESPONSES TO CUTANEOUS AND VISCERAL STIMULATION, A.P. Gokin*, X. Zhang, C.N. Honda, G.J. Giesler, Jr. Dept. of Cell Biol. & Neuroanat., Univ. Minnesota, Minneapolis, MN 55455.

Large numbers of neurons in the rat thoracic spinal cord project directly to the hypothalamus (H), thalamus or both. The aim of this study was to characterize the responses of these neurons to somatic and visceral stimuli. In lower thoracic segments of urethane-anesthetized rats, 17 SDT neurons were antidromically activated using current pulses $\leq$ 30 μA. Twelve neurons were antidromically activated from both the contralateral thalamus and H (STT/SHT) and 5 were activated from only the posterior thalamus (STT). The axons of all examined neurons passed through or terminated in posterior thalamus. Axons of half of the studied STT/SHT neurons crossed the midline and entered the ipsilateral H; three appeared to terminate in the contralateral H. 16 SDT neurons were recorded in deep dorsal horn (DDH) and 1 in lateral spinal nucleus. Based on their responses to innocuous and noxious cutaneous stimuli, 4 SDT neurons were classified as low threshold, 5 as wide dynamic range, and 7 as high-threshold (HT). Cutaneous receptive fields were located ipsilaterally over the posterior rib cage and anterior and ventral abdomen. Responses to distention of the bile duct were examined for 6 SDT neurons (3 STT/SHT, 3 STT). In 4 cases, stimulation evoked excitatory (3) or inhibitory responses. The range of thresholds for responses to bile duct distention was 40-50 mmHg. Responses incremented gradually as pressure was increased to 65-80 mmHg. Responsive neurons were located in the DDH. Neurons that responded to distention of the bile duct were classified as HT (2) or WDR (2). These data indicate that SDT neurons in rat thoracic spinal cord are capable of conveying somatic and visceral nociceptive information directly to targets in the contralateral and ipsilateral hypothalamus and thalamus. Supported by NS25932 and DA07234.

341.19

SOMATOSENSORY RESPONSE PROPERTIES OF SPINOHYPOTHALAMIC TRACT NEURONS IN THE UPPER CERVICAL SEGMENTS OF RATS. X. Zhang*, A.P. Gokin and G.J. Giesler, Jr., Dept. of Cell Biol. & Neuroanat., Univ. of Minn., Minneapolis, MN 55455.

Morphological studies in rats have shown that about 30% of the entire population of spinohypothalamic tract (SHT) neurons is located in upper cervical segments. The aim of this study was to characterize somatosensory response properties of these neurons. Eighteen neurons in segments C1-2 of urethane-anesthetized rats were antidromically activated using pulses of $\leq$30 μA in the contralateral hypothalamus. The recording points were located in the lateral reticulated area (16) and nucleus proprius of the dorsal horn (2). The mean antidromic latency from the hypothalamus was 2.5 ms (1.5-5.1 ms); the mean conduction velocity was 10 m/s (4.7-15.6 m/s). Cutaneous receptive fields (RFs) were determined using innocuous and noxious mechanical stimuli. Each of 15 tested neurons responded exclusively or preferentially to noxious stimuli. Twelve were classified as high threshold and 3 as wide dynamic range. Five neurons had RFs that were restricted to the head and neck. Four had large RFs that included the head, neck, forelimbs and parts of the trunk and hindlimbs. Six had RFs that covered the entire body. The receptive fields were generally bilateral with the most sensitive regions consistently located on the ipsilateral cheek. Bilateral antidromic mapping was done in the hypothalamus to determine the trajectories of 13 axons. Ten axons terminated in the contralateral hypothalamus, and 3 crossed the midline and terminated in the ipsilateral hypothalamus. These results indicate the somatosensory characteristics of SHT neurons in upper cervical segments differ from SHT neurons examined at all other levels of the cord. It appears that SHT neurons in upper cervical cord process and relay nociceptive information from widespread areas of the body to the hypothalamus. Supported by NS25932 and DA07234.

PAIN MODULATION: ANATOMY AND PHYSIOLOGY—NEUROPATHIC PAIN

342.1

THE DEVELOPMENT OF TACTILE HYPERSENSITIVITY VARIES AMONG STRAINS OF THE MICE AFTER A PERIPHERAL NERVE INJURY. K. Chung*, H. Noh and J. M. Chung. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77555.

The purpose of this study was 1) to determine whether neuropathic pain behaviors develop after peripheral nerve injury in mice and 2) if they do, whether there is any difference in neuropathic pain behaviors among different strains of mice.

Six different strains of adult male mice were used for this study. Under halothane anesthesia, neuropathic injury was produced by tightly ligating the spinal nerve (L4 or L5, left side) with 6.0 silk thread. Behavioral tests for evoked tactile sensitivity to von Frey hair (ranging from 0.03 to 5.5 g) stimulations were conducted for presurgery and 1 day (D), 4D, 7D, and 14D postoperatively. Tactile hypersensitivity was measured by determining the median 50% foot withdrawal threshold using the up-down method.

The data demonstrated that all tested strains of mice had lower (as compared to the normal) thresholds to tactile stimuli one day after the neuropathic surgery. However, the severity and duration of the tactile hypersensitivity varied greatly among different strains of mice. (Supported by NIH grants NS31680 and NS11255)

342.2

ENDONEURIAL INJECTION OF TNF-α OR CEREMIDE PRODUCES PATHOLOGIC CHANGES AND PAIN BEHAVIORS SEEN IN NEUROPATHIC PAIN STATES. R.R. Myers* and R. Wagner. Departments of Anesthesiology and Pathology, UC San Diego, La Jolla, CA 92093-0629

TNF is a proinflammatory cytokine produced by activated fibroblasts, endothelial cells, mast cells and macrophages in painful peripheral nerve injuries. Our recent observation that it is also produced by Schwann cells in these nerves prompted us to explore its role in primary nerve injury and pain. We injected 10 μl of a 2.5 pg/ml TNF-α solution or 10 μl of a 1mM ceremide solution into rat sciatic nerve. Ceremide is a second messenger produced by TNF receptor activation. Behavioral testing was performed 1, 3, and 5 days after injection. Separate animals were used for histology at the same time-points. One day after TNF injection, animals displayed marked thermal hyperalgesia that persisted until day 5 ($p<0.05$, day 3). Allodynia was significant at days 1 and 5. Ceremide injections produced less thermal hyperalgesia, but significant allodynia at 3 and 5 days. Vehicle-injected animals had only minor mechanical allodynia (9g) at day 1. These small injection volumes produced local neuropathic changes including edema initially, then myelin splitting, demyelination, and occasional axonal degeneration by days 3 and 5. Ceremide-induced pathology was slightly more severe, including more axonal degeneration. Vehicle-injected nerves showed only mild edema between days 1 and 3.

We conclude that local injections of TNF-α or ceremide into rat sciatic nerve produces behavioral and neuropathologic changes observed in several models of severe neuropathic pain.

Supported by the VA and NIH Grants NS 18715 and F32 NS 10071.

342.3

IMMUNOREACTIVITY FOR THE 55kD TNF-α RECEPTOR AT THE SITE OF PERIPHERAL NERVE INJURY PARALLELS THE DISPLAY OF NEUROPATHIC BEHAVIOR FOLLOWING CCI IN THE RAT R. Wagner*, J. Dolkas, and R.R. Myers. Department of Anesthesiology, UC San Diego, La Jolla, CA 92093-0629.

We recently observed an increase of TNF immunoreactivity (IR) at the site of injury in different neuropathic pain models. We chose to monitor the expression of the 55kD receptor of TNF following peripheral nerve injury, as the 55kD receptor for TNF (rTNF) is known to moderate many of TNF's cytotoxic functions.

Rats received a chronic constriction injury (CCI) [Bennett and Xie, 1988] to one sciatic nerve at mid-thigh level and were then sacrificed after 18 hours, 3, 5, 7, 14, or 28 days (n=3 each group). Tissue from the injury site, or from non-injured control animals, was removed and embedded in paraffin. Ten-micron-thick sections were stained immunocytochemically using DAB with Ni as a chromagen. rTNF-IR was present in endothelial cells, Schwann cells, and macrophages. Morphometric analysis of the entire tibial fascicle was used to quantify the ratio of rTNF-IR cells to total counts within the endoneurium. This analysis revealed that endoneurial cells demonstrating rTNF-IR significantly increased at 5, 7, and 14 days [12.8% ± 2, 15.39% ± 0.5, and 15.8% ± 0.7, respectively] compared to non-injured control sections [7.2% ± 1.9]. Measurements at 18 hours, 3 and 28 days were not significant from controls.

This study demonstrates that the site of injury exhibits significantly increased rTNF immunoreactivity at times associated with the display of thermal hyperalgesia in the rat following CCI.

Supported by the VA and NIH Grants NS 18715 and F32 NS 10071.

342.4

AN EXPERIMENTAL NEURITIS OF THE RAT SCIATIC NERVE PRODUCES IPSILATERAL HINDPAW ALLODYNIA AND HYPERALGESIA. E. Eliav, M.A. Ruda, and G.J. Bennett*. Neurobiol. & Anesthesiol. Br., NIDR, NIH, Bethesda, MD 20892.

The onset of painful peripheral neuropathies involves both axonal damage and an inflammation of the nerve. The role of the latter by itself was investigated by producing an experimental neuritis in the rat. The sciatic nerves were exposed at mid-thigh level and wrapped in 2mm-wide bands of hemostatic oxidized cellulose (Surgicel) saturated with either carrageenan (CARRA; 150 µl, 40 mg/ml) or undiluted complete Freund's adjuvant (CFA; 100 µl) on one nerve and saline on the other. In other rats, a myositis was created by implanting Surgicel/CFA into a pocket made in biceps femoris at a position adjacent to where the nerve was treated. The plantar hindpaws were tested daily thereafter for heat- and mechano-hyperalgesia (HH, MH), and mechano- and cold-allodynia (MA, CA). Microscopic examination indicated that the neuritis (CFA) produced little or no axonal damage. Statistically significant HH, MH, MA and CA were present on the CARRA- and CFA-treated sides for 1-5 days, with normal responses thereafter. No abnormal pain was detected on the saline-treated side, or in rats with the myositis. We conclude that inflammation of the sciatic nerve, but not adjacent muscle, produces abnormal pain in the hindpaw and that this may contribute to the genesis of painful neuropathies. We speculate that a chronic neuritis might produce a neuropathic pain-like syndrome.

342.5

PLASTICITY OF THE CIRCUITRY OF THE SUPERFICIAL DORSAL HORN OF THE RAT IN A MONONEUROPATHY MODEL OF NEUROPATHIC PAIN. Diane Daly Ralston*, Sandra Canchola, Xianwei Meng and Henry J. Ralston III. Department of Anatomy and the W.M. Keck Foundation Center for Integrative Neuroscience, University of California, San Francisco CA 94143-0452.

The loose-ligature model of Bennett and Xie (1988) was used to induce an experimental mononeuropathy in female rats, the animals surviving for one to several weeks following unilateral placement of ligatures around the sciatic nerve. Hindlimb posture and the animal's behavioral responses to mechanical stimuli were assessed throughout the pre and postoperative periods. Two days prior to euthanasia, the rats were reanesthetized and the sciatic nerves of the ligated and sham-operated sides were microinjected with either wheat-germ agglutinin or choleragenoid conjugated to HRP (WGA-HRP or B-HRP), for the transganglionic labeling of fine diameter or large diameter (respectively) primary afferent projections to the dorsal horn. Vibratome sections of appropriate segments of the lumbar enlargement were processed for the demonstration of the transported markers and for GABA immunoreactivity (GABA-ir), and were subsequently examined and quantified by light and electron microscopy.

Behavioral testing revealed that some animals exhibited only modest postural changes and little or no allodynia or mechanical hyperalgesia of the limb of the ligated nerve, compared to preoperative behavior or to the sham operated limb postoperatively. Other animals revealed substantial postural changes, allodynia and mechanical hyperalgesia. Both groups of animals revealed modest to marked sprouting of B-HRP labeled afferents into lamina II on the ligated side. Changes in GABA-ir, however, were closely matched to the behavioral state of both groups, the numbers of GABA-ir neurons in laminae I and II being markedly reduced ($p<0.05$) as were GABA-ir synaptic contacts on dendrites of dorsal horn neurons on the ligated side of those animals exhibiting pain-related behaviors. We conclude that changes in GABA circuitry, rather than sprouting of large diameter afferent fibers *per se*, play a fundamental role in this model of neuropathic pain. Supported by NIH/NS 21445.

342.6

CHANGE IN EXPRESSION OF AMINO ACID RECEPTORS AND NEUROPEPTIDES IN THE SPINAL CORD AND DORSAL ROOT GANGLIA IN A RAT MODEL OF NEUROPATHIC PAIN

T. Fukuoka, K. Miki, K. Noguchi* Dept. of Anatomy & Neuroscience, Hyogo College of Medicine, Nishinomiya, Hyogo 663, JAPAN

We examined the change in mRNA expression of γ-amino butyric acid $(GABA)_A$ receptor subunits and some neuropeptides in primary afferent neurons of an experimental rat model of neuropathic pain. The distribution of fibers containing neuropeptides' immunoreactivities in the dorsal horn was also studied in this model.

Male Sprague-Dawley rats (150-180g) received tight ligation of left L5 spinal nerves. Foot withdrawal latencies to radiant heat and sensitivity of the hind paw to mechanical stimulation with von Frey filaments were tested for the next 7 days. After the last evaluation was done, L4,5 dorsal root ganglia (DRG) and L4-L6 spinal cord were dissected out and processed for immunohistochemistry and *in situ* hybridization.

In normal DRG, most of the large neurons expressed $GABA_A$ receptor γ2 subunit mRNA. The level of this mRNA was significantly decreased, while the levels of neuropeptide Y (NPY) and preprotachykinin (PPT) mRNAs were increased in some large L5 DRG neurons ipsilateral to the ligation. Spinal nerve ligation had little effect on these mRNA levels in other DRGs. Immunohistochemistry with calcitonin gene-related peptide and NPY antibodies revealed that increased immunostaining in terminals and fibers in laminae III-IV of L4-6 segments of the spinal cord.

We report the decrease of presynaptic inhibition by downregulation of $GABA_A$ receptor and increased content of neuropeptides in primary afferent terminals in laminae III-IV of L4-6 spinal cord segments. This finding may be involved in the abnormalities of signal transmission through intact L4 primary afferent and contribute to the pathomechanisms of neuropathic pain.

(Supported by the MESC of Japan and Hyogo College of Medicine.)

342.7

ELECTOPHYSIOLOGICAL PROPERTIES OF DORSAL COLUMN NEURONS IN THE RAT WITH EXPERIMENTAL NEUROPATHY

K. Miki*, K. Iwata[1], Y. Tsuboi[1], R. Sumino[1] and K. Noguchi
Department of Anatomy and Neuroscience, Hyogo College of Medicine, Hyogo, 663, JAPAN, [1]Department of Physiology, School of Dentistry Nihon University, Tokyo, 101, JAPAN

We have previously reported that preprotachykinin mRNA is expressed in some large DRG neurons and that substance P immunoreactivity is increased in dorsal column (DCn) nuclei following peripheral nerve injury (J.Neurosci., 15,7633,95). Stimulus-evoked c-fos expression in gracile nucleus (GN) was also reported ipsilateral to the nerve injury. Therefore, in addition to the spino-thalamic tract, DCn pathway may have a functional role in the sensory abnormalities seen in neuropathic pain models. To examine this, Sprague Dawley rats had unilaterally chronic constriction nerve injuries (CCI) of their sciatic nerves. Later, single-unit activity was recorded from GN in rats while they were anesthetized with sodium pentobarbital (50 mg/kg. i.p.) and halothane (2-3%). A total of 89 neurons were record from the GN. WDR and LTM neurons were found to be spatially intermingled within the DCn. Five WDR neurons were identified contralateral and 9 were identified ipsilateral to the CCI. Twenty-five LTM neurons were detected contralateral and 34 neurons were detected ipsilateral to the CCI. Six neurons with no receptive field were identified ipsilateral to the CCI. Spontaneous activity of DCn neurons from the ipsilateral side (mean ± SE, 13.3 ± 2.3 Hz) was slightly higher than those for the contralateral side (9.1 ± 2.2 Hz). Forty of 89 DCn neurons were antidromically activated following electrical stimulation of the ventro-lateral thalamic nuclei (antidromic latency, ipsilateral: 3.6 ± 1.5 msec, contralateral: 2.2 ± 0.7 msec). Collectively, these data suggest that DCn neurons may be involved in CCI-produced sensory abnormalities by conveying their hyperactivity to the thalamic neurons. (Supported by the MESC of Japan and Hyogo Col. of Med.)

342.8

NOREPINEPHRINE CHANGES THE MECHANORECEPTOR ACTIVITY IN SYMPATHECTOMIZED NEUROPATHIC RAT. D. H. Lee* and J. M. Chung, Marine Biomed. Inst., Depts. of Anat. & Neurosci. and Physiol. & Biophys., Univ. of Texas Med. Br., Galveston, TX 77555.

The aim of this study was to investigate the physiological basis for the rekindling of mechanical allodynia elicited by subcutaneous injection of norepinephrine (NE) in sympathectomized neuropathic rats.

Neuropathic injury was produced by tightly ligating the left L5/L6 spinal nerves of the rats. One week after neuropathic injury, a bilateral lumbar sympathectomy was done. One week after the sympathectomy, single unit activity was recorded from fascicles of the L4 dorsal root (uninjured segment). After determining the type of mechanoreceptor and receptive field into the paw, NE (0.05 mg) was subcutaneously injected to the receptive field. Responses to mechanical stimuli were compared before and after NE injection.

There were numerous mechanoreceptors which showed an increased discharge rate and/or a change in discharge pattern after the subcutaneous injection of NE. The changes were observed in both slowly adapting and rapidly adapting mechanoreceptors.

The data suggest that NE interact with mechanoreceptors at the receptor level to rekindle mechanical allodynia in sympathectomized neuropathic rats. (Supported by NIH grants NS31680 and NS11255)

342.9

INDUCTION OF BRADYKININ BINDING SITES AFTER CHRONIC NERVE CONSTRICTION IN RAT SENSORY NEURONS. A.S. Eckert, G. Segond von Banchet, B. Heppelmann, M. Petersen and K.-D. Kniffki*. *Institute of Physiology, University of Würzburg, 97070 Würzburg, Germany.*

Bradykinin (BK), an endogenous pain producing nonapeptide, acts via specific receptors. It has been documented that only the B2 subtype is expressed under normal conditions whereas under chronic inflammatory conditions also the B1 subtype is expressed. We asked whether a chronic mechanical injury of a peripheral nerve affects the expression of BK receptors on sensory neurons. Therefore, we studied changes of the expression of BK binding sites in isolated dorsal root ganglion cells from adult rats following a unilateral tight ligation of the sciatic nerve at midthigh level. After two or ten days, the ganglia L4 and L5 were excised on both sides. As a control, corresponding ganglia from uninjured rats were used. Neurons were enzymatically isolated and grown on coverslips. After 0.8 or 1.8 days in culture BK binding sites were visualized by silver enhanced gold labeled BK and quantified using an image analyzing system. Data were pooled from three cultures and 293 to 542 cells each. From normal rats 47% of the neurons express BK binding sites at day 0.8 and about 85% at day 1.8 in culture. Following a tight ligation of 2 or 10 days the percentage of BK binding neurons from the ligated side was significantly increased to 83% or 90% at day 0.8 in culture and stayed at about this level at day 1.8. Surprisingly, on the contralateral side there was also an upregulation to 83% following a 2 day ligation on day 0.8 in culture. However, this upregulation was transient, because after a 10 day ligation on day 0.8 in culture only in 60% of neurons BK binding sites were detected. Density of BK binding sites increased on individual cells of both sides following a 2 day ligation but only of the ligated side following a 10 day ligation after 0.8 or 1.8 days in culture. These results indicate that a nerve injury increases the proportion of BK binding cells and the density of BK binding sites in sensory neurons. (Supported by DFG Pe 299/3-1 and DFG He 1919/2-3.)

342.10

INHIBITORY ROLE OF ABNORMAL DISCHARGES FROM INJURED LARGE-DIAMETER FIBERS IN THE DEVELOPMENT OF THE NEUROPATHIC SYMPTOMS IN A RAT MODEL H.S. Na*, K.A. Park[1], Y.I. Kim, H.J. Nam, H.J. Kim[2] and S.K. Hong Dept. Physiol., Korea Univ. Med. Coll., [1]Dept. Anat. and [2]Dept. Physiol., Yonsei Univ. Med. Coll., Seoul, KOREA.

The abnormal discharges from injured Aδ- and C-afferents are thought to produce the central sensitization that underlies the neuropathic pain. However, it was reported that about half of the units displaying abnormal discharges after injury were Aβ-fibers. In the present study, we investigated the role of injured large diameter afferents in the development of the pain behaviors in a rat model, produced by transection of the superior caudal trunk between the S3 and S4 spinal nerves. The fact that nerve injury near birth, unlike the one during adulthood, destroys most large diameter fibers was utilized to indirectly assess the role of large diameter fibers.

Nerve injury within 24 hours after birth (n=23, NEO group) destroyed most large, as well as small, diameter fibers. Whereas, nerve injury at 7 weeks of age (n=23, YOUNG group) preserved the majority both large and small diameter fibers. The behavioral tests were performed 2,6,7,8,10,14,18 weeks after the surgery in both groups. The signs of mechanical allodynia in response to mechanical stimuli applied to the tail with von Frey hairs (4.9-mN or 19.6-mN bending force) were more robust in NEO group than in YOUNG group. Also, the signs of thermal allodynia, inferred from the decrease in the tail response latency to the cold (4°C) or warm (40°C) water immersion, were more pronounced in NEO group.

These results are consistent with gate-control hypothesis for pain.
(Supported by Non-Directed Research Fund, KRF)

342.11

PERIPHERALLY AXOTOMIZED DRG CELLS EXHIBIT ALTERED INTRINSIC MEMBRANE PROPERTIES IN A RAT MODEL OF NEUROPATHIC PAIN. S.K. Hong*, Y.I. Kim, H.S. Na, S.L. Shin, M.G. Lee, S.H. Nahm and H.C. Han. Neurosci. Res. Inst. and Dept. of Physiol., Korea Univ. Coll. of Med., Seoul, KOREA

Several pieces of evidence suggest that abnormal electrical signals arising from axotomized DRG cells are critical for the induction and/or maintenance of neuropathic pain from peripheral nerve injury. In order to gain some insight into the neurophysiological mechanisms underlying the abnormal signals, we examined various membrane properties of axotomized DRG cells in a rat model of neuropathic pain.

This model, where the signs of neuropathic pain are localized in the tail, was produced by unilateral transection of the superior and inferior caudal trunks at the level between the S3 and S4 spinal nerves. The S1-3 DRGs ipsilateral to the injury were resected out 7-14 days postoperatively for *in vitro* electrophysiological experiments. The results of intracellular recordings show that the cells in these DRGs were not significantly different from control cells (i.e., cells in the S1-3 DRGs contralateral to the injury and S1-3 DRG cells of sham-operated animals) with respect to spike amplitude, threshold and AHP amplitude and duration. On the other hand, the results demonstrate that: (1) the cells in the ipsilateral DRGs had significantly less negative resting membrane potential (though not spontaneously active), higher input resistance, lower rheobase and longer action potential duration than control cells and (2) a large proportion of the cells in the ipsilateral DRGs (> 40%) exhibited "hump"-type action potential (i.e., spike having a shoulder in the repolarizing phase) unlike control cells (< 15%); in some additional experiments where the distance between the S1-2 DRGs and the injury site was reduced by transection of the caudal trunks at more rostral levels (i.e., levels between S1 and S2 and between S2 and S3 spinal nerves), the proportion of cells with "hump"-type spike was even greater (> 70%).

The present results suggest that, following partial peripheral nerve injury, profound changes occur in some intrinsic membrane properties of axotomized DRG cells making these cells more excitable and that emergence of a certain membrane property in a given axotomized cell is related to the distance between cell body and injury site. (Supported by KOSEF and Biotech 2000).

342.12

NITRIC OXIDE SYNTHASE INHIBITOR (L-NAME) DECREASES MECHANICAL ALLODYNIA IN A RAT MODEL OF NEUROPATHIC PAIN. Young Wook Yoon*[1], Backil Sung[1], and Jin Mo Chung[2] [1]Dept. of Physiol., Korea University College of Medicine, Seoul, 136-705, Korea, [2]Marine Biomed. Inst., Univ. Texas Medical Br., Galveston, TX, 77550, U.S.A.

The partial injury to a peripheral nerve may produce a painful abnormal sensations such as allodynia and hyperalgesia. The sensitization of spinal cord is thought as a basic mechanism to manifest abnormal pains. Recent data indicate that nitric oxide (NO) modulates mechanical, thermal and chemical nociceptive signals in somatosensory pathway at spinal cord level. The aim of this study was to observe whether NO modulates the behavioral signs of neuropathic pain in a rat model.

Neuropathic injury was made by tight ligation of L5 and L6 spinal nerves unilaterally in Sprague-Dawley rats under enflurane anesthesia. Behavioral tests were performed to examine the presence of mechanical allodynia. All drug tests were carried out from 3 days to 3 weeks after ligation of spinal nerves. During this period, mechanical allodynia was fully expressed and was not expect to diminish without any interventions according to our previous observation. In order to examine the effects of nitric oxide on the neuropathic pain, L-NAME (10 to 500 μM/kg), D-NAME (100, 500 μM/kg), and saline was injected intraperitoneally and behavioral tests were conducted.

Intraperitoneal injection of L-NAME reduced the behavioral signs of mechanical allodynia in dose-dependent manner, while D-NAME or saline did not change the mechanical allodynia.

The data show that mechanical allodynia observed in rat following partial peripheral nerve injury can be abolished by L-NAME. It is possible that manipulation of the L-arginine-NO system by blockade of the production of NO may be useful in alleviating neuropathic pain.
(Supported by Grant from Korea Univ. and NIH grants NS31680 and NS11255)

342.13

EVIDENCE FOR PERIPHERAL NITRIC OXIDE (NO) ACTIVITY IN A RAT MODEL OF CHRONIC NEUROPATHIC PAIN. D. Levy and D.W. Zochodne*. University of Calgary, Calgary, Alberta, Canada T2N 4N1

Partial injury to a peripheral nerve may result in severe chronic neuropathic pain. Recently, putative changes in dorsal horn NO have suggested a role for NO in an animal model of chronic neuropathic pain. In this study we sought to investigate a possible action of peripheral NO by examining local changes in nerve blood flow prior and following inhibition of nitric oxide synthase (NOS).

Chronic constriction injury (CCI) was induced in male Sprague-Dawley rats (250-350 g) by placing 4 x 4.0 chromic gut sutures around the sciatic nerve. Forty eight hours, 7 and 14 days postoperation, the rats had evidence of thermal hyperalgesia and mechanical allodynia. Changes in sciatic blood flow (NBF) were measured at these times, using Laser Doppler flowmetry (LDF) proximally and distally to the constriction site and from sham exposed nerve. Following baseline testing rats were injected either with L-NAME or D-NAME (10 mg/kg; IP injection) followed by repeated LDF measurements (15-20 min. postinjection). Further doses of L-NAME (0.1 and 1 mg/kg) were also studied.

CCI resulted in significant elevation in NBF distal to the constriction 48 hr. postoperation. Injection of L-NAME increased NBF in the sham operated side but reduced NBF in a dose dependent manner distal to the lesion. Injection of D-NAME did not have any effect.

The data may indicate a possible role for peripheral NO, perhaps locally elaborated by macrophages distal to the constriction, in producing changes in sciatic blood flow in CCI. Local NO might have other actions in this model as well.

(Supported by Muscular Dystrophy Association of Canada)

342.14

ATTENUATION OF TRANSSYNAPTIC ALTERATION OF SPINAL CORD DORSAL HORN NEURONS AND NEUROPATHIC PAIN IN RATS BY INHIBITING NITRIC OXIDE-ACTIVATED POLY(ADP-RIBOSE) SYNTHETASE J. Mao*, D.D. Price, J. Lu, J. Zhu* and D.J. Mayer, Depts. Anesthesiol. and Neurosurg#, Medical College of Virginia, Richmond, VA 23298

Transsynaptic alteration of spinal cord dorsal horn neurons (so-called 'dark' neurons) occurs in a rat model of neuropathic pain induced by chronic constrictive nerve injury (CCI). The incidence of dark neurons in CCI rats has been proposed to be mediated by neurotoxicity. We examined whether inhibiting the nitric oxide (NO)-activated poly(ADP-ribose) synthetase (PARS), a nuclear enzyme critical to glutamate-induced neurotoxicity, would reduce dark neurons and attenuate neuropathic pain in CCI rats. Dark neurons were observed bilaterally (with ipsilateral predominance) within the dorsal horn of CCI rats eight days after unilateral sciatic nerve ligation but not after sham operation ($P < 0.05$). The number of dark neurons was reliably reduced in CCI rats receiving once daily intrathecal treatment (x 7 days) with a PARS inhibitor benzamide (400, 200 nmol, but not 100 nmol benzamide or saline, $P < 0.05$). Concurrently, thermal hyperalgesia, mechanical hyperalgesia, and low threshold mechano-allodynia also were reliably reduced in CCI rats treated with either 200 or 400 nmol benzamide ($P < 0.05$). Once daily intrathecal treatment (x 7 days) with an NO synthase inhibitor N^G-nitro-L-arginine methyl ester (40 nmol) resulted in similar reductions of both dark neurons and signs of neuropathic pain in CCI rats (each $P < 0.05$). These results provide *in vivo* evidence indicating that NO-mediated PARS activation may be an underlying mechanism of nerve injury-induced 'dark neurons' and that such morphological changes contribute to behavioral manifestations of neuropathic pain in CCI rats. The present data provide new insights into mechanisms of intractable neuropathic pain syndromes in humans.

Supported by PHS grants DA 08835 and NS 24009.

342.15

INFRAORBITAL NERVE LIGATION SUPPRESSES JOR AND ELEVATES CALBINDIN AND PKC EXPRESSION. M. A. Matthews* M. L. Fogg and S.L. Liles. Dept. Anat., L.S.U. Med. Ctr, New Orleans, LA

Chronic constrictive injury (C.C.I.) of the rat's infraorbital nerve (ION) increases expression of CAL and second messengers in the trigeminal nucleus. Altered firing thresholds and activation of Ca++-sensitive protein kinase C (PKC) should occur but evidence for this is lacking in the present model. The jaw opening reflex (JOR) was tested in ION-ligated rats by stimulating the maxillary incisor pulp and recording evoked digastric EMG. Stimulus thresholds increased contralateral to the ION by 4 days but differences between control and experimental animals were reduced by 8 and 12 days. EMG response latencies increased ipsilaterally at 8-12 days. The response amplitude was suppressed at 8-12 days suggestive of descending inhibition. Frozen sections were exposed to antisera against either CAL or PKC B_{II}. CAL labeling was most intense in the ipsilateral Sp.V, contralateral thalamic reticular nucleus (TRN) and dentate hilar region. PKC labeling occurred bilaterally in the Sp. V, suggestive of potentiation of SpV neurons, and the anterior pretectal nucleus, a region known to inhibit the JOR (Chiang et al., '91). A concurrent colorimetric assay of PKC activity in Sp.V. revealed a 38% increase ipsilaterally and 22% increase contralaterally ($P<0.01$) in activity at 12 days following the ligation. These findings indicate that ION CCI causes NMDA receptor mediated translocation and activation of Ca++sensitive protein kinase C and descending modulation of nociceptive reflex function suggestive of initiation of a neuropathic central sensitization within the trigeminal complex. Supported by NIH grants DE 07237 and RR05704.

342.16

SPINAL MICROGLIAL ACTIVATION AND INCREASES IN IL-6 mRNA FOLLOWING PERIPHERAL NERVE INJURY: EVIDENCE FOR NEUROIMMUNE ACTIVATION IN ANIMAL MODELS OF NEUROPATHIC PAIN. R.W. Colburn (a), J.A. DeLeo*(a,b), A.J. Rickman(b). Dept. of Pharmacology(a) and Anesthesiology(b), Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756.

We have previously demonstrated increases in spinal immunoreactive-like proinflammatory cytokines (IL-1β, IL-6, TNF-α) following peripheral nerve injury that results in neuropathic pain-like behaviors. Cytokines induced both peripherally and centrally after nerve injury are likely to be involved in synaptic plasticity and subsequent spinal hyperexcitability, the pathologic correlate to neuropathic pain. It has been demonstrated that glial cells are capable of producing and/or releasing cytokines. Toward a better understanding of the spinal neuroimmunologic cascade of events following nerve injury, immunocytochemical detection of microglial activation using OX-42 monoclonal antibody and *in situ* hybridization of IL-6 mRNA were compared after sciatic nerve cryoneurolysis (SCN) or spinal nerve cryoneurolysis (SPCN) at various post-lesion time points (5-6 rats/group). Male Holtzman rats underwent SCN or SPCN where the sciatic nerve or L5 spinal nerve was frozen in a freeze-thaw-freeze cycle using a clinical cryoprobe. Preliminary data indicated that OX-42 like immunoreactivity peaked at 14 days in the dorsal horn and 7 days in the ventral horn after SCN using computer assisted image analysis. There was evidence of intense microglial proliferation and activation after SPCN specific to the region of lumbar innervation. IL-6 mRNA was increased in the spinal lumbar region following SPCN as compared with normal, unlesioned rats. These data indicate that cells within the spinal cord are capable of producing mRNA for cytokines in response to a peripheral nerve injury. It also suggests that microglia, the intrinsic immuneffector cell of the CNS, functions as an early sensor to nervous system injury. Interfering with microglia function and/or cytokine expression may provide a novel therapy in the prevention and/or attenuation of neuropathic pain in humans. Supported by NIH (DA10042).

342.17

STIMULATION-PRODUCED ANTINOCICEPTION FROM THE PERIAQUEDUCTAL GRAY MATTER IN NEUROPATHIC RATS. J.-H. Sohn*, B.H. Lee[1], J. Yi and S.H. Park. Dept. of Psychology, Chungnam Nat'l University, Taejeon 305-764, Dept. of Physiology, School of Medicine, Ajou University[1], Suwon 442-749, Korea.

It has been shown that there is opioid resistance in neuropathic pain. This indicates that descending opioid pain inhibition system may not be involved in neuropahtic pain. Recently we found that selective injury of the distal sciatic nerve branches produces neuropathic pain syndrome in rats. This present study sought to determine whether stimulation of descending pain inhibition system may produce analgesia in the animal neuropathic model we developed.

Under ketamine anesthesia, male Sprague-Dawley rats were chronically implanted with stimulating electrodes in the periaqueductal gray matter (PAG) and both tibial and sural nerves in the sciatic nerve were completely cut. Pain sensitivity was measured using von Frey filament and acetone applied to the sensitive area for 1 week postoperatively (p.o.). Rats with neuropathic pain syndrome after transection of the tibial and sural nerves were stimulated with bipolar electrodes in the PAG for additional 2 weeks.

Electrical stimulation of PAG was turned out highly effective in alleviating neuropathic pain. Mechanical allodynia to von Frey filament and cold allodynia to acetone were reduced by PAG stimulation.

These results suggest that activation of descending pain inhibition system including PAG can reduce neuropathic pain syndrome. (Supported by KOSEF)

PAIN MODULATION: ANATOMY AND PHYSIOLOGY—SPINAL CORD I

343.1

BIOPHYSICS OF RECEPTOR MEDIATED DENDRITIC TRANSFORMATION. V. S. Markin, T. H. Le* and D. L. Tanelian. Dept. of Anesthesiology and Pain Mgt., UT Southwestern Medical Center, Dallas, TX 75235

Stimulation of the nervous system by substance P, a G protein-coupled receptor, and subsequent receptor internalization causes dendrites to change their shape from homogeneous cylinders to a heterogeneous string of swollen varicosities (beads) connected by thin segments (Mantyh et. al., 1995). We have biophysically and mathematically analyzed this phenomenon and propose quantitative mechanisms for how dendritic transformation takes place. Our theory predicts the relationship between the initial dendrite radius and the average radii of the transformed dendritic varicosities and connecting segments, as well as the periodicity of the string of varicosities. The results of our model are in good agreement with published experimental observations of this beading phenomenon in the spinal cord and brain.

Biophysical analysis of action potential propagation along these small diameter beaded neuronal process reveals three possible outcomes: normal impulse propagation, action potential reflection, and conduction block. When action potential reflection is the prevailing condition, it can lead to both linear and nonlinear filtering of the incoming action potential train. Functionally, this process of dendritic transformation may serve as a mechanism for information processing along fine peripheral and central neuronal terminals.

Supported by the Sid W. Richardson Foundation.

343.2

MORPHINE DOES NOT REDUCE THE ENHANCEMENT OF NOXIOUS STIMULUS-EVOKED INTERNALIZATION OF THE SUBSTANCE P RECEPTOR THAT OCCURS IN SPINAL CORD OF RATS WITH PERSISTENT HINDPAW INFLAMMATION C. Abbadie*, J.A. Trafton, P.W. Mantyh and A.I. Basbaum. Dept. of Anatomy, UCSF, San Francisco and VAMC, Minneapolis.

We previously reported that noxious stimulation of the hindpaw induces internalization of the substance P receptor (SPR) in lamina I neurons of the dorsal horn and in dorsally-directed dendrites of lamina III neurons, but not in SPR immunoreactive (IR) cell bodies of lamina III-VI. Here we assessed the effect of hindpaw stimulation in rats that have persistent hindpaw inflammation, a condition associated with significant plasma membrane upregulation of the SPR. We produced hindpaw inflammation by injection of complete Freund's adjuvant (CFA). Three days later, under pentobarbital anesthesia, we stimulated the hindpaw with continuous noxious pinch, or non-noxious brush. Five min later, we perfused the rats and identified neurons with an immunofluorescence/confocal protocol. In some animals, we injected morphine (10 mg/kg s.c.) 30 min prior to stimulation. Noxious stimulation of the inflamed hindpaw produced internalization of the SPR in almost 100% of SPR-IR neurons of lamina I at the L4 segment; the extent of internalization did not vary with stimulus duration. Noxious stimulation also evoked internalization in about 50% of SPR-IR neurons in laminae III-VI of CFA rats. Even non-noxious stimulation, which is without effect in intact animals, evoked internalization, in approximately 75% of lamina I and 40% of lamina III neurons. Finally, morphine did not alter internalization evoked by noxious stimulation in CFA or intact rats. We suggest that diffusion of SP from terminals located in laminae I and II mediates the induction of SPR internalization in neurons of the deep dorsal horn by noxious stimulation of the inflamed paw. Peripheral sensitization of nociceptive afferents probably underlies the effects of non-noxious stimuli. Stimulation-evoked SPR internalization in the CFA rats may also be increased by central sensitization of dorsal horn neurons that occurs in the setting of persistent inflammation. The lack of effect of morphine argues against an opioid regulation of SP release from primary afferent nociceptors under the conditions of these experiments. Supported by INSERM (France) and NS21445 and DE23671.

343.3

ANTINOCICEPTION PRODUCED BY MICROINJECTING SUBSTANCE P NEAR THE A7 CATECHOLAMINE CELL GROUP MAY BE REDUCED BY STRUCTURAL CHANGES IN TYROSINE HYDROXYLASE IMMUNOREACTIVE DENDRITES. B.A. Heidenreich*, K. Nuseir, and H.K. Proudfit, Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60612.

Cell surface tachykinin receptors translocate to the cell interior in the presence of agonists such as substance P (SP; Mantyh et al., Science, 268, 1629, 1995). Receptor internalization is accompanied by structural changes in small dendrites which become varicose. These changes may reduce the effectiveness of SP and could explain the results of a previous report which demonstrated that the dose-response curve for the antinociception produced by microinjecting SP in the A7 cell group is attenuated at high doses (Yeomans and Proudfit, Neurosci., 49, 681, 1992).

The present experiments determined whether SP-induced structural changes in the dendrites of A7 noradrenergic neurons are related to the reduced antinociceptive effects of this peptide at high doses. More specifically, SP was microinjected in a range of doses just dorsal to the A7 cell group in lightly anesthetized rats. Antinociception was assessed using the tail flick test and structural changes in tyrosine hydroxylase-immunoreactive (TH-ir) A7 neurons were assessed by light microscopy. SP produced dose-dependent antinociception, but the antinociceptive effect of 32.5 pmol was less than that of a 3.25 pmol dose. The 32.5 pmol dose produced many varicosities in A7 cell dendrites, but the 3.25 pmol dose produced only a few dendritic varicosities. These results suggest that SP-induced receptor internalization and/or structural changes in the dendrites of A7 neurons reduces the responsiveness of these cells to SP. This work was supported by PHS grant DA03980 from the National Institute on Drug Abuse.

343.4

5-HT2 RECEPTOR IS INVOLVED IN THE NOCICEPTIVE AND HYPERALGESIC MECHANISMS OF SEROTONIN IN THE PERIPHERY. A. Tokunaga, M. Saika-Doi and E. Senba*, Dept. of Anatomy & Neurobiology, Wakayama Med. Coll., Wakayama, 640, Japan

Although it is generally believed that peripheral serotonin (5-HT) induced nociceptive responses are mediated by peripheral 5-HT3 receptors, there are still controversies. The present study was designed to investigate which subtypes of 5-HT receptors are involved in such noxious responses using c-fos expression as a marker of neuronal activation as well as behavioral assessment for hyperalgesia.

Each solution of 5-HT and various agonists for 5-HT receptor subtypes (5-HT1A, 2, 3) was intradermally injected into the rat hindpaw under light anesthesia. For the detection of Fos-like immunoreactivity (FLI) in dorsal horn neurons 2 hr after the injection, the spinal cord were immunostained using ABC method. For the behavioral assessment, paw-withdrawal latency to radial heat stimulation was examined every 15 min for 2 hrs.

Injection of 5-HT2 receptor agonists (DOI, a-methyl 5-HT) evoked FLI neurons in laminae I and II of the ipsilateral dorsal horn dose dependently, in the same manner as 5-HT did, while only a few neurons were detected after injection of other agonists including 5-HT3 receptor agonists (m-CPG, 2-methyl 5-HT) at any doses examined. Injection of 5-HT (30 μg) and 5-HT2 receptor agonist (α-methyl 5-HT; 0.86 mg/kg) significantly reduced the paw-withdrawal latency. On the other hand, injection of 5-HT3 receptor agonist (m-CPG; 8 mg/kg) showed a tendency to increase the latency. Furthermore, pre-treatment of 5-HT2 antagonist (ketanserin), but not 5-HT3 antagonist (tropisetron) reduced the expression of FLI neurons and attenuated the behavioral response after the injection of 5-HT.

This study provides morphological and pharmacological evidence to suggest that not 5-HT3, but 5-HT2 receptor subtype is involved in the 5-HT-induced activation of sensory nerve terminals and subsequently induced hyperalgesia in the rat.

343.5

SPINAL NEUROKININ2 RECEPTORS CONTRIBUTE TO THE INCREASED EXCITABILITY OF NOCICEPTIVE FLEXOR REFLEX DURING PERSISTENT PERIPHERAL INFLAMMATION. Yuping Jia* and V.S. Seybold. Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

The role of endogenous NKA in changes in the excitability of spinal neurons during adjuvant-induced, peripheral inflammation was examined by determining the effect of a selective neurokinin2 receptor antagonist, GR103537A, on the nociceptive flexor reflex.The flexor reflex was evoked in decerebrate, spinalized rats by stimulation of sural nerve with electrical current that maximally activated C-fibers. Action potentials were recorded in the ipsilateral hamstring muscles. Two days after injection of adjuvant the flexor reflex ipsilateral to the inflamed paw was enhanced two-fold compared to the reflex evoked in untreated animals, and the increased activity occurred in conjunction with thermal hyperalgesia. Intrathecal administration of GR103537A (5 nmol and 14.0 nmol in 10 µl) dose dependently attenuated the increased activity of the flexor reflex in CFA treated rats from 10-30 min after administration of antagonist, but had no effect in normal rats. The higher dose of GR103537A (14 nmol) enhanced the reflex in both groups of animals 5 min after drug injection. The preferential activity of GR103537A at NK2 receptors was confirmed by blockade of the facilitation of the reflex by NKA but not SP (7 nmol/10µl) in normal rats. These results indicate that endogenous NKA has a role in the increased excitability of spinal neurons during persistent peripheral inflammation. Supported by NIH NS17702.

343.6

RELATIONSHIPS OF NMDA, AMPA, AND METABOTROPIC TYPES OF GLUTAMATE RECEPTOR SUBUNITS TO SPINOTHALAMIC TRACT CELLS: LIGHT AND EM STUDIES. Z. Ye*, K.N. Westlund. Dept. of Neuroscience & Anatomy, UTMB, Galveston, TX 77555.

Glutamate and its receptors have been shown to play key roles in spinal nociception. Combined retrograde tracing and pre-embedding immunocytochemistry was used to localize NMDA, non-NMDA and metabotropic receptors in and on STT cells at the light and electron microscopic levels. A retrograde tracer, WGA-HRP, was injected into ventral posterolateral nucleus of thalamus of rats. After three days survival time and perfusion (2.5% glutaraldehyde, 1% paraformaldehyde, 0.1m PBS), lumber cord tissue was cut at 50µm. HRP was visualized by tetramethylbenzidine (TMB) and stabilized with ammonium paratungstate and 3,3'-diaminobenzidine hydrochloride (DAB). This procedure makes the peroxidase reaction product assume a large, dense crystalline appearance which can be easily distinguished from the amorphous peroxidase product generated in the subsequent immunostaining steps. Tissue sections were then stained for AMPA receptor subunit GluR2/3, NMDA receptor subunit GluNR1, or metabotropic receptors mGluR1α and mGluR2/3. Steps include primary antibody, biotinylated secondary antibody, avidin-biotin-peroxidase complex, and visualization with the DAB. After treatment with OsO4, embedding in epoxy resin and cutting, sections containing STT cells were examined under an electron microscope. Most of the double labeled cells were located in lamina IV, V and X. Staining for NR1 (NMDA) and R2/3 (AMPA) subunits were found in cytoplasm associated with rough ER, and postsynaptic membrane of STT cell bodies and dendrites. Staining for metabotropic receptors was associated with the membrane for the soma and proximal dendrites. The light level distribution of the mRGlu1 is concentrated in laminae I, II, and the distribution of mRGlu2/3 was in lamina III while almost devoid in laminae I and II. The postsynaptic localization for NR1 and R2/3 receptor types were found apposed by medium and large terminals with round, clear vesicles. Many NMDA positive terminals were found, particularly on STT dendrites. These data indicate that some STT cells express NMDA, AMPA, and metabotropic receptors and may receive input from excitatory amino acids. This work was supported by NS32778.

343.7

AN INCREASE IN FOS AND NITRIC OXIDE SYNTHASE EXPRESSION IN SPINOTHALAMIC NEURONS IN RATS AFTER COLON INFLAMMATION. Z. Ye, Y. Lu*, J. Wu, C.-C. Wang and K.N. Westlund. Dept. of Anatomy and Neurosciences, Univ. Texas Med. Branch, Galveston, TX 77555-1069

Immunofluorescent techniques in combination with retrograde tract tracing were used in an animal model of visceral inflammation to investigate changes in the expression of FOS and NOS in response to colon inflammation in spinal neurons, including spinothalamic tract neurons. The chemical irritant, mustard oil (2.5% in 0.5ml peanut oil), was introduced 7cm into the colon with PE90 tubing repeatedly over a one week period (2-6X). The ventral posterolateral nucleus in four rats was injected with fluorogold (3-4 µl total in 7 sites; 2-4%) and 3-4 days allowed for retrograde transport. Animals were perfused with 4% buffered paraformaldehyde and processed with standard immunofluorescent methods using rabbit anti-FOS (1:2,500; Oncogene) or anti-bNOS (1:20; Transduction Labs).

In control rats, a few cells immunoreactive for NOS were localized primarily in laminae X and no cells were immunoreactive for FOS. After colon inflammation, the number of cells in the sacral spinal cord (S1) expressing NOS increased by about 26%. Localization of NOS in spinothalamic tract cells was observed in lamina X of the sacral cord in the inflamed rat but not in the normal rat. Some spinothalamic tract cells localized in the superficial lamina I expressed FOS. NOS has not been co-localized with FOS after noxious hindlimb pinch (Lee et al., 1993). These results indicate that NOS is involved in visceral nociceptive transmission in the spinothalamic projection neurons in lamina X. The intermediate early gene, FOS, may be involved in some lamina I spinothalamic tract cells activated after prolonged colon irritation with mustard oil.

343.8

COMPARISON OF EFFECTS OF INTRADERMAL CAPSAICIN ON CELLS IN THE PRIMATE SPINAL TRIGEMINAL AND DORSAL COLUMN NUCLEI. P.M. Dougherty*, L.R. Rowland, A.T. Zirh, F.A. Lenz, and A.H. Schwartz. Dept. of Neurosurgery, Johns Hopkins University, Baltimore MD 21287-7509.

The acute and delayed responses of neurons in the *pars caudalis* of the trigeminal nucleus of the anesthetized primate (*Macaca fascicularis*, young adult, male, 4.8-8.0 kg) to intradermal injection of capsaicin (0.1ml 3%) were compared to those of neurons in the dorsal column nuclei (*N. gracilis, N. cuneatus*). A total of 22 cells have been surveyed, 13 in the dorsal column nuclei (9 in *N.g.* and 4 in *N.c*) and 9 in the spinal trigeminal nucleus. All dorsal column neurons were low threshold (LT) cells with baseline mechanical receptive fields over small areas of skin confined to the limbs best activated by innocuous stimuli. The trigeminal neurons included three types of neurons, LT, wide-dynamic range (WDR) and nociceptive specific (NS) cells. The baseline receptive fields of these cells were usually confined to an area of skin on the face in one division of the trigeminal nerve (maxillary or mandibular). Background activity was present in both groups of cells (trigeminal and lemniscal) but was widely variable in rate (0.3-22Hz).

Two dorsal column neurons showed responses to intradermal capsaicin characterized by an acute increase in discharge rate and an increase in the responses to mechanical stimulation of the receptive field. One dorsal column neuron showed evidence of an expansion in size of the receptive field. These changes following capsaicin were reversed by injection of 0.1ml 2% lidocaine to the capsaicin site. In contrast, all trigeminal WDR and 2 of 3 NS neurons but no LT cells, showed responses to capsaicin evidenced by an acute increase in discharge rate sustained over the entire post-capsaicin recording period, an increase in responses to stimulation of the RF, and an expansion in RF margins. These changes were also reversed by injection of lidocaine to the capsaicin site. Supported by a grant to PMD from the NIH (P01 NS32386-Proj. 2).

343.9

CAPSAICIN-SENSITIVE NERVES MEDIATE FORMALIN-INDUCED HYPERALGESIA AND SPINAL PROSTAGLANDIN RELEASE. N.A. Calcutt*[1] X-Y Hua[2] and A.B Malmberg[3] Depts. of Pathology[1] and Anesthesiology[2] UCSD, La Jolla, CA 92093 and Dept. of Anatomy[3] UCSF, San Francisco CA 94143.

This study was performed to investigate the contribution of capsaicin-sensitive neurons to formalin-evoked inflammation, behavior and the concurrent increase in spinal levels of amino acids and PGE_2. Adult Sprague-Dawley rats treated neonatally with capsaicin (NC) and age matched controls (C) were tested for paw thermal response latencies and then implanted with an intrathecal dialysis probe with the active site at the lumbar enlargement. Dialysis (10µl/min) was performed two days later and effluent collected in 10 min fractions. After 40 min baseline collection, 50µl of 0.5% formalin was injected into the dorsal surface of right hind paw and dialysate collected for a further 40 min along with counting of formalin-evoked flinching. NC rats showed thermal hypoalgesia (18.4±0.9sec: mean±sem) compared to C (14.2±0.6sec) and swelling of the paw following formalin injection was reduced (C=1.57±0.15mm vs NC=0.98±0.11mm). NC rats exhibited reduced flinching 0-9 min (23±2) and 20-40 min (11±4) after formalin compared to C (32±2 and 39±6 respectively). There was no change in basal or formalin-evoked levels of glutamate, aspartate citrulline or taurine, while formalin-evoked PGE_2 levels were reduced in NC rats (280±341 vs 1245±308pg/10 min) These data suggest that capsaicin-sensitive neurons contribute to formalin-induced flinching and increased spinal PGE_2 but not to formalin-evoked increases in spinal amino acids.

Supported by UCSD Academic Senate

343.10

GABA-A RECEPTOR BLOCKADE INHIBITS CAPSAICIN-INDUCED BRUSH RESPONSES OF NOCICEPTOR-SPECIFIC (NS) NEURONS: A ROLE FOR PRIMARY AFFERENT DEPOLARISATION IN ALLODYNIA ?

Jennifer M.A. Laird* and Fernando Cervero. Dept. of Physiology and Pharmacology, University of Alcalá de Henares, 28871 Madrid, Spain.

In areas of secondary hyperalgesia, innocuous mechanical stimuli evoke pain (allodynia). We have proposed that this is produced by a central pre-synaptic interaction whereby Aβ-fibers evoke activity (dorsal root reflexes) in nociceptive afferent C-fibers (Cervero & Laird 1996 *NeuroReport* **7**, 526). Here, we have tested this hypothesis by examining the effect of $GABA_A$ receptor antagonism on the responses to mechanical stimuli of NS spinal dorsal horn neurons after capsaicin injections in their receptive fields (RF).

NS neurons were recorded in the lumbar spinal cord of anaesthetised adult female Wistar rats. The RF was mapped, and the responses to 20s periods of brush, cotton bud, pinch and heat were tested. Capsaicin (50 µg) was injected intradermally in the RF, and excited 80% of neurons. Capsaicin-responsive neurons showed increased pinch responses (152 ± 37 to 358 ± 155 spikes) and novel responses to brush and cotton bud. Picrotoxin (0.5 mg/kg i.v.) reversed the enhanced pinch responses and attenuated the brush (35 ± 9 to 11 ± 5 spikes) and cotton bud responses (45 ± 8 to 10 ± 7 spikes). In NS neurons with no capsaicin response, picrotoxin produced small increases in pinch responses and in some cases, in RF area. In contrast, strychnine (3 mg/kg) enhanced the capsaicin-induced changes.

We conclude that the capsaicin-induced changes in the mechanical responses of dorsal horn neurons involve $GABA_A$ receptors. These results support our hypothesis that allodynia is mediated by pre-synaptic interactions whereby Aβ-fiber activation excites nociceptive afferent C-fibers.

(Supported by DGICYT grant PB-93-0491 and by Boehringer Ingelheim).

343.11

Aβ-FIBRE EVOKED WIND-UP OF WITHDRAWAL REFLEXES IN MONOARTHRITIC RATS: INVOLVEMENT OF $GABA_A$ RECEPTORS.

H.-R. Weng*[1], J.M.A. Laird[2], F. Cervero[2] and J. Schouenborg[1]. Dept. of Physiology and Neuroscience, University of Lund, Lund, Sweden[(1)] and Dept. of Physiology and Pharmacology, University of Alcalá de Henares, Madrid, Spain[(2)].

Activation of Aβ afferent fibres from areas of secondary hyperalgesia evokes pain (allodynia). This is due to a change in the central processing of inputs from low threshold afferents whereby their activity gains access to nociceptive systems. Here we have examined whether a process normally evoked only by afferent C-fibres (wind up of withdrawal reflexes) could also be triggered by Aβ afferents in arthritic animals.

We have recorded, in decerebrate-spinal rats, multiunit e.m.g. activity in the semitendinosus and peroneal muscles evoked by electrical stimulation of A-fibres in the sural nerve. The nerve volley was monitored continuously to ensure that only A fibres were activated. Responses were compared in control and monoarthritic rats (induced at least 4 hours before recording by injection of carrageenan and kaolin into the ipsilateral knee joint). Reflex wind up was examined by delivering trains of 16 stimuli to the sural nerve at 0.1 to 1Hz.

In control rats, wind up of the reflexes was never seen when afferent A-fibres were stimulated. In contrast, wind up could be evoked in monoarthritic animals by activation of Aβ afferents at 0.3 to 1Hz. Bicuculline (0.03-0.5 mg/kg i.v.) dose-dependently inhibited this A-fibre evoked wind up.

These results indicate that in arthritic rats, Aβ fibres gain access to spinal cord mechanisms normally activated only by C-fibres (such as wind up). The sensitivity of this process to $GABA_A$ receptor blockade suggests a role for primary afferent depolarization via a presynaptic link between A and C-fibres.

(Supported by MRC, Sweden, and by DGICYT, Spain).

343.12

VISCERAL PAIN AND HYPERALGESIA IN RATS WITH AN EXPERIMENTAL URETERIC STONE: PERIPHERAL AND SPINAL MECHANISMS

Carolina Roza, Jennifer M.A. Laird & Fernando Cervero*. Dept. of Physiology & Pharmacology, University of Alcalá de Henares, 28871 Madrid, Spain

Ureteric calculosis evokes intense pain and referred hyperalgesia in man. A similar syndrome is seen in rats with an artificial calculosis (Giamberardino *et al* 1995 *Pain* **61**, 459). Here we have investigated the underlying mechanisms.

An artificial stone was implanted in the left ureter in adult female rats under halothane anaesthesia. After 1-8 days the rats were reanaesthetised, the left ureter cannulated close to the bladder and its motility recorded as changes in intraureteric pressure. The amplitude of ureteric contractions was larger than in normal rats, reaching 25 - 30 mmHg (normal nociceptive threshold), even in rats in which the stone had been eliminated. In 17 rats the response of single dorsal horn neurons in the T13-L1 spinal cord to noxious distension of ureter (UD; 30s, 10-100 mmHg) was also tested. In rats with a stone, the proportion of neurons with ureteric input was higher than in control rats (52% vs. 42%), and 68% of them showed changes in their cutaneous receptive field area after UD. In rats with a stone, 3 of 28 neurons with ureteric input were low threshold (class 1) and 2 of them also showed changes in their input properties after UD. In contrast, none of the neurons with ureteric input in control rats were class 1 and none showed changes in their input properties after UD.

We conclude that spinal neurons processing ureteric input are more excitable during and after the passage of a stone as shown by the greater proportion excited by ureteric stimulation and increased numbers with low threshold cutaneous inputs. The hypermotility of the ureter evoked by the stone, which persists after it has passed, is a peripheral event that contributes to maintaining this increase in central excitability.

(Supported by DGICYT grant PB-93-0491 and by Boehringer Ingelheim).

343.13

CEREBRAL C-FOS EXPRESSION IN UNANAESTHETIZED RATS EXPERIENCING CAPSAICIN-INDUCED ACUTE CARDIAC PAIN.

G.J. Ter Horst,* B.J. Arkies, F. Postema, R.W.M. Hautvast and M.J.L. De Jongste. Biol. Psychiat. & Cardiol., Univ. & Acad. Hospital Groningen, The Netherlands.

Spinal cord stimulation in angina pectoris increases exercise capacity and reduces both anginal attacks and ischemic electrocardiographic signs, but the underlying mechanisms are not understood. To show effects of neurostimulation in the brain we need an acute cardiac pain rat model. The aim of this study is to validate a rat model that is based on infusion of capsaicin (45 µl, 0.3 ng/µl; 2 µl/min.) into the pericardial space of freely moving rats. Cerebral c-fos expression was employed to analyze the effects of cardiac pain in the brain. The pericardial space of male Wistar rats was catheterized via the diaphragm. The catheter was fixed to the head to allow easy access. The rats were allowed post-operative survival times of 3 days. Solvent infusions into the pericardial space and capsaicin infusions into the thoracic cage served as controls. During the infusion of capsaicin into the pericardial space the animals scratched the chest and then they immobilized for 15 - 20 min. Two hours after the infusion the animals were fixed to allow immunocytochemical detection of c-fos like proteins in brain and spinal cord sections. Increased c-fos expression was found in laminae I and II of the thoracic spinal cord, the solitary tract nucleus, the peri-ambiguus area, the parabrachial nucleus, the peri-aqueductal gray, the dorsal thalamus, the hypothalamus, the central amygdala and the insular and prelimbic cortices. Controls exhibited significantly less c-fos expression. Many of the affected regions participate in cardiovascular regulation. The behavioral and immunocytochemical data show that the model can be used to induce acute cardiac pain in unanaesthetized rats and may serve as a model to study effects of neurostimulation in the brain.

343.14

KNEE JOINT INFLAMMATION: SPINAL C-FOS EXPRESSION AND THE EFFECT OF SECONDARY NOCICEPTIVE STIMULATION.

[1]**L.Ch. Yang***, [2]**J. Orendacova**, [3]**M. Marsala**, [3]**T. L.Yaksh** [1]Department of Anesthesiology, Chang Gung Memorial Hospital, Taiwan, [2]Institute of Neurobiology, Kosice, Slovakia, [3]Anesthesiology Res. Lab., Univ. of California San Diego, CA 92093-0818

In the present study, the spinal expression of c-fos products after knee joint inflammation and the effect of a superimposed secondary nociceptive stimulus (ipsilateral paw formalin injection) was studied. In male Sprague-Dawley rats after induction with 1.5% halothane the right knee joint was injected with 3% kaolin/carrageenan mixture (C/K). Animals then survived for 1, 2, 6, or 24 hrs. In a separate group of animals, as a subsequent stimulus, formalin (50µl/5%) was injected subcutaneously into the dorsum of the paw ipsilateral to the C/K injection 4 hrs after initial C/K injection. After formaline injection animals survived for an additional 2 hrs. After perfusion fixation with 4% paraformaldehyde, frozen transverse sections (40µm) were prepared and processed for the presence of c-fos-protein-like immunoreactivity. Carrageenan-induced knee joint inflammation evoked a transient c-FOS protein expression in the neurons localized in the apical region of laminae I-III with the peak activity observed 2 hrs after inflammation (12±3 cells; mean±sd). No c-Fos-positive neurons after longer (i.e. 6 or 24 hrs) survival periods were detected. Ipsilateral paw formalin injection, if administered 4 hr after knee inflammation, evoked significantly fewer c-FOS positive neurons in laminae I-V as compared with formalin injected animals without knee joint inflammation (46±15 cells vs. 120±8). These data indicate that primary acute or subacute nociceptive input may evoke central processes that are characterized by an induceable form of central inhibition. (This work was supported by NIH NS32794, M.M.)

343.15

NALOXONE INCREASES *C-FOS* EXPRESSION IN THE SPINAL TRIGEMINAL NUCLEUS FOLLOWING OROFACIAL INFLAMMATION. Y.-O. Lee[1]* and A. I. Beitz[2]. Graduate Programs in Oral Biology[1] and Neuroscience[2], University of Minnesota, St. Paul, MN 55108.

We have utilized an animal model of orofacial inflammation (Haas *et al.*, 1992) to assess the effect of the opioid receptor antagonist, naloxone, on the level of c-Fos expresssion in the spinal trigeminal nucleus (STN) (Hathaway et al., 1995). Orofacial inflammation was induced by paraperiosteal injection of mustard oil (10 µl of 20 % allyl isothiocyanate in mineral oil: Aldrich Chemicals Co.) into the periarticular temporomandibular joint (TMJ) tissue of anesthetized rats.

Sixteen male and 16 female Sprague-Dawley rats were randomly assigned into one of the following four groups: 1) naloxone and TMJ mustard oil (MO) injection group, 2) saline and TMJ/MO group, 3) naloxone and TMJ saline (SAL) injection group, and 4) saline and TMJ/SAL group. Naloxone or saline was given *i.p.* 10 min before TMJ injection and again every 20 min after the initial injection until sacrifice. Two hours after injection of mustard oil or saline into the TMJ, animals were perfused transcardially with 4 % paraformaldehyde and subsequently processed for immunocytochemical staining using an antiserum against c-Fos (Santa Cruz Biotechnology, Inc.). Additional animals with only peritoneal injections and animals with anesthesia only were processed in parallel to rule out any effect of repeated *i.p.* injection or anesthesia.

There was a statistically significant effect of treatment (naloxone *vs* saline) ($p<0.01$). The naloxone group contained a greater number of c-Fos labeled neurons in the STN subnucleus caudalis than the saline control group after TMJ injection of mustard oil. There was also a statistically significant effect of gender (male *vs* female) ($p<0.01$). The female TMJ/MO group exhibited a greater number of c-Fos labeled neurons than the male TMJ/MO group. It is likely that the endogenous opioid system is active during orofacial inflammation induced by injection of mustard oil into the TMJ. In addition, there seems to be a gender difference in the response of cells in the STN to the injection of mustard oil into the TMJ. (Supported by NIH grant DE06682 & NS31318)

343.16

CORNEAL STIMULATION BY MUSTARD OIL: EFFECTS OF NK1 AND NK2 RECEPTOR ANTAGONISM ON FOS-LI WITHIN SPINAL TRIGEMINAL NUCLEUS, ON PLASMA ACTH AND ON AUTONOMIC RESPONSES IN THE RAT. D.F. Bereiter, D.A. Bereiter, B.H. Tonnessen and D.B. MacLean*. Depts. of Neuroscience & Surgery, Brown Univ./RI Hospital, Providence, RI 02903 and Pfizer Central Research, Groton, CT 06340

The cornea is well-supplied by afferent nerves that contain neuropeptides. To assess the role of neuropeptide receptors in mediating the responses to corneal stimulation, chloralose-anesthetized male rats were pretreated with the NK1 antagonist, CP99,994 (0.1-50 nmol icv), or the NK2 antagonist, MEN10,376 (0.01-1 nmol icv) 20 min prior to topical application of mustard oil (5 µl, 20% sol.). After 2h survival, brainstems were processed for Fos-like immunoreactivity (Fos-LI). Fos-LI increased ($P<0.01$) in two distinct transition regions of the spinal trigeminal nucleus (Vi/Vc and Vc/C1) in saline controls and after the inactive NK1 enantiomer, CP100,263. The highest dose of CP99,994 reduced Fos-LI ($P<0.05$) among neurons in laminae I-II at Vc/C1, but had no effect at Vi/Vc. NK2 antagonism caused a significant dose-related decrease in Fos-LI ($P<0.001$) in laminae I-II at Vc/C1 and had no effect at Vi/Vc. In parallel experiments, arterial plasma ACTH, blood pressure and heart rate were assessed after corneal stimulation. Both NK1 and NK2 antagonism blocked the plasma ACTH response to corneal stimulation, but neither drug affected the plasma ACTH response to hemorrhage. Neither the NK1 nor NK2 antagonist altered cornea-evoked blood pressure or heart rate responses. These results suggest that multiple parallel pathways control the pituitary and autonomic responses to corneal stimulation. Both NK1 and NK2 receptors act afferent to the hypothalamus to mediate an increase plasma ACTH, whereas neither receptor subtype is necessary for the cardiovascular responses to corneal stimulation. NK1 receptor activation has only minor effects on expression of c-fos in central trigeminal neurons after selective small fiber irritant stimulation of the cornea, whereas NK2 receptors contribute only to Fos produced in the most caudal portions of the spinal trigeminal nucleus. Supported by RI Hospital.

343.17

Dose dependent changes in behaviour and trigeminal c-fos expression after intracisternal capsaicin infusion.

R.H.A. Kemper, M.B. Spoelstra, L. Vosmeijer, M.G. Postma, M.J.L. De Jongste*, W.J. Meyler, G.J. Ter Horst. *Biol. Psychiat., Anaesthesiol. & Cardiol., Academic Hospital Groningen, The Netherlands*

The mechanisms causing migraine are still under debate. Animal models therefore focus on direct stimulation of the principal afferent nerve system of the head: the trigeminal nerve system. We demonstrate c-fos expression in the trigeminal nucleus caudalis, lamina I and II_0 (TNC) and behavioural data caused by capsaicin infusion into the cisterna magna (CM) of unanaesthetised rats. All rats received a cannula that was inserted into the CM via the cranium. After 3 days, capsaicin or vehicle solution was infused over a 2 minutes period. Behaviour was recorded on video tape. Two hours after infusion, rats were perfused and brain sections were stained for c-fos. Expression of c-fos was quantified at levels -1,-2,-3,-4,-5 and -6 mm. from obex and averaged.

	conc. (nM)	amount (ul)	n	cfos TNC I,II (cells/section)	exploring (seconds)	head grooming (seconds)	head scratching (seconds)	head immobilisation (seconds)
Vehicle	0	100	5	10,3 ± 2,1	112,4 ± 4,5	0,0 ± 0,0	0,0 ± 0,0	4,0 ± 3,8
Capsaicin	10	100	6	11,3 ± 1,9	92,7 ± 5,7	0,2 ± 0,2	1,8 ± 1,8	26,8 ± 5,9
Capsaicin	100	100	10	55,7 ± 18,5	80,2 ± 6,1*	4,9 ± 3,4	2,1 ± 1,4	33,8 ± 7,1*
Capsaicin	1000	100	5	723,2 ± 63,0**	48,8 ± 6,7**	16,8 ± 4,3*	17,4 ± 10,5	10,0 ± 1,8**
Capsaicin	100	150	5	162,1 ± 40,3**	71,2 ± 9,1	10,8 ± 4,7	0,0 ± 0,0	44,4 ± 6,1

*P<0,05 compared to vehicle **p<0,05 compared to Capsaicin 100nM (100 ul) - One Way Anova

Capsaicin infusion in the cisterna magna of freely moving rats dose-dependently induces c-fos expression in the TNC. Exploring behaviour dose dependently decreases and rats predominantly demonstrate passive coping behaviour (head immobilisation) at lower capsaicin concentrations and active coping behaviour (head grooming/scratching) at the highest concentration. *Supported by Glaxo-Wellcome*

343.18

L5 SPINAL NERVE INJURY INDUCES RAPID SPROUTING OF A-FIBER PRIMARY AFFERENTS INTO SUBSTANTIA GELATINOSA OF THE ADULT RAT SPINAL CORD. P. Shortland[1], E. Kinnman[2]* & C. Molander[1,2]. Depts. of (1) Neuroscience & (2) Dept. of Surgery, Divn. of Rehabilitation, Neurogenic Pain Unit, Karolinska Institutet, Stockholm, Sweden.

Recently, an animal model has been developed for the study of neuropathic pain mechanisms that involves the section or ligation of the spinal nerves (Kim & Chung, '92, Pain 50:355). These rats show behaviour interpreted as spontaneous pain, allodynia & hyperalgesia. We have shown previously that A-fibers sprout into lamina II after spinal nerve section (Shortland et al., '95 Neurosci. Abstr. 21:356.13). We have now examined the time-course of spinal nerve section-induced sprouting in adult male rats. BHRP was injected into the L5 DRG (to label injured afferents). The contralateral, uninjured L5 DRG, used for control injections, showed labelling in laminae I and III-V, leaving lamina II devoid of labelling. Changes in the laminar distribution of A-fibers began 24 hours following spinal nerve section. Labelling was seen throughout laminae I-V in the L4-5 dorsal horn by 3 days post-injury. The results indicate that injured Aß afferents begin sprouting into lamina II very shortly after nerve injury. The changes in the laminar distribution after spinal nerve injury occur with a similar time-course as changes in mechanical hypersensitivity (Kinnman & Levine, '95, Neurosci.64:751) suggesting that injured Aß fibers may directly contribute to the state of central sensitisation which gives rise to mechanical hypersensitivity. Supported by Åke Wibergs stiftelse.

PAIN MODULATION: ANATOMY AND PHYSIOLOGY—SPINAL CORD II

344.1

CENTRALLY ADMINISTERED MORPHINE SUPPRESSES C-FOS EXPRESSION FOLLOWING A NOXIOUS THERMAL STIMULUS IN THE DEVELOPING RAT. D.K. Yi* and G.A.Barr, Biopsychology Doctoral Program, Dept. of Psychology, Hunter College-CUNY, NY, NY 10021and New York State Psychiatric Institute, 722 W. 168th Street, NY, NY 10032.

Mechanisms involved in antinociception are not well understood in the developing animal. In this study, morphine-induced analgesia following a noxious thermal stimulus in the neonatal rat was examined using behavior, and the expression of the c-*fos* gene as a marker for neuronal activity. Three- and 14 - day old rat pups were given morphine (0, 0.1, 0.3, 3.0, and 10 µg) intracerebroventrically 15 minutes prior to the application the stimulus. The rat's forepaw or the hindpaw was immersed in a hot water bath (52° C), allowing the pups to withdraw the paw. If the withdrawl latencies were less than 5 seconds, then the paw was held in the water bath for a total of 5 seconds. The pups were tested in this manner every 5 minutes for 20 minutes, and were sacrificed 2 hours after the end of the testing session. Either the cervical or the lumbar cord was processed for Fos immunohistochemistry. The behavioral results showed that at both ages the forepaw was analgesic in the thermal test, with the 3-day-old pups being more analgesic than the 14-day-old animals. In the hindpaw, however, no analgesia was observed at either age at any morphine dose. Anatomically, morphine suppressed the Fos immunoreactivity in both the cervical and the lumbar spinal cord, reducing the number of Fos labeled nuclei in the cervical cord in a dose dependent manner at the two ages. The Fos labeled nuclei in the superficial layer were more resistent to morphine than were in the deeper layers in the 14-day-old lumbar cord. Although there was a positive correlation between behavior and Fos immunoreactivity when the forepaw was tested, in the hindpaw the suppression of Fos appearance occurred in the absence of analgesia. It is speculated that mechanisms of morphine-induced analgesia are different in developing animals than adults, and that morphine may induce analgesia using different mechanisms at different segmental levels in the maturing animal. (Supported in part by DA07646).

344.2

THE TIME-COURSE OF Aß-EVOKED C-FOS EXPRESSION IN NEURONS IN THE DORSAL HORN AND GRACILE NUCLEUS AFTER PERIPHERAL NERVE INJURY. C. Molander[1,2]* & P. Shortland[1]. Depts. of (1) Neuroscience, & (2) Surgery, Divn. of Rehab. & Phys. Med. Karolinska Inst. Stockholm, Sweden.

Peripheral nerve injury alters the laminar distribution of myelinated and unmyelinated primary afferents in the spinal cord. It also alters the expression of c-fos in dorsal horn cells. Normally, only noxious stimulation is effective in producing fos protein in second order cells. After injury, however, low-threshold electrical stimulation can evoke fos expression in the dorsal horn and gracile nucleus (GN). Here we have investigated the time-course of this abnormal expression. Electrical stimulation of the sciatic nerve at Aß-fiber strength (0.1mA, 0.5ms, 5Hz, 10 mins) was performed 3 days to 1 year following either sciatic nerve section or sciatic nerve crush in adult rats. Stimulation of the intact contralateral sciatic nerve was made for comparison. Stimulation of the normal sciatic nerve at Aß strength resulted in no labeled neurons in the dorsal horn or GN. Stimulation of the crushed sciatic nerve resulted in c-fos expression in cells in laminae I-V of the dorsal horn and GN only from 1-3 weeks post injury. Stimulation after sciatic section also resulted in fos expression in the dorsal horn after 1 week and this expression persisted at all timepoints studied. In the GN fos expressing cells were seen at 1-4 weeks but not at longer survival times. The results indicate that abnormal c-fos expression may be correlated with the onset of Aß sprouting which occurs after nerve injury. When nerve regeneration is successful, Aß-evoked fos expression returns to normal in both the cord and GN. When regeneration is prevented then Aß-evoked fos persists in the dorsal horn but not in the GN. This suggests that (1) abnormal fos expression in the dorsal horn may be regulated by the loss of some retrograde signal from the periphery and (2) abnormal fos expression in the GN may be under some other control since its expression is independent of whether or not regeneration occurs. Supported by Tore Nilsons fond.

344.3

CHEMICAL CYSTITIS INDUCED FOS PROTEIN EXPRESSION IN ADULT RAT BRAIN: EFFECTS OF CAPSAICIN AND SPINAL CORD INJURY. M.A. Vizzard*, Univ. of Vermont, Depts. of Neurol./Anat. and Neurobiol., Burlington, VT.

Immunohistochemical detection of the protein product (Fos) of the c-fos immediate early gene was used to study neuronal activation in the brain of unanesthetized rats following induction of chemical cystitis by cyclophosphamide (CYP). CYP is metabolized to acrolein, an irritant eliminated in the urine. Injection of CYP (n=10; 75 mg/kg, i.p.) three hrs prior to euthanasia significantly increased Fos expression in a variety of supraspinal sites, including: the pontine micturition center, locus coeruleus, subcoeruleus, parabrachial nucleus, ventrolateral periaqueductal gray, A5, A7, ventrolateral medulla, hypothalamus and visceral regions in the cerebral cortex. In vehicle-treated animals (n=10), Fos expression was minimal except in the inferior colliculus where the greatest numbers of Fos-immunoreactive cells were present. To determine if the Fos expression was mediated by A-delta and/or C-fiber bladder afferents, animals were pretreated with capsaicin (Cap), a C-fiber neurotoxin (20 mg/ml; 125 mg/kg; s.c.; n=5). Control animals (n=5) received a corresponding volume of vehicle. Three to four days after the last Cap injection, animals were treated with CYP. To check the effectiveness of Cap pretreatment, an eye-wipe test was conducted before CYP injection. In control animals, the number of defensive eye-wiping movements induced by topical application of Cap solution (100 µg/ml) to the eye averaged 29.5±1.3 in 2 min. No eye-wiping movements were detected in Cap-pretreated animals. In both Cap and control (vehicle-treated) animals, CYP increased Fos expression in the same supraspinal sites as observed in untreated (no Cap or vehicle) animals injected with CYP. To determine if CYP-induced Fos expression in the brain was mediated by spinal pathways, CYP was injected in chronic (3-4 week) spinal cord transected (SCT; T10) animals (n=3). CYP injection in SCT animals did not elicit Fos protein in any supraspinal site. These results demonstrate: (1) CYP-induced chemical cystitis elicits Fos expression in supraspinal sites previously identified as playing a role in bladder function; (2) CYP-induced Fos expression in brain is mediated by spinal pathways originating below T10; (3) CYP sensitive, bladder A-delta fibers play a role in CYP-induced Fos expression in brain. [PVA-SCRF (1461-01), APA (VB1-9402-1), ICA]

344.4

NMDA AND 5-HT2 RECEPTOR ANTAGONISTS REDUCE FOS-LI WITHIN SPINAL TRIGEMINAL NUCLEUS AFTER INFLAMMATION OF THE TMJ REGION IN THE RAT. D.A. Bereiter*, J.F. Crary and S.L. Masia. Depts. of Neuroscience & Surgery, Brown Univ./RI Hospital, Providence, RI 02903

Injury of the temporomandibular joint (TMJ) region produces persistent pain that is poorly localized. The neural basis for TMJ pain is not well defined, but glutamatergic and serotonergic mechanisms may be involved. To assess the role of NMDA and 5-HT2 receptors in activation of central trigeminal neurons after inflammation of the TMJ region, barbiturate-anesthetized male rats were pretreated with the NMDA antagonist, MK-801 (0.02-2 mg/kg iv), or the 5-HT2 antagonist, ketanserin (0.03-3 mg/kg iv) 15 min prior to mustard oil (25 µl, 20% sol.) injection into the TMJ region. After 2h survival, brainstems were processed for Fos-like immunoreactivity (Fos-LI) and neural activation was estimated from the number of Fos-positive neurons in spinal trigeminal n. (Vsp). Fos-LI was found mainly at two distinct transition regions of Vsp (subnucleus interpolaris/caudalis, Vi/Vc and caudalis/upper cervical cord, Vc/C1) after inflammation. MK-801 and ketanserin affected differentially the magnitude and pattern of Fos-LI produced within the Vsp. MK-801 caused a dose-related decrease in Fos-LI among laminae I-II neurons at the ipsilateral Vc/C1 transition (-5 to -6.5mm to obex) and at mid-caudalis levels (-2 to -3.5mm to obex) with high dose causing 71±5% and 78±10% reduction, respectively, vs saline, (P<0.01). Ketanserin did not affect the number of Fos-positive neurons in these caudal regions. By contrast, at periobex levels of Vsp, all doses of ketanserin reduced Fos-LI (P<0.01) bilaterally at the Vi/Vc transition (Vi/Vc-DM, Vi/Vc-VL, and Pa5 regions, +0.5 to -1mm to obex), whereas only higher doses of MK-801 reduced Fos-LI in these regions. These results suggest that NMDA receptors preferentially contribute to activation of neurons in the most caudal portions of Vsp, whereas 5-HT2 receptors selectively affect activation of neurons at the periobex levels of Vsp after inflammation of the TMJ region. Supported by RI Hospital.

344.5

EVOKED ACTIVITY IN NOCICEPTIVE DORSAL HORN NEURONES AFTER 5-HT_{1A} AND 5-HT_{1B} RECEPTOR STIMULATION. J. Gjerstad, A. Tjølsen* and K. Hole. Department of Physiology, University of Bergen, Årstadveien 19, 5009 Bergen, Norway.

The role of spinal 5-HT_{1A} and 5-HT_{1B} subreceptors in regulation of nociception was investigated. Extracellular recordings were made from single WDR neurones in spinal laminae III-VI of intact anaesthetized rats. The skin was stimulated electrically (16 pulses, 0.5 Hz) at 1.5 x threshold for C-fibre response (1.95-4.50 mA). The Aβ-, Aδ- and C-fibre responses and post-discharge were recorded. The 5-HT_{1A} agonist 8-OH-DPAT, the 5-HT_{1A} antagonist WAY100635 and the 5-HT_{1B} agonist CP93,129 were applied directly onto the spinal cord. 8-OH-DPAT (50-500 nmol) mainly affected the Aδ- and C-fibre responses and the post-discharge. C-fibre response was reduced in 71%, increased in 6% and little affected in 23% of the cells. The inhibition was weaker with increasing control C-fibre response, and increased with increasing wind-up in the control measurements ($p<0.001$). The inhibitory effect of 8-OH-DPAT was totally blocked by coadministration of WAY100635 (50 nmol). CP93,129 (50 nmol) inhibited the Aδ-fibre response to approximately 60 % of the control response.

In conclusion, the effect of spinal 5-HT_{1A} receptor stimulation was mainly an inhibition of the response to Aδ- and C-fibre stimulation. This effect seems to be related to the wind-up properties of highly convergent neurones. 5-HT_{1B} receptor stimulation specifically inhibited the response to Aδ-fibre stimulation. The effects of 5HT_1 receptor stimulation may be partly due to presynaptic mechanisms.

Supp. by the Norwegian Res. Council and the European Commission.

344.6

TYROSINE HYDROXYLASE- AND SEROTONIN-IMMUNOREACTIVE VARICOSITIES APPOSE PROPRIOSPINAL NEURONS IN THE RAT C_1-C_2 SPINAL SEGMENTS. K.E. Miller*, M.J. Chandler, and R.D. Foreman. Dept. Anatomical Sciences, Dept. Physiology & Biophysics, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

Physiological and anatomical studies from our laboratories have demonstrated that neurons in upper cervical spinal segments have descending projections to thoracic and lumbar spinal cord segments. Many of these propriospinal neurons appear to mediate inhibition of thoracic and lumbar dorsal horn neurons produced from somatic and visceral afferent input. In order to further understand the neurochemical circuitry involved in regulating or modulating this propriospinal system, the present study examined if norepinephrine or serotonin fibers contacted upper cervical, propriospinal neurons. Fluorogold was injected into the rat lumbosacral spinal cord to retrogradely label propriospinal neurons in the C_1-C_2 spinal segments. Following retrograde transport, tissue sections from the C_1-C_2 segments were processed for tyrosine hydroxylase (TH) or serotonin (5-HT) immunofluorescence. Retrogradely labeled neurons were abundant on both ipsilateral and contralateral sides in the lateral cervical and spinal nuclei, nucleus proprius, ventral horn, and the central gray region (lamina X). Many retrogradely labeled neurons were apposed by TH- and 5-HT-immunoreactive fibers. We conclude from these results that descending monoaminergic pathways from the brainstem are situated anatomically to modulate the activity of C_1-C_2 propriospinal neurons. These brainstem pathways could influence the propriospinal mediation of spinal neurons in lower spinal segments. Supported by NIH#27213 (KEM), NIH#22732 (RDF) and Presbyterian Health Foundation.

344.7

AUTORADIOGRAPHIC EVIDENCE THAT m4 MUSCARINIC RECEPTORS MODULATE NOCICEPTION IN THE HUMAN SPINAL CORD. MT Borenstein, M Santiago, HH Valentine and LT Potter*, Molecular and Cellular Pharmacology, U of Miami School of Medicine, P.O. Box 016189, Miami, FL.

Three lines of evidence indicate that M1 (m1 and/or m4) receptors are involved in the modulation of nociception. (1) Autoradiography with 20 nM ^{3}H-pirenzepine demonstrates that M1 receptors are concentrated in lamina II (substantia gelatinosa) of the human spinal cord (Villiger and Faull, Brain Res 345,196,1985). (2) Muscarinic agonists and esterase inhibitors administered into the lumbar subarachnoid space in animals cause a dose-dependent increase in the latency of hot-plate and tail-flick responses. Antinociceptive effects are reduced by atropine and pirenzepine, but not by naloxone, strychnine, picrotoxin, curare or methysergide and phentolamine (Yaksh *et al*, Eur J Pharmacol 117,81,1985; Iwamoto and Marion, JPET 266,329,1993). (3) A new orally-active muscarinic agent developed by Novo Nordisk and Eli Lilly is as effective as, but 10-40 times more potent than morphine in reducing nociception in animals, and analgesia does not appear to be mediated by m1 or M3 receptors (Sauerberg *et al*, Life Sciences 56,807,1995). To go further, we have utilized m1-toxin and m4-toxin to delineate the subtypes of muscarinic receptors most prominent in the human spinal cord. Sections of fresh lumbar cord were prepared in a cryostat, exposed to radioligands, washed, dried and apposed to film. Studies with one nM ^{3}H-N-methylscopolamine confirmed the work of many previous investigators showing muscarinic receptors in lamina II. m4-Toxin markedly reduced this binding, whereas m1-toxin reduced binding slightly. ^{125}I-m4-Toxin was prepared by a chloramine-T method. Studies with this ligand firmly establish a dense concentration of m4 receptors in lamina II. [Supported by AG 06170].

344.8

AN NMDA RECEPTOR ANTAGONIST BLOCKS THE INCREASE IN [^{3}H]-PHORBOL 12,13-DIBUTYRATE BINDING IN SPINAL CORD DORSAL HORN INDUCED BY NOXIOUS CHEMICAL STIMULATION. T.J. Coderre* and K. Yashpal. Pain Mechanisms Lab, Clinical Research Institute of Montreal, 110 Pine Ave. West, Montreal, QC, Canada, H2W 1R7.

We have previously suggested that both spinal excitatory amino acids (EAAs) and protein kinase C (PKC) contribute to persistent pain in the formalin test. The present study examines the interactive effects of EAAs and PKC by determining the effects of an NMDA antagonist, MK-801, on the increase in membrane-associated PKC in spinal cord dorsal horn induced by injection of formalin into the rat hind paw. Changes in the levels of membrane-associated PKC, as assayed by quantitative autoradiography of the specific binding of ^{3}H-phorbol-12,13-dibutyrate (^{3}H-PDBU) in lumbar spinal cord sections, were assessed in rats given a hind paw injection of 50 µl of 2.5% formalin, following intrathecal (i.t.) treatment with MK-801 (150 nmoles) or vehicle. The specificity of the assay, at measuring noxious stimulus-induced changes in membrane-associated PKC, was also assessed by determining the effects of GM_1 ganglioside (80 nmoles, i.t.), which inhibits the translocation of PKC, on formalin-induced [^{3}H]-PDBU binding in spinal dorsal horn. Intrathecal treatment with either MK-801 or GM_1 ganglioside produced a significant suppression of the increases in [^{3}H]-PDBU binding in spinal dorsal horn induced by hind paw injection of formalin. The data provide support for a role of NMDA receptors in noxious stimulus-induced translocation of PKC in spinal cord dorsal horn (*Supported by an MRC of Canada grant to T.J.C.*).

344.9

PROLONGED DECREASE OF SUBSTANCE P BINDING IN RAT SPINAL CORD DORSAL HORN INDUCED BY NOXIOUS CHEMICAL STIMULATION OF THE HIND PAW WITH FORMALIN. K. Yashpal*, J.L. Henry and T.J. Coderre. Pain Mechanisms Laboratory, Clinical Research Institute of Montreal, and Department of Physiology, McGill University, Montreal, Quebec, Canada, H2W 1R7.

Previous data (Yashpal et al., *NeuroReport* 5:2101; 1994) indicate that noxious thermal stimulation leads to a brief decrease in [^{125}I]BH-substance P binding in spinal cord dorsal horn. It was suggested that the decreased binding was due to occupation of receptors by endogenous ligand, following a noxious stimulus-evoked release of substance P. The present study used a similar technique to examine whether the persistent nociceptive responses to hind paw formalin injection are associated with a prolonged release of substance P in the spinal cord in rats. Rats were decapitated either 5, 25 or 60 min following an injection of 50 µl of either 1% or 5% formalin into the plantar surface of the hindpaw, and 20 µm sections from the lumbar spinal cord were incubated with [^{125}I]BH-substance P. Compared with unstimulated control rats, substance P binding was significantly reduced in rats given hind paw injections of formalin. Decreased substance P binding was most pronounced in the superficial dorsal horn. Both 1% and 5% formalin injections produced significant decreases in substance P binding at each of the three measured time points. Assuming that decreased radioligand binding reflects increased competition by endogenous ligand, these data suggest that both low (1%) and high (5%) concentrations of formalin produce a prolonged release of substance P within the dorsal horn of the spinal cord. *(Supported by MRC of Canada grants to T.J.C. and J.L.H.).*

344.10

DIFFERENTIAL EFFECTS OF DORSAL RHIZOTOMY ON MU AND DELTA OPIOID RECEPTOR-LIKE IMMUNOREACTIVITY (LI) IN THE CERVICAL SPINAL CORD OF THE RAT. M.-C. Lombard*, C. Abbadie, R. Elde, J.-M. Besson and A.I. Basbaum. INSERM U161, Paris, France, Dept. Anatomy, UCSF, San Francisco and Depts. Cell Biology and Anatomy, U. Minnesota, Minn. MN.

To address the pre and postsynaptic distribution of mu and delta opioid receptors, we used quantitative ICC in 1) rats that underwent dorsal rhizotomy (C4-T2); 2) rats that underwent dorsal rhizotomy C4-C6 and C8-T2, sparing C7; 3) sham-operated rats that underwent laminectomy without rhizotomy. Seven days after surgery, we immunostained lumbar spinal cord with antisera that distinguish MOR and DOR and then densitometrically measured immunostaining in laminae I-II. To normalize immunostaining, we compared the density ipsilateral and contralateral to the lesion. In laminae I-II, MOR-LI is found in axon terminals and in somatic and dendritic profiles; DOR-LI is in varicose axons. Dorsal rhizotomy (C4-T2) reduced MOR-LI in all segments, most notably in lamina I and outer II, dense labelling persisted in inner II. By contrast, residual DOR-LI was evenly distributed in lamina I and II. In the most deafferented segment (C7) we recorded a 44.2% reduction of MOR-LI. For DOR-LI. the decrease was 64.2%. We estimate that C7 MOR-LI afferents distribute as follows: C4-7.5%, C5-14.3%, C6-16%, C7-22.9%, C8-13.7%, T1-11.5%, T2-14.1%. For DOR-LI we found: C4-5.8%, C5-19.8%, C6-14.6%, C7-25.1%, C8-11.9%, T1-10.9%, T2-11.9%. For both receptors there is a greater rostral than caudal arborization. Finally, we estimate that 27% and 34% of the primary afferent-derived MOR and DOR-LI, respectively, derive from dorsal roots rostral and caudal to C7. Besse *et al.* (1990) found that up to 75% of mu and 65% of delta opioid receptor binding in dorsal horn is primary afferent-derived. Conceivably, we found more postsynaptic MOR-LI, because antibodies readily stain cytoplasmic and membrane receptor. The equivalent delta numbers suggest a predominant axonal location for this receptor. If there is no induction of opioid receptor in deafferented cord, our results and those of Besse *et al.* indicate that the dorsal horn contains both pre and postsynaptic mu *and* delta opioid receptors and suggest that incompletely overlapping populations of small diameter primary afferents generate these different labelling patterns. Supported by DA08377, NS14627 and INSERM.

344.11

SYNAPSES BETWEEN CAPSAICIN-SENSITIVE PRIMARY AFFERENT CENTRAL TERMINALS AND GABA- AND MET-ENKEPHALINERGIC INTERNEURONS IN THE SUBSTANTIA GELATINOSA AND SPINAL TRIGEMINAL NUCLEUS CAUDALIS. A. Hiura*, F. Nasu, M. Kuwahara and H. Ishizuka. Dept. of 2nd Oral Anat., Tokushima Univ. Sch. of Dent., Tokushima 770, Japan.

Investigation of the synaptic relations between primary afferent central endings and interneurons in the superficial dorsal horn is a key study to understand the nociceptive transmission. Recently, we have shown that capsaicin-sensitive primary afferent central terminals (CI-terminals) make presynaptic contacts with interneurons in the substantia gelatinosa (Hiura & Ishizuka, 1995).

In order to directly demonstrate the synapses between CI-terminals and GABA- and Met-Enkephalinergic interneurons, capsaicin treatment and immunolabeling methods were utilized. Superficial layer of the lumbar spinal cord dorsal horn and spinal trigeminal nucleus candalis of the mouse were examined by electron microscopy after post-embedding immunogold labeling procedures. Double-labeling with different size of IgG gold was used for GABA (15nm) and Met-Enk(5nm).

Glomerular CI-terminals made presynaptic contacts with surrounding dendrites exhibiting GABA and Met-Enk immunoreactivities(IR). Nonglomerular CI-terminals also made presynaptic contacts with GABA- and Met-Enk-IR soma. Frequently a single dendrite and somata showed double labeling, i.e. GABA and Met-Enk might coexist in some of the interneurons. The present findings strongly suggest that nociceptive primary afferent neurons modulate the pain transmission by themselves via inhibitory GABAergic and Met-Enkephalinergic interneurons in the superficial layer of dorsal horn and spinal trigeminal nucleus caudalis.

344.12

ESTROGEN RECEPTORS ARE PRESENT IN DORSAL HORN ENKEPHALINERGIC NEURONS. Å. Amandusson , O. Hermanson & A. Blomqvist* Dept. of Cell Biology, Faculty of Health Sciences, Univ. of Linköping, S-581 85 Linköping, Sweden.

There is increasing evidence that pain sensitivity is influenced by gonadal hormones such as estrogen, but the mechanisms by which estrogen modulates pain-processing are not known. In a previous immunohistochemical study we demonstrated that large numbers of neurons in the superficial dorsal horn of rats expressed estrogen receptors (Amandusson et al., Neurosci. Lett. 196:25-8, 1995). This finding raised the possibility that estrogen may influence dorsal horn enkephalin expression, since enkephalinergic neurons are localized to the same part of the dorsal horn. To explore this possibility we used a double-labeling procedure, combining immunohistochemical staining with in situ hybridization, to demonstrate estrogen receptor-like immunoreactivity (ER-IR) and preproenkephalin-A (ppENK) mRNA in the medullary and spinal dorsal horn of female rats. Both markers labeled large numbers of neurons in the substantia gelatinosa and its trigeminal homologue. Many of these neurons were double-labeled, displaying both ER-IR and ppENK mRNA. Cell counts showed that 60-70% of the enkephalinergic neurons in the superficial dorsal horn expressed ER-IR, whereas enkephalinergic neurons in other parts of the spinal and medullary gray matter were single-labeled.

Previous studies have shown that estrogen receptors can bind to the promotor region of the ppENK gene, and studies on the hypothalamus have demonstrated that estrogen regulates enkephalin expression in select neuronal populations. The present results demonstrate that enkephalinergic neurons in the superficial dorsal horn contain estrogen receptors and suggest that estrogen may play an important role for the modulation of sensory and nociceptive processing. Supported by the Swedish Medical Research Council (#7879).

344.13

PARAVENTRICULAR HYPOTHALAMUS, THE SOURCE OF SPINAL VASOPRESSIN FIBERS IN THE RAT

Martin Hallbeck*, Ola Hermanson, Anders Blomqvist. Dept. Cell Biology, University of Linköping, S-581 85 Linköping, Sweden.

Vasopressin (VP) has been proposed to be involved in pain modulation, which is thought to be mediated by vasopressinergic fibers in lamina I of the spinal cord. To clarify the origin of these fibers we have used retrograde tract tracing combined with in situ hybridization for VP.

Rats were injected into the cervical spinal cord with the retrograde tracer cholera toxin B, which was demonstrated by immunocytochemistry. In situ hybridization was performed with a ^{35}S-labelled 0.6 kb RNA probe complementary to preproVP (ppVP). The sections were pretreated with proteinase K, hybridized for 18 h at 59°C and then posthybridized under high stringency conditions followed by autoradiography.

Several areas contained ppVP, such as the suprachiasmatic n., supraoptic n., and bed nucleus of stria terminalis. However, ppVP was not found in DRG, n. tractus solitarius, or the locus coeruleus, which all have been suggested to contain spinally-projecting VP neurons. The only area with spinal projections that contained ppVP was the paraventricular hypothalamic nucleus (PVH). The posterior parts of PVH displayed large numbers of double-labeled neurons, located primarily in the lateral parvocellular division and in the ventral part of the medial parvocellular division. Almost no double-labeled neurons were seen in the posterior magnocellular division, rich in vasopressin, or in the dorsal parvocellular division, rich in descending neurons.

These findings suggest that PVH is the sole source of spinal VP. Further, they show that the spinally-projecting VP neurons in PVH are parceled into anatomically distinct cell groups that constitute a subset of the spinally-projecting PVH neurons. In conclusion these findings show that a putative role for VP in pain modulation must involve the PVH.

Supported by SMRC grant 7879.

344.14

NOCICEPTIVE AND NEUROPATHIC PAIN ARE REDUCED IN MICE LACKING THE GAMMA ISOFORM OF PROTEIN KINASE C (PKCγ). A.B. Malmberg[1]*, C. Chen[2], S. Tonegawa[2] and A.I. Basbaum[1], [1]Depts of Anatomy and Physiology and W.M. Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143, and [2]HHMI, MIT, Cambridge, MA 02139.

A variety of second messenger systems are implicated in the tissue and nerve injury-evoked hyperexcitability of dorsal horn neurons that underlies nociceptive and neuropathic pain, respectively. Here, we studied mice that carry a null mutation in the gene encoding for the neuronal specific isoform PKCγ. We evaluated pain behavior after intraplantar injection of 10 µl 2% formalin and studied capsaicin-induced plasma extravasation of Evan's Blue dye in skin punches. To induce nerve injury, we made a partial ligation of the sciatic nerve and assessed mechanical allodynia with von Frey hairs and thermal hyperalgesia in the Hargreaves model. To study the anatomical consequences of nerve injury we performed immunocytochemistry for the Fos protein, substance P (SP), SP receptor (SPR) and neuropeptide Y (NPY) on spinal cord sections.

Baseline mechanical and thermal withdrawal thresholds were similar in PKCγ knock-out and wild-type mice. In the knock-out mice, we recorded a significantly less (56%) second phase paw licking behavior in the formalin test, less paw swelling (21%) and reduced ipsilateral superficial dorsal horn Fos-ir (32%) compared to wild-type mice; capsaicin-evoked plasma extravasation was also less than in wild-type mice (44%). The magnitude of allodynia and hyperalgesia was also significantly reduced in the knock-out mice as were the anatomical changes (decreased SP, and increase SPR and NPY) that characterize this neuropathic pain model. These results indicate that the PKCγ isoform contributes to the development of both nociceptive and neuropathic pain and to the anatomical consequences of nerve injury.

Supported by: NS 14627 and 211445 and the Swedish Cancer Foundation.

344.15

CHARACTERIZATION OF ALPHA-2A RECEPTOR IMMUNOREACTIVITY IN THE RODENT SPINAL CORD: RELATIONSHIP TO NORADRENERGIC TERMINALS AND NEUROPEPTIDE CONTENT. L.S. Stone*[1-3], C. Broberger[4], G.L. Wilcox[1-2], T. Hökfelt[4] and R. Elde[1-3]. [1]Graduate Program in Neuroscience, Department of [2]Pharmacology and [3]Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455. [4]Department of Neuroscience, Karolinska Institutet, 17177 Stockholm, Sweden.

Activation of α_{2A}-adrenergic receptors mediates many physiological effects including analgesia. mRNA for the receptor has been detected in many regions of brain and spinal cord as well as in small diameter dorsal root ganglia (DRG) neurons. We have developed an anti-peptide antiserum in rabbits directed against the carboxy-terminal portion of the rat RG20 (α_{2A}) clone. The resultant serum revealed α_{2A}-immunoreactivity (α_{2A}-ir) on the membranes of MDCK cells transfected with α_{2A}, but not the α_{2B} or α_{2C} subtypes, or untransfected cells. Staining was blocked by pre-absorption of the serum with the cognate peptide in both cells and tissue sections. We have investigated the relative distribution of α_{2A}-ir in the spinal cord and brainstem of both rats and mice. Staining was observed in the superficial layers of the dorsal horn of the spinal cord and the spinal tract of the trigeminal nerve, both of which receive input from primary sensory neurons. In order to determine if the receptor is located on the terminals of DRG neurons or on second order spinal neurons, we double-labeled tissue sections with antibodies to either Substance P (SP) or CGRP, both of which are synthesized in DRG neurons. A high degree of co-localization was observed between α_{2A}-ir and SP-ir in both spinal cord and brainstem, whereas CGRP-ir co-localized only partially with α_{2A}-ir. In addition, dorsal rhizotomy decreased both α_{2A}-ir and SP-ir ipsilateral to the severed roots. This result suggests that α_{2A} receptors are synthesized and trafficked into the spinal cord almost exclusively by SP-containing neurons, some of which are likely to be nociceptors. Interestingly, an antibody directed against dopamine β-hydroxylase, which is involved in the synthesis of norepinephrine, did not exhibit a close association with α_{2A}-ir. This suggests a spatial mismatch between the endogenous ligand and this receptor. (Supported by R01-DA-04274 to GLW, DA-06299 to RE, Swedish MRC 04X-2887 to TH and DK 43879 to Lee E. Limbird, Vanderbilt University)

344.16

LOCALIZATION OF NK2 AND NK3 RECEPTOR-LIKE IMMUNOREACTIVITY IN RAT SPINAL CORD. B.R. Southwell[1], V.S. Seybold[2]*, A. Portbury[1] and J.B. Furness[1]. [1]Dept. of Anatomy and Cell Biology, Univeristy of Melbourne, Parkville 3052,VIC, Australia and [2]Dept. of Cell Biology and Neuroanatomy, University of Minneapolis, MN 55455.

Although receptor autoradiography has provided information on the regional distribution of NK2 and NK3 receptors in the spinal cord, the cellular localization of these receptors has not been resolved. Antisera generated against unique sequences in the C-terminal region of each receptor were used in conjunction with immunofluorescence and confocal microscopy to examine the distribution of NK2 and NK3 receptor-like immunoreactivity in spinal cords from rats perfused with Zamboni's fixative. NK2 receptor-like immunoreactivity was localized primarily to short, fine, processes in the superficial regions of the white matter both medial and lateral to the dorsal root entry zone. Immunofluorescent processes also occurred in the pia. Within the gray matter, immunoreactive processes were most prominent in the superficial laminae of the dorsal horn. Immunoreactive perikarya were rare. This pattern of staining suggests that NK2 receptors occur on axons. In contrast, the pattern of NK3 receptor-like immunoreactivity was consistent with a distribution on dendrites and perikarya. Immunofluorescence in perikarya was greatest at the cell membrane. Immunoreactive perikarya were sparsely distributed in the dorsal horn and immunoreactive processes were dense in the superficial laminae of the dorsal horn. Thus NK2 and NK3 receptors may mediate effects of tachykinins at different cellular sites. This work was support by NIH NS17702 (VSS) and NH&MRC 963213 (JBF).

344.17

AN IMMUNOHISTOCHEMICAL STUDY OF LUMBAR SPINAL DURA AND POSTERIOR LONGITUDINAL LIGAMENT. S Kallakuri, JM Cavanaugh*, DC Blagoev. Bioengineering Center, Wayne State University, Detroit, MI 48202.

Understanding the pattern and type of innervation of the lumbar dura may offer more insight into the etiology of low back pain. Our objective was to investigate the innervation in lumbar dura and posterior longitudinal ligament (PLL) using immunocytochemistry. Methods: Primary antibodies included the general neuronal marker protein gene product (PGP 9.5), substance P (SP), calcitonin gene related peptide (CGRP) and the sympathetic nervous system marker tyrosine hydroxylase (TH). The tissues were processed for immunochemistry based on the Avidin Biotin Peroxidase Complex method. Nine lumbar dorsal dura, ventral dura and PLL were obtained from adult male New Zealand White rabbits. Results: *PGP 9.5*: In dura, large bundles and very small diameter immunoreactive fibers concentrated in nerve root sleeves and along the lateral margins were observed. PLL showed an extensive longitudinal network of parallel bundles and interconnected fibers. Some transverse fibers at the intervertebral region of PLL suggest a supply into the disc. *SP*: In the dura very intensely stained small diameter immunoreactive fibers were seen at the nerve root sleeves and on the lateral margins. SP immunoreactivity in PLL included large bundles and small diameter fibers along the entire length and also at the intervertebral region. *CGRP*: A sparse distribution of immunoreactive fibers were found in all the three tissues and these fibers appeared to innervate the deep layers. *TH*: Small diameter immunoreactive fibers and bundles were seen in both dura and PLL. Their density and distribution appeared to be similar to that of CGRP immunoreactive fibers. *Controls*: In tissues stained without the primary antibody no nerve fibers were observed. Discussion: Our results indicate that the dura is well innervated near the nerve root sleeves and on lateral margins. As reported by others the PLL is also well innervated. An extensive distribution of SP immunoreactive fibers implicate dura and PLL in the pathogenesis of low back pain. TH immunoreactivity support a role for the sympathetic system in some cases of low back pain. Supported by NIH grant AR41739 (JMC).

344.18

PATCH CLAMP CHARACTERIZATION OF SYNAPTIC PLASTICITY IN DEEP DORSAL HORN NEURONS IN TRANSVERSE SLICES OF NEONATAL RAT SPINAL CORD. S.M. Garraway*, S. Pockett, & S. Hochman. Dept. Physiology, University of Manitoba, Winnipeg, MB, Canada R3E 0W3.

Chronic pain syndromes may arise from long-term alterations in excitability of neurons in the spinal cord. Neurons possibly involved include ascending tract cells, which send nociceptive information to the brain, and wide-dynamic range neurons, which receive inputs both from nociceptive and non-nociceptive afferents. Since both cell types are found in laminae IV-VI, we sought to determine whether long-lasting alterations of the synaptic properties of neurons in these regions of the cord could be induced by bursts of conditioning primary afferent activity.

Whole-cell patch clamp recordings were made from neurons in laminae IV-VI in 500-1000 μm slices of the neonatal rat lumbar spinal cord (P3-P6). One stimulating electrode placed in the dorsal root entry zone was used to evoke primary afferent excitatory postsynaptic currents (epscs) and another in the contralateral anterior commissure or ventrolateral funiculus was used to identify antidromically ascending tract or long propriospinal neurons. Only recordings where series resistance and leak conductance values were unaltered for the duration of data collection were accepted for analysis (n=9). The lowest stimulation intensity which could evoke an epsc was 100-500 μs and 500 μA. Test epscs were evoked at threshold stimulation strength. In the absence of support solution we observed significant run-down of evoked synaptic currents. In the presence of a support solution (4 mM ATP, 1 mM GTP) the test epsc displayed a stable amplitude which could be maintained for over an hour. Conditioning stimuli at threshold or higher stimulation strengths were capable of evoking either LTP or LTD. Adjustment of conditioning stimulation parameters could interconvert synaptic depression and potentiation in individual cells. *Supported by the Thorlakson Foundation Fund and the Manitoba Medical Services Foundation.*

344.19

EFFECTS OF STIMULUS PARAMETERS ON BEHAVIOUR AND RESPONSES OF TRIGEMINAL BRAINSTEM NEURONES TO TOOTH-PULP STIMULATION IN CATS. D. Banks*[1] and B. Matthews. (SPON: Brain Research Association). Department of Physiology, University of Bristol, BS8 1TD and [1]Department of Physiology, UMDS, St Thomas' Hospital, London SE1 9RT, England.

In our laboratory, bipolar electrical stimulation of tooth-pulp (TP), at 0.5Hz with stimuli up to 1mA intensity, 1ms duration in chronically-prepared, conscious cats produces no signs of aversive behaviour. This result was unaffected by the type of anaesthetic used during the initial surgery, the type and timing of surgical procedures or the type of conditioning of the animals during pre-operative laboratory visits (*Soc. Neurosci. Abstr.* **24**, 320.5, 1994). We have now investigated the behavioural effects of brief trains of higher frequency TP stimulation and recorded the activity of neurones in the trigeminal brainstem under anaesthesia using the same stimulus parameters. Three male cats were prepared for chronic recording as described previously (*J. Neurosci. Meth.* **38**, 35-40, 1991; **46**, 83-88, 1993). With 100ms trains of stimuli, aversive responses were observed with stimulus intensities of 100-200μA and stimulus durations of 0.1-0.5ms at frequencies above 30Hz (range 30-1000Hz). Recordings were made from 43 neurones with oro-facial receptive fields in the rostral part of the trigeminal spinal nucleus in the same cats under light anaesthesia. Of these, 14 responded to TP stimulation but all responded to single shocks of below 100μA and 0.1ms duration and none required the temporal summation provided by a train of stimuli to evoke a response. With 7, a 100ms train of stimuli near threshold evoked more spikes than single shocks of maximum intensity. It is concluded that, in the cat, temporal summation is required to produce aversive responses to TP stimulation but the site of this summation has not been identified. In man (McGrath *et al., Pain* **15**, 377-388, 1983), temporal summation has less effect on the threshold of pain produced by TP stimulation.

Supported by the Medical Research Council, U.K.

344.20

COMPARISONS OF RESPONSES OF DORSAL HORN CELLS TO TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION (TENS) IN CATS WITH INTACT AND TRANSECTED SPINAL CORDS. D.W. Garrison * and R.D. Foreman, Depts. of Physical Therapy and Physiology, Univ. of Okla. HSC, Oklahoma City, Okla 73190.

We have shown that TENS applied to somatic receptive fields decreases spontaneous and noxiously evoked dorsal horn cell activity. It is proposed "gating" is contributing to the decrease which may involve segmental and/or supraspinal mechanisms. We hypothesized that TENS would equally decrease activity of dorsal horn neurons in animals with intact and transected cords.METHODOLOGY: Using alpha chloralose anesthetized cats with intact or T-12 transected cords(x-cut), spontaneous and noxiously (nox.) evoked (mechanical clamp/left hind limb) activity was recorded from the lumbar cord. Stimulating variables in the "Norm" mode were frequency (10-125 Hz), intensity (10-60 mAmps) and pulse duration (100 usec). TENS application was continuous for either 30 s. or 5 m. RESULTS are given in % decrease in mean activity (imp/s) from control levels. Of the four comparison categories between intact and cord cut animals, only nox. 30 s was statistically significant (p <.04).

	Spont. 30s Intact	Spont. 30s X-Cut	Spont. 5 m Intact	Spont.5 m X-Cut
% Reduction during TENS	63% n = 48	40% n = 49	58% n = 30	42% n = 14
	Nox. 30 s Intact	Nox. 30s X-cut	Nox. 5 m Intact	Nox. 5 m X-Cut
% Reduction during TENS	72% n = 36	44% n = 25	62% n = 21	42% n = 8

CONCLUSION: The data support the involvement of a spinal modulatory mechanism during TENS reduction of cell activity but not a significant supraspinal one. (HL722732 & OCAST HR1-063)

345.1

EFFECT OF INTRATHECAL SOMATOSTATIN AND ITS ANALOGS ON MECHANICAL ALLODYNIA IN THE RAT

J.S. Kroin*, A.V. Maioriello, R.D. Penn. Dept. of Neurosurgery, Rush Medical College, Chicago, IL 60612

Somatostatin-14 (SOM) and its stable analog octreotide provide pain relief when administered intrathecally in patients, and SOM inhibits firing in nociceptive dorsal horn neurons when applied locally. Using an animal model of neuropathic pain (the chronic constriction injury model of Bennett and Xie) SOM and analogs were tested intrathecally for their effectiveness to reduce tactile allodynia. Male Sprague-Dawley rats were implanted with an intrathecal catheter and 5 days later the sciatic nerve was ligated to produce a withdrawal response to the light touch of von Frey hairs on the plantar surface of the foot. Octreotide (2μg) caused a moderate suppression of allodynia, with the withdrawal threshold increasing 2.2g (%MPE = 18%). Lower doses of octreotide did not produce significant blockage of allodynia. Neither SOM (up to 4μg) nor lanreotide (up to 3μg) reduced allodynia. Higher doses produced transient hindlimb motor block. In conclusion, the clinical pain relief seen in patients cannot be explained by the results here, which only show a moderate effect of octreotide and no effect of SOM in this rat model of allodynia.

345.2

HYPERALGESIA INDUCED BY PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) AND ITS RELATIONSHIP TO IMMUNOHISTOCHEMICAL LOCALIZATION IN THE MOUSE SPINAL CORD.

M. Narita[1]*, S. L. Dun[2], N. J. Dun[2] and L. F. Tseng[1]. [1]Dept. of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226, [2]Dept. of Anatomy and Neurobiology, Medical College of Ohio, Toledo, OH 43699.

Pituitary adenylate cyclase activating polypeptide (PACAP) is a newly discovered neuropeptide belonging to the secretin-, glucagon-, vasoactive intestinal polypeptide-family of peptides. The aim of the present study was to evaluate the distribution of PACAP-like immunoreactivity in the mouse spinal cord using an antibody against PACAP38 and to determine the behavioral profiles, particularly with respect to hyperalgesia, of PACAP38 given intrathecally (i.t.) in male ICR mouse. Immunoreactivity to PACAP38 was detected in numerous nerve fibers in the superficial layers of the dorsal horn of cervical, thoracic, lumbar and sacral segments and a few fibers extended into the deeper layers of the spinal cord. In addition, PACAP-like immunoreactivity were seen in the intermediolateral cell column of the thoracic and sacral segments. In behavioral studies, PACAP (0.05 to 0.5 µg) produced a dose-dependent decrease of the tail-flick latency when given i.t. At higher doses (1 to 10 µg), PACAP38 given i.t. elicited scratching, biting and licking behaviors immediately after i.t. injection. Similar to substance P, these behaviors produced by PACAP can be considered as pain-like syndrome. I.t. pretreatment with a protein kinase A (PKA) inhibitor, KT5720, and a protein kinase C (PKC) inhibitor, calphostin C, 10 min prior to i.t.-challenge of PACAP38 dose-dependently attenuated the PACAP38-induced scratching, biting and licking behaviors. These findings suggest that PACAP may be one of the putative sensory neurotransmitter involved in nociceptive signaling in the mouse spinal cord. Furthermore, the i.t.-administered PACAP38-induced hyperalgesia is mediated by the activation of PACAP receptors, which are linked to PKA and PKC systems (Supported by NIH grant, DA 03811, NS18710 and HL51314).

345.3

ACTIVATION OF PROTEIN KINASE C OR cAMP TRANSDUCTION CASCADES AUGMENT PEPTIDE RELEASE FROM RAT SPINAL CORD SLICES.

L.A. Barber*, S. Frayer, M.D. Southall, R.L. Michael, M.R. Vasko. Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN, 46202.

Activation of either the cAMP or the protein kinase C (PKC) transduction cascade enhances nociceptive behaviors in animal studies and augments neuropeptide release from isolated sensory neurons. To address whether these transduction pathways regulate peptide release from sensory nerve terminals in the spinal cord, we examined if activators of adenylyl cyclase or PKC alter capsaicin-induced release of substance P (SP) and calcitonin gene-related peptide (CGRP) from rat spinal cord slices.

Spinal cords from adult Sprague Dawley rats were removed, chopped and each half of the lumbar cord placed into separate perfusion chambers. The tissue was superfused with Kreb's buffer in the presence or absence of various drugs and peptide release stimulated using 500 nM capsaicin. Superfusates were assayed for SP and CGRP using radioimmunoassay.

Exposing spinal cord tissue to 100 nM phorbol 12,13 dibutyrate (PDBu) increased capsaicin-evoked release of SP from 1.9 ± 0.3 to 4.3 ± 0.8 % of total content and CGRP release from 4.9 ± 0.5 to 10.5 ± 1.4% of total content. This augmentation of peptide release was attenuated by pretreating tissue with 1 µM of the PKC inhibitor, bisindolylmaleimide I. An inactive PDBu analog, 4α-PDBu (1µM) did not alter peptide release. In the presence of 10 µM forskolin, capsaicin-evoked release of SP increased from 1.4 ± 0.2 to 3.0 ± 0.4 % of total content, whereas CGRP release increased from 2.7 ± 0.5 to 5.0 ± 0.7 % of total content.

These results indicate that activation of cAMP or PKC transduction cascades augment evoked release of peptides from spinal cord slices and this could contribute to hyperalgesia. (Supported by NS 34159)

345.4

Heregulin Modulates Capsaicin-evoked Release of iCGRP from Sensory Neurons.

N.O. Dybdal*, Y. Ulrich+, C. Harding-Rose+, B. Huerter, K. Hagler, C.M. Flores+, M.X. Sliwkowski∞, D. Mortensen, R. Clark, K.M. Hargreaves+. Depts. of Pathobio. and ∞Pro. Chem., Genentech, Inc., S.S.F., Calif 94080 and +Dept. of Rest. Sci., Univ. of Minnesota, MPLS, MN 55455

Neuregulins are a family of alternatively spliced gene products that serve as ligands for ErbB3 and ErbB4. A number of pleiotrophic activities are now known to be attributed to this family of polypeptide growth factors. Two of these isoforms, glial growth factor (GGF) and heregulin (HRG), have been shown to be potent mitogens for Schwann cells. We have observed that chronic administration of HRG has effects on small diameter peptidergic neurons of sensory origin. In the present study, we evaluated whether chronic administration of rHRGß1177-244 modulated the activity of capsaicin-sensitive peptidergic neurons. Adult female Sprague Dawley rats (n=4-5/group) were implanted sc with miniosmotic pumps which provided vehicle, or heregulin at 0.1 or 1.0 mg/kg/d for 14 days. At the end of the administration period, animals were sacrificed and their trigeminal ganglia (TGG) removed. TGG were chopped into 200 µm slices and placed into superfusion chambers (4 TGG per chamber) which were pumped with oxygenated Krebs buffer. After a recovery period, the TGG were then pulsed with capsaicin (30 µM). Superfusate levels of iCGRP were measured by RIA. Capsaicin stimulation evoked a 2-3 fold increase in release of iCGRP in vehicle-treated animals. In contrast, HRG treated animals demonstrated an attenuated iCGRP release in response to capsaicin at both the 0.1 and 1.0 mg/kg/d doses. Collectively, these data indicate that HRG modulates capsaicin-sensitive peptidergic neurons. The mechanism for this effect may be either direct, e.g., reduction in peptide content or indirect, e.g., release of an inhibitory factor from Schwann cells. Supported in part by Genentech, Inc., DE09860, DE11277 and F32DE5659.

345.5

SPINAL NK1 RECEPTOR MEDIATES THERMAL HYPERALGESIA AND PROSTAGLANDIN RELEASE

X.-Y. HUA*, P. CHEN, M. MARSALA & T.L. YAKSH Dept. of Anesthesiology, Univ. of California, San Diego, CA 92093-0818, USA.

Previous study has indicated that acticvation of spinal tachykinin NK1 receptor-induced nociception may be mediated by cyclooxygenase products[1]. We have recently carried an *in vivo* study in the rats with intrathecal loop dialysis catheters[2] to correlate intrathecal substance P (SP)-induced thermal hyperalgesia with the levels of prostaglandin and glutamate in spinal fluid. Intrathecal application (i.t.) of SP 20nmol in 10µl artificial cerebral spinal fluid (ACSF) induced a significant reduction in the latency of thermal paw withdrawal response. The peak effect occurred at 10 min (Baseline: 11.8±0.8 vs. SP: 7.6±0.6 sec, n=7) and returned to baseline around 30 min. SP-induced thermal hyperalgesia was associated with an increase in the levels of prostaglandin E2 (PGE2) (342±43% of baseline, n=8) and glutamate (285±95% of baseline, n=7) in intrathecal space. I.t. application of same volume of ACSF produced neither hyperalgesia nor spinal PGE2/glutamate release (n=5-7). A selective NK1 receptor antagonist RP 67580 3µg (n=8) applied (i.t.) 10 min prior SP 20nmol (i.t.) completely blocked the SP-induced hyperalgesia, whereas an inactive form of the NK1 antagonist, RP 68651, at the same dose (n=8) did not cause any inhibition. RP 67580 10µg (n=5) also blocked the SP-induced PGE2 release, but had no effect on the glutamate release evoked by SP. These results further support the hypothesis of the involvement of NK1 receptor in spinal nociceptive transmission, and provide direct evidence that activation of spinal NK1 receptor and subsequent generation of cyclooxygenase products mediates SP-induced hyperalgesia.

[1] Science 257: 1276, 1992; [2] J Neurosci Meth 62: 43, 1995.

(This work is supported by NIH NS16541 and NIH R29 HL 50403).

345.6

THE NEUROTENSIN ANTAGONIST SR48692 ALTERS PAIN MODULATION BY NEUROTENSIN IN THE RAT.

D.J. Smith*, D.L. Smith, D. Gully and P.J. Monroe. Dept. of Anesth., Robert C. Byrd HSC, Morgantown, WV 26506, & Sanofi Recherche, Toulouse Cedex, France.

Neurotensin (NT) has a dose dependent biphasic role on pain modulation following its microinjection into the rostroventral medial medulla (RVM). High doses are antinociceptive, while lower doses (< 30 pmol) are either inactive or produce hyperalgesia, but are antinociceptive in the presence of a NT receptor antagonist (JPET. 265:580, 1993). In the current study, the ability SR48692 to discriminate between these actions of NT was evaluated in awake rats chronically prepared with guide cannula over the RVM. In the presence of 30-3000 fmol of SR48692, a marked antinociceptive effect (tail flick test) was observed with an otherwise inactive (30 pmol) dose of NT. In contrast, lower doses (0.1-0.3 fmol) of SR48692 blocked antinociception produced by 10 nmol NT. The NT response returned to control levels as the dose of antagonist was increased, and was again depressed at still higher doses of antagonist. Thus, at least two NT receptors exist in the RVM: one with a high affinity for NT, a low affinity for SR48692, and which mediates a hyperalgesic response; the other has a lower affinity for NT, a higher affinity for SR48692, and is associated with antinociception. A third NT receptor may explain the effect of high antagonist doses, however, other explanations are possible. Physiologically, it appears that the antianalgesic component of NT action may be most prominent, since the activation of NT neuronal processes from the PAG to the RVM in response to systemically or PAG administered morphine is potentiated by SR48692 injected into the RVM. On the other hand, the highest dose of the antagonist continues to have diminished effectiveness.

Supported by Sanofi Recherche, and the WVU Medical Corporation.

345.7

EVIDENCE FOR ENDOGENOUS SUBSTANCE P (SP) NH_2-TERMINAL ACTIVITY IN THE FORMALIN AND WRITHING ASSAYS IN MICE. V.M. Goettl* and A.A. Larson. Graduate Program in Neuroscience, University of Minnesota, St. Paul, MN 55108 USA.

Intrathecal (i.t.) injection of SP(1-7) produces short- and long-term antinociception in the acetic acid-induced writhing assay and long-term hyperalgesia in Phase I of the formalin assay. To investigate whether endogenous SP NH_2-terminal fragments modulate nociception acutely, the effect of the SP(1-7) antagonist, D-SP(1-7), injected i.t., was examined in these two nociceptive assays. Although low doses (5-100 pmol) of SP(1-7) inhibited writhing and 1 nmol of D-SP(1-7) blocked this effect, D-SP(1-7) produced no effect on writhing when administered alone until relatively high doses (10-100 nmol). At these doses, D-SP(1-7) was antinociceptive, suggesting that endogenously SP NH_2-terminal activity is pronociceptive at a site that is relatively insensitive to D-SP(1-7). In contrast, in the formalin assay D-SP(1-7) increased Phase I responses at relatively low doses (2-1000 pmol) whereas higher doses had no effect. Endogenous SP NH_2-terminal activity appears to be antinociceptive at sites that are relatively sensitive to D-SP(1-7). The U-shaped dose-response curve for D-SP(1-7) in this assay suggests possible additional hyperalgesic activity of the SP NH_2-terminus at sites that are relatively insensitive to D-SP(1-7). Mixed activity of the SP NH_2-terminus in the formalin assay would explain the lack of effect of acute, exogenous SP(1-7) on Phase I responses at any dose tested. Although SP NH_2-terminal activity is the same in both the writhing and formalin assays, i.e. antinociceptive at D-SP(1-7)-sensitive sites and pronociceptive at D-SP(1-7)-insensitive sites, acetic acid appears to induce only pronociceptive activity endogenously whereas formalin induces both. These data are consistent with [^{3}H]SP(1-7) binding studies in which 2 binding sites are described in the spinal cord. [NIDA DA07234 to VMG & DA04090 to AAL]

345.8

PHOSPHOINOSITIDE SECOND MESSSENGER RESPONSES IN THE SPINAL CORD DURING HYPERALGESIA J.L. Fuchs, K.J. Blanton and H.D. Schwark*, Dept. Biological Sciences, University of North Texas, Denton TX 76203.

The search for central changes underlying hyperalgesia has focused on neurotransmitters and their receptors. Substance P receptors (NK-1), which are concentrated in the upper laminae of the dorsal horn, increase as hyperalgesia develops (Stucky et al., 1993). However, stimulation of substance P receptors can result in their rapid internalization (Mantyh et al., 1995), presumably making them less available for neurotransmission. One approach to determining the functional implications of receptor changes is to examine the second messenger responses, such as phosphoinositide (PI) turnover initiated via NK-1 receptors.

To localize PI responses, we used a modification of the method developed by Hwang et al. (1990). Slices of lumbar spinal cord were taken from rats untreated or 2 days after injection of complete Freund's adjuvant in one hindpaw. Slices were submerged in oxygenated Krebs buffer and incubated with [3H]cytidine as precursor to membrane-bound [3H]CDP-DAG. In the presence of LiCl, neurotransmitter agonists for substance P or ACh (carbachol) were added to initiate PI turnover. Slices were then sectioned for film autoradiography.

PI labeling in response to substance P was coextensive with the distribution of NK-1 receptors. PI labeling decreased in the upper dorsal horn ipsilateral to the inflamed paw (15%, $p<0.03$, $n=6$ rats), in contrast to the increased substance P binding. It is unlikely that there was a general down-regulation in PI turnover, because carbachol-induced PI labeling increased ipsilaterally (29%, $p<0.02$, $n=6$). The results are compatible with a functional decrease in responsiveness to substance P in the ipsilateral dorsal horn during the development of hyperalgesia.

(Supported by MH41865 & IBN9221956)

345.9

CALCIUM MEDIATION OF HYPERALGESIC EFFECTS OF SUBSTANCE P IN RAT SPINAL CORD. J.L. Henry, K. Yashpal, J. Reid and D.D. Samulack*, Depts. of Physiology & Psychiatry, McGill University, McGill University, Montreal, QC H3G 1Y6

Evidence from *in vitro* electrophysiological studies has implicated both a Ca^{2+} current and intracellular Ca^{2+} release in excitatory responses of spinal nociceptive neurones to substance P (Murase, Brain Res. 365: 369, 1986; Womack et al., Nature 334: 351, 1988). The present study determined the effects of L-type Ca^{2+} channel blockers and of depletion of intracellular stores of Ca^{2+} on the hyperalgesic response to intrathecal (it.) administration of substance P in the tail-flick test. Male Sprague-Dawley rats (200-250 g) were implanted with chronic it. catheters to the lower lumbar spinal cord (L5-L7). Substance P (6.5 nmol) decreased the tail-flick latency to approx. 25% of baseline at 1 min after administration. This effect was followed by an overshoot of the response at 4 and 7 min. The L-type Ca^{2+} channel blockers verapamil (50 μg) and diltiazem (100 μg) blocked these effects of substance P, while the vehicle, artificial CSF, had no effect on the substance P response. Thapsigargin (2 μg), an inhibitor of the endoplasmic reticulum Ca^{2+} ATPase, reduced the responses to substance P; DMSO, the vehicle, was without effect on this substance P response. The results implicate both a Ca^{2+} current and intracellular Ca^{2+} release in excitatory responses of spinal nociceptive mechanisms to substance P. Thus, the findings from the *in vitro* studies translate to the *in vivo* systems level. *Supported by the Canadian MRC*

345.10

NEONATAL CAPSAICIN ABOLISHES MORPHINE'S MULTIPHASIC EFFECT ON SUBSTANCE P RELEASE FROM RAT TRIGEMINAL SLICES. H. Suarez-Roca*, G. Cano, J.L. Arcaya, G. Gomez and W. Maixner. Instituto de Investigaciones Clinicas, Univ. of Zulia; INBIOMED, Maracaibo, Apartado 1151, Venezuela and Dental Research Center, The Univ. of North Carolina, Chapel Hill, NC 27599-7455.

We previously found that morphine produces a dose-dependent multiphasic effect (inhibition or facilitation) on K^+-evoked substance P release from trigeminal slices, dissociated sensory neurons and on capsaicin-evoked substance P release from trigeminal slices. In the present study, we evaluated morphine's effect on K^+-evoked substance P release from trigeminal slices of rats neonatally treated with capsaicin. Neonatal capsaicin, at 25-50 mg/kg i.p., produced depletion of trigeminal substance P, a dose-dependent prolongation of hotplate latencies, and loss in the ability of capsaicin to evoke substance P release. Both 25 and 50 mg/kg capsaicin abolished the multiphasic effect of morphine on substance P release. In contrast, in rats treated with the vehicle, 0.1 nM, 1uM and 10 uM morphine inhibited (56 ± 6%), enhanced (133 ± 25%) and inhibited (69 ± 9%) K^+-evoked substance P release, respectively. These results further suggest that the multiphasic effect of morphine is exerted on type-C primary afferents and thus, is of relevance in the modulation of nociceptive transmission. (Supported by TW00305, CONDES171595 & DE07509).

345.11

OPPOSITE MODULATION OF CAPSAICIN-EVOKED SUBSTANCE P (SP) RELEASE FROM TRIGEMINAL SLICES BY SUBTYPE SELECTIVE GLUTAMATE RECEPTOR AGONISTS. M.C. Cuesta*, H. Suarez-Roca, G. Cano, J.L. Arcaya, G. Gomez and W. Maixner. Inst. Investigaciones Clinicas. Univ. of Zulia; INBIOMED, Maracaibo, Apartado 1151, Venezuela and Dental Research Center, The Univ. of North Carolina, Chapel Hill, NC 27599-7455.

We previously reported that 10 nM glutamate (GLU) inhibits capsaicin-evoked SP release from trigeminal slices by a mechanism sensitive to 5 uM (±) MCPG, a selective metabotropic GLU receptor antagonist. We now report the effect of the selective GLU receptor agonists: L-CCG-IV (NMDA subtype agonist) and L-AP4, DHPG and 1S,1R-ACPD (metabotropic subtype agonists), on capsaicin-evoked SP release from trigeminal slices. L-CCG-IV (10 uM) enhanced (323 ± 95%) capsaicin-evoked SP release. This facilitatory effect was blocked by 0.3 uM MK-801, a selective NMDA receptor antagonist. Both L-AP4 and DHPG (1-30 uM) inhibited capsaicin-evoked SP release and showed similar efficacy and potency. ACPD (30 uM) did not alter capsaicin-evoked SP release. Thus, SP released from primary afferents may be under inhibitory and excitatory control by metabotropic and NMDA GLU receptors, respectively. (Supported by TW00305, CONDES171695 & DE07509).

345.12

SYSTEMIC RESINIFERATOXIN DECREASES mRNAs ENCODING SP IN LUMBAR DRGs OF THE RAT BUT NOT THE NUMBER OF SP-POSITIVE SENSORY NEURONS. A. Szallasi*, T. Farkas-Szallasi, J. B. Tucker, J. M. Lundberg, T. Hökfelt and J. E. Krause. Dept. Pharmacology and Dept. Neuroscience, Karolinska Institute, 171 77 Stockholm, Sweden; Dept. Anatomy & Neurobiology, Washington Univ. Med. Sch., St.Louis, MO 63110.

Capsaicin is believed to deplete the sensory neuropeptide substance P (SP) in the adult rat due to a combination of neuron loss and decreased synthesis in the surviving cells. In this study, the effects of resiniferatoxin (RTX), an ultrapotent capsaicin analog, were examined on the expression of SP in lumbar DRGs and the corresponding segment of the spinal cord of the adult rat. The number of DRG neurons showing an *in situ* hybridization signal for preprotachykinin mRNAs encoding SP was not altered following RTX (300 µg/kg s.c.) treatment (up to 8 weeks), though the signal was perceptibly weaker. In accord, 2 weeks after RTX administration a 60% decrease was observed in the steady-state levels of SP-encoding mRNAs using Northern blot analysis, leaving the ratio of β- and γ-preprotachykinin mRNAs unchanged. SP-like immunoreactivity was likewise reduced in the spinal cord of RTX-treated animals. The present findings suggest that, unlike capsaicin, RTX does not kill SP-positive neurons, though it suppresses the synthesis of SP. This reduced SP synthesis is likely to play a central role in the known analgesic as well as antiinflammatory actions of RTX (Supported by the Swedish MRC and by NIH grant NS21937)

345.13

EFFECT OF TETRODOTOXIN ON EXPERIMENTAL TACTILE ALLODYNIA IN THE RAT: ALTERATION BY NEONATAL CAPSAICIN TREATMENT. Chaplan SR*, Scott BP, Hua X-Y, Yaksh TL, Anesthesiology Research Lab, UCSD, 9500 Gilman Drive, La Jolla CA 92107-0818

Spontaneous activity in the peripheral nervous system, attributed to alterations in Na+ channel distribution, density and function, has been hypothesized as a basis for persistent anomalous pain states after nerve injury. Functionally differing Na+ channels may be predominantly associated with specific fiber types and with the regenerating state. We have used a model of allodynia, as paw withdrawal thresholds (PWT) to von Frey filaments, generated by tight nerve ligation (Kim and Chung 1992) to investigate the effects of treatments affecting Na+ channel function. Expt 1: IV tetrodotoxin (TTX) 30 μg/kg (maximum sublethal dose) had a small but statistically significant effect on PWT compared to saline (15.9 ± 5% maximum possible effect, MPE). Lumbar intrathecal TTX in the maximum dose not causing motor flaccidity had no effect. Expt 2: Rats were neonatally treated with capsaicin, 50 mg/kg, SQ, or vehicle, and underwent nerve ligation at appropriate body weight. Resulting PWT were significantly higher in the capsaicin-treated rats. The effect of TTX was significantly increased in capsaicin-treated rats (70.9 ± 19% MPE). No diminution of previously described lidocaine effect was seen due to capsaicin treatment (74.5 ± 16% MPE). CGRP and substance P levels were significantly lower in lumbar dorsal spinal cords of capsaicin-treated rats ($P<.05$) by RIA. We conclude that capsaicin treatment results in increased suppression of allodynia by TTX, presumably by a mechanism affecting the population of Na+ channels present, without altering effects of systemic lidocaine.

(Support: NIH NS 017691)

345.14

EVIDENCE THAT ENDOGENOUS CAPSAICIN-LIKE SUBSTANCE IS INVOLVED IN GENERATING INFLAMMATORY PAIN. Ju Young Jung, Ji Yeon Kwak, Young-Shin Park, Kwang-Jin Kim*, Uhtaek Oh, Seoul National University, College of Pharmacy, Kwanak-Gu, Seoul 151, Korea.

Capsaicin (CAP) plays an important role in pain transmission. CAP receptors in sensory neurons were largely predicted by the presence of CAP-specific agonists and antagonists or high-affinity binding sites found in sensory neurons. Recently, we characterized a non-selective cation channel activated by CAP and its analogs. The presence of CAP-activated channel further implies the existence of CAP-like substance endogenous to the periphery. This study was, therefore, directed to test whether endogenous CAP-like substance played a role in pain transmission during inflammation. To test this, we hypothesized that Fos immunoreactivity as a marker for nociceptive neural signals induced by inflammation would be reduced by pretreatment of capsazepine (CZP), a CAP receptor antagonist. Carrageenan (CAR, 6 mg, 150 μl) injected to the rat hind paws intradermally induced Fos-like immunoreactivity (Fos-LI) in the dorsal horn of the lumbar spinal cord. CZP (25 mM, 150 μl) pretreatment, however, reduced the Fos-LI in the spinal cord induced by CAR. Intradermal injection of CAR together with CZP showed 29 ± 3 (n=13) Fos-LI per section of the ipsilateral spinal cord while injection of CAR and vehicle to the contralateral hind paw showed 66 ± 17 Fos-LI per section of the spinal cord. The reduction by CZP of CAR-induced Fos-LI was dose dependent. Intradermal pretreatment of CZP in 1, 5, 10, and 25 mM showed -11 ± 8, 30 ± 5, 46 ± 8, and 56 ± 4% reduction, respectively, in Fos-LI when compared to the vehicle pretreatment. CZP pretreatment also exhibited a significant increase in paw-withdrawal latency, showing a significant analgesic effect. These results thus suggests that capsaicin-like substance plays a role in producing nociceptive signals in the inflamed tissue. Supported by KOSEF-RCNDD of Korea.

345.15

CAPSAICIN (CAP) BINDS TO THE INTRACELLULAR SURFACE OF CAPSAICIN-ACTIVATED ION CHANNEL. U Oh*, SW Hwang, JY Kwak, and J Yang[1], Seoul National University, College of Pharmacy, Kwanak-Gu, Seoul 151-742, Korea and [1]Dong-A Central Research Institute, Seoul, Korea.

CAP activates sensory neurons by increasing ion conductances. Recently, we identified a CAP-activated ion channel. The channel was gated and blocked by CAP and capsazepine (CZP), a competitive antagonist of CAP receptor. Although kinetic properties of the channel have been characterized, location of the binding site of CAP to the channel complex was not yet known. Because CAP is a lipophilic substance, this lipophilicity of CAP raise a question whether specific binding site of CAP is located in the extracellular face of the channel complex. The present study was thus directed to identify the location of the binding site of CAP to the CAP receptor or channel. Extracellular application of CAP activated single-channel currents in cultured DRG neurons. This activation by extracellular CAP was blocked by CZP applied to the intracellular surface of patch membrane. CAP applied to the bath (intracellular side) in inside-out patches also activated single-channel current greatly. The activation of the channel by intracellular CAP was blocked by co-application of CZP. These results indicate that biding sites of CAP or CZP are located in the intracellular surface of the cell membrane. Since CAP and CZP are highly soluble to lipid, these results are not conclusive. We, thus further studied the location of CAP-binding sites by using a soluble form of CAP analogue, DA-5018•HCl (DA). DA greatly activated the ion channel when applied to the intracellular surface of inside-out patches. The activation of the channel by intracellular DA was blocked by CZP. In contrast, DA in the pipette (extracellular side) failed to activate the single-channel currents in inside-out patches. In the same patches, application of DA to the bath (intracellular side) greatly activated single-channel currents. These results thus clearly indicate that ligands bind to the intracellular structures of CAP-receptor or channel. Supported by RCNDD-KOSEF and Genetic Engineering Grant of Korea.

345.16

RESPONSES OF THE NON- (OR LESS)- PUNGENT CAPSAICIN ANALOGUE, OLVANIL, ON RAT TRIGEMINAL GANGLION NEURONS L. Liu and S.A. Simon*. Departments of Neurobiology and Anesthesiology, Duke University Medical Center, Durham, NC 27710.

Capsaicin, the pungent ingredient in chili pepper that activates polymodal nociceptors is used clinically as an antinociceptive and anti-inflammatory compound. One clinical disadvantage of using capsaicin is that its initial application produces marked pain. Various capsaicin analogues were synthesized with the goal of finding one that will reduce the initial pain. Olvanil (N-(3-methyoxy-4-hydroxybenzyl)oleamide is the best studied less-pungent capsaicin analogue. Olvanil was mostly characterized behaviorally and using whole nerve DRGs recordings but the reasons for its lower pungency were not elucidated. To address this question we performed whole cell patch-clamp studies on rat trigeminal neurons. We previously showed (J. Neurophysiol. 75: 1503 1996) that capsaicin activates a variety of currents that can activate with a time to peak (tp) of about 4 s and also more slowly with tp's to 40 s. In neurons held at -60 mV, 1 μM olvanil also activated a variety of kinetically distinct currents, but unlike for capsaicin the majority of these were the slowly-activating type. As with capsaicin, the olvanil-activated currents were reversibly inhibited by 10 μM capsazepine and had an apparent dissociation constant of ≈ 0.7 μM. Olvanil induced tachyphylaxis to a significantly larger extent than 1 μM capsaicin. That is, after three 30 s applications followed by 2.5 min washes the peak current was inhibited about 40% and 90% for capsaicin and olvanil, respectively. We speculate that the lower pungency of olvanil arises because of its slower activation kinetics which gives it more time to inhibit voltage-dependent ion channels while only slowly activating vanilloid receptors. This work was supported in part by NIH grant DC-01065.

345.17

DIFFERENTIAL ACTIVATION AND DESENSITIZATION OF SENSORY NEURONS BY RESINIFERATOXIN Acs, G., Biro, T., Acs, P. and Blumberg, P. M.*, Molecular Mechanism of Tumor Promotion Section, LCCTP, NCI, NIH, Bethesda, MD 20892

The ability of capsaicin to deactivate primary sensory neurons, a phenomenon termed desensitization, provides a basis for the use of vanilloids as analgesics. Recently, using rat dorsal root ganglion (DRG) neurons we have been able to dissociate the binding affinities of vanilloids from their potencies to induce ^{45}Ca uptake into the cells, suggesting the existence of distinct classes of receptors (Acs, G. *et al*, Mol Brain Res, 35, 173-182, 1996). Further support for this conclusion is that vanilloids bind to DRG neurons with positive cooperativity but induce ^{45}Ca uptake in a noncooperative manner. In the present study, we have demonstrated that the ultrapotent capsaicin analog resiniferatoxin (RTX) is able to desensitize the rat DRG neurons to the subsequent induction of ^{45}Ca uptake by capsaicin. The ED_{50} for desensitization, 80 ± 10 pM (mean ± SEM), was similar to that for [^{3}H]RTX binding (48 ± 3.5 pM) and contrasted with the 10-fold higher RTX concentration required for induction of ^{45}Ca uptake. Furthermore, RTX desensitized the cells in a positive cooperative fashion with Hill coefficient 1.51 ± 0.11 (mean ± SEM). At 100 pM RTX, the half-time for desensitization was approximately 1 hour. The effect of RTX pretreatment could not be attributed to receptor down-regulation, since receptor density remained constant over this time period. We conclude that the ^{45}Ca uptake induction and the desensitizing effects of vanilloids are mediated by distinct sublasses of vanilloid receptors with distinct pharmacology. Optimization of ligands for the receptor coupled to desensitization provides a promising strategy for design of novel vanilloids for human therapy.

This work was supported by the National Cancer Institute.

346.1

5-HT_3 RECEPTOR ANTAGONIST, ONDANSETRON, FAILED TO BLOCK ANTERIOR PRETECTAL NUCLEUS INDUCED ANTINOCICEPTION IN RATS. P.S. Chen*, H. Rees, and W.D. Willis, Marine Biomedical Institute, University of Texas Medical Branch,.Galveston, Texas, 77555-1069, U.S.A.

We have shown in previous experiments that APtN activates noradrenergic bulbospinal neurons that modulate the flow of nociceptive information at the level of the spinal cord. This is supported by the fact that microdialysis administration of alpha-2 noradrenergic receptor antagonists reversed APtN-evoked antinociception as shown by paw withdrawl latency to a radiant heat source and dorsal horn cell responses to cutaneous stimulation. The purpose of this study was to determine the importance of 5-HT_3 receptors in anterior pretectal nucleus (APtN) evoked antinociception by administering a specific 5-HT_3 receptor antagonist, Ondansetron, through a microdialysis fiber placed in the spinal dorsal horn of rats. Electrophysiological recordings were used to measure changes in dorsal horn cell responses to brushing, pressing and pinching the peripheral receptive field. Male Sprague-Dawley rats (270-350g) were used for this study. APtN stimulation was achieved using a 333 Hz DC current through a monopolar stimulating electrode. Extracellular recordings of dorsal horn cell responses to brush, press and pinch stimuli were used to measure changes of cell responses during APtN-evoked antinociception during microdialysis administration of artificial CSF, 0.1 mM, 1.0 mM, and 5.0 mM Ondansetron. Responses of dorsal horn cells to cutaneous brush, press and pinch stimuli were inhibited by electrical stimulation of APtN. 5-HT_3 receptor antagonist, Ondansetron, did not block the APtN-evoked antinociception at any dosage. The baseline responses remained unaffected by Ondansetron as compared with ACSF control responses. The role of 5-HT_3 receptors in spinal dorsal horn to APtN-evoked antinociception remains unclear. A second 5-HT_3 receptor specific antagonist needs to be tested to further elucidate the possible role of 5-HT_3 receptors. (Supported by NIH grants NS509743 and NS11255 and by NIMH Fellowship MH10322)

346.2

DIFFERENTIAL CONTRIBUTIONS OF SPINAL, MEDULLARY, THALAMIC AND AMYGDALOID SEROTONIN TO THE ANTINOCICEPTIVE ACTION OF MORPHINE ADMINISTERED INTO THE PERIAQUEDUCTAL GRAY. G. S. Borszcz*, C. P. Johnson, M. V. Thorp and D. H. Williams. Department of Psychology, Dartmouth College, Hanover, N.H. 03755.

Increases in the thresholds of spinal motor reflexes (SMRs), vocalizations during shock (VDSs), and vocalization afterdischarges (VADs) generated by the injection of morphine into the ventrolateral periaqueductal gray (vPAG) were challenged by the administration of the serotonin receptor antagonist methysergide. Responses were generated by applying graded electric current to the tail. Methysergide was injected either intrathecally (IT) or into the rostral ventromedial medulla (RVM), amygdaloid central nucleus (ACe), or nucleus parafascicularis thalami (nPf).

The minimum effective doses of vPAG administered morphine that elevated VAD, VDS and SMR thresholds were 1μg, 2.5μg and 5μg, respectively (BNS, 1995, 109, 502). Antagonism of morphine-induced threshold increases was found to depend on: 1) the dose of morphine (1μg - 10μg) administered into the vPAG, 2) the response being assessed, and 3) the site at which methysergide (2.5μg - 40μg) was injected. Increases in SMR thresholds generated by 5μg and 10μg morphine were antagonized by administration of methysergide either IT or into RVM. Increases in VAD and VDS thresholds generated by 10μg morphine were partially antagonized by IT methysergide. Increases in VDS thresholds generated by 2.5μg and 5μg morphine were antagonized by administration of methysergide into either RVM, ACe or nPf. Increases in VAD thresholds generated by 5μg morphine were partially antagonized by methysergide administered into RVM. Increases in VAD thresholds generated by 1μg, 2.5μg and 5μg morphine were antagonized by administration of methysergide into ACe and nPf.

As these pain-elicited behaviors are organized at different levels of the neuraxis the results reveal vPAG morphine-induced, serotonin-mediated antinociception at spinal (SMRs), medullary (VDSs), and forebrain (VADs) levels.

Supported by NS27668 from NINDS.

346.3

5-HT_{1B} AGONIST ANALGESIA IN GENETIC MOUSE MODELS OF OPIATE ANALGESIC MAGNITUDE. **H.S. Hain, J.S. Mogil, D.J. Feller*, R. Hen, and J.K. Belknap. Dept. Of Behavioral Neuroscience, Oregon Health Sciences University, Portland, OR 97201 and Center for Neurobiology and Behavior, Columbia University, New York, NY 10032.**

A quantitative trait locus for morphine analgesic magnitude was mapped to chromosome 9 near the *Htr1b* gene which encodes the 5-HT_{1B} receptor (5-HT_{1B}R), suggesting that it may play a role in sensitivity to opiate analgesia. To examine this hypothesis, 5-HT_{1B} agonists were administered to genetic mouse models differing in opiate analgesic sensitivity: DBA/2J (D2) vs. C57BL/6J (B6), HAR vs. LAR, HA vs. LA, and *Htr1b* gene knockout (KO) mice vs. their wild-type (WT) littermates. Nociceptive sensitivity was assessed using the 49°C tail-withdrawal (TW) test, before and 20, 50, 120, and 240 min after drug injection. The putative 5-HT_{1B} agonist, CGS-12066A (i.p.; 0-75 mg/kg), was administered. Mice exhibiting high and low opiate (morphine) analgesic sensitivity also exhibited high or low CGS-12066A analgesic sensitivity, respectively. The opposite pattern was observed in the KO vs. WT mice suggesting that CGS-12066A may be producing its effects through the 5-HT_{1A} receptor in the KO mice. Anpirtoline, another 5-HT_{1B} agonist (i.p.; 0-40 mg/kg), produced effects in the expected direction. These results further support a role for the 5-HT_{1B}R in sensitivity to opiate analgesia. The data also question CGS-12066A's specificity as an agonist at the 5-HT_{1B}R. Supported by an NIDA training grant (HSH), a NRSA post-doctoral fellowship (JSM), and a VA Merit Review (JKB).

346.4

ALTERATIONS IN SWIM STRESS-INDUCED ANALGESIA FOLLOWING SEROTONERGIC ANTAGONISM IN THE ROSTROVENTROMEDIAL MEDULLA OF RATS. E. Hopkins, M. Spinella, Z.W. Pavlovic and R.J. Bodnar*. Dept. of Psychol. and Neuropsych. Doc. Prog., Queens Col., CUNY, Flushing, NY 11367.

Microinjections of general, $5HT_2$ or $5HT_3$ serotonergic antagonists into the rostroventromedial medulla (RVM) significantly decreases morphine analgesia elicited from the periaqueductal gray without altering basal nociceptive thresholds. Analgesia elicited by continuous cold-water swims (CCWS, 2°C, 3 min) is mediated by spinal delta$_2$ opioid receptors and supraspinal $5HT_2$ receptors, while analgesia elicited by intermittent cold-water swims (ICWS, 18 10 s swims and recoveries) is mediated by supraspinal mu opioid receptors and spinal $5HT_1$ receptors. The present study examined whether microinjection of the general serotonergic antagonist, methysergide (5-10 ug) into the RVM altered CCWS and ICWS analgesia on the tail-flick and jump tests in rats. CCWS produced analgesia on the tail-flick (30-60 min) and jump (30-120 min) tests which was significantly and dose-dependently enhanced for both peak (tail-flick: 41%; jump: 43%) and total (tail-flick: 72%; jump: 21%) effects by RVM microinjections of methysergide. In contrast, ICWS analgesia on the tail-flick (30-120 min) and jump (30-120 min) tests was minimally reduced by RVM microinjections of methysergide. Thus, medullary serotonergic mechanisms differentially modulate pharmacologically-distinct analgesic responses possibly through collateral inhibition. (Supported by PSC-CUNY Grants 666237 & 667248)

346.5

OPIATE SUPRASPINAL ANALGESIC SYNERGY BETWEEN THE AMYGDALA AND PERIAQUEDUCTAL GRAY IN RATS. Z.W. Pavlovic* and R.J. Bodnar. Dept. of Psychol. and Neuropsych. Doc. Prog., Queens Col., CUNY, Flushing, NY 11367.

Functional supraspinal analgesic interactions have been demonstrated between the periaqueductal gray (PAG) and rostroventromedial medulla using both antagonist and synergy studies. Microinjections of opiates and opioids into the amygdala elicit analgesia which is blocked by microinjection of general and delta$_2$ opioid antagonists into the PAG. The present study examined whether sub-threshold or near-threshold doses of morphine administered into the amygdala and PAG produced an analgesic synergistic interaction on the tail-flick and jump tests in rats. Morphine (1-5 ug) microinjections into either the amygdala alone or PAG alone dose-dependently increased nociceptive latencies and thresholds. Simultaneous administration of morphine into the amygdala (1 ug) and PAG (1 ug) produced profound analgesia on the jump, but not tail-flick tests that was significantly greater than analgesia elicited from either the PAG alone (1 or 2 ug) or amygdala (1 or 2 ug) alone. Analgesic interactions were significantly greater following a PAG morphine dose of 1 ug and amygdala morphine doses of 50 or 250 ng than following a PAG morphine dose of 250 ng and an amygdala morphine dose of 1 ug. These data are consistent with a direct role of the PAG in inhibiting nociceptive input, and demonstrate primarily a modulatory role for the amygdala in descending supraspinal brainstem pain-inhibition. (Supported by PSC/CUNY Grants 666237 & 667248)

346.6

ALPHA$_2$ ADRENERGIC TONIC CONTROL IN RATS WITH CARRAGEENAN-INDUCED HYPERALGESIA. SPON: Brain Research Association.
Juan F. Herrero & Fernando Cervero. Dept. de Fisiología y Farmacología, Universidad de Alcalá de Henares, 28871 Madrid, Spain

Involvement of α_2 adrenergic systems in the processing of nociception is well documented. It is not clear whether an endogenous tonic α_2 adrenergic control is active in animals with an intact central nervous system. We have studied this possibility in normal and hyperalgesic conditions.

Single motor units (SMU) were recorded from hind limb muscles of male Wistar rats (300-450g) under α-chloralose anaesthesia. Three groups were compared: i) control, ii) carrageenan-inflammation (intraplantar) and iii) carrageenan monoarthritis (knee joint).Thermal and electrical stimulation (wind-up) was applied to the peripheral receptive field of the SMUs in 3.5min cycles. The α_2 adrenoceptor antagonist Idazoxan was injected iv in cumulative doses from 20 to 320μg/kg. The μ opioid fentanyl (2 to 16/32μg/kg) was also tested before and after Idazoxan.

In control animals, responses to either heat or electrical stimulation remained unchanged after the administration of Idazoxan. In animals treated with carrageenan, however, a dose-dependent increase of the activity evoked by heat was seen, with a maximum effect of 38±9.2% ($p<0.01$). The enhancement was significant respect to the results obtained in the control group ($p<0.01$) but there was no difference between the 2 groups of animals with carrageenan. Also in these animals, the antinociceptive effectiveness of fentanyl was reduced by 2.5 fold after the injection of Idazoxan.

We conclude that in anaesthetised animals with an intact central nervous system, an endogenous control involving α_2-adrenergic systems is activated by carrageenan-induced hyperalgesia. Since the potency of fentanyl is also modified, the control may involve endogenous opioids.
(Supported by DGICYT PB-93-0491).

346.7

SYMPATHETIC EXCITATION OF SENSORY NEURONS INDUCED IN THE DRG BY PERIPHERAL NERVE INJURY IS MEDIATED BY ALPHA-2 ADRENOCEPTORS. J.W. Leem*, E.J. Choi, Y.S. Gwak, T.S. Nam and K.S. Paik. Dept. of Physiology, Yonsei Univ. Col. Med., Seoul, 120-752, Korea.

The aim of this study is to determine which subtype of adrenoceptor is involved in sympathetically elicited excitation of sensory neurons induced after spinal nerve ligation.

Rats that received a unilateral ligation of L5-L6 spinal nerves 10-15 days previously were used. Under anesthesia, dorsal roots with ligated spinal nerves were cut near the root entry zone, and recording was made from afferents in microfilaments teased from the distal cut end of dorsal roots. Sympathetic excitation of the recorded afferents was elicited by stimulating T13 and L1 ventral roots.

All afferents that responded to stimulation of ligated spinal nerve produced spontaneous discharges. About one-fourth of active afferents responded with increased discharges to sympathetic stimulation. This sympathetically evoked afferent excitation was mimicked by clonidine, blocked by yohimbine (not by prazosine nor by propranolol), and was not affected by conduction block between the DRG and ligated site.

The results suggest that after spinal nerve ligation, sympathetic postganglionic fibers sprout in the DRG where adrenaline released from sympathetic endings excites sensory neurons by acting on alpha-2 adrenoceptors. (Supported by KOSEF 961-0701-008-2)

346.8

EFFECTS OF YOHIMBINE ON NALOXONE-INDUCED ANTINOCICEPTION IN A RAT MODEL OF UNILATERAL INFLAMMATION. M. Tsuruoka* and W.D. Willis, Marine Biomedical Institute, UTMB, 301 University Blvd, Galveston, TX 77555-1069

In order to explain the analgesic effects induced by low doses of opiate-antagonist naloxone, the existence of autoreceptors for some endogenous opioid peptides has been proposed. The present study addresses the involvement of noradrenergic mechanisms in producing naloxone-induced antinociception in a rat model of inflammatory hyperalgesia.

Male Sprague-Dawley rats were divided into the following two groups: 1) rats receiving an administration of the α2-adrenoceptor antagonist yohimbine (3 mg/kg, i.p.) prior to naloxone injection and 2) rats receiving the administration of saline. In each group, rats had a single s.c. injection of carrageenan (6 mg in 0.15 saline), and effects of a low dose of naloxone (5 µg/kg, i.p.) on thermal nociception were examined for 28 days following the induction of unilateral hindpaw inflammation. The analgesic effect was assessed by the prolongation of the paw withdrawal latency (PWL) to noxious thermal stimuli.

In rats receiving the administration of saline prior to naloxone injection, the low dose of naloxone significantly prolonged PWL in both the inflamed and the non-inflamed paws 4 h and 7 days after carrageenan. In rats receiving the administration of yohimbine, the low dose of naloxone failed to induce prolongation of PWLs 4 h after carrageenan, whereas naloxone produced analgesia 7 days after carrageenan. At 28 days, in both groups of rats, the low dose of naloxone induced hyperalgesia in the inflamed paw but not in the non-inflamed paw. The result supports our previous finding that the locus coeruleus is involved in naloxone-induced antinociception during the acute phase of inflammation. (Supported by NS09743, NS11255 and a grant from Bristol-Myers Squibb Co.)

346.9

ANALGESIC EFFECT OF OXOTREMORINE ON BRIEF REFLEXIVE AND PERSISTENT NOCICEPTIVE MODELS. F.Pavone*, F.Capone°, A.M.Aloisi^, D. Pullia Inst. of Psychobiology & Psychopharmacology, Via Reno 1, 00198 Roma, Italy; °Dept. Genetics & Molecular Biology, , "La Sapienza" University, Roma, Italy; Inst. Human Physiology,University of Siena, Siena, Italy.

The neural pathways involved in the modulation and transmission of noxious stimuli, as well as the effects of drugs on nociception seem to be different depending on the nature of the stimulus and its duration. The aim of this study was to investigate the effects induced by the muscarinic cholinergic agonist oxotremorine on the response to a long-lasting painful stimulus induced by formalin in comparison with the well known antinociceptive properties of this drug observed after a brief thermal stimulus measured in the tail-flick test. The modulation of the effects of oxotremorine by morphine was successively assessed in both tests. Different groups of CD1 mice were used. For the formalin test mice were injected with saline solution or oxotremorine (from 0.01 to 0.05 mg/kg i.p.) fifteen minutes before they received a subcutaneous injection in the dorsal part of the right hind paw of a diluted solution of formalin (5%) or a sham injection. The time spent in licking of the injected paw was measured for 30 minutes following injection. Oxotremorine induced a dose and time dependent analgesic effect on both early and late phases. A simultaneous injection of 1 mg/kg of morphine with 0.03 mg/kg of oxotremorine, doses not effective by themselves in this test, significantly reduced the time of licking. An interaction of morphine with the effects of cholinergic stimulation was also observed in the acute pain induced by thermal stimulus in the tail-flick test. Oxotremorine (0.005 and 0.01 mg/kg) in combination with morphine (2.5 mg/kg) induced a clearcut and long lasting analgesic effect in mice in comparison with mice injected with these drugs alone. These results suggest that oxotremorine is able to modulate both phasic and tonic pain and that this action is at least in part modulated by the opioid system.

346.10

EFFECT OF CHRONIC INTRATHECAL ADMINISTERED GLYCINE ON NERVE INJURY-INDUCED NEUROPATHIC DISORDER. W. Huang and R. K. Simpson*, Dept of Neurosurgery, Baylor Col. of Med., TX., 77030.

We studied the effects of chronic intrathecal administered glycine on the mechanical and thermal hyperalgesia, and neuropathic gait and posture following unilateral ligation of the sciatic nerve in rats. Glycine or saline was intrathecally administered to low lumbar region immediately after the nerve lesion, by an osmotic pump (Alzet model 2002) 0.5 µL per hour for 14 days. The rats which were treated with saline (0.9%, pH7.4) displayed typical ipsilateral mechanical and thermal hyperalgesia with a typical neuropathic gait and posture from day 3 to16. The rats with intrathecal glycine (0.1, 0.2 µg / 0.5 µL, pH7.4) displayed a significant reduction in mechanical hyperalgesia on injuried paw through day 3 to12, as compared to the animals with intrathecal saline ($n = 9$, $p < 0.05$), and became normal sensation to mechanical stimuli on day 16 (2 days after absence of glycine administration, $n = 9$, $p < 0.05$). About 50% of these rats showed an abnormal gait and posture on the injuried paw on day 6 and became recovered on day 9 to 12. By the day 16, 90% of them regained their normal gait and posture, which were significantly different than animals treated with saline ($n = 9$, $p < 0.05$). The animals in the same group showed typical ipsilateral thermal hyperalgesia from day 3 to 16, similar to animals treated with saline ($n = 9$, $p > 0.05$). The result supports the hypothesis that spinal glycinergic activity prevents development of the mechanical hyperalgesia as well as accompanied neuropathic changes on gait and posture, but not thermal hyperalgesia. This study also suggests that the loss of spinal glycinergic receptors or neurotransmission may be the underlying mechanism of nerve compression-induced neuropathic disorder. The study is supported by Medtronic Inc.

346.11

PRIMARY AFFERENT INHIBITION OF SPINAL LAMINAE I AND II NEURONS, J. Bao, J. Li* and E. R. Perl, Department of Physiology, CB# 7545, Univ. North Carolina, Chapel Hill, NC 27599.

Primary afferent activation produces inhibitory effects on some neurons of the superficial spinal dorsal horn (Laminae I and II). To obtain evidence on synaptic mediators of such inhibition, we prepared transverse spinal cord slices with dorsal rootlets (DR) attached from deeply-anesthetized hamsters. The slices were maintained *in vitro* by superfusion with an artificial cerebral spinal fluid, equilibrated with O_2/CO_2, to which agents were added. In tight-seal, whole cell voltage- clamp recordings at a holding potential of -60 mV, 30 of 78 neurons from Laminae I and II exhibited mixed fast inward and outward evoked currents differentially related to DR stimulation intensity; a rare neuron mainly showed an outward current. Bicuculline (10µM), an established $GABA_A$ channel antagonist had no effect on either outward currents or on overall DR-evoked responses in neurons recorded within Lamina II. In contrast, the glycine action blocker, strychnine (10µM), consistently eliminated outward current components and enlarged the fast inward currents evoked by DR stimuli from neurons within Lamina II. In some Laminae I-II_o cells, DR-evoked outward currents were partially suppressed by both bicuculline and strychnine. We conclude that DR-evoked inhibitory actions in Lamina II are principally mediated by glycine whereas for some Laminae I-II_o neurons both glycine and $GABA_A$ channels are involved. Supported by grant NS-10321 from the NINDS.

346.12

EFFECTS OF L-PHENYL-ISOPROPYL ADENOSINE ON RATS SENSITIZED BY MUSTARD OIL. T. Sumida, M.A. Smith, Y. Maehara, J.G. Collins, and L.M. Kitahata*. Dept. of Anesthesiology, Yale Univ. School of medicine, New Haven, CT 06510

Recent studies suggest that spinally located adenosine receptors may be involved in pain signalling pathways within the spinal cord. This study was carried out to assess the spinal effects of the adenosine receptor agonist L-phenyl-isopropyl adenosine (L-PIA) on dorsal horn nociceptive neurons in rats sensitized by mustard oil.

This protocol was approved by our institutional Animal Care and Use Committee. Male Sprague-Dawley rats (340-420g) were anesthetized with halothane (2.0-3.0% during surgery, 0.75-1.0% during measurement) and paralized with pancuronium bromide (0.5mg/kg/h, i.v.). Extracellular activity from spinal wide dynamic range (WDR) neurons was recorded through a tungsten microelectrode. Evoked responses to noxious radiant heat stimuli (51°C, 8sec) were recorded as impulses per second at 5 minute intervals. After baseline measurements, mustard oil (N-allyl isothiocyanate, 5% in mineral oil) was applied topically to an area 10-20 mm above the ipsilateral ankle joint (i.e., outside the receptive field). 30 minutes after the application of mustard oil, L-PIA was administered intrathecally at doses of 0.625nmol, 2.5nmol, 10.0nmol, and 50.0nmol in 50µl saline. As a control, 50µl saline alone was also administered intrathecally.

Mustard oil produced a long-lasting sensitization of spinal WDR neurons. L-PIA (0.625nmol, 2.5nmol, 10.0nmol, and 50.0nmol) caused 48.5+/-10.7%, 64.6+/-14.0%, 75.7+/-13.5%,and 84.1+/-7.2% suppression of evoked activity, respectively (Mean+/-SEM; $p<0.05$ compared to saline control). This finding suggests that further studies concerning the involvement of adenosine receptors in pain modulation at the spinal level is indicated.

(Supported by NIH grant NS-09871)

346.13

L-PHENYLISOPROPYL-ADENOSINE MEDIATED SUPPRESSION OF SPINAL NOCICEPTIVE NEURONS RESPONDING TO VISCERAL PAIN: ANTAGONISM BY 8-PHENYLTHEOPHYLLINE. Y.Maehara*, M.A.Smith, T.Sumida, J.G. Collins, L.M. Kitahata, Department of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510.

We have previously demonstrated that the A_1 adenosine receptor agonist L-phenylisopropyl adenosine (L-PIA) mediates dose dependent supression of spinal dorsal horn nociceptive neurons (DHNN) responding to noxious visceral stimulation in rats. This study was designed to investigate the ability of the adenosine receptor antagonist 8-phenyltheophylline (8-PT) to attenuate this action of L-PIA on DHNN.

This protocol was approved by our institutional Animal Care and Use Committee. Twenty three male Sprague-Dawley rats were laminectomized from T12 to L1 vertebral levels for extracellular recording of lumbar spinal dorsal horn activity under pentobarbital anesthesia. After baseline evoked activities (ips) to CRD were recorded, 10nmol of 8-PT or saline in a volume of 50µl was administered intrathecally. 2.5 nmol L-PIA (50µl volume) was administered 15 min following 8-PT application. Evoked neuronal activities to CRD were recorded every 5 min thereafter for 60 min. All evoked activities were converted to percent change of baseline value.

Intrathecal application of 8-PT alone caused a 16.5% increase of in the mean evoked activity of DHNN for the 5-60 min recording period.The mean suppression of evoked activity of DHNN between 45 and 60 min after i.t. application of 2.5 nmol L-PIA in the absence of 8-PT was 39.8%; in the prescence of 8-PT, the mean suppression by i.t. 2.5 nmol L-PIA during the same time period was only 10.4%.

This study demonstrates that 1) endogenous adenosine may tonically modulate activity of DHNN, and 2) suppression of spinal DHNN responding to noxious visceral stimulation by i.t. L-PIA is antagonized by the adenosine receptor antagonist 8-PT. (Supported by NIH grant NS-09871.)

346.14

THERMAL NOCICEPTION IS ATTENUATED BY NICOTINIC AGONISTS IN TWO MODELS OF PERIPHERAL INFLAMMATION IN THE RAT. S. Kilo*, K.M. Hargreaves, C.M. Flores. Lab. of Neuropharmacol., UMN. Mpls, MN 55455.

Previous studies showed that nicotine is able to enhance the capsaicin induced release of calcitonin gene-related peptide (CGRP) from peripheral primary afferent terminals in an *in vitro* superfusion model. The purpose of the present study was to assess the effects of nicotine or its potent congener epibatidine on peripheral thermal nociception *in vivo*.

In one experiment, male Sprague Dawley rats were coinjected into one hindpaw with capsaicin (20µg/40µl) and nicotine bitartrate (0.165-16.5mM). In a second experiment, rats got an additional injection of the same dose of capsaicin into the other paw. In a third experiment, animals received a unilateral epibatidine injection (1-100µmol) and ~20 min. later a second injection with carrageenan (2mg/100µl) into the same paw. To assess thermal nociception, paw withdrawal latencies (PWL) to radiant heat were measured before the first injection and up to 120 min after the last injection.

Capsaicin alone showed a decrease in PWLs (-7.1+/-1.7s) within minutes after injection. Cotreatment with nicotine dose-dependently attenuated this decrease in PWL ipsilateral to the capsaicin/nicotine injection, but was less effective in the contralateral paw (capsaicin only). Epibatidine pretreatment dose dependently reduced the carrageenan induced hypersensitivity. Additionally, the highest dose of epibatidine was able to substantially block the edema associated with the injection of carrageenan. Injection with epibatidine alone evoked a transient reddening of the paw skin but did not lead to a significant reduction in PWLs.

These data indicate that nicotine exhibits antinociceptive activity in two models of peripheral inflammation. These studies further suggest that nicotine in addition to its previously documented effects in the central nervous system has a peripheral site of action to inhibit nociceptive neurotransmission.

Supported by: the Smokeless Tobacco Research Council grant #0490-01, NIH DE09860 and the Deutsche Forschungsgemeinschaft.

346.15

SIGNIFICANCE OF OPIOID AND HISTAMINERGIC MECHANISMS IN SKF92374-INDUCED ANTINOCICEPTION, B.Y. Li*, J.W. Nalwalk, and L.B. Hough, Dept. Pharmacology and Neuroscience, Albany Medical College, Albany, NY 12208.

A recent study showed that SKF92374, a structural analog of the H_2 antagonist cimetidine, induces hot plate- and tail flick- antinociception after intraventricular (ivt) injection in the rat (Li et al., JPET 276:500, 1996). This compound lacked significant activity on H_1 or H_2 receptors, and had only weak activity (Kd = 5.2 µM) on H_3 receptors. To test the hypothesis that SKF92374-induced analgesia is mediated by an action on H_3 receptors, the effects of the H_3 agonist R-α-methylhistamine (RAMH) and the H_3 antagonist thioperamide (both given by ivt administration) were investigated on SKF92374 antinociception in rats. Male Sprague-Dawley rats chronically implanted with lateral ventricular cannulas were maintained in a reversed light cycle (lights on 1900 - 0700) and tested by hot plate (52° C) and tail flick nociceptive tests. Animals were tested for baseline responses, received ivt injections (5 µl), and were re-tested 5, 10 and 30 min later. On both hot plate and tail flick tests, SKF92374-induced antinociception was slightly enhanced by thioperamide (30 µg), but was unaffected by a range of doses of RAMH (up to 2 µg). Furthermore, SKF92374-induced analgesia (10 min) was not reduced by large doses of systemically-administered antagonists (30 min pre-treatments) of H_1, H_2, H_3, or opiate receptors (pyrilamine: 10 mg/kg, i.p.; zolantidine: 20 mg/kg, s.c.; GT-2016: 30 mg/kg, i.p.; naltrexone: 5 mg/kg, i.p., respectively). These findings show that the novel agent SKF92374 induces antinociception by a non-opiate analgesic mechanism that does not utilize H_1, H_2 or H_3 receptors.

Supported by DA-03816

346.16

SKF92374 AND BURIMAMIDE INDUCE ANTINOCICEPTION IN MICE J.W. Nalwalk, J.N. Finkell, and L.B. Hough*, Dept. Pharmacology and Neuroscience, Albany Medical College, Albany, NY 12208.

Previous studies have shown that the H_2 antagonist cimetidine and a structural analog (SKF92374) induce antinociception in rats when given into the lateral ventricle (ivt, Li et al., JPET 276:500, 1996); the effect does not depend on H_1, H_2, H_3 or opiate receptors (Li, et al, this volume). Presently, the analgesic actions of SKF92374 and another analog, burimamide (BUR) were assessed in male Swiss-Webster mice. Animals were housed in either a normal light cycle (lights on 0700-1900) or reversed cycle (lights on 1900-0700) and tested with the hot water (55° C) tail immersion nociceptive test (cutoff 8 sec). Following baseline testing, subjects received an ivt injection under ether anesthesia, and were re-tested 10, 20 and 40 min later. Under reverse-cycle conditions, SKF92374 and BUR produced time-dependent (peak effects at 10 & 20 min, respectively) and dose-dependent (ED50s of 3.0 and 3.45 µg, respectively) antinociception. Under normal lighting conditions, SKF92374 also induced complete antinociception, but with a lower potency (ED50 = 7.35 µg). These findings show that: 1) SKF92374 is a potent antinociceptive agent in mice, similar to results previously found in rats; 2) BUR inhibits spinally-mediated nociceptive responses in mice, extending recent findings with supraspinally-mediated tests (Lamberti et al., Pharm. Biochem. Behav. 53:567, 1996); 3) SKF92374 antinociception in mice exhibits diurnal changes. Further studies to elucidate the mechanism of action of this novel non-opiate analgesic are in progress.

Supported by DA-03816

346.17

STIMULUS PROPERTIES AND ANTINOCICEPTIVE EFFECTS OF CANNABINOIDS IN MODELS OF INFLAMMATORY NOCICEPTION IN RATS. J.T. Roach[1]*, S.L. Broom[1], S.M. Graham[2], M.A. ElSohly[3,5], A.B. Jones[3,5], S.A. Ross[4,5] and K.J. Sufka[1,2]. Depts. of Psychol.[1], Pharmacol.[2], Pharmaceutics[3], Pharmacognosy[4], Res. Inst. of Pharmaceutical Sci.[5], University of Mississippi, Oxford, MS 38677.

Research has documented the role of cannabinoids in the mediation of inflammatory nociception and has suggested that Δ-9-THC possesses therapeutic potential against chronic inflammatory pain. The present study examined the antinociceptive and reinforcing properties of low-potency marijuana (2.5% Δ-9-THC/cigarette; smoke delivered over a 10 min interval; ADL/II Smoking System) in models of inflammatory nociception. Exposure to 1 cigarette produced antinociception on both early (0-5 min) and late (30-50 min) phases of the formalin response (50 µl of 2.5% formalin). Marijuana-treated rats displayed significantly fewer nociceptive behaviors compared to no-smoke controls (both formalin phases) and compared to placebo (< .01% Δ-9-THC) controls (late phase only). In the conditioned place preference paradigm incorporating the Freund's adjuvant (100 µl, ipl) model of chronic inflammatory pain, multiple exposures to low-potency marijuana cigarettes did not produce significant reinforcing or aversive effects in either inflamed or non-inflamed rats. Comparison of preference scores for marijuana-treated rats across inflammation treatment conditions indicates a modest negative reinforcing effect (analgesia) in inflamed rats. Taken together, these data suggest that low doses of THC may offer therapeutic effects against inflammatory pain while possessing little addiction liability.

346.18

The analgesic effects of R(+)-WIN 55,212-2 Mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. U. Herzberg*[1], G. J. Bennett[2], E. Eliav[2] and I. J. Kopin[1]. [1]Clinical Neuroscience Branch, National Institute of Neurological disorders and Stroke. [2]Neurobiology and Anesthsiology Branch, National Institute of Dental Research, 9000 Rockville Pike, Bethesda, MD 20892.

The effects of a high affinity cannabinoid receptor agonist were evaluated in rats subjected to chronic constriction injury of the sciatic nerve (CCI) as well as sham operated animls. Intraperitoneal (i.p) injections of the active, but not the inactive enantiomer, alleviated the pain behavior exhibited by animals subjected to CCI in a dose dependant manner. Moreover, at doses ranging from 0.43 to 4.3 mg/kg effects on sensitivity to a heat stimulus were observed neither in the paw contralateral to the sciatic ligation, nor in animals subjected to sham surgery. Animals subjected to CCI and treated with the high drug dose exhibited hypoalgesia in the paw ipsilateral to the ligated sciatic, i.e. heat hyperalgesia was completely reversed and a presumed sensory deficit due to nerve injury was revealed. Interestingly, even this high dose showed no effects on animals subjected to sham surgery. Although side effects were present in some animals subjected to the high dose treatment, a moderate dose (2.14 mg/kg) exhibiting complete alleviation of thermal hyperalgesia, with minimal side effects was identified. Using this dose, the sensitivities to von Frey hairs, pin prick and cold stimulus are being tested in animals subjected to CCI and sham surgery. In addition to identifying a potential drug treatment for painfull mononeuropathy, this study points to possible changes in cannabinoid receptors in animals subjected to unilateral mononeuropathy. (Supported by NINDS and NIDR)

346.19

SR 141716A, A CANNABINOID RECEPTOR ANTAGONIST, INDUCES NMDA-DEPENDENT HYPERALGESIA. J.D. Richardson*, L.M. Aanonsen[1], K.M. Hargreaves. Depts. of Restorative Sciences and Pharmacology, Univ. of MN, Mpls, MN 55455 and [1]Dept. of Biology, Macalester College, St. Paul, MN 55105.

We have recently reported that intrathecal administration of the cannabinoid receptor antagonist SR 141716A produces hyperalgesia in mice with an ED_{50} of 0.0012 fmol (Abs. Soc. Neurosci, 20:128, 1994). It has also been reported that cannabinoids inhibit glutamatergic synaptic transmission in the hippocampal cultures (Shen et al, J. Neurosci, in press). Because glutamate is a well-established modulator of hyperalgesia, we tested the hypothesis that there is a glutamatergic component of SR 141716A-induced hyperalgesia.

Baseline hot plate latencies were recorded from male Swiss-Webster mice. One of the following was administered intrathecally in a 5µl volume: saline, SR 141716A (0.005 fmol), SR 141716A (0.005 fmol) + AP5 (5 pmol), or SR 141716A (0.005 fmol) + MK-801 (0.025-2.5 pmol). Hot plate latencies were re-tested 5 min after drug administration. Data were analyzed with ANOVA and Duncan's test.

SR 141716A induced significant hyperalgesia when compared with the saline controls (-6.1 sec vs 0 sec, $F[7, 88] = 7.10$). This was reversed by 5 pmol AP5, an NMDA antagonist (-1.1 sec, $F[7, 88] = 7.10$). The SR 141716A-induced hyperalgesia was also reversed dose-dependently by 0.025, 0.25, and 2.5 pmol MK-801, another NMDA antagonist (-6.3, -3.4, -0.4 sec respectively, $F[1, 22] = 7.48$).

These results suggest that there is an NMDA component of SR 141716A-induced hyperalgesia. One potential mechanism for SR 141716A-induced hyperalgesia is tonic activation of the cannabinoid receptor resulting in the inhibition of glutamate release from some component of the spinal nociceptive system. According to this hypothesis, injection of the cannabinoid antagonist would result an increase in glutamate release leading to activation of central mechanisms of hyperalgesia.

This research was supported by a Predoctoral Fellowship from the Howard Hughes Medical Institute, DE9860, and DE11277.

RETINAL RECEPTORS AND CHANNELS

347.1

THE MGLUR6 L-AP4 RECEPTOR IS EXPRESSED IN BOTH ROD AND CONE ON BIPOLAR CELLS IN PRIMATE RETINA. R.M. Duvoisin*[1] and N. Vardi[2]. [1]Dyson Vision Research Institute, Dept. Ophthalmology, Cornell Med. College, New York, NY10021; [2]Dept. Neuroscience, U. Penn., Philadelphia, PA19104.

The depolarization of ON bipolar cells in response to light is mediated by an L-AP4-sensitive metabotropic glutamate receptor. This receptor (now known as mGluR6) has been cloned from rat retina and localized to dendritic terminals of rat bipolar cells. It remained unclear whether mGluR6 is expressed also by certain or all ON cone bipolar cell types. We wished to address this question in primate retina where ON cone bipolar cells are well identified and their connections with cones are well described. However, antisera raised against rat mGluR6 carboxy-terminus (Nomura et al., 1994) did not stain primate retina, suggesting that the primate carboxy-terminal sequence is different. We therefore cloned the human mGluR6 cDNA by screening a human retina cDNA library (generously provided by J. Nathan) with the rat mGluR6 as probe, determined the deduced carboxy-terminal human sequence and raised antisera against this sequence in two rabbits. Staining was highly localized to bipolar dendritic tips of both rod and cone bipolar cells in the OPL. Faint staining could be seen in somas located high in the INL, where ON cone bipolar cells reside. Roughly half of the cone bipolar cells were stained, indicating that most or all cone bipolar cell types express this receptor. Thus we conclude that the same L-AP4 receptor is expressed in both rod and cone bipolar cells. (Supported by EY11105, EY08124, EY09534, and RPB)

347.2

Ligand-gated currents of the rod dominant A17 amacrine cell of the cat retina E.D. Cohen* Dept. of Ophthalmology and Visual Science, Yale Univ. Med. School, New Haven, CT 06520-8061

We have examined the ligand- gated conductances of A17 amacrine cells in a retinal slice preparation. This large wide-field amacrine cell forms reciprocal synapses to rod bipolar axon terminals and is thought to contain both serotonin and GABA. Retinal slices were prepared similar to the methods of Cohen et al. J. Neurophysiol.72:1260-1269,1994. A17 amacrine cells were voltage-clamped using Cs^+-filled low chloride containing electrodes at a holding potential of -70mV. Lucifer yellow was included in the patch pipette for cellular identification. Cadmium (1mM) and TTX (200nM) were used to block synaptic transmission. Drugs were bath applied. Large inward currents were seen to GABA (200µM), while glycine (200µM) produced small currents. The GABA current reversed near the reversal potential for chloride. A large portion (45-80%) of the GABA-induced current was blocked by 20-100µM bicuculline, while 120µM picrotoxin blocked any remaining current. Application of the excitatory amino acid agonists kainate 30µM and AMPA 30µM both evoked prominent inward currents that reversed near 0mV. In contrast, application of 200µM NMDA even under Mg^{2+}-free (200µM Cd^{2+}) conditions evoked little or no inward current. Thus the glutamatergic bipolar inputs to this amacrine cell are predominantly from AMPA/kainate receptors. Supported by NIH EY10617 to EDC.

347.3

PET-SUBSTITUTED cGMP ANALOGS: NOVEL ANTAGONISTS OF ROD PHOTORECEPTOR cGMP-GATED CHANNEL

J.-Y. Wei, E. D. Cohen, Y.-Y. Yan, H.-G. Genieser# and C. J. Barnstable* Dept. Ophthalmol. & Vis. Sci., Yale Univ. Sch. of Med., New Haven, CT, #BIOLOG Life Sci. Institute, Bremen, Germany

The exocyclic N1 and N2 of the guanine ring of cGMP are thought to play a key role in activating the rod CNG channels. To test this hypothesis, studies have been undertaken with four synthetic cGMP analogs: PET-cGMP, 8-Br-PET-cGMP, Rp-8-Br-PET-cGMPS and Sp-8-Br-PET-cGMPS, all of which contain a phenyl-substituted 5-membered ring system fused to the amino group in position 2 and the nitrogen in position 1 of the purine ring. The compounds were tested on excised patches from *Xenopus* oocytes expressing α-subunit of the rat rod channel and in *Xenopus* rod outer segments. Both Rp-8-Br-PET-cGMPS and Sp-8-Br-PET-cGMPS acted as potent antagonists of rod cyclic GMP-gated channels. PET-cGMP at millimolar concentrations did not lead to detectable currents. When co-applied with subsaturating concentrations of cGMP, it increased the responses of cGMP to the maximal response. 8-Br-PET-cGMP acted as a very weak partial agonist and an antagonist when co-applied with cGMP. Together the results suggest that the cGMP sites altered directly by PET substitution play a major role in rod CNG channel activation. The oxygen atoms of the cyclic phosphate moiety have a small but measurable effect on channel activity. Sp-8-Br-PET-cGMPS will be a useful tool for studying the effects of cGMP because although it acts as a potent CNG channel antagonist, it is a good activator of cGMP-dependent protein kinase. Supported by the NIH and the Kemper fund.

347.4

ACTIVATION OF NMDA OR KAINATE RECEPTORS MODULATES CGMP-LIKE IMMUNOREACTIVITY IN TURTLE RETINA: INVOLVEMENT OF NITRIC OXIDE SYNTHASE. W.D. Eldred*, T.A. Blute and J. De Grenier. Department of Biology, Boston University, Boston, MA 02215.

In retina, cGMP gates ion channels and modulates gap junction conductivity in many cell types. Because excitatory amino acids are the transmitter used by many retinal neurons, we examined the effects of stimulating NMDA or kainate (KA) receptors on levels of cGMP-like immunoreactivity (cGMP-LI). Following *in vitro* incubations with KA or NMDA, immunocytochemistry with a cGMP antibody was used to examine cGMP-LI in specific retinal neurons. KA and NMDA increased cGMP-LI in bipolar and amacrine cells, and in somata in the ganglion cell layer. In response to each ligand, there were clear regional differences seen in the labeled cell types, in the numbers of labeled cells and in the intensity of labeling. In the inferior retina, NMDA stimulated intense cGMP-LI in bipolar and amacrine cells, and in three bands in the inner plexiform layer (IPL). In superior retina, NMDA increased cGMP-LI less intensely in amacrine cells and in two bands in the IPL. In contrast, KA stimulated cGMP-LI in bipolar and amacrine cells, and in three bands in the IPL in all retinal regions. To test the involvement of nitric oxide synthase (NOS), we used the NOS inhibitors 7-NI and L-NNA. Both inhibitors blocked almost all of the increases in cGMP-LI seen in response to KA or NMDA receptor activation. KA and NMDA increased cGMP-LI in different cell types, which suggests more than one postsynaptic cell was involved. These results indicate that activation of ionotropic glutamate receptors can lead to the stimulation of the cGMP second messenger system in a wide variety of select retinal neurons.

This research supported by EY04785 to W.D.E.

347.5

SOMATOSTATIN RECEPTOR SUBTYPES (SSTR2) IN THE RABBIT RETINA. A.Vasilaki, E. Hatzilaris#, G.Liapakis, Z.Georgoussi# and K.Thermos*. Lab. of Pharmacology, Univ. of Crete, Sch. of Med., 71110 Iraklion, and #Inst. of Biology, N.C.S.R. "Democritos",153 10 , Athens, GR.

To address the role of somatostatin and its mechanism of action in the retina, we have engaged in studies to identify and characterize somatostatin receptors in the rabbit retina. In the present study we employed selective ligands for the recently cloned receptors (SSTR1-5) and examined their ability to inhibit [125I]Tyr11-somatostatin binding to rabbit retinal membranes. SMS201-995, MK678, and BIM23014, more selective for the SSTR2, displayed IC50 values of 0.026±0.002, 0.09±0.06 and 1.0±0.6 nM, respectively, while BIM23052 more selective for SSTR5, displayed an IC50 value of 18±3 nM. GTPγS reduced the [125I]Tyr11-somatostatin binding to rabbit retinal membranes, and shifted the somatostatin inhibition dose-response curve to the right. Using a series of anti-peptide antisera generated against the C-terminal decapeptides of Gi / Go proteins, the pertussis-toxin sensitive G-proteins Gi2α, Gi1α, and Goα, were immunoprecipitated. In addition, employing photocrosslinking methodology, the somatostatin receptor was studied further, using isoelectric focusing and lectin chromatography. The results from the above studies suggest that the somatostatin receptor in the retina is a slightly acidic N-glycosylated protein, linked to pertussis toxin sensitive G-proteins, with pharmacological characteristics similar to those of SSTR2. To substantiate the pharmacological findings, RNA blotting studies are in progress to examine with more precision at the molecular level, the differential expression of mRNAs for the somatostatin receptor subtypes in the retina. (This study was funded by a grant from the Greek Ministry of Research and Development, PENED 91ED665).

347.6

ELECTROPHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF A K^+ CHANNEL IN PHOTORECEPTORS OF THE HORSESHOE CRAB, *LIMULUS POLYPHEMUS*. Chaves, D.*[1], Jeziorski, M.C.[2], Battelle, B.-A.[2], Renninger, G.H.[1]. [1]Department of Physics, University of Guelph, Guelph, Canada, N1G 2W1. [2]Whitney Laboratory, University of Florida, St. Augustine, FL 32086.

Light depolarizes the photoreceptor cells of the horseshoe crab, *Limulus polyphemus*. Voltage-gated ion channels shape this electrical response. One ion channel found by patch-clamp recordings of photoreceptor cells dissociated from the compound eye is a voltage-dependent native K^+ channel characterized by fast transient openings. The channel has a mean patch conductance in the cell-attached configuration of 9.0 ± 3.4 pS (n = 18). Solution exchange experiments performed on inside-out excised patches confirmed that this is a K^+ channel. The kinetics of this channel are reminiscent of Shaker-type K^+ channels.

Using RT-PCR with degenerate primers designed against the Shaker family of K^+ channels, we isolated a 1 kb fragment from ventral eye RNA that exhibits homology with known Shaker-type K^+ channels. Subsequent cloning strategies were used to isolate the 5′ and 3′ fragments of this cDNA from ventral eye. Preliminary studies suggest this sequence is also represented in the lateral eye.

By expressing the channel in *Xenopus laevis* oocytes and performing two-electrode voltage clamp and patch clamp experiments, we hope to compare the functional properties of the cloned K^+ channel to the native K^+ channel. Characterization of this ion channel in the *Limulus* photoreceptors will contribute to a complete understanding of phototransduction.

Research sponsored in part by NSERC Canada, and by U.S. funding agencies: NIMH (MH10625) and NSF (IBN9211327).

347.7

KINETIC ANALYSIS OF THE L-TYPE CALCIUM CHANNEL OF THE HORIZONTAL CELL BASED ON TIME AND FREQUENCY DOMAIN MEASUREMENTS. E.C. Hymel*, C.R. Murphey, L.E. Moore, and B.N. Christensen. Dept. of Physiology & Biophysics, Univ. of Texas Medical Branch, Galveston, TX 77555-0641.

In enzymatically isolated catfish cone horizontal cells, the L-type calcium channel was investigated by fitting both time and frequency domain voltage-clamp responses. Under conditions where all other channels were pharmacologically blocked, the cell membrane potential was stepped from rest (-65 mV) to various test potentials. After obtaining the large-step current response, small signal sinusoidal stimuli (2-3 mV RMS, 0.3 to 1000 Hz) were used to measure the frequency-dependent response of the cell at each test potential. The frequency domain admittance functions and the time domain step responses for a range of potentials were fitted with a Hodgkin-Huxley type kinetic model that allows for activation and inactivation of the Ca^{2+} current. Passive characteristics of the cell were also estimated.

Results obtained show that L-type channel inactivation is not strongly voltage dependent in this cell, while activation characteristics are consistent with previous results. The methods here differ from conventional analyses since linear voltage perturbations (frequency domain responses) are used to extract kinetic information, with non-linear voltage step responses used to constrain the estimated parameters to fits which produce appropriate step clamp responses. Supported by grants NEI-01897 from the NIH and F30-MH11169-01 from the NIMH (to E.H.).

347.8

EVIDENCE FOR A DIHYDROPYRIDINE-INSENSITIVE CALCIUM CURRENT IN DISSOCIATED RETINAL GANGLION CELLS OF THE TIGER SALAMANDER. D. Henderson*[1], R. Nitzan[2], and R. F. Miller[1,2]. [1]Graduate Program in Neuroscience, [2]Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

Calcium imaging techniques, based on ratiometric FURA-2 fluorescence changes, were used to study the mechanisms of calcium accumulation in retinal ganglion cells. Ganglion cells were labeled with a retrograde dye (tetramethylrhodamine - "TMR") prior to dissociation and FURA-2 loading. An increase in $[Ca^{++}]_{in}$ in TMR labeled cells was observed in response to an elevation in external K^+ (25-50 mM). This K^+ induced change in $[Ca^{++}]_{in}$ was blocked by Co^{++}/Cd^{++}. However, 1-10 μM nimodipine, which completely blocked the K^+ induced increase in photoreceptors, did not significantly affect the K^+ induced change in ganglion cells. The type of calcium channel that supports this pharmacology in ganglion cells is under study using both calcium imaging and electrophysiological techniques.

Research supported by NIH grants EY07133 to D.H. and EY03014 to R.F.M.

347.9

STIMULATION OF AMACRINE CELLS ELICITS SYNAPTIC INPUTS TO GANGLION CELLS MEDIATED BY GABAa RECEPTORS. C.R. Shields, P.D. Lukasiewicz* Depts. of Ophthalmology and Anatomy & Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

GABA-evoked responses in retinal ganglion cells have been reported to be mediated primarily by GABAa and to a lesser extent by GABAc receptors (Zhang & Slaughter, 1995, J. Neurophysiol. 74:1583). We determined the types of GABA receptors that mediate *synaptic* inputs to ganglion cells by electrically stimulating amacrine cell processes with a concentric bipolar electrode placed at the inner plexiform layer of the tiger salamander retinal slice preparation. GABAergic synaptic currents were measured in ganglion cells with whole-cell patch clamp techniques. Glycinergic synaptic inputs were blocked with 2μM strychnine and glutamatergic inputs were blocked with 10μM CNQX and 40μM D-APV. The GABAergic synaptic currents reversed polarity near ECl and were completely blocked by the GABAa receptor antagonist bicuculline (100μM), indicating that these synaptic inputs were mediated exclusively by GABAa receptors. In contrast, amacrine cell inputs to bipolar cells were composed of both bicuculline sensitive and insensitive components, indicating that these synaptic inputs were mediated by both GABAa and GABAc receptors. Puffing GABA onto ganglion cell dendrites or bipolar cell terminals yielded similar results: responses in ganglion cells were blocked by bicuculline whereas only a component of the responses in bipolar cells was sensitive to bicuculline. These data show that amacrine cell synaptic inputs to ganglion cells are mediated by GABAa receptors and that GABAc receptors play little or no role.

Supported by NIH Grants EY08922, EY02687 and RPB, Inc.

347.10

EXPRESSION OF SOMATOSTATIN RECEPTOR SUBTYPE 2A (SSTR2A) IN THE RABBIT RETINA. J. Johnson*, H. Wong, J.H. Walsh and N.C. Brecha. Depts. of Neurobiol. and Med., CURE, UCLA and WLA-VAMC, LA, CA 90073

The neuropeptide somatostatin is likely to act as a neuromodulator by interacting with specific G-protein coupled membrane receptors SSTR1 to SSTR5. Somatostatin is widely expressed in the central nervous system including the retina. The cellular expression pattern of SSTR2A in the rabbit retina was determined using an affinity purified polyclonal antibody directed to the C-terminus of SSTR2A. Antibody specificity was tested by preadsorption of the primary antibody with 10^{-5} M $SSTR2A_{361-369}$. SSTR2A immunoreactivity is predominantly localized to rod bipolar cells and it is also observed in sparsely occurring amacrine cells. SSTR2A immunostaining in rod bipolar cells is strongest in the axon and axon terminals, and weak in the cell body and dendrites. Double-labeling experiments with the rod bipolar cell selective marker protein kinase C showed that all rod bipolar cells express SSTR2A immunoreactivity. In horizontal sections of retina, SSTR2A immunostained cell bodies have a dense distribution in agreement with the reported distribution of rod bipolar cells. SSTR2A immunoreactive amacrine cells occur very infrequently. They have a round cell body and are located at the border of the INL and IPL. They are likely to be multi-stratified and give rise to thin processes that ramify in laminae 2 and 4 of the IPL. These observations indicate that somatostatin, which is known to be localized to displaced amacrine cells, modulates the rod pathway through its interaction with SSTR2A expressed by rod bipolar cells.

Supported by EY04067 and DK41301 and VA Research Funds.

347.11

IDENTIFYING A SUBREGION CRITICAL FOR HOMOMERIC EXPRESSION OF FUNCTIONAL GABA ρ SUBUNITS. Dongxian Zhang*, Zhuo-Hua Pan, Johanna A. Koolen, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital, and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

We have previously shown that rat GABA ρ1 and ρ2 subunits can form heteromeric receptors with properties similar to $GABA_C$ receptors in the rat retina. However, unlike the rat ρ1 subunit and other GABA ρ subunits in human and fish, the rat ρ2 subunit is unable to form functional homomeric receptors. In order to understand the subunit interaction of the putative pentameric receptor, we identified regions that are critical for the homomeric expression of rat GABA ρ subunits. Chimeric subunits were constructed by substituting 5 different subregions of the rat ρ1 subunit with corresponding subregions of the ρ2 subunit. These chimeras were tested for their ability to form functional homomeric receptors mediating GABA-evoked currents in *Xenopus laevis* oocytes. By testing six chimeric subunits, we show that the subregion of the ρ1 subunit that is located 5' to the cys-cys loop (residues 94-214) is the only region absolutely required for homomeric expression. Since this subregion of the rat ρ2 subunit is highly homologous to the human ρ2 subunit (97% identity), we substituted this subregion of the human for the rat. This rat ρ2 mutant gained the ability to form functional homomeric receptors. In contrast, two additional rat ρ2 mutants, engineered to contain a few amino acid residues from this subregion in the rat ρ1 subunit, failed to express functional receptors. Thus, the integrity of the subregion may be critical for homomeric expression, and further mutants are being constructed to test this hypothesis.

Funded by NIH grants R01 EY05477 and P01 HD29587 (to S.A.L.).

347.12

METABOTROPIC GLUTAMATE RECEPTORS IN THE RAT RETINA. J.H. Brandstätter[1*], P. Koulen[1], R. Kuhn[2], and H. Wässle[1]. [1]Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, D-60528 Frankfurt am Main, Germany; [2]CNS Research, CIBA, CH-4002 Basel, Switzerland.

In recent years, there has been an increased awareness of the important role that metabotropic glutamate receptors (mGluRs) play in the synaptic events that underlie the processing of visual information.

We have studied the cellular and subcellular distribution of **mGluR2**, **3**, **4** and **7** in the rat retina using specific anti-peptide antisera. Of the four receptors examined, **mGluR3** was not found in the retina. **MGluR2** was expressed by amacrine cells. At the subcellular level, mGluR2 was localized to amacrine cells postsynaptic to both rod and cone bipolar cells. **MGluR4** was distributed throughout the inner plexiform layer (IPL) and was found postsynaptically in amacrine cell and ganglion cell processes. **MGluR7** labelling was localized to discrete strata in the IPL. At the subcellular level, mGluR7 was found at ribbon synapses, presynaptically in cone bipolar cells, and postsynaptically in amacrine cells. Presynaptically, mGluR7 was found at only one part of the active zone of the ribbon synapse, facing only one of the postsynaptic neurons.

During development, a gradual shift from receptor labelling of somata and processes to a preferential labelling of processes ocurred. This redistribution of mGluRs during development might correspond to a clustering of the receptor proteins during the formation of synapses.

Our data suggest that each mGluR plays a distinct role in retinal synaptic circuitry. The pre- and postsynaptic distribution of mGluRs at ribbon synapses in the IPL, suggests that the release of glutamate is highly regulated.

Supported by the Deutsche Forschungsgemeinschaft (SFB 269/B4).

347.13

SYNAPTIC LOCALIZATION OF GLYCINE AND $GABA_A$ RECEPTORS IN THE DEVELOPING RAT RETINA. M. Sassoè-Pognetto[1,2], P. Panzanelli*[2] and H. Wässle[1]. [1]Max-Planck-Institut für Hirnforschung, D-60528 Frankfurt am Main, Germany; [2]Department of Anatomy, Pharmacology and Forensic Medicine, University of Turin, I-10126 Turin, Italy.

The mechanisms by which neurotransmitter receptors are clustered at postsynaptic sites of neurons are largely unknown. The 93 kDa peripheral membrane protein gephyrin has been shown to be essential for the formation of postsynaptic glycine receptor clusters, and there is now evidence that gephyrin can also be found at GABAergic synapses. In this study, we have analysed the synaptic localization of glycine receptors, $GABA_A$ receptors, and the anchoring protein gephyrin in the inner plexiform layer of the developing rat retina, using immunofluorescence with subunit specific antibodies.

At early postnatal stages, the antibodies produced a diffuse staining, suggesting that early retinal neurons can express glycine and $GABA_A$ receptors. A clustered distribution of the subunits in "hot spots" was also observed. The number of the hot spots increased during development and reached adult levels in about two weeks. Electron microscopy showed that synapses of the conventional type are present in the inner plexiform layer of the postnatal retina and that the hot spots correspond to an aggregation of receptors at postsynaptic sites. Gephyrin was also localized to hot spots, and double immunofluorescence revealed a colocalization of gephyrin with the α2 subunit of the $GABA_A$ receptor.

These results indicate that clustering of receptor subunits occurs in parallel with the formation of morphologically identifiable synaptic specializations and suggest that gephyrin may be involved in clustering of $GABA_A$ receptors at postsynaptic sites.

Supported by grants from the DFG (SFB 269) and MURST (40% and 60%).

347.14

IMMUNOCYTOCHEMICAL ANALYSIS OF NEURONAL TRANSPORT OF GABA ANALOGUES IN THE DEVELOPING RABBIT RETINA. D.V. Pow, D.K. Crook and D.I. Vaney*. Vision, Touch and Hearing Research Centre, Dept. of Physiology and Pharmacology, University of Queensland, Brisbane 4072, Australia.

We have generated antibodies against non-endogenous GABA analogues including γ-vinyl GABA and have shown that in the adult retina γ-vinyl GABA is specifically accumulated by all the GABAergic amacrine cells. In this study we examined the transport of γ-vinyl GABA into neurons in the developing retina and compared this transport with the distribution of GABA and glycine in serial semithin sections. We demonstrate that at 1 dpn retinal ganglion cells can take up γ-vinyl GABA, but lose this capacity later in development, concomitant with a decline in their content of endogenous GABA. At all postnatal ages amacrine cells which contained glycine but not GABA failed to accumulate γ-vinyl GABA. γ-vinyl GABA was accumulated into many GABAergic amacrine cells at 1 dpn, however there were clear mismatches, some cells accumulated γ-vinyl GABA but did not contain detectable endogenous GABA and vice-versa. In addition some cells accumulated γ-vinyl GABA weakly whilst others showed strong accumulation, irrespective of the amount of GABA in these neurons, indicating that re-uptake of GABA plays a variable role in overall GABA homeostasis in different cell populations. These mismatches were markedly reduced by 10 dpn. To assess whether our results might be influenced by some amacrine cells changing from a GABA-containing to a glycine-containing phenotype we quantified the ratio of glycine-immunoreactive to GABA-immunoreactive (or γ-vinyl GABA - accumulating) neurons in retinae from 1 dpn to adult. Surprisingly, our data shows that by as early as 5 dpn the ratio of GABA-containing to glycine-containing amacrine cells is the same as that observed in the adult. We are now examining by immunocytochemistry whether the capacity of glycinergic neurons to transport glycine analogues shows analogous developmental changes. Funded by grants from NH&MRC (Australia) to DVP.

347.15

GLYCINE TRANSPORTER AND $GABA_A$ RECEPTORS COLOCALIZE IN DAPI-3 CELLS IN RABBIT RETINA. C. L. Zucker*, B. Ehinger, A. Bruun, N. C. Danbolt, J. Storm-Mathisen. Department Of Ophthalmology, University Of Lund, Sweden; Department Of Anatomy, University Of Oslo, Oslo, Norway; Schepens Eye Research Institute, Harvard Medical School Boston, MA 02114.

DAPI-3 cells are a recently described type of amacrine cell in the retina. They have a characteristic distribution of processes in two discrete sublayers in the inner plexiform layer of the retina (Ehinger and Zucker 1996). Their uniquely high concentration of $GABA_A$ receptors (subtypes α_1 and β_2/β_3) can be used as a reliable identifier, and we have used this to analyze their content of glycine transporter membrane protein (GLYT1) which is considered a marker for glycinergic neurons. $GABA_A$ receptors were visualized with a commercially available antibody (BD17) in conjunction with an antibody directed against the C-terminus of the glycine transporter protein (GLYT1).

GLYT1 immunoreactive cell bodies had the distribution characteristic of glycinergic amacrine cells, and emitted varicose processes throughout the IPL without discernible sublayering. Strong immunoreactivity for $GABA_A$ receptors appeared in amacrine cells of the DAPI-3 type. These cells were always seen to be immunoreactive for GLYT1 as well. Cell bodies and processes with weak $GABA_A$ receptor immunoreactivity were glycine transporter immunonegative.

With Lucifer Yellow microinjection experiments we have previously shown that strongly $GABA_A$ receptor immunoreactive cells are DAPI-3 cells. The present demonstration of GLYT1 immunoreactivity in their cell membrane suggests that glycine is a DAPI-3 cell transmitter in the rabbit retina. Independently, Wright and Vaney have reached a similar conclusion, using glycine immunohistochemistry (personal communication Nov. 1995).

347.16

DISTRIBUTION OF CONNEXIN43 IMMUNOREACTIVITY IN THE RETINAS OF DIFFERENT VERTEBRATES U. Janssen-Bienhold, R. Dermietzel and R. Weiler * Neurobiology, FB 7, Carl von Ossietzky University of Oldenburg, D-26111 Oldenburg, F.R.G.

In the retina, the main subclasses of cells employ gap junction channels as means of intercellular communication, and homologous as well as heterologous coupling has been demonstrated. However, while many informations have been collected regarding the regulation of gap junctions in the retina mediated by neurotransmitters and second messengers, only little is known about the expression of the hemichannels forming connexin proteins in the different retinal cells. Here we have used polyclonal antibodies (rb) against a synthetic oligopeptide of the C-terminal of connexin43 (rat, corresponding to AS 362-382) to investigate the occurence and distribution of connexin43 in the retinas of fish (carp and zebrafish), turtle, chicken and rat. The specifity of the antibody was analyzed by immunoblotting, where it localized two broad bands of 43-47 kDa (monomer) and 92-98 kDa (dimer) in samples of rat heart homogenates. The 43 kDa component also occured in retina samples of all tested species, while the dimer was more prominent only in the fish and rat retinal homogenates. In the retinas of all species a substantial immunolabeling of the margins of neighbouring pigment epithelium cells was observed. Both fish retinas also showed an intense punctuate connexin43 immunoreactivity (IR) at the level of the outer limiting membrane and the outer plexiform layer, most likely resulting from the presence of connexin43 in Mueller glia cells. In addition, a population of amacrine cells ramifying at layers one and three of the inner plexiform layer revealed an intense punctuate immunolabeling in the zebrafish retina. In accordance with the presence of connexin43 IR in Mueller glia cells, the rat retina also showed immunoreactive puncta at the level of the inner limiting membrane, which is formed by Mueller glia cells. In summary, our data demonstrate the expression of connexin43 in the vertebrate retina.

Supported by the Deutsche Forschungsgemeinschaft

347.17

CLONING OF A GAP JUNCTION GENE FROM THE RETINA OF THE GIANT DANIO T.L.E. Wagner*, D.G. McMahon, and D.K. Vaughan 1. Department of Physiology, University of Kentucky, Lexington, KY 40536; 1. Department of Biology, Utah State University, Logan, Utah 84322

Gap junctions are closely apposed areas of cell membranes which contain gap junctional channels, or connexons. Gap junctions in the retina serve a variety of functions and are an excellent model for electrical synapses found throughout the nervous system.

We have cloned from the retina of the giant danio (*Danio aequipinnatus*) a full length cDNA (DACX43) which shares high sequence homology and similarly predicted structural features with previously cloned connexins. A partial clone was isolated from retinal RNA using reverse transcriptase polymerase chain reaction (RT-PCR). We then created a giant danio retinal cDNA library and used the partial clone to isolate a full length clone. The cDNA predicts an amino acid sequence of 382 residues with a molecular weight of 43.4 kDa. The cloned sequence was compared to those of previously sequenced connexin genes in nucleic and amino acid forms (PCGENE, IntelliGenetics). Identity to rat CX43 at the nucleotide level is 75%, and at the amino acid level is 78%. Consensus phosphorylation sites for PKA, PKC, PKG, and CAKII were found in the C terminus.

We synthesized a 14 aa peptide corresponding to the cytoplasmic loop sequence of DACX43. Eight copies were joined to form a MAP (multiple antigenic peptide) and injected into mice to create antibodies. In addition to the positive response of the hyper-immune serum, several monoclonals reacted with horizontal cells, as well as other cells in the inner nuclear layer. We are continuing to characterize these monoclonals for use as antibodies. Supported by NIH EY09256, NS01734, and EY09038 to DKV.

347.18

PARTIAL SEQUENCE OF A GAP JUNCTION FROM THE HYBRID STRIPED BASS RETINA C. Lu, D.G. McMahon and D. Speck*. Department of Physiology, University of Kentucky, Lexington, KY 40536.

Gap Junctions are widely distributed in all synaptic layers of the retina and are of fundamental importance in transmitting and shaping visual signals. Retinal gap junctions serve as an excellent model for gap junctions found throughout the nervous system.

We have detected expression of connexin 43 in retina and heart tissue of both rat and bass by Northern analysis using rat connexin 43 cDNA as probe. We have successfully cloned a partial cDNA (HBRET-1) from hybrid striped bass retina which shares high sequence homology and predicted structural features with previously cloned connexins. This 434 base pair clone was isolated from bass retinal RNA using reverse transcriptase polymerase chain reaction (RT-PCR) and a set of degenerate primers based on conserved areas of previously cloned connexins. This clone showed a positive hybridization to rat connexin 43 cDNA. Sequence analysis (PCGENE, IntelliGenetics) revealed that HBRET-1 is most similar to rat connexin 43. In comparison the equivalent partial sequence of rat connexin 43, HBRET-1 exhibits identity at the nucleotide level is 77.1%, and 79.2% homology at the amino acid level. HBRET-1 has also been confirmed as part of the hybrid bass genome by Southern analysis. We are currently localizing further the cell-specific expression of HBRET-1 in the hybrid bass retina using single cell PCR and in situ hybridization. Supported by NIH EY09256 and NS01734.

347.19

IMMUNOBLOT AND IMMUNOCYTOCHEMICAL ANALYSES OF CATFISH RETINAL PROTEINS USING ANTIBODIES RAISED AGAINST RAT CONNEXINS: CX43,CX32 AND CX26. L.J. Giblin and B.N. Christensen* Dept. Physiology & Biophysics, Univ. of Texas Medical Branch, Galveston, TX 77555.

Gap junctions have been identified throughout the teleost retina; most notably connecting horizontal cells, photoreceptors, amacrine cells and, presumably, Mueller cells. While the electrophysiology and modulation of the gap junctions present in teleost retina have been extensively studied, the connexin proteins forming them have yet to be isolated or cloned. To determine whether catfish retinal connexins might be recognized as homologues of known connexins, we performed immunoblot and immunocytochemical (IC) analyses of catfish retina using polyclonal (PAb) and monoclonal (MAb) antibodies directed against rat connexins: Cx43, Cx32 and Cx26.

PAb's directed against the C-terminal region of rat heart Cx43 recognized a 43kD protein in the catfish retina with great specificity. IC staining resulted in punctate labeling of the outer nuclear layer.

PAb's directed against the second cytoplasmic domain of Cx26 also recognized proteins in the catfish retina, with apparent molecular weights of ~70kD and ~19kD. IC staining resulted in labeling of the Mueller cells, especially the outer limiting membrane. A MAb directed against the same region recognized only the 70kD protein.

PAb's directed against the second cytoplasmic domain of Cx32 recognized many different proteins in the catfish retina. Most notable were proteins having apparent molecular weights ~15kD, ~26kD, ~32kD ~60kD and ~200kD. IC staining resulted in diffuse signal throughout the inner retina. A monoclonal antibody directed against the same region failed to recognize any proteins from the catfish retina.

Our results suggest that the 43 kD protein found in the teleost retina may be a gap junction protein present in photoreceptor cell bodies. Furthermore, the Mueller cells may also contain a Cx26 homologue. Supported by Grant NEI-01897 from the NIH.

VISUAL PSYCHOPHYSICS AND BEHAVIOR II

348.1

DEFICITS AND RECOVERY OF FIRST- AND SECOND-ORDER MOTION PERCEPTION IN PATIENTS WITH UNILATERAL PARIETAL LESIONS. D. I. Braun* (1), M. Fahle (1) & P. Schönle (2) (1) Sektion Visuelle Sensorik, University Eye Clinic, Waldhörnlestr. 22, D-72072 Tübingen, Germany, (2) Neurological Rehabilitation Clinic Schmieder, D-78473 Allensbach, Germany.

Unilateral lesions in the posterior parietal cortex can degrade motion perception in the contralateral visual field. Our aim was to test whether the effects may be differential for first- and second-order motion, and to investigate the time scale of any potential recovery. We tested nine patients with circumscribed lesions in the parietal cortex. Thresholds were measured for the discrimination of the direction of motion of stimuli presented 5.5 deg peripherally in the visual hemifields ipsi- and contralateral to the lesion. Subjects had to indicate whether a small rectangular region (1.6 deg x 3 deg) embedded in a dynamic random noise background moved up or down. The region contained moving dots (signal) and flickering dots (background) and moved for one second at a speed of 2.36 deg/sec. Signal dots were either (a) coherently moving in the same direction as the region (1st-order), (b) stationary (2nd-order), or (c) coherently moving in the opposite direction (theta). Thresholds were defined as the percentage of signal dots within the region yielding 75% correct responses.

All patients had higher thresholds for second-order motion than for first-order motion. When contra- and ipsilesional thresholds were compared, three patients showed proportional threshold elevations for all three types of motion stimuli in the field contralateral to the lesion. Two of these three patients were tested again five months later. Both showed considerable recovery: In one patient the deficit was no longer present, in the other reduced by about half. None of our patients had lesions differentially affecting first- or second-order motion; lesions always affected first- and second- order motion in the same way. Due to the surprisingly good recovery, deficits might be detectable only for a short time after the lesion.

Supported by the German Research Council (DFG, Klinische Forschergruppe)

348.2

MOTOR CONTROL PRINCIPLES CONTRIBUTE TO THE APPARENT MOTION OF A HUMAN BODY. R. Osu[1], Y. Uno[1], T. Inui[2], H. Ando[1]*, & M. Kawato[1]. [1]ATR Human Inf. Processing Res. Labs., Kyoto, Japan. [2]Dept. of Psychol., Fac. of Letters, Kyoto Univ.

We examined the apparent motion of non-rigid articulated objects like the human arm. Subjects saw pairs of alternating simple stick figures of a human upper arm viewed from above. The subjects adjusted the positions of dots displayed between the figures to remain on the path along which they saw the motion of a hand. For comparison, they also judged the apparent motion path of rigid objects, which were composed only of the forearm part of two joint figures.

According to the previous purely perceptual theory of apparent motion, motion paths are independent of the object. The following two hypotheses have been derived from the theory: 1) the shortest possible (straight) hand path, and 2) a) rotation or b) translation and rotation of the rigid part (forearm). We examine another class of theories in which models utilized in generating arm movements are also utilized in perceiving arm movements. According to motor control theory, arm trajectories are planned in body space so that intrinsic variables such as joint angles or joint torques are at their smoothest possible. The following two hypotheses are possible along this line. If the vision system considers the kinematics of arm movements, one possible hypothesis is 3) the smoothest possible movement in joint angle space. If the vision system additionally considers the dynamics of the arm, one possible hypothesis is 4) the minimum-torque-change trajectory, which is the optimum trajectory considering the dynamics of the musculoskeletal system. In the case of rigid sticks, the subjects reported paths closest to those expected by hypothesis 2) b). In the case of human arm stick figures, however, the subjects reported paths closest to 4). The results suggest that information on motor control contributes to the motion perception of a human body.

348.3

CONTRAST SENSITIZATION IN HUMAN MOTION PERCEPTION: PSYCHOPHYSICAL EVIDENCE. C. Wehrhahn*. Max-Planck-Institut für biologische Kybernetik, Tübingen, Germany.

In an attempt to identify the neuronal mechanisms underlying human motion perception, I investigated whether human observers are able to detect a subthreshold line, when a suprathreshold stimulus is presented next to it.

Foveal contrast detection thresholds for a vertical line were measured on a color monitor with 60 Hz frame rate using a two-alternative forced-choice procedure. Dark or bright lines were shown on a grey ground at ±5 arcmin eccentricity for 1 frame. Line length and width were 20 and 1 arcmin, respectively. These thresholds served as control values throughout.

An 'inducing line' of twice the detection threshold (either bright or dark) was presented at the centre of gaze and contrast thresholds were measured for test lines shown at variable distances to the left or right of the inducing line. Simultaneous presentation of inducing and test lines at 3 arcmin distance with equal contrast sign elevated detection thresholds of the test line. A delay of 1 frame between inducing and test line yielded thresholds at the control value. When line distance was increased, synchronous presentation of inducer and test lines decreased thresholds to 70-80% of the control. A delay further decreased thresholds to values below 50% of the control. When line contrasts had the same sign, this value was reached within a delay of 1 frame. With opposite sign of contrasts, 2-3 frames were required to reach that value.

These results are consistent with earlier experiments in which temporal thresholds were measured using high contrast apparent motion stimuli [Wehrhahn & Rapf (1992) J. Neuroscience 12: 2247-2250]. The experiments described, therefore, provide evidence for contrast sensitization, which may underly human motion perception [Exner 1894]. Supported by Deutsche Forschungsgemeinschaft (SFB 307/A5 and SPP 'Physiologie und Theorie neuraler Netze').

348.4

OBJECT ENUMERATION IN EQUILUMINANT AND LOW CONTRAST VISUAL DISPLAYS.

G.Patel*, S.Peterson, K.Sathian & T.J.Simon Neuroscience Program (GP) and Dept. of Neurology (KS), Emory University; School of Psychology, Georgia Institute of Technology (SP,TJS); Atlanta, GA.

Humans can rapidly enumerate (*subitize*) small sets of four or fewer objects, their reaction time (RT) increasing by about 50 ms per object in this range. For sets numbering over four items, RT increases more steeply, by about 300 ms per item. We studied the effect on object enumeration of selectively interfering with the function of either the magnocellular (M) or parvocellular (P) visual pathway.

To selectively impair the M system, we presented green objects on a perceptually equiluminant red background. RT was significantly prolonged relative to a control condition where the objects were much brighter than background. This effect was specific to the subitizing range, suggesting that subitizing may preferentially depend on the M system. In another experiment, the objects were gray on a white background, with Michelson contrasts of 20% or 5%. At the lower contrast, where the P system is relatively inactive compared to the M system, RT was significantly longer than at high contrast. This effect was present both within and outside the subitizing range and may result from a non-specific decrease in object visibility or dependence on the P system for object individuation. *Supported intra-murally.*

348.5

AN ELECTROPHYSIOLOGICAL CORRELATE OF THE 'FILEHNE ILLUSION' IN MAN. T. Haarmeier*, P. Thier. *Sekt. f. Vis. Sensomotorik, Neurologische Universitätsklinik, 72076 Tübingen, Germany.*

During smooth pursuit eye movements made across a stationary background an illusionary motion of the background is perceived (*Filehne illusion*=FI). This illusionary movement of the background is widely assumed to result from a neuronal comparison of retinal image slip and an imperfect estimate of the eye movement. We have recently demonstrated that the FI can be modified in a predictable way by visual motion unrelated to the pursuit eye movement and, moreover, preceding it (Vis. Res., 36: 741-750). In the present event-related potential (ERP) study we made use of the fact that identical constellations of retinal image slip and eye movement can yield different percepts of illusionary background motion in order to locate the neuronal substrate underlying the comparison of the two neuronal signals. Towards this end, ERPs obtained under two conditions, which were identical physically but gave rise to very different FIs (high vs low Filehne condition), were compared. ERP responses were collected from ten naive subjects, who performed 12°/s rightward smooth pursuit eye movements in front of a stationary background (a 27°x27° random dot pattern, presented for 300ms). Under both conditions ERPs showed an initial positive peak (P1; peak latency about 120ms), a first negative peak (N2; 180ms) followed by a second negative component (N3; 300ms) and a huge positive peak (P3; 450ms). While the P1, the N2, and the P3 were the same for the low and the high FI condition, only the amplitude of the N3, which was most prominent in the parietooccipital leads, depended significantly ($p<0.01$) on the size of the illusion with higher peaks for higher FIs. It is generally accepted that the N2 reflects activity in motion processing area MT. The fact that the N3, the first potential whose amplitude reflects the size of the FI, follows the N2 by 120ms suggests that the N3 arises from a later stage in the hierarchy of cortical motion processing. *Supported by DFG 'KFG: Neuroophthalmologie'.*

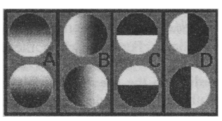
348.6

IS PRE-SACCADIC VERNIER DISTORTION RELATED TO PERI-SACCADIC MISLOCALIZATION? R.H.Cai*, A.Pouget, M.Schlag-Rey & J. Schlag, Brain Research Institute, UCLA, Los Angeles, CA 90095-1761

In a 3-dot vernier alignment test, if the outer dots are continuously lit while the middle dot is flashed shortly before a saccade, a distortion in vernier alignment is perceived (Cai, et.al. ARVO 1996). Is this phenomenon related to pre-saccadic mislocalization? It is known that a spot of light flashed before a saccade is mislocalized in the direction of the saccade. However, such mislocalization is suppressed if the visual stimulus is continuously lit. If we hypothesize that in the three-dot vernier test as described, the mislocalization of the two outer dots is suppressed, whereas the mislocalization of the middle dot is not, a relative distortion in the relationship among the 3 dots can result. Under this hypothesis, one would predict that if all three dots were flashed at the same time before a saccade, they would all be mislocalized by the same amount, the vernier distortion would disappear. Here, we tested this prediction. All three dots for vernier alignment were flashed at the same time for 4ms shortly before a 4° rightward saccade. The results were compared with those obtained when only the middle dot was flashed before saccade and when no saccade occurred. For all subjects, there was no difference in the vernier bias between the all-3-dot-flash trials and the no-saccade trials. Such results are consistent with the hypothesis that pre-saccadic vernier distortion is related to peri-saccadic mislocalization of visual stimuli. *(Supported by USPHS grants EY05879 and EY02305)*

348.7

PERCEPTUAL STABILITY CORRELATES WITH ACTIVITY IN EARLY VISUAL AREAS. G.K. Humphrey, M.A. Goodale*, C.V. Bowen, J. S. Gati, B.K. Rutt and R.S. Menon. Departments of Psychology and Medical Biophysics, University of Western Ontario; Advanced Imaging Labs, Robarts Research Institute, London, Ontario, Canada.



Vertically oriented shading gradients (as in the disks in A) produce a strong and stable percept of 'concave' and 'convex' elements. If the shading gradients are rotated 90°, as in B, the depth percept is reduced and much more ambiguous. In a 3-D fMRI study at 1.5T, we found significantly less activation in area V1 and neighbouring low-level visual areas of cortex when subjects viewed displays that led to a stable depth percept (composed of disks as in A) than when they viewed displays that led to weak and ambiguous depth percepts (as in B). There was no reliable difference in activation for the control stimuli that lacked depth structure and ambiguity (C and D). The study demonstrates that the difference in strength and stability of a depth percept that occurs when a shading gradient is rotated 90° is accompanied by a corresponding change in the level of activation in early visual areas of the human brain. The reduced activation with stable visual displays may reflect top-down modulation of early visual processing or an intrinsic bias in neural networks in V1 and neighbouring areas.

Supported by grants from MRC and NSERC (Canada).

348.8

3D VECTOR MODEL FOR EGOCENTRIC SPACE PERCEPTION. L. Matin* and W. Li. Dept. of Psychology, Columbia University, New York, NY 10027

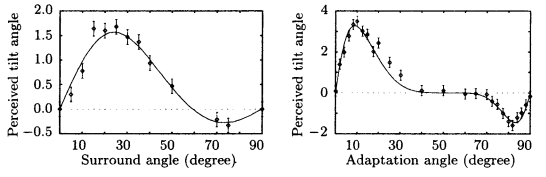
The orientation of a single dim line in darkness is sufficient to generate large systematic (nearlinear) influences on the perception of elevation (small circular target set to appear at eye level; VPEL) and of orientation within a frontal plane (small test line set to appear vertical; VPV). These influences on visual spatial localization and orientation (as well as manual reaching) are almost as large as are measured with complexly-structured, well-illuminated, pitched and rolled visual fields, respectively. The influences on both VPEL and VPV are negatively accelerated exponentials of total line length with space constants in the 10° - 20° range. The influence on VPEL is essentially independent of head and eye orientation. The central vertical meridian (CVM) and circumference of the midfrontal plane (CMFP) of a projection sphere centered at the monocularly-viewing eye provide the two qualitative dimensions of projected line orientation; line length provides the intensitive dimension. These three dimensions are the bases of a vector structure that encloses the two major opponent processes for perceived elevation and perceived orientation within a frontal plane. The similarity of the treatment to the treatment of brightness and hue within color space is striking. Several experiments support the view that processing of VPEL is mediated in parietal cortex.

Supported by NIH EY10534 and AFOSR F49620-94-1-0397.

348.9

TILT ILLUSIONS AND DYNAMIC DECORRELATION: A THEORY OF LATERAL INTERACTIONS OF THE ORIENTATION SELECTIVE CELLS IN STRIATE CORTEX (V1). Dawei W. Dong*. California Institute of Technology, 139-74, Pasadena, CA 91125.

Natural images possess significant correlations. We explore the hypothesis that visual information in V1 is adaptively transformed into a decorrelated representation through lateral interactions of the orientation selective cells. Although retina and LGN do spatial and temporal decorrelation to incoming images (Dong and Atick 1994), only the lowest order correlations are eliminated. Therefore, the signal sent to V1 is still correlated and the activities of the orientation selective cells are correlated because of the high order correlations in the signal. Using some recently measured high order statistical properties of natural images, we predict the form of the lateral interactions in V1 which reduces the high order correlations. The predicted lateral interactions are anisotropic. The theory not only agrees with the physiological experiment by Gilbert and Wiesel (1990), but also gives a unified and quantitative explanation of the psychophysical experiments on tilt or orientation illusions as shown below. The solid curves are the theoretical predictions: the data points are from Westheimer (1990) on orientation contrast (left) and Campbell and Maffei (1971) on orientation adaptation (right).



(Supported in part by DOE DE-AC03-76SF00098)

348.10

VISUAL PERCEPTION PROBLEMS IN MS. E. Vandenbussche[1]*, L. Vleugels[2], C. Lafosse[1], A. van Nunen[2] and P. Ketelaer[2]. [1]Lab. Neuropsychologie, KULeuven, Campus Gasthuisberg, B-3000 Leuven, Belgium, [2] Nat. Center for MS, Melsbroek, B-1820, Belgium.

Purpose. To study prevalence and nature of behavioral consequences of visual CNS disorders in a MS population using neuropsychological and psychophysical testing. Methods. 112 MS patients were randomly selected. An effort was made to exclude any ophthalmological, neurological, psychiatrical or other neuropsychological condition that might hamper interpretation or test performance. 49 subjects meeting all criteria were submitted to a comprehensive neuropsychological test battery of 33 representative visual indices including tests such as the Hooper VOT, the Benton Facial Recognition Test and Form Discrimination and the Judgment of Line Orientation Test. Temporal resolution capacity of early visual pathways was evaluated with a Double Flash Threshold determination (DFT). Here, the neuropsychological part is highlighted. Results. MS patients showed a significantly ($p<0.005$) greater number (mean 3.68, SD 3.42) of failed tests than a group of properly matched normal controls (NC) (n=30) (mean 1.8, SD 2.41). True rate of visual disorders in our sample is estimated at 32%. Among MS DFT proved to be a significant predictor of this number of failures. Based on the neuropsychological testing 37% of the MS and 12% of the NC could be classified as impaired. The four most sensitive tests assessed color perception and aspects of object or of space perception. Among these the Farnsworth Munsell 100 Hue Test was the most sensitive (39% failures). Conclusion. Although the increased DFTs, suggesting impairment of the magnocellular system. The MS patients failed in the neuropsychological tests boundary on the dorsal as well as on the central pathway in the cortex. Data further suggest that MS damage may primarily affect both dorsal and ventral stream functions. As much surprisingly, the predictive value of presence of magnocellular pathway disturbances seems to extend to either kinds of higher visual problems.

348.11

THE CONTRIBUTION OF 3-DIMENSIONAL INFORMATION DURING VIEWPOINT DEPENDENT OBJECT RECOGNITION. S. Nishina and T. Inui*. Dept. of Psychol., Fac. of Letters, Kyoto Univ., Kyoto 606-01 JAPAN

Recent research has suggested that humans can recognize a novel view of an object by interpolating known views of it. On the other hand, Shepard & Metzler (1971) suggested that there is a process called mental rotation, which rotates the 3-dimensional structure of objects. In order to separate these two processes, we performed a recognition experiment using two types of distractors. One type of the distractor was 3-dimensionally different from the target, but a 2-dimensional silhouette of it was equivalent to the target's view presented during testing. The other type was created to be completely unrelated to the target. Each trial consisted of one learning and one testing. A stimulus shown during testing was either one type of the distractors or a rotated target. Under the "short" condition, the stimuli was only shown for one second during testing; the subjects had to respond within that duration. There was no time limit for the "long" condition.

The correct ratio over a rotated angle can be modeled as the combination of 2-dimensional view matching and 3-dimensional structure matching. The calculated range(σ) of view matching was about 20°, which is narrower than the result of Logothetis et al. (1994); it is possible that 3-dimensional matching was included in their result. The result from a simulation using GRBF (Poggio and Edelman, 1990), which theoretically could not be affected by 3-dimensional information, is close to our result. These results show that 3-dimensional structure matching would be performed especially for larger rotation.

Supported by JSPS Research Fellowships.

348.12

HEIGHT AND DISTANCE IN THE VISUAL FIELD: CALIBRATING A MONOCULARLY GUIDED REACH J.J. Marotta* and M.A. Goodale. Vision and Motor Control Laboratory, Univ. of Western Ontario, London, Ontario, Canada, N6A-5C2.

It remains unclear as to which of the many available depth cues humans use to calibrate manual prehension. If a target sphere is presented in the same location in one of two predictable arrays (varying height or varying distance) would subjects learn to use relative position information to calibrate their reach and grasp? Thirteen subjects reached out in the dark to one of 3 illuminated styrofoam spheres presented one at a time on a rod placed in a board. In one block of trials, spheres were placed at one of 3 different distances at eye-level (20, 30 and 40cm from the board). In the other block of trials, spheres were placed at one of 3 different heights (eye-level, 5cm above and 5cm below) at a distance of 30 cm from the board. Grasping movements directed at spheres placed at eye-level, 30 cm from the board, in both series were compared. Reaches to the spheres in the series in which distance was varied took longer, reached lower peak velocities, and had longer deceleration periods than reaches made to spheres in the series in which distance was varied. The visuomotor system is able to use relative height better than it can use relative distance to program and control reaching and grasping movements under monocular viewing. Supported by NSERC grant A6313.

348.13

EVIDENCE FOR BLINDSIGHT IN PATIENTS WITH HOMONYMOUS HEMIANOPIA. A.J. Butler*, W.G. Darling, M. Rizzo, T.B. Shoen. Departments of Exercise Science and Neurology, University of Iowa, Iowa City, IA 52242.

"Blindsight", the perception of and/or action upon visual stimuli in the absence of acknowledged awareness, has been used to explain residual abilities in the fields of a visual cortical scotoma. However, the extent of these abilities requires further definition. We investigated these issues by studying residual reaching into the blind hemifield in 2 patients with MRI defined visual cortical lesions. The subjects reached to visual targets in 4 different experimental conditions: (1) Visual presentation (brief flash of target light), reach to remembered target location with no vision of hand; (2) Visual presentation (brief flash of target light), reach to remembered target location with vision of hand; (3) Visual presentation, reach to actual target with no vision of hand; (4) Visual presentation, reach to actual target with vision of hand. Subjects were instructed to focus on a stationary, green light emitting diode (LED) positioned directly in front of them, and to utilize only peripheral vision to observe the target light. Movements were performed in complete darkness. Visual feedback on hand position was provided by illumination of a LED on the hand. Optoelectronic (WATSMART) records of fingertip positions were analyzed for movements to both hemifields. The results were that both subjects made much larger errors in direction when pointing to visual target locations in the blind visual hemifield. Yet, there was a statistically significant relationship between target location and fingertip location in the blind hemifield and performance even improved when vision of the limb was available. The findings indicated residual vision in the blind hemifields of both subjects that was, however, of insufficient quality to permit target acquisition. (supported by NIH/NIA grant AG00214-05 to AB and NIH PO NS 19632 to WD and MR)

349.1

FORM FROM COLOR DISCRIMINATION FOR BOTH ISOLUMINANT AND ISOBRIGHT STIMULI IN A SUBJECT WITH CEREBRAL ACHROMATOPSIA. R.W.Kentridge[1]*, C.A.Heywood[1] & A.Cowey[2]. 1:Psychol. Dept., Univ. of Durham, Durham DH1 3LE, UK. 2:Psychol. Dept., Univ. of Oxford, South Parks Road, Oxford OX1 3UD, UK.

The cerebral achromatopsic subject MS can discriminate equiluminant forms defined by color even though he cannot discriminate colors themselves, luminance being defined by flicker photometry. This ability persists even in the presence of random luminance masking. Moreover, his chromatic contrast sensitivity was indistinguishable from that of normal subjects. We tested the hypothesis that this may be because of differences in the subjective brightnesses of colors at low temporal frequencies in comparison to those obtained by flicker photometry. In particular, red/green mixtures (yellow to us) may be perceived as less bright than photometrically equiluminant reds or greens at low temporal frequencies. In an isoluminant red/green sinusoidal grating of spatial frequency f such red/green subadditivity can be compensated for by adding luminance isochromatically at a frequency of $2f$ at a phase of 90°. Using a motion slowing paradigm we show that both MS and normal subjects perceive red/green 3.8 cycles per degree gratings, moving at 1.84 Hz as being most uniform in brightness with the addition of a 5% luminance contrast compensation grating to a photometrically isoluminant stimulus. We show that the ability of MS to discriminate grating orientation is not diminished when using this compensated isobright stimulus in comparison to an isoluminant stimulus. We conclude that evidence for separate form from color and color discrimination processes cannot be accounted for by subjective brightness artefacts.

This research was funded by MRC GRANT G9024591

349.2

ROD MODULATION OF COLOR-OPPONENT CHANNELS. T.E. Frumkes*, E. Lembessis, and J. Vollaro. Department of Psychology, Queens College of CUNY, Flushing, NY 11367

Three general theories could account for the rod contribution to color vision: a) rod-photic stimulation provides an excitatory input to the "blue-cone" trichromatic zone (Trezona, 1970); b) rod-photic stimulation provides an excitatory input to the "luminosity" channel (Lie, 1963; Stabell and Stabell, 1967); and c) rod-adaptation modulates the output of cone-mediated, color-opponent mechanisms. In the parafoveal retina of normal psychophysical observers, we compared the influence of rod-light and rod-dark adaptation upon chromatic vision using monochromatic stimuli chosen to approximate the unique hues of blue (472 nm), green (512 nm), yellow (577 nm), and red (632 nm). Observers obtained specific (hue-identification) thresholds, and additionally estimated hue and saturation of 5 td stimuli using the "4+1" scaling procedures of Abramov Gordon, and Chan (1994). Our results suggest that dark-adapted rods slightly reduce the output of the blue/yellow opponent mechanism, and much more strongly reduce the output of the red/green opponent mechanism. Although rods may additionally provide a direct contribution to color, our results are entirely consistent with a modulatory influence upon cone-mediated color channels.

349.3

USING ASSOCIATIVE LEARNING TO TEST ZEBRAFISH COLOR VISION, L.A. Fetsko, E. Cohen, J.B. Sheffield*, M.I. Mote. Dept. of Biology, Temple University, Philadelphia, PA 19122.

In an attempt to determine if zebrafish can learn and if they are capable of using their four types of cones to learn, an associative learning paradigm was designed. Zebrafish were first trained to choose a color in the visible spectrum vs. dark and were then trained to choose between two color stimuli. Results show that they can discriminate red, orange, and blue from dark. They can also discriminate blue from yellow, blue from orange, blue from green, blue from UV, blue-green from UV, and yellow from orange. They do not seem to discriminate blue from blue-green. Results also support that the zebrafish are discriminating on the basis of color and not brightness.

349.4

SPATIAL FREQUENCY TUNING FUNCTIONS AND CONTRAST SENSITIVITY IN THE HUMAN VISUAL FIELD: MEG MEASUREMENTS. H.-W Chen, C. Aine*, E. Flynn, and C. C. Wood. Biophysics Group, MS D454, Los Alamos National Laboratory, Los Alamos, NM 87545

The human luminance spatial frequency contrast sensitivity function (CSF) has been well studied using psychophysical measurements by detecting spatial frequency (SF) grating patterns at threshold. Since the CSF is measured near threshold, it is quite possible that only the most sensitive retinal or LGN M (magnocellular) cells may contribute to the measured CSF. Therefore, measurements of SF tuning functions at different contrast levels (from near threshold to suprathreshold) may reveal information on the relative contributions between M and P (parvocellular) cell groups. It is still a controversial issue whether the ratio of M to P cells in the retina and LGN of macaque varies with eccentricities of the visual field. Some results suggest that the human M cells should have a higher contrast sensitivity and a lower spatial resolution than those in macaques. It is of interest to investigate these problems in humans using noninvasive MEG techniques.

In this study, we measured the SF tuning functions as well as the CR functions at different suprathreshold contrast levels and different eccentricities of the visual field. Evoked magnetic fields were recorded with a BTi 7-channel SQUID-coupled gradiometer system in a magnetically-shielded chamber. Four subjects with normal vision participated in this study. Transient target stimuli (circular sinusoid, 266 ms duration and ~1 Hz rate of presentation) were presented at 5 different contrast. The estimated SF tuning curves were fitted by a two-stream (M & P) model. These results are consistent with the two stream (M and P) theory: at low contrast levels, the majority of responding cells may be M-type (which prefer low contrast and low SFs), resulting in lower peaks in the SF tuning curves. With an increase in contrast level, more P-type cells (which prefer high contrast levels and high SFs) may be activated, resulting in curves with peaks shifted in the direction of higher SFs. As predicted by the two-stream model, the M system shows a narrow-band low-pass SF tuning property, whereas the P system shows a broad-band band-pass SF tuning property, which is consistent for all the 4 subjects. The results also indicate that the techniques developed in this study may provide useful information on the relative involvement of magno-like and parvo-like systems at different eccentricities.

Supported by NIH grant (EY08610) and DOE contract W-7405-ENG-36.

349.5

JUDGING DIFFERENCES BETWEEN VISUALLY TEXTURED MATERIALS. R.Y. Cho*, V. Yang, and P.E. Hallett. Dept. of Physiology, U. of Toronto, Toronto, Ont., Canada M5S 1A8.

The behavioral differentiation between textured materials could, in principle, be based on very detailed representations, considering the diversity of receptive field types in the primary visual cortex at each point. Our results suggest otherwise.

Subjects made two types of comparisons of pairs of textures: judgements of overall dissimilarity (Δ); judgements of specific dissimilarity according to each of six candidate attributes (Δ^A), e.g. "coarseness", "regularity", "lightness", etc. Regression and cross-validation methods have demonstrated that we could make good predictions of the Δ judgements with 3 to 4 of the Δ^A judgements as predictors. Multidimensional scalings of Δ judgements are also low-dimensional. Dimensionality was reduced by increased viewing distance or low lighting.

These results suggest that, despite the rich early neural representation, the behavioral discrimination of textures is actually based on a much sparser representation.

(Supported by MRC Canada and NSERC Canada)

349.6

INTEGRATION ACROSS SPATIAL FREQUENCY CHANNELS IN SPEED DISCRIMINATION. D. Ascher[1], L. Welch[2], N. M. Grzywacz[3]*
[1]Department of Cognitive and Linguistic Sciences and [2]Department of Psychology, Brown University, Providence RI 02912, [3]Smith-Kettlewell Eye Research Institute, 2232 Webster Street, San Francisco CA 94115.

There is converging evidence from psychophysics (Anderson & Burr, 1985; Watson, 1986) and physiology (Holub & Morton-Gibson, 1981) that the channels responsible for single grating detection have relatively narrow spatial-frequency bandwidths (1 to 2 octaves). These channels are widely assumed to provide the information used to compute retinal speed. Existing data indicate that in detection tasks information from multiple channels is not combined to increase performance. We examined whether the visual system performs such a combination when computing speed.

We report the results of experiments testing speed discrimination as a function of the spectral content of the stimuli. Subjects performed forced-choice discriminations in one of three blocked conditions: a 1 cycle per degree (cpd) sinusoidal grating, a 6cpd grating, or both gratings superimposed. In each 150-trial block, subjects viewed gratings moving at one of five speeds centered around $2^{\circ} \cdot s^{-1}$, and judged whether the stimulus speed was faster or slower than the mean speed. By fitting a cumulative Gaussian to the proportion of "fast" responses for each speed, an estimate of the discrimination threshold was obtained for each condition. Low contrasts (randomized between 4% and 8%) and short stimulus durations (120ms) were used to avoid ceiling effects.

Our results indicate that speed discrimination is better for displays containing two gratings of equal speeds but differing by 2.5 octaves in spatial (1cpd and 6cpd) and temporal frequencies (2Hz and 12Hz) than for displays containing only the component gratings. These results are compatible with several models of speed perception and are predicted by models that posit integration of speed measures across multiple spatial scales. Our data are compared to a version of the Grzywacz & Yuille (1990) model, modified by the incorporation of filter functions derived from psychophysical data.

NMG is supported by Air Force Grant AFOSR F49620-95-1-0265.

349.7

INTERACTIONS BETWEEN SPATIAL FREQUENCY, LUMINANCE CONTRAST AND BINOCULAR DISPARITY IN MOTION COHERENCY

Gene R. Stoner* and Thomas D. Albright, Salk Institute, La Jolla, CA 92037.

Plaid patterns composed of component gratings that differ in spatial frequency (**SF**), luminance contrast (**LC**) or binocular disparity (**BD**) tend not to cohere (Adelson and Movshon, *Nature*, **300**: 523-525, 1982; Adelson and Movshon, *J. Opt. Am. A*, **1**:1266, 1984). Moving plaid patterns configured to mimic one occlusive grating overlying another also fail to cohere (Stoner et al., *Nature*, **244:**153-155, 1990; Trueswell and Hayhoe, *Vis. Res.*, **33**:313-328, 1993). We hypothesized that plaids with dissimilar components fail to cohere because these components are interpreted as occlusive surfaces lying in different depth planes.

It is known that when depth-from-occlusion and depth-from-binocular disparity cues support the same depth-ordering, both segmentation in depth and motion non-coherency are more likely to be perceived than when these two cues conflict. To test our hypothesis, component gratings of either different SF or different LC were superimposed and the BD between them varied from trial-to-trial. Subjects judged whether the gratings occupied the same depth plane on one set of trials. Motion coherency was judged in a second set. We predicted that if relative SF and LC elicited a particular occlusion-based depth-ordering, both types of judgments should depend upon which grating was stereoscopically in front of the other.

We found that whether subjects were likely to report the component gratings to lie in the same plane depended upon which grating (i.e. high vs. low LC or high vs. low SF) was stereoscopically closer. Perceptual motion coherency exhibited a parallel dependency. We conclude that although these types of plaid patterns are not intentionally configured to mimic occlusive superimposition, the visual system does its best to interpret such stimuli in terms of real-world surfaces. Channel and non-fourier motion accounts of motion coherency are rendered insufficient by these results.

Supported by MH 50706.

349.8

EVIDENCE FOR STRIATE CORTEX SITE FOR BINOCULAR RIVALRY.

J.Beusmans,* Nissan Cambridge Basic Research, 4 Cambridge Center, Cambridge, MA 02142, USA.

When shown different images to each eye, humans experience "binocular rivalry," a continual alternation between perceiving the left and right eye image. I present evidence that rivalry is caused by competition between neuronal populations that are not in extrastriate cortex (as suggested by Leopold et al., ARVO, 1996) and that are not exclusively monocular.

Let P_1 be a clockwise (/) diagonal, blue-white square-wave grating, and let P_2 be a counter-clockwise (\) diagonal, orange-white grating (both gratings 1cpd, 10 deg in diameter, with a contrast of 0.3). A vertical chimera $V(P_1P_2)$ combines the left half of P_1 with the right half of P_2. Shown $V(P_1P_2)$ to the left and $V(P_2P_1)$ to the right eye, subjects alternately perceived the P_1 and P_2 patterns, but not the vertical chimeras. The same holds for horizontal chimeras, although some subjects now perceived the chimeras occasionally. This eliminates competition between exclusively monocular channels.

To determine whether competition is at or before extrastriate cortex, I used drifting gratings. Now P_1 drifts towards the upper-left (UL) and P_2 towards the upper-right (UR). Shown P_1 and P_2 dichoptically through circular apertures, subjects perceived either P_1 with UL motion or P_2 with UR motion, never upward motion (the pattern or plaid motion if the motion signals of P_1 and P_2 had been combined). With the corresponding drifting vertical chimeras, subjects also perceived either P_1 with UL or P_2 with UR. They never perceived upward motion, even though the motion in each vertical chimera was unambiguously upward. Assuming that humans have an MT-like extrastriate area, that is involved in motion perception and whose neurons are virtually all binocular and receive their input mainly from striate cortex, these results suggest that competition is at the level of striate cortex.

349.9

THE EXTRAORDINARILY RAPID DISAPPEARANCE OF ENTOPTIC IMAGES.

D. M. Coppola* and D. Purves, Department of Neurobiology, Duke University, Durham, NC 27710.

It has been known for more than 40 years that images fade from perception when they are kept at the same position on the retina by abrogating eye movements. The time course of image degradation, typically studied by close-fitting contact lenses with reflectors to stabilize the retinal image, has generally been reported to be a few seconds to a minute or more, depending upon the conditions. We have recently examined a different sort of stabilized image, the shadows of retinal vessels that can be generated by illumination with an appropriate source. As described in detail by Helmholtz, vascular shadows can be visualized by rapid movement of a point source of light at the focal point of the eye, but disappear when shadow motion across the retinal surface is stilled. To explore the duration of such images, we constructed a device that translated a light over a 6 mm horizontal path at frequencies between 1-15 Hz; a computer program started and stopped the movement of the light source, controlled its velocity, and recorded the subjects' responses. Our results show that: (1) images of entoptic vascular shadows in central vision disappear less than 80 msec after the movement required for their perception ceases; (2) the retinal image motion necessary to maintain the percept of entoptic images is several times greater than that which can be generated by eye movements. The disappearance of these perceptions implies an active and extraordinarily rapid (>10 Hz) mechanism of image erasure and creation

[Supported by NIH grant NS29187].

349.10

EFFECT OF EARLY ENUCLEATION ON THE VISUOMOTOR CONTROL OF GRASPING IN PIGEONS

D.Musumeci, G.Cesaretti, C.Kusmic and M.Matteoni Dep. of Physiol. and Bioch., Univ. of Pisa, Italy. (SPON: Eur. Brain and Behav. Soc)

The pecking in pigeons is characterized by head fixation stops interspersed with rapid forward head thrusts, during which animals evaluate position, depth and size of the target, since the last pecking phase is ballistic. It is assumed that binocular vision enhances the accuracy and the precision of pecking. In the present research we compare the features of reaching bidimensional (2D) targets and of grasping three dimensional (3D) targets, in both reversible (intact) and chronically (early enucleated) monocular viewing. Posthatced pigeons were enucleated under general and local anesthesia and they were trained and tested by adult. The experimental set-up consisted of a modified Skinner box in which the frontal panel was a 9" b/w VGA monitor sloped by 45° out. A "behavioral fixation" procedure ensured a constant head fixation. A single seed (∅ = 4 or 7,5 mm) was delivered after a peck to a 2D target (∅ = 7,6; 3,5; 2 mm), displayed on the monitor. Pecking sequences at spots and seeds were videorecorded and off-line analyzed. Time, distance of fixation, grasp efficiency and bill gape were measured. The main results concerning 2D targets show a remarkable impairment of early enucleated pigeons in calibrating the opening of the beak to the target size, whereas pigeons tested in reversible monocular condition did not behave very differently than in binocular vision. As regard to 3D targets, only the early enucleation caused a heavy reduction of the grasp efficiency even if the animals restored their ability to calibrate the bill gape as function of the target size. In addition, enucleated pigeons developed a peculiar feature during target fixation, in fact they showed head movements (about 5 mm) of approaching to the seed. Summarizing, the occurence of binocular interactions seems to be essential for a suitable visuomotor control in the reaching and the grasping. However, monocular strategies are able to compensate some few aspects of the deficit, namely the control on the bill gape in relation with three dimensional features of the target.

This research was supported by M.U.R.S.T. 1995

349.11

CONFIGURATION-DEPENDENT PHYSIOLOGICAL SUMMATION IN HUMAN FOVEAL VISION.

U. Polat* and A. M. Norcia Smith-Kettlewell Eye Research Institute San Francisco, CA. 94115

Collinear (co-axial and co-oriented) long-range spatial interactions predominate over other iso-orientation interactions psychophysically (Polat & Sagi, 1993, 1994) and neurophysiologically in humans (Polat & Norcia, 1996) and in single units of cat V1 (Mizobe et al, ARVO 1996). In these studies, facilitation for collinear configurations was found only near contrast threshold and suppression occurred for higher contrasts. Herein, we explored the shape and extent of the spatial summation region governing contrast thresholds for collinear and non-collinear patterns using the human VEP. The stimuli were either symmetrical or elongated Gabor signals that were temporally modulated at 4.1 Hz. Contrast thresholds were estimated using a swept contrast technique. The Gabor envelope orientation was either vertical, oblique or horizontal and the carrier grating orientation was vertical. Thresholds improved more as the Gabors were elongated for collinear combinations than they did for the non-collinear ones. The response was also larger and faster for collinear configurations, but only near threshold. The pattern of results is consistent with an elongated mechanism that pools signals preferentially along the orientation axis. This mechanism may enhance the saliency of extended contours.

Supported by EY06579.

350.1

CHARACTERIZATION OF POTASSIUM CURRENTS IN THE RAT INFERIOR COLLICULUS USING THE *IN VITRO* BRAIN SLICE. M. E. Wisgirda*, L.-Y. Wang and L.K. Kaczmarek. Depts. of Pharmacol., Cell. & Mol. Physiol., Yale University School of Medicine, New Haven, CT 06520.

The inferior colliculus (IC) is a major center for integration of information in the auditory system. Several members of the *Shaker* and *Shaw* potassium channel gene families have been shown to be highly expressed in this nucleus. We have used the whole cell patch clamp recording method to study potassium currents in the rat IC. Brain slices (200 μm) containing the IC were obtained from P8-14 rats and maintained at room temperature in oxygenated saline until use.

Current clamp recordings revealed three response patterns to a series of current steps (-0.5 to 2 nA, 150 ms). In one class of cells, suprathreshold current steps evoked only a single action potential (AP). In a second class, increasing current steps evoked multiple APs near the beginning of the step. In the third class, increasing current steps evoked increasing numbers of regularly spaced APs. Similar firing patterns have been described in intracellular recordings and are termed onset, adapting, and regular patterns, respectively (D. Peruzzi, pers. comm.) In all cells tested, application of 1 mM TEA broadened the AP, reduced the afterhyperpolarization, and reduced the total number of APs.

IC neurons express large outward currents that begin to activate at between -80 and -60 mV. Voltage steps to as high as +60 mV were never sufficient to saturate the current. Application of 1 mM TEA produced a blockade of between 15-60% of the total current. In 8 of 16 cells, the TEA-sensitive component was very slowly activating, suggesting the presence of a Ca-activated potassium current. 100 nM DTX produced a 40-60% blockade of current in 3 of4 cells tested, and had no effect in one cell. These results indicate that IC neurons express multiple potassium currents, and suggest that different neurons within the IC express different complements of potassium channels. Supported by NIH grant GM 48851.

350.2

EFFECTS OF ACUTE SPIRAL GANGLION LESIONS ON THE TUNING PROPERTIES AND TONOTOPIC ORGANIZATION OF NEURONS IN THE CAT INFERIOR COLLICULUS. R.L. Snyder, D.G. Sinex*, E.J. Walsh, and J.D. McGee. Dept. of Otolaryngology, Univ. of California, San Francisco, CA 94143, Dept. of Speech and Hearing Science, Arizona State Univ., Tempe, AZ 85287, Boys Town National Research Hospital, Omaha, NE 68131.

Many studies have reported plastic changes in the frequency organization of central auditory areas. These studies have employed mechanical or acoustic disruption the organ of Corti, altering its mechanical and physiological characteristics. In this study mechanical lesions were made in a restricted region of the spiral ganglion, leaving the organ of Corti intact. In addition, the consequences of the lesions were assessed immediately after their production, without prolonged recovery periods. Initially, CAP audiograms were constructed, and then the tonotopic organization of the inferior colliculus (IC) was mapped by obtaining single and multiunit response areas at 100 micron intervals. At each location, response areas were acquired to contralateral, ipsilateral, and binaural tones. Following this initial mapping, a 1 mm segment of the contralateral spiral ganglion was then destroyed. The extent and completeness of the lesion was confirmed by reconstructing the CAP audiogram. Then the IC was re-mapped. As expected, IC neurons were less sensitive to frequencies represented within the lesioned area as demonstrated by broadened frequency tuning curves and elevated minimum thresholds. However, they were more sensitive to edge frequencies, i.e., those represented within areas adjacent to the lesion, as indicated by broader tuning curves without elevated minima, more neurons tuned to edge frequencies, broader spatial tuning curves and steps in the normally smooth frequency gradient. These results suggest that the 'plastic' changes reported by previous studies require little of no experience and occur within 24-36 hrs following the lesion.

Supported by NIDCD grants DC00982 to BTNRH and DC00341 to DGS.

350.3

DISCRETE IBOTENATE LESIONS OF RAT INFERIOR COLLICULUS ALTER PERFORMANCE OF A SOUND-GUIDED NAVIGATION TASK. M.C. Zrull*, R.E. Bowman, and A.J. Trivette. Department of Psychology, Appalachian State University, Boone, NC 28608.

Perceptual or proximal cued locomotor tasks are often used to examine sound localization behavior and its neural control. The present study examines the role of inferior colliculus (IC) in mediating spatial navigation when distal sound cues provide salient information about the location of a goal objective. Adult, male hooded rats learned to navigate through a modified Hebb-Williams maze to obtain a water reward. Sighted (V+A, N=12) and surgically blinded (AUD, N=12) rats received sequential, spatially distinct noise bursts (125 ms, 2-16 kHz, 50 dB) as cues to avoid blind alleys. A control group (VIS, N=12) had only intra-maze visual cues to guide navigation. After a series of 22 sessions with 8 trials each, half of the rats were anesthetized and bilateral ibotenate (IBO, 63 mM, 1.0 μL) lesions targeted at the central nucleus of IC were made. A second series of 22 sessions was then conducted. Performance was quantified as the percent of trials with at least 1 blind alley error for the final 2 expected and final 6 sessions unexpected maze sessions. AUD and V+A rats with IC lesions ran more error trials than during pre-lesion sessions, and all sham operated controls and VIS rats with lesions ran fewer post-surgery trials with errors in both expected (p<.05) and unexpected mazes (p<.05). Trials with errors increased over baseline by 4.8% and 12.1% for the AUD lesion group and by 69.0% and 58.4% for V+A rats with lesions in expected and unexpected mazes, respectively. Cell counts of Nissl stained material indicate similar IC lesions in all groups receiving IBO injections. These results suggest that the IC may contribute to integration of localized acoustic information into a useful neural representation of the environment. Supported by NIDCD grant DC02476.

350.4

PHARMACOLOGICAL STUDIES OF ACTIVITY-DEPENDENT INHIBITION IN THE INFERIOR COLLICULUS OF THE RAT USING A PAIRED-CLICK PARADIGM : A NOVEL MECHANISM OF INHIBITION IN THE AUDITORY SYSTEM?

W.S. Szczepaniak and A.R. Moller*, Dept. of Neurological Surgery, Univ. of Pittsburgh Sch. of Med., Pittsburgh PA, 15213.

It is well known that neurons of the inferior colliculus (IC) receive a considerable amount of both GABAergic and Glycinergic inhibitory influence, and that these inhibitory mechanisms play an essential role in the processing of auditory information by neurons of the IC. We studied activity-dependent inhibition on neurons of the IC by recording evoked potentials from the IC to paired click stimuli (94dB,Pe SPL), and varying the interval between the first and second click (interstimulus interval (ISI)). We show that the amplitude of a peak (generated post-synaptically in the IC) in the response that is elicited by the second click is depressed by about 80% of the amplitude of the peak that is elicited by the first click when the ISI is 1ms, and that this depression decreases slowly with increasing ISI to about 20% when the ISI is 100ms. A microinjection of the $GABA_A$ antagonist bicucullene, did not reduce this depression, and in fact, caused a small, but statistically significant, increase in this depression. Microinjection of the $GABA_B$ antagonist phaclofen, and the glycine antagonist strychnine, did not affect this relationship. We interpret these findings to show that very brief acoustic stimuli can elicit inhibitory mechanisms within the IC that are active for a very long time and influence subsequent input, and that the mechanism(s) responsible for this phenomenon do not involve any of the major inhibitory systems known to influence neurons of the IC. We are currently studying the effects of other pharmacological agents on this phenomenon.

This work was supported by grants from the American Tinnitus Association.

350.5

GABA IN INFERIOR COLLICULUS OF AWAKE, FREELY MOVING RAT: MICRODIALYSIS AND STEREOTAXIC COORDINATES. D.C.Jackson, Y.Hu and K.L. Drew* Institute of Arctic Biology, Univ. of Alaska Fairbanks, Fairbanks, AK 99775.

Significant age related changes in GABAergic neurotransmission have been observed previously in rat inferior colliculus (IC) and may be related to neural presbycusis. However, little is known about how sound or other neurotransmitters regulate GABA release in this central auditory nucleus and implantation of microdialysis probes into the IC are complicated by its proximity to the transverse sinus. The purpose of the present study was to establish a technique for using microdialysis to sample extracellular GABA in the IC of awake, freely moving rats. Stereotaxic coordinates which spared rupture of the sinus and placed cannula in the IC 6 out of 6 times in 390 to 490 g rats were as follows: With the incisor bar raised to 9.7 mm and the micromanipulator arm tilted 13° lateral to perpendicular probes were implanted 2.1 mm anterior to interaural line, 3.0 mm lateral to lambda and 5.0 mm ventral to brain surface. Basal perfusate concentrations of GABA in awake rats sampled 48 and 72 hrs following implantation of 2.0 mm CMA/11 probes perfused at a rate of 1.0 μl/min with aCSF were (mean +/-SEM) 0.055+/-0.03 and 0.025+/-0.008 pmol/10 μl. Basal levels reported here are 50 times lower than reported by Goldsmith et al., 1995 in ketamine/xylazine anesthetized guinea pigs. Use of microdialysis to sample from the IC without confounding effects of anesthetics will contribute to the study of sound and drug-induced changes in neurotransmitter release in young and aged rats. Funded by: NIDCD Partnership Program, NSF(OPP)REU, Univ. of Alaska Natural Resource Fund, University of Alaska Fairbanks.

350.6

MONAURAL EVOKED RESPONSES IN THE RAT'S INFERIOR COLLICULUS AFTER BLOCK OF THE CONTRALATERAL DORSAL NUCLEUS OF THE LATERAL LEMNISCUS AND INFERIOR COLLICULUS. L. Li* and J. B. Kelly. Lab. of Sensory Neuroscience, Psychology Dept., Carleton Univ., Ottawa, Canada K1S 5B6

We have previously shown that binaural inhibition in the central nucleus of inferior colliculus (ICC) is due in part to a GABAergic projection from the contralateral dorsal nucleus of the lateral lemniscus (DNLL). In the present study we have investigated the effects of suppression of neural activity in the contralateral DNLL and contralateral inferior colliculus (IC) on monaural evoked potentials (EPs) recorded in the ICC with microelectrodes, before and after local injection of kynurenic acid (KYNA) into the contralateral DNLL or contralateral IC. Following KYNA injection into the contralateral DNLL, the EPs recorded in the ICC to monaural ipsilateral stimulation were enhanced. In contrast, KYNA injection into the contralateral IC decreased the responses in the ICC to ipsilateral stimulation. Responses to contralateral monaural stimulation were not substantially affected by injections into either the contralateral DNLL or the contralateral IC. The data suggest that in the rat's ICC evoked responses to ipsilateral stimulation are generally facilitated by neural activity in the contralateral IC and suppressed by activity in the contralateral DNLL.

Supported by the Natural Sciences and Engineering Research Council of Canada.

350.7

AUDITORY SPATIAL TUNING OF INFERIOR COLLICULUS NEURONS IN THE GUINEA PIG

S.J. Sterbing*, K. Hartung~, K.-P. Hoffmann*~, J. Blauert~. Lehrst. f. allg. Zoologie und Neurobiologie*, Lehrst. f. allg. Elektrotechnik und Akustik~, Ruhr-Universität Bochum, 44780 Bochum, Germany

The head related transfer functions (HRTFs) of pigmented guinea pigs were measured under free-field conditions with miniature microphones placed in the external ear canal. The complete upper hemi-field was sampled (-10° to 90° elevation, 10° resolution, and 0° to 345° azimuth, 15° resolution). The interindividual differences of the monaural and binaural cues of the HRTFs made it necessary to create the stimuli (virtual sound sources) for the electrophysiological experiments by filtering white noise bursts with individual HRTF. The stimuli were pseudorandomly presented via earphones. The single-unit activity of the central nucleus of the inferior colliculus (ICc) was recorded with glass micropipettes in parallel dorsal to ventral tracks. In addition to the recordings of the spatial tuning characteristics, the frequency and sound pressure level tuning as well as coventional bin- or monaural characteristic were determined by using pure tone stimulation. More than 90% of the ICc units were sharply tuned in azimuth and elevation. Although the majority of the neurons preferred directions in the central and lower contralateral hemi-field, preference for frontal or back positions and upper elevation positions also occurred frequently. It turned out that the classification based on binaural pure tone stimulation (best interaural delay, best interaural level difference, EE-, EI-, E0-classification etc.) cannot predict the space position tuning of an ICc neuron. One exception, however, is when individual HRTFs are taken into account.

(Supported by DFG, Graduiertenkolleg "KOGNET - Kognition, Gehirn und Neuronale Netze")

350.8

MODULATION OF AUDITORY RESPONSE BEHAVIOR IN THE RAT INFERIOR COLLICULUS BY BEHAVIORAL CONTEXT.

C.Felsheim*, J.Ostwald. Dept. of Animal Physiology, University of Tuebingen, D-72076, Germany.

Evidence from recent studies in the auditory cortex suggest that the auditory system is dynamic and depends on the behavioral context. Modulation of neuronal responses on subcortical levels due to the behavioral context, however, is still unknown. To test for such modulations, we recorded from the IC of behaving rats in two different behavioral paradigms.

The influence of learning was studied in an operant conditioning task in which the animals learned to respond to a pure tone stimulation and to ignore independently presented noise pulses. Unit responses after learning were compared to those recorded before and during the learning phase.

In the second paradigm, attentional effects on neuronal responses was investigated by quickly changing the behavioral significance of auditory stimuli. This could be achieved by presenting the same stimulus first in a discrimination task and second, shortly thereafter, as an behaviorally irrelevant stimulus after animals were rewarded for their response.

In both tests it could be shown that already on a subcortical level auditory evoked neuronal responses are modulated by the behavioral context of a stimulus.

Supported by Deutsche Forschung Gemeinschaft (SFB 307) and Graduierten Kolleg Neurobiologie, Tuebingen

350.9

DIRECTIONAL MECHANISMS AND SPATIAL PREFERENCES OF SINGLE UNITS IN THE CAT'S INFERIOR COLLICULUS (IC).

P.Poirier*, F.K.Samson and T.J.Imig, Dept. of Physiology, Kansas Univ. Med. Ctr., Kansas City, KS, 66160.

Directional mechanisms and spatial preferences of high-frequency azimuth-sensitive cells to broad band noise burst stimulation are similar in the primary auditory cortex (A1) and medial geniculate body (MGB) of barbiturate-anesthetized cats. Binaural-directional (BD) cells were azimuth-insensitive or unresponsive to monaural stimulation. BD tuning derives from binaural inhibitory (EI), facilitatory (FAC) or inhibitory/facilitatory (FI) interactions. Monaural directional (MD) cells were azimuth-sensitive to monaural noise stimulation. MD cells received either exclusively monaural (EO) or EI inputs. To elucidate where those mechanisms appear in the ascending auditory pathway and to assess colliculo-thalamic transformations in spatial preferences, recordings were obtained in the IC under conditions similar to those used in the MGB or A1. MD and BD proportions in the IC were similar to those in the MGB and A1, indicating a segregation of these mechanisms in the IC. IC BD cells exhibited binaural interactions similar to those found at higher levels. In addition to MD-EO and MD-EI cells, some IC MD cells exhibited FAC or FI interactions. IC BD-EI cells preferred exclusively contralateral positions whereas at higher levels some preferred ipsilateral positions. Moreover, spatial preference of IC MD cells was limited to contralateral or midline positions whereas in the MGB or A1, a few MD cells also exhibited ipsilateral preference. Correspondingly, all IC cells were predominantly excited by contralateral ear whereas MGB or A1 cells were excited principally by the contra- and, less frequently, by the ipsilateral ear. Finally, a similar proportion of BD-FAC cells preferring midline positions was found in the IC compared to the MGB or A1. This result suggests that high-frequency midline preference is created at a lower level than previously thought (supported by FCAR, FRSQ and NIDCD #DC00173).

350.10

EFFECTS OF EAR PLUGGING ON RESPONSES OF AZIMUTH-SENSITIVE NEURONS IN MEDIAL GENICULATE BODY (MGB) AND PRIMARY AUDITORY CORTEX (AI) OF BARBITURATE-ANESTHETIZED CATS.

F.K. Samson, P. Poirier, W.A. Irons and T.J. Imig*.

Directionality of high-frequency neurons to broad band noise bursts in AI depends upon monaural and binaural interactions. Monaural directional (MD) cells are sensitive to the azimuth of monaurally presented stimuli. Some of them are monaural (MD-E0) and others exhibit binaural inhibition (MD-EI). Binaural directional (BD) cells are unresponsive to monaurally presented noise bursts or insensitive to their azimuth. They exhibit binaural inhibition (BD-EI), or facilitation in the presence or absence of inhibition (BD-FAC). The MGB contains neurons with the same MD/BD characteristics as AI and they are distributed in about the same proportions (i.e., MD, 30%; BD, 70%). However, MD-EI and BD-FAC cells were more numerous in AI than in the MGB (MD-EI: AI, 64%; MGB, 36% of all MD cells. BD-FAC: AI, 66%; MGB, 51% of all BD cells). A recent report from this laboratory shows that AI contains a higher proportion of azimuth-sensitive cells with nonmonotonic level tuning (NM) than the MGB and suggests that there is synthesis of NM azimuth-sensitive responses in AI. To find out whether this difference in proportions is common to all azimuth-sensitive neurons or is related to a particular MD/BD response category, we have compared the level tuning of MD and BD cells in the MGB and AI. We found that AI contains a greater proportion of NM cells than the MGB in all cell categories, suggesting that the proposed synthesis is not related to a particular MD/BD response category. Supported by FCAR, FRSQ and NIDCD grant # DC00173.

350.11

FORWARD MASKING DIFFERENTIALLY AFFECTS RESPONSIVENESS IN RAT INFERIOR COLLICULUS.

P.G. Finlayson*. Dept. of Surgery, Rotary Hearing Centre, University of British Columbia, Vancouver, B.C. Canada, V6T-2B5.

Extracellular single unit responses of neurons in the central nucleus of the inferior colliculus (ICC) of anaesthetized Long-Evans rats were examined with a forward masking paradigm. Pure tone "probe" stimuli were presented at the same frequency and intensity as preceding "masking" pure tone stimuli. The intervals between monaural and binaural masking and probe stimuli were 2, 8, 32, 128, 512, and 2014 ms. The effect of masking on responses of ICC neurons was dependent on the temporal response pattern of discharge to 200 ms tones.

The majority (~2/3) of ICC neurons exhibited short-term adaptation, as responses to probe stimuli were decreased following an equal intensity masking tone. The level of responsiveness recovered slowly over time. These ICC neurons exhibited transient excitatory responses.

Probe tone responses were facilitated at delays of 32 or 128 ms, and minimal at long delays following 200 ms masking stimuli in ICC neurons with bimodal response patterns. These cells exhibited a transient response, a pause in activity, followed by sustained activity. Shortening the duration of the masking tone produced predictable effects: i) following 50 ms maskers, no responses to probe tones were present at short delays (2 to 32 ms), and responses increased at longer delays. ii) following 100 ms maskers, responses to probe tones were facilitated at short delays.

Masking shapes ICC neuron responses over periods of hundreds of milliseconds. These processes may be important for detecting patterns in complex sounds and may be involved in noisy listening conditions.

Research supported by the Deafness Research and BC Health Research Foundations

350.12

INTEGRATION OF SYNAPTIC INPUTS IN IDENTIFIED NEURONS IN THE INFERIOR COLLICULUS.

G. Reetz*, G. Ehret. Dept. Comparative Neurobiology, University of Ulm, 89069 Ulm, Germany.

One important function of the central nucleus of the inferior colliculus (ICc) is the integration of ipsi- and contralateral afferent inputs from almost every auditory brainstem nucleus. We recorded intracellularly from neurons of the ICc in brain slice preparations to investigate the relative contribution of excitatory and inhibitory inputs from the ipsilateral lemniscus lateralis (iLL), the commissure of Probst (CP), and the commissure of the IC (CoIC). Bipolar stimulation electrodes were placed in the iLL, the CP, and the CoIC. Morphology of the cells was revealed by iontophoretic injection of biocytin and subsequent staining using HRP and DAB.

Neurons in all regions of the ICc predominantly responded to depolarisation by intracellular current injection with a phasic-tonic spike pattern (onset-sustained, 20 out of 40 cells). Few neurons with an onset response (N=8) were most often found in the ventral ICc. Neurons with a tonic (N=5) or with a delayed response (N=7) were located in the dorsal or rostral ICc. All neurons were identified as multipolar cells, with variations of shapes of somas and of dendritic trees. The axons projected either into adjacent laminae of the ICc, to the brachium of the IC, into the ICx, to the CoIC, or to the LL.

All neurons received excitatory inputs. A composed excitatory/inhibitory postsynaptic potential (E/IPSP) was observed in 47% of the cells. In general, EPSPs or IPSPs upon stimulation of all of the three fibre bundles arrived with latencies of 1.5 to 10 ms, indicating that the multipolar neurons receive both direct synaptic inputs and/or indirect inputs via interneurons. Our data indicate that these neurons are involved in intrinsic and ascending information processing.

Supported by the DFG (Eh 53/14-1)

351.1

THE POST-MOVEMENT EVOKED MAGNETIC FIELD: A MANIFESTATION OF SOMATOSENSORY FEED-BACK OR MOTOR FEED-FORWARD? JT Davis*, JD Lewine, R Thoma, D Hill, K Paulson, WW Orrison, Jr. The New Mexico Institute of Neuroimaging, The New Mexico Regional Federal Medical Center, 2100 Ridgecrest Dr, SE, Albuquerque, NM, 87108

Self-paced movements are associated with a complex movement-related neuromagnetic signal with several components including a pre-movement readiness field, and a large amplitude post-movement evoked signal that has been demonstrated previously to originate from somatosensory cortex. The post-movement signal is generally interpreted as generated by peripheral feedback, but direct evidence in support of this interpretation is weak. Recently, we have used a whole-head, 122-channel sensor array to record movement related activity while subjects perform self-paced thumb extensions. As has been reported previously, the movement related field shows multiple sub-components. At approximately 50 msec prior to movement the premovement signal peaks as the primary motor field. The motor field is unilateral and has its origin in area 4 of the precentral gyrus contralateral to the side of movement. The post-movement field typically peaks 50-70 msec after movement. In contrast to the primary motor field, the post-movement field is BILATERAL in many subjects. This activity is best described by two equivalent current dipoles, one located in each of the right and left primary somatosensory cortices, with the contralateral source activity peaking approximately 15 msec before the ipsilateral activity.

The median nerve was electrically stimulated and the response used for comparison with post-movement field. Following electrical stimulation of the median nerve, contralateral somatosensory cortex is activiated at and after 20 msec post-stimulus. The neuromagnetic signal remains specifically unilateral until 70-90 msec post-stimulus at which time ipsilateral SII activity is seen. Ipsilateral SI activity is not seen. The implication of the movement-related and somatosensory results is that the post-movement activity seen originating from SI on the side ipsilateral to movement does not reflect somatosensory feedback, but rather, some type of motor feedforward. Given this, the origin of the contralateral SI activity is also likely to reflect, at least in part, feedforward mechanisms.

351.2

EFFECT OF TRAIN OF STIMULI ELICITED BY WHOLE HAND (MESH GLOVE) AFFERENT INPUT BELOW CONSCIOUS SENSATION ON EXCITABILITY OF PARIETAL AND MOTOR CORTEX MM Dimitrijevic*, FE Pollo, MR Dimitrijevic. Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030

A recent study reported on how daily stimulation with a mesh-glove can modify altered motor control and improve voluntary wrist extension movement in stroke subjects with chronic neurological deficits (1). Mesh-glove stimulation targets the whole hand as a common anode to a pair of cathodes over the wrist flexors and extensors. Earlier work into the electrophysiological characteristics of single stimuli with mesh-glove afferent stimulation demonstrated that at the subthreshold level, somatosensory evoked potentials (SSEPs) could not be elicited. Extending this work in 4 healthy subjects, we examined the effects of a conditioning train (0.5 sec @ 50 Hz) below conscious sensation on a test SSEP elicited by a stimuli above sensory and motor level. The effects of an identical conditioning stimulus was also tested on motor cortex evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS). In both series of investigations, there were no profound changes in the size of the tested SSEP and MEP responses. In order to further identify underlying mechanisms involved in mesh-glove stimulation, pilot work is in progress utilizing functional MRI (fMRI) for evaluation of brain cortical activity immediately after this form of stimulation. Preliminary results have indicated that mesh-glove afferent input is projected to the posterior portion of the parietal lobe, close to the visual cortex. This discrepancy in the projection of exteroceptive and proprioceptive input to the parietal lobe might contribute to the understanding of the electrophysiological and fMRI results.

1. Dimitrijevic, MM, et al., Modification of motor control of wrist extension by mesh-glove afferent stimulation in stroke patients. Arch Phys Med Rehabil, 77:252-258, March 1996.
This work was supported by the V.L. Smith Foundation for Restorative Neurology, Houston, Texas USA.

351.3

EFFECTS OF REACTION TIME ON VISUOMOTOR INTERACTION: SPATIO-TEMPORAL PATTERNS OF INTRA- AND INTERHEMISPHERIC CORTICAL ACTIVATION. C.D. Saron*, H.G. Vaughan, Jr., G.V. Simpson, and J.J. Foxe, Depts. of Neurosci. and Neurol., Albert Einstein Coll. of Med., Bronx, NY. 10461.

This study extended our previous work which suggested differing activations of interhemispheric routes (motor or visual) for fast vs. slow reaction times (RTs) during a lateralized visuomotor task. We used a large number of responses (1512/condition) to allow examination of the effects of small differences (10 ms) in response speed. Event-related potentials (ERPs) from 62 scalp sites were elicited from 2 right-handed males using wedge-shaped checkerboards in the left or right lower visual fields in a simple (left or right hand response) RT task. Control conditions used stimuli without responses and responses without stimuli. The 16 ms stimuli were 2° from fixation, 3° in extent and had an ISI of 1-4 s. Subsequent analyses included topographic mapping of scalp current density (SCD) and spatio-temporal dipole analysis. Initial analyses for one subject were based upon twelve sets of ERPs per condition associated with RTs in 10 ms bins from 220 to 330 ms (mean n=95). Spherical spline interpolated Laplacian waveforms from left and right central sites showed that responses contralateral to the responding hand had consistent increasing negativity with decreasing RT peaking at approx. 150 ms post-stimulus (ps). Interestingly, a second wave of activity 150-250 ms ps was only present for RTs *above* the median suggesting two neural response modes for fast vs. slow RTs. Occipital waveforms revealed smaller effects, with prolonged N160 components for the slower responses, and for RVF stimuli particularly, decreased P100 amplitudes. Individual complexity of the spatio-temporal patterns of visuomotor activation is exemplified by this subject who manifests activation of the right motor cortex by way of central transcallosal projections when performing a left hand response to right field stimulation. By contrast, in the opposite condition activation of left motor cortex prior to right hand response appears mediated by dual inputs from the contralateral (right) frontal and occipital cortices. The temporal patterns of motor cortex activation are also complex, and can manifest distinct surges of activation that may be propagated via different routes from visual to motor cortex for fast and slow responses within the same condition. These include paradoxical increases in central activation of the non-output hemisphere for slower responses, suggesting possible transcallosal mediation of motor output commands. *(Supp. by NIDCD 95261122, NINDS 27900, and Hum. Front. Sci. Prog.)*

351.4

Event related desynchronization studied in an auditory SRTT paradigm P. Zhuang*, N. Dang, L.G. Cohen, C. Gerloff, M. Hallett. Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892.

To investigate cortical physiology during motor learning of sequences, we studied 6 right-handed, normal hearing subjects who performed a variation of the auditory SRTT. The stimuli consisted of 4 pure tones of 4 different frequencies, 500, 1000, 1500, and 2000 Hz, lasting 200 ms. The stimuli were presented binaurally at 60 dB intensity with a constant interstimulus interval of 2 sec. The subjects were instructed to push one of four buttons with a different finger of the right hand corresponding to the 4 different tones. After practice, when the subjects were able to discriminate 4 tone signals (indicated by < 5% errors), the experimental procedure started. The first and the last blocks had a random sequence as a control. The test sequence, which began with the second block, was a repetitive cycle of 10 tones. ERD was calculated within the alpha (8-12 Hz) band from EEG recording of 30 scalp positions from -1 to 1 sec around keypresses. During the task performance, all subjects developed implicit knowledge which was reflected by decreasing reaction time and explicit knowledge, reflected by ability to generate the sequence.

In a previous study using a visual-motor SRTT task, we demonstrated that ERD maps revealed localized alpha power changes overlying the contralateral central or central-parietal-occipital areas with a peak of desynchronization when knowledge became explicit. In the present study, an alpha ERD was found which localized over central-parietal and temporal regions. A peak of desynchronization was found when the subjects first demonstrated full explicit knowledge. These electrophysiologic data suggest that transient functional "plasticity" in association with learning is related both to primary motor cortex and sensory association areas. Both visual and auditory stimuli activate the parietal region during the SRTT suggesting that the parietal lobe plays a crucial role in sensorimotor integration of sequences.

351.5

CORTICAL DYNAMICS OF THE HUMAN EEG ASSOCIATED WITH BEHAVIORAL PHASE TRANSITIONS IN AN AUDITORY-MOTOR TASK

J.R. Meaux, G.V. Wallenstein, A.J. Nash*, S.L. Bressler, J.A.S. Kelso
Program in Complex Systems and Brain Sciences, Center for Complex Systems, Florida Atlantic University, Boca Raton FL 33431.

Cortical rerouting strategies were investigated as a function of changes in behavioral performance. 61-channel EEG recordings were collected from six subjects during performance of a coordinative auditory-motor task. Subjects (N = 6) were instructed to flex their right finger *in between* periodically-delivered tones (syncopation). The presentation rate of the tones increased from 1.0 to 3.0 Hz in 0.25 Hz increments after every 10 cycles. Spontaneous behavioral transitions were observed in which syncopation changed to quasi-synchronization (i.e. finger flexion coinciding with tone onset).

Fourier analysis indicated a concentration of EEG power at the frequency corresponding to the stimulus presentation rate. Relative phase measures between the EEG at the stimulus frequency and the stimulus (calculated from the negative peak in the EEG signal to tone onset) showed similar transitions from around 180 degrees (syncopation) to around 0 degrees (synchronization). Topographical maps of EEG power at the stimulus frequency revealed that these phase changes were associated with spatial shifts of neural activity. Differences in topographical pattern between syncopated and synchronized cycles suggest a reorganization of cortical activity. Motor-only and auditory-only control conditions revealed that the topography associated with performance of the sensorimotor task involves additional areas than would be expected from the task's individual component behaviors (listening and flexing).

Supported by NIMH Grant MH42900 and NIMH Training Grant MH19116.

351.6

EEG BRAIN ACTIVITY: STATIC TORQUE DIRECTION AND MAGNITUDE DIFFERENCES IN THE UPPER EXTREMITY OF HUMAN SUBJECTS.

A.L. Ricamato*, J.D. Given and J.P.A. Dewald. SMPP, Rehab. Inst. of Chicago, and Dept. of PM&R, Northwestern University Medical School, Chicago, IL 60611.

We have identified differences in 3D topographic voltage maps of EEG activity as a function of torque direction and magnitude using statistical analysis. We sought to determine whether there was a relationship between cortical activity and static joint torque magnitude and direction by combining EEG and EMG recordings during mechanically controlled static torque exertions in four directions and two magnitudes in the upper extremities of three subjects.

Subjects were seated with their wrists casted to a 6 degree of freedom load cell which measured the respective elbow and shoulder torques. Subjects were trained to produce elbow and shoulder torque ramps of 2 seconds duration followed by a 2 second steady state hold using visual torque feedback. Four target directions, separated by 90° and corresponding to elbow flexion-extension and shoulder abduction-adduction torques, were used in our paradigm. For each target direction, two torque load levels were used: 25% and 40% of the corresponding maximum elbow or shoulder torque. EMGs were recorded from four elbow and six shoulder muscles using surface electrodes. EEG signals were recorded from a total of 121 electrodes placed in a cap on the subjects' head. 80-100 trials of recorded EEG epochs were aligned using torque onsets and then ensemble averaged for each direction yielding SNR's > 15.

Cross-correlation analysis confirmed the validity of using t-tests on the voltage maps. Subsequently, a statistical assessment of subject specific 3D voltage maps showed statistically significant differences ($p < .05$) in topographic brain activity as a function of target direction (i.e. between the various elbow/shoulder torque directions). In addition, statistically significant differences in EEG responses were observed between the two torque magnitudes in the same direction. These results appear to indicate cortical activity is related to both static joint torque direction and magnitude.

Supported by NIDRR H133P20016 & H133B0024.

351.7

EEG-BASED BRAIN-COMPUTER INTERFACE (BCI) COMMUNICATION: THE ROLE OF FEEDBACK IN PERFORMANCE. D.J. McFarland*, L. McCane, L.A. Miner, T.M. Vaughan and J.R. Wolpaw, Wadsworth Center, NY State Dept of Health and State University of New York, Albany, NY 12201.

Individuals can learn to control the amplitude of the 8-12 Hz mu rhythm or related higher frequency activity in the EEG recorded over sensorimotor cortex and use it to move a cursor to a target on a video screen (Electroenceph clin Neurophysiol 78:252-259, 1991 and 90:444-449, 1994). In the standard trial sequence, a target appears, the cursor appears and moves controlled by the subject's EEG, the cursor hits (or misses) the target, the target flashes for a hit (or disappears for a miss), and, after a brief pause, the next target appears. This design provides two types of feedback to the subject: cursor movement during the trial and the target flashing (or disappearing) at the end of the trial. We are studying the role of this feedback in performance.

To date, we have studied two subjects who had begun to master one-dimensional cursor control over 10 40-min sessions, each consisting of 8 3-min runs (of 20-30 trials each) separated by 1-min breaks. In the two subsequent sessions, runs of three different types were interspersed: 1) standard feedback, 2) no cursor, and 3) no cursor and no target flash (or disappearance). Trials of type 3 terminated after 4 sec. Accuracy was 79.6% with the cursor present, and 80.1% with the cursor absent. Values for R^2 (EEG variance associated with up vs. down targets) were 0.232 for cursor present, 0.265 for cursor absent, and 0.246 for no feedback.

These initial results indicate that, at least for short periods, trained subjects can perform satisfactorily without feedback. Thus, their EEG control is not dependent on specific visual stimulation. The importance of feedback in maintaining performance over longer periods remains to be determined. (Supported in part by NIH HD30146.)

351.8

EEG-BASED BRAIN COMPUTER-INTERFACE (BCI) COMMUNICATION: OFFLINE ASSESSMENT OF ALTERNATIVE CONTROL ALGORITHMS. H. Ramoser*[1], D.J. McFarland[2], and J.R. Wolpaw[2], [1]Graz Univ of Tech, Graz, Austria and [2]Wadsworth Center, NYS Dept of Health & SUNY, Albany, NY 12201.

People can learn to control the amplitudes of certain EEG components (i.e.,specific frequency bands at specific scalp locations) and use them to move a cursor in one or two dimensions to a target on a video screen (Electroenceph clin Neurophysiol 78:252-259, 1991 and 90:444-449, 1994). We are trying to improve the rapidity and accuracy of cursor control by improving the online algorithms that translate EEG components into cursor movement. Present algorithms use electrodes over sensorimotor areas, common average or LaPlacian spatial filtering methods, autoregressive frequency analysis, 8-12 Hz or 20-24 Hz frequency bands, and linear equations to calculate cursor movement. Online evaluation of alternatives is extremely time-consuming. Thus, we are developing offline methods for estimating the online performance of alternative algorithms.

Sixty-four channels of EEG are digitized and stored for later offline analysis while subjects control cursor movement using current algorithms. Offline analyses simulate the performances that would have occurred had other algorithms been employed online. The simulated performances are compared to each other and to the algorithm that was actually used online. Algorithms tested include nonlinear equations, histogram methods, neural nets, and learning vector quantization.

This simulated performance has several limitations. First, it cannot predict the effect on performance of change in feedback caused by the new algorithm. Second, it is limited to the body of EEG data provided by the online algorithm, so that some simulated trials may end inconclusively. Nevertheless, it allows assessment of a much wider range of alternatives than could be assessed online and should prove to be an important tool in BCI development. (Supported by FFWF Project P9043 (Austria) and NIH HD30146 (USA).)

351.9

EEG-BASED BRAIN-COMPUTER INTERFACE (BCI) TRAINING IN A MAN WITH ADVANCED AMYOTROPHIC LATERAL SCLEROSIS (ALS). L.A. Miner*, L. McCane, T. Vaughan, D.J. McFarland, and J.R. Wolpaw, Wadsworth Center, New York State Dept of Health and State Univ of New York, Albany, NY 12201.

Humans can learn to use the amplitude of the mu rhythm or related higher frequency EEG components to control movement of a cursor on a computer screen in one or two dimensions (Electroenceph clin Neurophysiol 78:252-259, 1991 and 90:444-449, 1994). Brain-computer interface (BCI) technology may provide a new means of communication and control for individuals with paralysis or other severe motor disorders.

The subject is a 55-year-old man almost completely paralyzed by amyotrophic lateral sclerosis (ALS). He is ventilator-dependent and currently communicates by eye movements or an eyebrow switch. He is learning BCI communication with our standard training protocol. In this protocol, 64 EEG channels are digitized and stored while he learns to use mu-rhythm activity over the sensorimotor cortex to control cursor movement. He completes 2-3 one-hour sessions per week. Over his initial sessions, high-resolution EEG frequency and topographic analysis of the stored data was used to select optimal EEG channels, spatial filtering method, and frequency band. Changes in the online algorithm derived from this analysis, combined with his gradual mastery of EEG control, are producing a progressive improvement in the speed and accuracy of cursor movement. His rate of learning is comparable to that of non-disabled subjects.

These data, and comparable results from other disabled subjects, suggest that, with further development, EEG-based BCI communication could be of substantial value for those with severe movement deficits. We are currently exploring options for converting one-dimensional cursor control into a useful single-switch control system. (Supported by The National Center for Medical Rehabilitation Research, NICHHD, NIH (HD30146).)

BASAL GANGLIA: ANATOMY II

352.1

NITROTYROSINE IMMUNOLABELING IS PRESENT IN AXONS AND GLIAL PROCESSES IN THE GLOBUS PALLIDUS OF NORMAL ADULT RAT BRAIN.

RR Trifiletti*, EA Bolan and VM Pickel. Department of Neurology and Neuroscience, Cornell University Medical College, New York, NY 10021.

The globus pallidus is the major target of striatal efferents essential for normal motor activity involving close functional interactions between nitric oxide (NO) and GABAergic neurons. Nitric oxide derived free radicals, such as peroxynitrite, are thought to be involved in nitration of proteins at tyrosine residues. Accordingly, the product nitrotyrosine (NT) is present in regions containing neurons that produce NO, these include the caudate-putamen nuclei and globus pallidus. We examined the cellular sites that might locally accumulate NT in the globus pallidus by light and electron microscopy using an anti-nitrotyrosine monoclonal antibody generated against tetranitromethane treated KLH. The antibody was localized by the avidin-biotin horseradish peroxidase method. In coronal sections through the globus pallidus examined by light microscopy, a punctate and diffuse immunoperoxidase label was selectively absorbed with nitrated bovine serum albumin. Electron microscopy showed intense labeling in selective glial processes and unmyelinated axons and axon terminals. Many of these labeled terminals formed symmetric synapses characteristic of GABAergic neurons. The NT-like immunoreactivity (NT-LI) in these terminals was mainly seen in aggregates near the plasma membrane resembling endosomes. We postulate that NT may locally accumulate in axon terminals where NO is known to presynaptically modulate the release of GABA from striatopallidal neurons. Such accumulations may be important in various movement disorders. (Supported by grants from NIMH, MH 40342)

352.2

DIFFERENTIAL DISTRIBUTION OF GAD_{65} AND GAD_{67} mRNA IN THE ENTOPEDUNCULAR NUCLEUS OF THE RAT. A.E. Johnson*, P.-Q. Yuan, C. Gråsäs, L. Källström, J. Yu, K. Huhman, D. Larhammar, H. E. Albers and F.-A. Wiesel, Psychiatry Department, Uppsala University, S-75017 Uppsala, Sweden.

The entopeduncular nucleus (EP) is one of the major output nuclei of the basal ganglia which plays an important role in the expression of dyskinetic motor functions associated with the interruption of dopaminergic (DA) transmission. Anatomical studies have shown that the EP is also interconnected with limbic structures and thus is involved in cognitive function. The limbic and motor neurons of the EP are topographically distributed with limbic neurons in rostral subregions and motor circuit neurons located caudally. The synthesis of the primary transmitter of the EP, GABA, is regulated by the enzyme glutamic acid decarboxylase (GAD) which exists in two isoforms, GAD_{65} and GAD_{67}. Previous studies have indicated that both isoforms of GAD were evenly distributed throughout the EP. However, these studies examined a limited region of the EP. In the present experiments, we examined the distribution of GAD_{65} and GAD_{67} mRNAs throughout the entire rostro-caudal extent of the EP using oligodeoxyribonucleotide probes and *in situ* hybridization techniques. In agreement with previous studies, Northern analysis of whole rat brain preparations showed that the GAD_{65} and GAD_{67} probes used in the present studies labeled mRNAs of 5.8kb and 3.7kb, respectively. Film autoradiographic analysis revealed that GAD_{65} and GAD_{67} mRNAs were differentially distributed in the EP with GAD_{65} predominantly in rostral regions and GAD_{67} restricted to more caudal regions. Cellular analysis demonstated a significant region-dependent difference in the density of labelling by the two GAD probes ($p<.0002$, two-way ANOVA). In the rostral EP, the percentage of cell body area covered by silver grains was 47.8±1.0 for GAD_{65} and 17.2±2.7 for GAD_{67}. In the caudal EP, GAD_{67} labelling increased to 39.6±3.0 while GAD_{65} labelling was reduced to 22.4±3.2. No differences were detected in the number of neurons labeled by the two probes at any level of the EP. These results provide evidence that GABAergic transmission in limbic and motor subregions of the EP may be differentially regulated. Supported by grant #8318 from the Swedish MRC.

352.3

THE ALPHA4 NICOTINIC RECEPTOR SUBUNIT IS FOUND AT SYNAPSES ON TYROSINE HYDROXYLASE IMMUNONEGATIVE NEURONS IN THE RAT SUBSTANTIA NIGRA. E.M. Sorenson*, T. Shiroyama, and S.T. Kitai, Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

Cholinergic neurons in the pedunculopontine nucleus have been shown to project to the substantia nigra which contains cholinergic axons and axonal boutons throughout. Cholinergic neurotransmission can be mediated by muscarinic and/or nicotinic receptors and evidence indicates that the dopaminergic neurons in the substantia nigra pars compacta (SNc) express both. In particular, we have previously shown that the majority of dopamine neurons express the alpha4 nicotinic receptor subunit. We now report that non-dopaminergic neurons in the SN also express the alpha4 nicotinic receptor subunit.

The alpha4 and beta2 nicotinic receptor subunits where immunohistochemically localized using mAb 299 and mAb 270 (gifts of Dr. Jon Lindstrom), respectively. Single label experiments showed that both subunits are expressed in SNc and SNr neurons. In sections labelled for TH, the synthetic enzyme for dopamine,and the alpha4 subunit, it was found that a number of TH immunonegative neurons, particularly in the reticulata, were labeled for the alpha4 subunit. At the ultrastructural level, reaction product for the alpha4 subunit was located in TH immunonegative cell bodies and dendrites in the nigra, including at some synapses.

Since TH immunonegative neurons in the SN are likely to be GABAergic, we conclude that alpha4 containing nicotinic receptors are found on presumed GABAergic neurons and suggest that they are likely to be involved in cholinergic neurotransmission at these neurons.

This work was supported by USPHS grants NS20702 and NS26473.

352.4

Dopamine and Glutamate Co-localized in Substantia Nigra Neurons: Immunocytochemical Evidence. T.Shiroyama, C.D.Richards* and S.T.Kitai. Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

Previous evidence has suggested that neurons in the substantia nigra (SN) which are immunopositive for tyrosine hydroxylase (TH), i.e. dopamine neurons, are also immunopositive for glutaminase, a marker for glutamate and GABA neurons. Separate studies have indicated that most, if not all, TH-positive neurons in the substantia nigra are immunonegative for glutamic acid decarboxylase (GAD), a marker for GABA neurons. In addition, it has been suggested that TH-positive neurons are also immunopositive for glutamate. In order to ascertain whether neurons in the substantia nigra co-localize glutamate and dopamine, we have employed immunocytochemical double- and triple-labelling techniques in the rat, using antibodies against TH, glutaminase and GAD, and both fluorescent and light-level markers.

We found that many TH-positive neurons throughout the substantia nigra pars compacta (SNc) were also immunopositive for glutaminase. However, we found that no TH-positive SNc neurons were also labelled for GAD. Within the SN, and particularly in the pars reticulata, glutaminase-positive neurons were found which were also GAD-positive, i.e. GABA neurons. These results indicate that the TH-positive neurons in the SNc which are also immunopositive for glutaminase are glutamate and not GABA neurons.

We therefore conclude that a sub-population of neurons within the substantia nigra co-localize dopamine and glutamate.

Supported by USPHS grants NS 20702 and NS 26473.

352.5

THE IMMUNOCYTOCHEMICAL LOCALIZATION OF TYROSINE HYDROXYLASE IN THE HUMAN STRIATUM: AN ULTRASTRUCTURAL STUDY. L. Kung*, M. Force, D.J. Chute, J. Smialek and R.C. Roberts, Maryland Psychiatric Research Center, Departments of Psychiatry and Pathology, University of Maryland School of Medicine, Baltimore, MD 21228.

Electron microscopic observations of human brain are scarce due to the difficulty in obtaining tissue with very short postmortem intervals [PMI]. The goal of this study was to describe the ultrastructural organization of tyrosine hydroxylase (TH), the synthesizing enzyme for dopamine, in the human striatum. Striatal tissue from four normal control males [ages 35-79, PMI < 4.5hrs] was obtained from the Maryland Brain Collection. Tissue was placed in 4% paraformaldehyde and 1% glutaraldehyde in 0.1M phosphate buffer for 7-14 days. Free floating vibratomed sections [40μm] were processed for the immunocytochemical localization of TH [1:1000, 36-44 hrs] and flat embedded for electron microscopy. TH-immunoreactivity [TH-i] was present in myelinated and unmyelinated varicose axons. Both varicosities [usually 0.75-1.5μm] and intervaricose segments [typically 0.2-0.3μm] formed synapses with spines and dendrites. Most synapses formed by TH-i profiles were symmetric axospinous or symmetric axodendritic. An occasional asymmetric axodendritic synapse was observed. Synapses formed by TH-i profiles were short in length [typically 0.12-0.25μm], often being present in only 2-4 serial sections, and had non-perforated postsynaptic densities. TH-i profiles formed synapses with both the head and neck of spines. Typically, the TH-i bouton was apposed to both a spine and a nonlabeled terminal which formed an asymmetric synapse with that spine. Appositions, characterized by parallel synaptic membranes and a density within the cleft, were seen between TH-labeled and nonlabeled boutons forming asymmetric synapses. These results indicate that apart from immunoreactivity in some myelinated axons, the pattern of TH-i in the human striatum is very similar to that of other species. Supported by MH40279 & Foundation grants from Stanley & Scottish Rite.

352.6

MULTIARCHITECTONIC STEREOTACTIC ATLAS OF THE HUMAN BASAL GANGLIA: FUNCTIONAL COMPARTMENTALIZATION OF CALCIUM-BINDING PROTEINS IN THE STRIATUM AND PALLIDUM. F. Loup, D. Jeanmonod, M. Magnin and A. Morel*, Lab. for Functional Neurosurgery, University of Zurich, CH-8091 Zurich, Switzerland.

Stereotactic surgery at the pallidal level has proven beneficial in the treatment of Parkinson's disease. To provide accurate target localization, we are developing a multiarchitectonic atlas of the basal ganglia. Human brains obtained at autopsy were cut stereotactically into blocks comprising the basal ganglia and neighboring structures. Serially cut sections in the frontal or horizontal plane were stained for Nissl, myelin, or acetylcholinesterase, or immunohistochemically for calretinin (CR), calbindin (CB), or parvalbumin. The differential neuropil distribution of these calcium-binding proteins allowed delineation of distinct striatal and pallidal territories. Within the rostral striatum, staining for CR showed a low-to-high dorsolateral to ventromedial gradient. A complex and highly heterogeneous pattern of immunostaining was present in the ventral striatum where regions with high CR were in close spatial register with patches low in CB. This intense CR immunoreactivity (IR) extended to most of the basal forebrain and hypothalamus. By contrast, CB-IR was confined largely to the basal ganglia. Staining of the striatal extrastriosomal matrix was most intense in the ventral striatum and less intense, but still significant, in most of the caudate nucleus and rostral putamen. Within the globus pallidus, CB-IR was highest in the ventral pallidum, rostral pole of the external segment (GPe), and ventromedial part of the rostral internal segment (GPi). It was also high in dorsomedial GPe and GPi. Thus, immunostaining for CR and CB permits delimitation of limbic and associative territories from the sensorimotor region in the human striatum and pallidum. Atlas reconstruction of these data should facilitate the determination of selected targets for the treatment of motor disorders. Supported by SNSF grant 31-36330.92.

352.7

PERMANENT PREPARATIONS OF DYE-INJECTED NEURONS IN RAT AND HUMAN POSTMORTEM BRAIN, IN COMBINATION WITH STAINING FOR ACETYLCHOLINESTERASE. L.D. Loopuijt*, H.J. Groenewegen and F. G. Wouterlood, Department of Anatomy, Free University Amsterdam, the Netherlands.

Intracellular injection of Lucifer yellow (LY) into neurons in slices of fixed brain is used with success to study neuronal morphology. We developed a method to investigate the morphology of human striatal neurons in postmortem brain tissue in sections stained also for acetylcholinesterase (AChE) in order to mark striatal compartments. Preparations had to be permanent for the purpose of building up a library. Thus we introduced immunocytochemical stabilization using anti-LY antibodies. Because bloodvessel-borne erythrocytes interfere with immunostaining, a bleaching procedure using UV-irradiation of the tissue prior to the intracellular LY-injection and AChE staining was conducted. The procedure was developed in postmortem rat brain and then transferred to human postmortem brain tissue. Brain tissue was immersion-fixed for 30 min in a phosphate-buffered aldehyde fixative. Slices (350 µm thick) were stained with 4',6-diamino-2-phenylindole and exposed for 20 minutes to UV-irradiation via a standard 10X Neofluar objective in a Zeiss epifluorescence microscope (100W mercury bulb). Then, selected neurons were intracellularly injected with 4% LY or 2% LY / 2% biocytin according to the method published by Buhl and Schlote [Acta Neuropathol. 75(1987):140-146]. After postfixation for 1 hr, the slices were cut into 40 µm thick sections and histochemically stained for AChE [Hardy et al. Neurosci. Lett. 3(1976):1-5]. Afterwards, the injected LY was visualized immunocytochemically or via treatment with avidin/biotin/peroxidase (ABC) complex.

We obtained satisfactory results with this method, i.e., good visualization of LY-injected neurons together with AChE staining, without erythrocyte-associated background staining. Outside the irradiation spots in irradiated sections as well as in non-irradiated control sections, we noticed dense erythrocyte-associated staining. Supported by LDL Research Funds.

352.8

PHOSPHATASE CONTROL OF DYNORPHIN EXPRESSION IN ORGANOTYPIC CULTURES OF DEVELOPING STRIATUM. F.-C. Liu* and A.M. Graybiel, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139; Institute of Neuroscience, National Yang-Ming University, Taipei, Taiwan 11221, R.O.C.

Our previous work has shown that inhibition of protein phosphatase-1 and -2A (PP-1, PP-2A) results in phosphorylation of the cAMP response element (CRE)-binding protein (CREB) in organotypic cultures of neonatal striatum (Liu and Graybiel, 1995). Okadaic acid and calyculin, inhibitors of PP-1 and PP-2A, induced immunodetectable Ser^{133} phosphorylated CREB (PCREB) throughout the cultured striatum. Among candidate downstream striatal targets of CREB phosphorylation is prodynorphin, a CRE-containing gene that can potentially be regulated by CREB activation and is expressed in the developing as well as the mature striatum. In the present study we asked whether protein phosphatases could regulate dynorphin expression in slice cultures of developing striatum. Immunostaining showed that dynorphin was down-regulated in neonatal striatal cultures by 3 days *in vitro*. Incubation of such striatal cultures with okadaic acid (0.1, 1 µM) or calyculin (0.1, 1 µM) for 2 hr strikingly induced dynorphin immunoreactivity in numerous dynorphin-positive neurons in both striosomes and matrix of the cultured striatum. This homogenous pattern of dynorphin induction paralleled that of CREB phosphorylation. By contrast, inhibition of calcineurin (protein phosphatase-2B) with FK506 (10, 100 µM) did not increase dynorphin immunostaining in the cultured striatum, nor did it induce CREB phosphorylation. Our findings suggest that striatal dynorphin expression is negatively regulated by constitutively active PP-1 and/or PP-2A, possibly through dephosphorylation of PCREB. Thus striatal neuropeptide expression is under rigid dephosphorylation control by specific protein phosphatases. Supported by NIH 2 R01 HD28341 and the Science Partnership Fund at MIT.

352.9

THE STRIATONIGRAL NEURON: ITS DA RECEPTOR EXPRESSION PATTERN. M. Ray, K.L. Noblett, J.Y. Wang, and M.A. Ariano*. Dept. Neuroscience, Finch University Health Sciences/The Chicago Medical School, North Chicago, IL 60064.

Patterns of gene expression and receptor protein for the five DA receptor subtypes were detected in identified striatonigral neurons. The mRNA was marked by enzymatic incorporation of fluorescent dUTP (FIST) following hybridization of a unique sequence for oligonucleotide primers derived from previous in situ hybridization studies. Immunofluorescence using subtype specific anti-peptide antisera assessed protein staining (*Meth. Neurosci.* 25:455, 1995). Striatonigral neurons were identified by retrograde transport. Both the gene and protein expression were visualized simultaneously in individual medium sized neurons of the striatal region. Separate examination of gene and protein expression confirmed that mRNA and protein translation for each receptor subtype occur in neurons of the striatonigral pathway. In additional experiments, simultaneous staining of gene and protein expression was visualized on identified striatonigral neurons. These findings demonstrated a high level of homologous coincidence for gene and protein expression for all five subtypes of DA receptors in striatonigral neurons. The level of staining for the D_{1A} and D_2 signals were robust, whereas D_{1B}, D_3, and D_4 were less intense. Acutely isolated striatonigral neurons were used to analyze further functional attributes of the different DA receptor subtypes. This work was supported by USPHS NS 33277.

352.10

ULTRASTRUCTURAL CHARACTERIZATION OF AROMATIC L-AMINO ACID DECARBOXYLASE (AADC)-POSITIVE CELLS IN THE STRIATUM OF RATS WITH 6-OHDA LESIONS. A.A. Alcantara*, A. Mura, G.N. Ato, J.C. Linder, S.J. Young and P.M. Groves. Departments of Neurosciences and Psychiatry, University of California, San Diego, La Jolla CA 92093-0603.

The most common treatment for Parkinson's disease (PD), a disorder characterized by the loss of the nigro-striatal dopaminergic pathway, is administration of L-3,4-dihydroxyphenylalanine (L-DOPA). An apparent paradox is that exogenous L-DOPA is effective in PD despite the severe loss of the nigral afferents which are a main source of striatal AADC, the enzyme that converts L-DOPA to dopamine. Recently, a small population of striatal neurons immunoreactive (IR) for AADC has been discovered in the rat that may be relevant to PD since dopamine-IR cells similar in morphology and location were observed in the dopamine deafferented striatum following L-DOPA administration (Mura et al., 1995).

To characterize the ultrastructure of these neurons, we combined immunocytochemical with electron microscope (EM) techniques. AADC-IR cells identified at the light level were selected for EM. Cell somata were round or oval and ~10 microns in diameter. In some cases, axo-somatic contacts were observed. Cell nuclei were round or elongated and centrally localized with occasional shallow or deep invaginations and surrounded by a scant amount of cytoplasm. AADC immunoreaction product was present in the cell cytoplasm and dendrites, but absent from the nucleus. AADC-IR cells displayed EM features which may distinguish them from common medium spiny neurons, including their smaller size, less cytoplasm, and more prominent nuclear indentations. These EM features most resembled calretinin-positive interneurons; however, the discrete localization of these cells in the dorso-medial striatum in combination with LM double labeling results (Mura et al., Soc. Neurosci., 1996) indicate that these AADC-IR cells are a previously unidentified class of striatal neurons. To further define the morphology of these neurons and possibly differentiate them from other striatal classes, we are currently using serial section EM to characterize synapses and axons, and to examine dendrites for such features as varicosities and spines. Supp. by NIH AG00216, DA02854, DA00079 and S06GM47165-05.

352.11

STRIATAL CELLS CONTAINING AROMATIC AMINO ACID DECARBOXYLASE (AADC): A NEW NEURONAL CLASS. A. Mura, V.A. Andrus, J.C. Linder*, S.J. Young and P.M. Groves. Dept. Psychiatry, Univ. Calif. San Diego, La Jolla , CA 92093-0603

We recently described a group of cells that were immunolabeled for AADC in the rat neostriatum and obtained evidence that these neurons may produce dopamine in the presence of exogenous L-DOPA (Mura et al., Brain Res. 704:51-60, 1995). Thus, these cells may be significant for the pharmacotherapy of Parkinson's disease. Here we further characterize these cells in an attempt to determine whether they form a subclass of one of the previously-described categories of striatal neurons, or if these AADC cells represent a novel cell type. Sections of striatum from rats with unilateral 6-hydroxydopamine MFB lesions were single- or double-immunolabeled for a variety of neurochemicals (AADC, dopamine, calretinin, somatostatin, parvalbumin, VIP, calbindin, GABA) using standard methods. The morphology of the AADC cells (small, uni- or bipolar, aspiny) does not correspond to that of the spiny projection neurons or to the larger or more polygonal classes of interneurons, such as those containing acetylcholine, parvalbumin/GABA, somatostatin/neuropeptide Y/nitric oxide synthase, or VIP. The distribution of the AADC cells (dorsal striatum, just below the corpus callosum) did not overlap that observed for parvalbumin/GABA or calbindin-containing cells or that reported for cholecystokinin. The AADC cells most closely resemble the small calretinin interneurons, but we did not find any cells double-labeled for calretinin and AADC. However, we did find that the majority of AADC cells were double-labeled for GABA. These cells were among those with the most intense GABA labeling and therefore appear distinct from projection neurons and most interneurons that are reported to stain weakly for GABA. While parvalbumin interneurons do label intensely for GABA, their morphology and distribution differs from that of the AADC cells. We conclude that AADC cells contain GABA but not calretinin or calbindin and probably represent a new class of striatal interneurons.
Supported by PHS DA02854 and DA00079.

352.12

STRIATAL REGIONALIZATION AS DETERMINED BY DIFFERENTIAL DISPLAY. W.J. Rushlow*, N. Rajakumar, C.C.G. Naus and B.A. Flumerfelt. Dept. of Anatomy & Cell Biology. Dept. of Psychiatry, University of Western Ontario, London, Canada, N6A 5C1.

Differential display is a powerful new approach that can be used to identify mRNA species uniquely expressed in different regions of the brain. It is a reverse transcriptase-polymerized chain reaction (RT-PCR) based technique that allows for the visual identification of candidate cDNA's by directly comparing banding patterns present on a polyacrylamide gel. In the present study, differential display was used to identify novel, regionally expressed mRNA's in the rat striatum. Total cellular RNA was isolated from punches obtained from the caudate-putamen of adult Sprague-Dawley male rats using a guanidine isothiocyanate technique. Following the RT-PCR reaction, samples were run on a 6% polyacrylamide gel, and differentially expressed bands were identified. Reamplified cDNA bands were used to generate probes for in situ hybridization and to screen a rat striatal or rat brain cDNA library. More than 75 candidate genes have been identified thus far using different primer combinations. Screening of several of these candidates has yielded 5 novel cDNA's. These cDNA's are currently being characterized and sequenced. Supported by the Medical Research Council of Canada.

352.13

GLUTAMATE-ENRICHED CHOLINERGIC TERMINALS IN THE BASAL GANGLIA OF THE RAT. N. P. Clarke, M. D. Bevan, C. Cozzari, B. K. Hartman & J. P. Bolam*. MRC Anatomical Neuropharmacology Unit, Mansfield Road, Oxford, OX1 3TH, U.K.; Instituto Biologia Cellulare CNR, Rome Italy (CC); University of Minnesota, Minneapolis, Minnesota 55455, U.S.A. (BKH).

The output nuclei of the basal ganglia i.e. the entopeduncular nucleus (EP) and substantia nigra (SN), and the subthalamic nucleus (STN) receive a cholinergic innervation derived from the mesopontine tegmentum (MTg). It has been proposed that at least some of the cholinergic neurons of MTg also contain glutamate, implying that acetylcholine and glutamate are co-released. The object of the present study was to determine whether the terminals of cholinergic neurons in the basal ganglia have elevated levels of glutamate. Vibratome sections of the basal ganglia of perfuse-fixed rat brain were reacted to reveal choline acetyltransferase (ChAT) immunoreactivity using peroxidase methods with diaminobenzidine as the chromogen. After re-embedding, ultra-thin sections were subjected to post-embedding immunocytochemistry to reveal GABA and glutamate. The density of immunogold particles overlying ChAT-immunopositive terminals and non-labelled terminals forming asymmetrical synapses was then normalised by expressing them as a ratio of that overlying non-labelled GABA-positive terminals forming symmetrical synapses. In the STN, GABA-positive terminals forming symmetrical synapses had an index of glutamate immunoreactivity of 1.0347±0.103 (n=67), ChAT-immunopositive terminals had a significantly greater index (2.8554±0.185; n=75; $p < 0.0001$) as did non-labelled GABA-negative terminals forming asymmetrical synapses (4.6906±0.27; n=52; $p < 0.0001$). These results demonstrate that ChAT-immunopositive terminals in the STN, which are probably derived from the MTg, are enriched in glutamate suggesting that at least some of these terminals co-release glutamate as a neurotransmitter. Similar experiments are in progress to examine the levels of glutamate-immunoreactivity in cholinergic terminals in the EP and SN.
Supported by the Medical Research Council

352.14

THE ONTOGENESIS OF NITRIC OXIDE SYNTHASE IN THE BASAL GANGLIA OF THE MOUSE. X. Chen*, J. W. Langston, and M. W. Jakowec. The Parkinson's Institute, Sunnyvale, CA, 94989.

We are interested in understanding the role of nitric oxide in the developing basal ganglia and in models of neurodegeneration and toxicity. We used immunocytochemistry with antibody probes against both neuronal and inducible forms of nitric oxide synthase (NOS) to determine the pattern of expression in the developing mouse basal ganglia. Our results indicate that the neuronal NOS protein is expressed in a subset of interneurons scattered throughout the adult striatum with strong staining of cell bodies and their processes. Neuronal NOS is also expressed at high levels in the subthalamic nucleus, and at low, but detectable, levels in the substantia nigra pars compacta especially along the lateral aspect of the region. A similar pattern of expression was seen in the neonate except neuronal NOS was seen at higher levels in corresponding regions. Using the antibody probe against the inducible form of NOS our results indicate that this protein is not detectable on cell bodies in either the striatum, subthalamic nucleus, or substantia nigra of the adult. However, neuropil staining in the striatum was present.
This work is supported by a grant from The Mather and Lookout Foundations.

352.15

ULTRASTRUCTURAL EXAMINATION OF 5' NUCLEOTIDASE ACTIVITY IN PATCH/MATRIX COMPARTMENTS OF RAT NEOSTRIATUM. M. E. Martone*, K. Tang, Z. Nebyelul, Y. Jones, S. J. Young and M. H. Ellisman. Natl. Ctr. for Microscopy and Imaging Research, Univ. California San Diego, 92093-0608.

5'-nucelotidase (5NT) is a glial-associated ectoenzyme that delineates the patch/matrix compartments in adult rat neostriatum. We examined the ultrastructural distribution of 5NT in the patch and matrix compartments of rat neostriatum using conventional and high voltage electron microscopy (HVEM). Vibratome sections of neostriatum fixed with 4% paraformaldehyde were processed for 5NT histochemistry using the method of Schoen and Graybiel (J. Comp. Neurol., 322: 566, 1992). In the light microscope (LM), 5NT activity appeared as discrete dark patches against a lighter staining background. Regions containing clearly delineated patches were selected for further electron microscopic examination. Alternate thick (3-5um) and thin (100nm) sections were cut from the same block and mounted on high transmission grids. The thick sections were first photographed under LM to provide correlated images for electron microscopy. The dark patches of 5NT activity were visible under LM in 3-5 um thick sections but not in thinner sections. The thick sections were then examined directly using HVEM at 400 keV or 1 meV. Adjacent thin sections were examined at 80 keV, using the light micrograph of the thick section as a guide to identify patch regions. In the electron microscope, 5NT reaction product was found extracellularly associated with the plasma membrane of neuronal and glial processes. Stereopairs of thick sections revealed that 5NT activity was distributed in a fine filigree pattern, forming nets around neuronal processes, cell bodies and blood vessels. 5NT activity was found in both patch and matrix compartments but the staining in patches was more intense and the amount of labeling also appeared to be greater than outside the patches. The distinction between patch and matrix was subtle, however, and was most clearly visible in 3-5 um sections viewed at low magnifications with HVEM. A difference in staining between patch and matrix was still detectable in thin sections when the LM images were used to identify the border between patch and matrix. Work is underway to characterize further the staining patterns in patch/matrix and the 3-dimensional organization of the border region. Supported by NIH grant RR04050 to MHE.

352.16

LOCALIZATION OF N-METHYL-D-ASPARTATE (NMDA) RECEPTOR SUBUNIT PROTEINS IN THE NIGROSTRIATAL PATHWAY

D.G. Standaert* and S.W. Weiss. Dept. of Neurology, Mass. Gen. Hosp., and Harvard Medical School, Boston, MA 02114.

NMDA glutamate receptors in the substantia nigra pars compacta (SNpc) regulate the bursting activity of dopamine neurons, while in the neostriatum NMDA receptors appear to influence the release of dopamine from presynaptic terminals. We have used dual-label immunofluorescence and confocal laser microscopy to study the localization of NMDAR1, NMDAR2A, and NMDAR2B proteins within dopaminergic neurons identified by the presence of immunoreactivity (-ir) for tyrosine hydroxylase (TH) in the rat. In the SNpc, NMDAR1-ir was prominent in the cytoplasm and proximal dendrites of TH-ir neurons, and moderate amounts of NMDAR2A-ir and NMDAR2B-ir were present. Immunoreactivity for the variably spliced segments of NMDAR1 (N1, C1, C2) was scarce. In the neostriatum, NMDAR1-ir, NMDAR2A-ir, and NMDAR2B-ir were present within neuronal cytoplasm, proximal dendrites, and fine punctate varicosities within the neuropil. Neurons and the neuropil also contained abundant NMDAR1 C1-ir and C2-ir. No colocalization of NMDA subunit immunoreactivity with TH-ir processes in the striatum was observed. Following unilateral 6-OHDA lesions, TH staining of the striatum was absent, but the expression of NMDA subunit immunoreactivity in the neuropil was not altered.

These data demonstrate that dopaminergic neurons of the nigrostriatal pathway express NMDAR1, NMDAR2A, and NMDAR2B receptor subunit proteins, and that these proteins are restricted to the somatodendritic compartment. *Supported by the National Parkinson Foundation & NS31579.*

352.17

MORPHOLOGY AND IMMUNOCYTOCHEMISTRY CHARACTERISTICS OF THE MARGINAL DIVISION IN MONKEY STRIATUM, S.Y. Shu* and X.M. Bao, Dept. Neurobiology, Zhu-Jiang Hospital, Guangdong, China.

In our previous study, a new area, the marginal division(Mr.D.), was observed in the rat striatum. The Mr.D. is located at the caudal border of the striatum and is more densely filled with many neuropeptidergic terminals. The neuronal somata in Mr.D. are mostly fusiform. The function of Mr.D. is supposed to correlate to the learning, memory and nociceptive responses. The present study described the Mr.D. in the monkey(Macaca Mulatta) striatum. Using Nissl staining, A band of packed small fusiform neurons with medially located scattered large fusiform neurons were observed at the caudomedial border of the monkey striatum. Some of them are AchE positive, or SP-,NPY-, Enk-, NT-immunohistochemistry positive reacted. Two bands of SP-, M- & L-Enk-, NPY- and NT- immunoreacted fibers and terminals were found at the caudomedial edge of the striatum and the rostolateral edge of the globus pallidus respectively. Pedunculopontine fibers were reported projecting to the caudomedial portion of the putamen in the squirrel monkey. It is proposed that the Mr.D. is better developed in the monkey brain than that in the rat brain and has special afferent connections, so that the Mr.D. probably plays more important role in the monkey brain.(supported by NSFC).

BASAL GANGLIA: FUNCTION II

353.1

NEURONAL GENE EXPRESSION ASSOCIATED WITH DOPAMINE SUPERSENSITIVITY IN RAT STRIATUM. G.J. LaHoste[1]* and J.F. Marshall[2]. Departments of [1]Physical Medicine & Rehabilitation and [2]Psychobiology, University of California, Irvine, CA 92717-4550.

Dopaminergic systems within the mammalian brain are characterized by a striking degree of plasticity. For example, following extensive injury to dopamine (DA)-containing cell bodies within the mesencephalon, DA receptors in telencephalic target regions become supersensitive to agonists with respect to behavioral, electrophysiological, neurochemical and genetic response. In addition, the density (but not the affinity) of D2 receptors is increased following injury. For many years this increase in D2 receptor density was believed to be the cause of agonist supersensitivity. However, several lines of evidence demonstrate convincingly that receptor up-regulation cannot account for the high degree and early appearance of supersensitivity. Assuming that changes in gene expression may underlie the changes in receptor sensitivity, we have attempted to elucidate the mechanism(s) of supersensitivity using differential display of mRNA. In this method, subsets of mRNA species are labelled, amplified and displayed in adjacent lanes of a high resolution polyacrylamide gel. Adult male rats received injections of 6-hydroxydopamine (6-OHDA; 8 µg) into the ascending medial forebrain bundle. Rats with confirmed extensive lesions were sacrificed several weeks after 6-OHDA and their caudate-putamens (CPu) rapidly dissected. RNA was isolated, reverse transcribed into cDNA and amplified using polymerase chain reaction (PCR) in the presence of ^{33}P-dATP. Primers for PCR were from the GenHunter RNAimage kit. To date, several cDNA fragments have been identified that are differentially expressed in control and 6-OHDA-treated CPu. Some are expressed in 6-OHDA tissue but not control tissue, while others are expressed in control but not 6-OHDA tissue. We are in the process of confirming and characterizing the differentially expressed genes. *Funded by MH 49690, NS 22698.*

353.2

ADENOSINE A_{2a} ANTAGONISTS MODULATE DOPAMINE DEPENDENT TURNING BEHAVIOR AND C-FOS EXPRESSION IN 6-HYDROXYDOPAMINE LESIONED RATS M.Morelli,* A.Pinna, S.Fenu, S.Cabiddu, G.Di Chiara. Department of Toxicology, University of Cagliari, Italy.

The interaction between adenosine A_1 and A_{2a} antagonists and dopamine (DA) D_1 and D_2 agonists was evaluated in rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the dopaminergic nigrostriatal pathway, by studying the contralateral turning behavior induced by DA receptor agonists and the activation of the early-gene c-fos, as measured by Fos-like-immunoreactivity (FLI). Selective blockade of adenosine A_{2a} receptors by SCH 58261 strongly potentiated the contralateral turning behavior and increased FLI induced by the D_1 agonist SKF 38393 in the 6-OHDA lesioned striatum. At variance, the A_1 receptor antagonist DPCPX induced a small potentiation of D_1-mediated turning behavior while FLI was not modified. SCH 58261 positively modulated also turning behavior induced by stimulation of D_2 receptors by quinpirole or by stimulation of D_1/D_2 receptors by L-DOPA. The degree of potentiation of D_2-mediated turning behavior was however smaller as compared to that obtained after D_1 receptor stimulation. FLI induced by quinpirole and L-dopa in the globus pallidus and striatum was also potentiated by SCH 58261. Blockade of A_{2a} receptors positively influences the functional expression of D_1 and D_2-mediated responses in dopamine denervated rats, suggesting new therapeutic approaches for the treatment of Parkinson's disease.

353.3

DOPAMINE DEPLETION IN THE RAT ALTERS RESPONSES OF SUBTHALAMIC NUCLEUS NEURONS TO APOMORPHINE AND THE D_1 RECEPTOR AGONIST SKF 38393.

D.S. Kreiss*, K.A. Allers, M.J. Twery, and J.R. Walters. ETB, NINDS, Bethesda MD 20892.

The subthalamic nucleus (STN) plays a critical role in the regulation of basal ganglia output. Evaluation of STN neuronal activity during basal stimulation as well as during exogenous dopaminergic (DAergic) agonist stimulation is essential for understanding the role of the STN in Parkinsonian symptomology. Previous studies from this laboratory characterized the regulation of STN neuronal activity in the normal rat. The present study examines the STN in the DA-depleted rat 6-16 weeks post treatment with 6-OHDA. The basal firing rate of STN neurons in the DA-depleted rat (18.1 ± 1.7 spikes/sec, n = 47) was markedly elevated relative to basal rates in normals (8.4 ± 0.7 spikes/sec, n = 54). This was probably not a result of altered NMDA receptor-mediated glutamatergic tone as the glutamate antagonist MK 801 (0.1 mg/kg) did not alter firing rates. Whereas the D_1/D_2 agonist apomorphine (APO, 0.3 mg/kg) increased firing in the normal rat, APO *decreased* STN neuronal firing rates to 59% (n = 9) of basal values in the DA-depleted rat. Alteration of APO's effects in the DA-depleted rat could not be explained by responses to selective D_1 and D_2 receptor agonists. The excitatory effects of SKF 38393 seen in normals were *potentiated* by DA-depletion: a dose that was ineffective in normals (10 mg/kg) significantly increased firing in the STN of lesioned rats. The D_2/D_3 receptor agonist quinpirole (0.3 mg/kg), however, did not alter firing rates in normal or DA-depleted rats. Understanding the mechanisms mediating the regulation of the STN under conditions of DAergic deficiency may lead to improved treatments for Parkinson's disease using pharmacotherapy and/or neurosurgery.

(Funding: NINDS)

353.4

SELECTIVE INCREASES IN 5-HT1B AND 5-HT2 BINDING SITES IN THE RAT BASAL GANGLIA FOLLOWING SEROTONERGIC LESIONS.

V. Compan[1], M.-C. Buhot[2], L. Segu[2]* and A. Daszuta[1]. Lab. Neurobiologie Cellulaire et Fonctionnelle., CNRS, Marseille[1], and Lab. Neurosci. Comportementales & Cognitives, CNRS-URA 339, Univ. Bordeaux I, Talence[2], France.

The distribution and regulation of 5-HT1B and 5-HT2 binding sites labelled with S-CM-G[^{125}I]TNH2 and [^{125}I]DOI, respectively, were examined by autoradiography in the basal ganglia of adult rats. In some of the animals, the serotonergic (5-HT) neurons were lesioned by injections of 5,7-dihydroxy-tryptamine in the dorsal and medial raphe nuclei (three weeks survival).

In controls, the 5-HT1B binding sites were homogeneously distributed in the core and shell of the nucleus accumbens, as well as in the rostro-caudal levels of the caudate putamen (head, body and tail), the higher densities were observed in the ventral part of the body. 5-HT2 binding sites density increases progressively from the head to the tail of the caudate putamen. In the body of the caudate putamen, higher densities were found in the medio-dorsal region. In the nucleus accumbens, higher densities were detected in the shell than in the core.

In lesioned animals, there is a lack of changes in 5-HT binding sites density after a large but still incomplete lesion of 5-HT neurons, inducing a 80% decrease in 5-HT tissue level in the structures examined. Following a complete lesion of 5-HT neurons, a 20% increase in 5-HT1B binding sites was observed in the substantia nigra, while no changes were detected in the globus pallidus or the striatum. This kind of lesion induced a 40% increase in 5-HT2 binding sites in the medial part of the body of the caudate putamen. Weaker increases were observed in the dorsal regions, and no significant changes were detected in the head or tail of the caudate putamen. Taken together, these results suggest the existence of selective up-regulations of 5-HT receptors in the basal ganglia following complete 5-HT denervation.

Supported by Centre National de la Recherche Scientifique, France.

353.5

CONSEQUENCES OF NIGROSTRIATAL DENERVATION ON THE FUNCTIONING OF THE BASAL GANGLIA.

M. Vila, R. Levy, M.-T. Herrero†, M. Ruberg, B. Faucheux, J.A. Obeso†, Y. Agid* and E. C. Hirsch. INSERM U289, Hôpital de la Salpêtrière, 75013 Paris, France. †University of Murcia, 30071 Murcia, Spain. ‡Quiron Clinic, 20012 San sebastian, Spain.

In Parkinson's disease (PD), the loss of nigral neurons provokes a cascade of changes in the basal ganglia which may account for the motor parkinsonian syndrome. To examine the consequences of nigrostriatal denervation and chronic levodopa (L-dopa) treatment on functional activity of the basal ganglia, we analysed, using *in situ* hybridization, the cellular expression of the mRNA encoding for cytochrome oxidase (subunit I, COI mRNA), a metabolic marker for functional neuronal activity, in the basal ganglia. This analysis was performed in patients with PD and in monkeys rendered parkinsonian by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication, some of which had been receiving L-dopa. In MPTP-intoxicated monkeys compared to control animals, COI mRNA expression was increased in the subthalamic nucleus (STN, +85%) and in the output nuclei of the basal ganglia: internal segment of the globus pallidus (GPi, +70%) and substantia nigra pars reticulata (SNpr, +82%). This increase was partially reversed by L-dopa treatment. In PD patients, who had all been chronically treated by L-dopa, COI mRNA expression was unchanged in the output basal ganglia nuclei but increased in the STN (+71%). In both, MPTP-intoxicated monkeys and PD patients, COI mRNA expression in the external segment of the globus pallidus (GPe) remained unchanged. These results are in agreement with the accepted model of basal ganglia organization, in which the output nuclei of the basal ganglia are considered to be overactive after nigrostriatal denervation, partly due to increased activity of excitatory afferents from the STN. Yet, our results are also in contradiction with this model since the overactivity of the STN does not seem to be due to an hypoactivation of the GPe. Thus, the hyperactivity of the STN might perhaps be due to a hyperstimulation of fibres originating in the cerebral cortex and/or in the thalamus. *Supported by INSERM, NPDF, MESR and MEC.*

353.6

SUBSTANTIA NIGRA PARS RETICULATA (SNpr) NEURONS EXHIBIT SLOW OSCILLATIONS IN FIRING RATE THAT PERSIST AFTER A 6OHDA-INDUCED LESION OF MIDBRAIN DOPAMINE NEURONS.

M. J. Twery*[1], D. A. Bergstrom[1], Y.-X. Li[2], J. Rinzel[2], and J. R. Walters[1]. [1]ETB, NINDS and [2]MRB, NIDDK, NIH, Bethesda, MD 20892.

The expression of motor behaviors associated with thalamo-cortical circuitry has been closely linked to the *level* of output from basal ganglia centers including the SNpr. Thalamic and collicular targets of SNpr neuronal projections exhibit oscillatory activity patterns which may be a principal mode of regulating neurotransmission. The present study reveals that prominent oscillatory firing patterns also occur in SNpr neurons and that the basal expression of these patterns does not appear to be solely dependent on endogenous dopamine. SNpr neuronal activity was recorded extracellularly in locally-anesthetized, paralyzed rats and was characterized by plotting instantaneous spike frequencies and power spectra obtained from 3-10 min spike trains. In normal rats, all neurons (n=18) exhibited a slow oscillation in firing rate with periods of 15-63 sec. This rhythmic pattern was superimposed on faster oscillations (0.8-2 sec). In 6OHDA-lesioned rats (10-17 weeks post lesion), 24 of 28 neurons exhibited the long period oscillations (6-50 sec). Only 14 of 24 neurons clearly exhibited the short period oscillations (0.6-1.8 sec). A combination of fast and slow oscillatory patterns occurred in 12 of the 24 neurons from 6OHDA-lesioned rats. Intravenous administration of quinpirole and/or SKF 38393 to lesioned rats altered the regularity of oscillations compared subjectively with baseline in 14 neurons. The presence of spike frequency oscillations in SNpr neurons raises the possibility that temporal relationships in firing pattern as well as the overall level of neuronal output govern the expression of motor behavior through basal ganglia circuitry.

(Funding: DIR, NINDS).

353.7

NEUROPEPTIDE mRNA EXPRESSION IS SELECTIVELY AFFECTED BY SUICIDE TRANSPORT LESIONS OF STRIATONIGRAL AND STRIATOPALLIDAL NEURONS.

M.B.Harrison* and R.G.Wiley,[1] Univ. of Virginia, Charlottesville VA 22908 and [1]DVAMC, Nashville TN 37212.

Injections of volkensin (VOLK) into the SN or OX7-saporin (OX7) in the GP produce lesions of striatal projection neurons ,and demonstrate differential distribution of receptor subtypes between striatonigral or striatopallidal neurons (Harrison 1990, 1992a and b, 1993, 1994; Pollack 1993). Histologic evaluation of projection and interneurons showed appropriate specificity; however the area of maximal effect was not examined separately (Roberts 1993). In the present study, effects on preproenkephalin (PPE) mRNA in striatopallidal and preprotachykinin (PPT) mRNA in striatonigral neurons were compared at 10 days survival. Rats received stereotactic injections of VOLK (2.5 ng) in the SN (n=5) or OX7 (0.5µg) in the GP (n=4). *In situ* hybridization was performed on alternate sections and film autoradiographs analyzed for optical density in the maximally affected region of the striatum. In a separate series of animals treated with VOLK (n=7), cell counts of PPE neurons were performed on emulsion dipped slides to assess if an increase in PPE by remaining neurons could mask cell loss. In the VOLK lesioned animals, PPT mRNA decreased by 60% (0.075 ± 0.01 vs 0.188 ± 0.03*; mean± se, ipsi- vs contralateral to the injection), while PPE was unchanged (0.464 ± 0.02 vs 0.460 ± 0.03). Cell counts of PPE-positive neurons showed a slight *increase* on the lesioned side (282±70 vs 229±42/150µm²*). In OX7 lesioned animals, PPE decreased by 41% (0.300 ± 0.06 vs 0.508 ± 0.11*); PPT was unchanged (0.163 ± 0.03 vs 0.166 ± 0.03). In these animals, D1 and D2 receptor binding changes were similar to those reported previously (VOLK: D1 49% decrease, D2 16% decrease; OX7: D1 12% decrease, D2 28% decrease). These results indicate that the effects of suicide transport toxins on striatal projection neurons are selective and suggest that 40-60% of the targeted neurons in the maximally affected area are lost at 10 days. *$P < 0.05$, *t* test.

Supported by NINDS NS 01454; American Parkinson Disease Assn.; Veterans Admin.

353.8

THE EFFECTS OF GLOBUS PALLIDUS LESIONS ON DOPAMINE D1- AND D2-RECEPTOR MEDIATED MOTOR BEHAVIOUR IN RATS.

W. Hauber*, S. Lutz and M. Münkle. Biologisches Institut, Abt. Tierphysiologie, Universität Stuttgart, Pfaffenwaldring 57, D-70550 Stuttgart; Germany.

The striatum is the main input structure of the basal ganglia and projects via two different pathways to the basal ganglia output nuclei. A direct pathway involves striatal projections to the substantia nigra pars reticulata and to the entopeduncular nucleus. An indirect pathway projects from the striatum via the globus pallidus (GP) to these output nuclei. Dopamine (DA) plays a critical role in regulating the activity of striatopallidal and striatonigral projection neurons which involves different receptor subtypes, respectively. In the striatum, the D1- subtype of DA receptors is almost exclusively expressed by striatonigral neurons, whereas the DA D2-receptor subtype is selectively expressed by striatopallidal neurons. Little is known about the relative functional significance of striatonigral and striatopallidal neurons with regard to motor control. Therefore we investigated the effects of bilateral GP lesions on motor behaviour mediated by DA D1- or D2-receptors. Results show that (1) GP lesions (AP:-0.4 mm; L: 2.8 mm; V: 4 mm) induced by quinolinic acid reduced spontaneous and d-amphetamine (1 mg/kg, i.p.)-induced locomotor activity as well as spontaneous and d-amphetamine (3 mg/kg, i.p.)-induced sniffing. (2) GP lesions reduced catalepsy induced by the DA D1-antagonist SCH 23390 (0.75 and 1 mg/kg, i.p.) and the DA D2-antagonist raclopride (1.25 and 5 mg/kg, i.p.). Based upon recent models of basal ganglia functions, it could be predicted that pallidal destruction should lead to motor hypoactivity. Accordingly, GP lesions reduced spontaneous locomotion and sniffing and amphetamine-induced hyperactivity. However, modified models of basal ganglia functions will be required to incorporate the finding that GP lesions reversed catalepsy induced by a DA D1- or D2-receptor blockade.

Supported by the Deutsche Forschungsgemeinschaft (HA 2340/1-1).

353.9

EFFECTS OF l-DOPA-THERAPY ON DOPAMINE D_1 AND D_2 RECEPTOR mRNAs IN THE STRIATUM OF MPTP PARKINSONIAN MONKEYS. M.-T. Herrero*, S.J. Augood†, H. Asensi, E.C. Hirsch†, Y. Agid†, J.A. Obeso*, P.C. Emson†. Experimental Neurology. University of Murcia, Spain. †Babraham Institute, Cambridge, UK. †INSERM U289, Hôpital de la Salpêtrière, Paris, France. *Quirón Clinic, San Sebastian, Spain.

The cellular expression of both dopamine D_2 receptor (DA D_2) and dopamine D_1 receptor (DA D_1) mRNAs was examined in striatal (caudate nucleus and putamen) neurons of 12 *Macaca fascicularis* monkeys by quantitative *in situ* hybridization using human specific ^{35}S-labelled oligonucleotides. Nine monkeys were rendered parkinsonian by systemic injection of MPTP, 3 of them also received chronic l-DOPA-therapy. Three control monkeys were untreated and received neither MPTP nor l-DOPA. In these control brains, a substantial DA D_2 and DA D_1 receptor hybridization signal was detected overlying on the medium-sized striatal neurons. Moreover, some large (putatively cholinergic) neurons, in both the caudate nucleus and putamen, were hybridized with DA D_2 ^{35}S-labelled probe. Neurons from the lateral and medial regions of the caudate nucleus, and from the dorsal and ventral regions of the putamen were analysed separately. A significant increase ($p < 0.05$) in the cellular abundance of DA D_2 receptor mRNA was seen in the striatum of all MPTP-treated monkeys: this increase being restricted to the population of medium-sized striatal cells. However, no such increase in DA D_2 receptor mRNA was observed in (dyskinetic) l-DOPA-treated monkeys. In constrast, the significant decrease ($p < 0.05$) observed in the cellular abundance of DA D_1 receptor mRNA in the striatum of all parkinsonian monkeys was reversed after l-DOPA-therapy. The implications of these findings in relation to the dissociation of D_2 and D_1 coupling in the striato-pallidal pathways must be important for the development of l-DOPA-induced dyskinesias. *Supported by CICYT (SAF94-1392).*

353.10

MOTOR AND SOMATOSENSORY DEFICITS INDUCED BY UNILATERAL AND BILATERAL THERMOCOAGULATORY LESIONS OF THE CORTEX IN THE ADULT RAT.
J.A. Napieralski*, R.J.A. Banks, and M-F. Chesselet. Dept. of Pharmacology, Univ. of Pennsylvania., Phila, PA 10104

Previous studies in our laboratory have shown that contralateral cortical neurons undergo axonal sprouting in the denervated striatum of the adult rat following a lesion of the sensorimotor cortex induced by thermocoagulation of pial blood vessels (Napieralski et al., 1995). We have examined the behavioral effects of either unilateral or bilateral thermocoagulatory lesions of the sensorimotor cortex in adult rats. Male Sprague-Dawley rats were tested to determine limb use asymmetry by analyzing 1), coordinated forelimb placement and 2), paw use preference when rearing. Their responsiveness to somatosensory stimulation was tested by analyzing 1), the latency to remove sticky tape on the ventral surface of the paw and 2), vibrissae-stimulated forelimb placing. The following day, rats received either a unilateral or bilateral thermocoagulatory lesion of the cortex, or were sham operated controls. The tests performed prior to surgery were re-administered on 4, 8, 12, 16, and 20 days after the surgery. Animals with a unilateral lesion showed a deficit in contralateral limb use and responsiveness to somatosensory stimulation which was maximal at 8 days after the surgery and returned to or near control responsiveness by day 20. Animals with a bilateral lesion showed a greater deficit in both categories with less recovery than the unilaterally lesioned animals. The data indicate that unilateral thermocoagulatory lesions of the sensorimotor cortex induce transient sensory and motor deficits in the contralateral forelimb. The recovery of function seen in the animals with unilateral lesions may be due to morphological changes in neurons of the homotypic contralateral cortex as previously indicated by the presence of sprouting axons after this type of lesion. Supported by PHS grant NS-29130.

353.11

EFFECTS OF CORTICAL LESIONS IN RAT PUPS ON PSA-NCAM EXPRESSION AND AXONAL PLASTICITY. Kunihiro Uryu*, Amy Butler, Victoria Morehouse, Geneviève Rougon and Marie-Françoise Chesselet, Dep. of Pharmacol., Inst. of Neurol. Science, U. of Pennsylvania , Philadelphia, PA 19104 and U. of Aix-Marseille, France

The developmental conversion of the neural cell adhesion molecule (NCAM), from its highly polysialiated (PSA-NCAM) to its mature form, follows synapse formation in rat striatum (Szele et al. '94). We have shown that PSA-NCAM loss is delayed in the striatum after lesions of the cerebral cortex on postnatal day 14 (P14), prior to the formation of cortico-striatal synapses (Butler et al., 1994). We have now examined the cellular location of PSA-NCAM immunoreactivity in the striatum ipsilateral to the lesion and determined whether axonal sprouting and compensatory synaptogenesis occur in the denervated striatum. Rat pups received a thermocoagulatory lesion of the sensory-motor cortex at P14 and were sacrificed at P25. Ultrastructural analysis showed that PSA-immunoreactivity was maintained in both pre and post-synaptic elements in the striatum on the side of the lesion. The number of asymmetric synapses (the type formed by corticostrial projections) was similar in control and denervated striatum, indicating that reactive synaptogenesis had occured. PSA-NCAM positive growth cone-like structures were more numerous in lesioned pups, suggesting axonal growth. Injections of the anterograde tracer fluoro-ruby into the contralateral cortex revealed a marked increase in contralateral corticostriatal projections in lesioned pups. Based on the results of Di-I tracing which revealed that, during normal development, crossed corticostriatal projections are minimal after P7, the data indicate that P14 cortical lesions, which delay PSA-NCAM loss in striatal neurons, induce compensatory sprouting from the contralateral cortex and reactive synaptogenesis, suggesting that corticostrital inputs play a role in the regulation of PSA expression. Supp. by NS-29230 and F-31-MH10794.

353.12

PSA-NCAM IN THE DEVELOPING STRIATUM: REGULATION BY MK-801 DURING A CRITICAL PERIOD OF DEVELOPMENT.
A.K. Butler*, G. Rougon, and M-F Chesselet, Inst. of Neurol. Sci., and Dept of Pharmacol., U. of Penn. Phila, PA19104 and U. Aix-Marseille, France.

Polysialic acids associated with the Neural Cell Adhesion Molecule (PSA-NCAM) play a critical role in the development of the nervous system. In the rat striatum, loss of PSA-NCAM immunoreactivity occurs after the formation of corticostriatal synapses, which use the neurotransmitter glutamate (Szele et al, '94). Peripheral injections of the non-competitive antagonist of the NMDA type of glutamate receptor, MK-801, from postnatal day 14 (P14), before corticostriatal synapse formation, until P24, after the number of corticostriatal synapses has reached the adult level, result in a precocious loss of PSA-NCAM in the striatum and cortex, but not hippocampus (Butler et al '95). We have now determined the critical period of MK-801 actions by examining shorter treatment periods. Sprague Dawley rat pups were injected with MK-801(0.25 mg/kg once daily, ip), either starting at P14 (before cortico-striatal synapse formation) or P18, (when corticostriatal synapses reach a peak). Striatal sections were processed for immunohistocehmistry with an antibody recognizing specifically PSA residues associated with N-CAM in mammalian brain (Rougon et al, 1986). No loss of PSA-NCAM expression was observed when rats were treated with MK-801 from P14 to P16 or P18. In contrast, treatment with MK-801 from P18 to P20 or P22, but not P18 and P19 only, resulted in a precocious loss of PSA-NCAM in the striatum and cerebral cortex. The data identify periods of NMDA-independent and NMDA-dependent PSA expression in rat forebrain. The latter corresponds to the emergence of spontaneous activity in corticostriatal afferents and a decrease in the number of corticostriatal synapses, suggesting that PSA-NCAM may play a role activity-dependent synaptic plasticity during striatal development. Supp. by PHS grants NS-29230 and 1 F31 MH10794-01.

353.13

ROLE OF THE SUBTHALAMIC NUCLEUS IN CANNABINOID ACTIONS IN THE BASAL GANGLIA OF THE RAT M. Clara Sañudo-Peña* and J. Michael Walker. Schrier Research Laboratory, Departments of Psychology and Neuroscience, Brown University, Providence, RI 02912.

Receptors for cannabinoids (active principles of marijuana) are known to be highly concentrated in brain areas that control movement. The highest concentrations are located in the substantia nigra reticulata (SNR). To date, the only known source of cannabinoid receptors to the SNR is the striatum. Previous work from this laboratory (*Neurosci. Lett. 1996, 206(1):21–24*) led us to propose the subthalamic nucleus as a source of cannabinoid receptors to the SNR. This hypothesis was supported by the fact that the subthalamic nucleus (major excitatory input to the SNR) has moderate levels of mRNA for the cannabinoid receptor. Also, the subthalamic input to the nigra is tonically active, in contrast to the striatonigral pathway (major inhibitory input to the SNR) which is mainly silent. The last fact might suggest the subthalamic nucleus is the main site of cannabinoid action in the SNR under basal conditions, and would agree with the study cited above. To study the possibility of the subthalamic nucleus as a second source of cannabinoid receptors in the SNR, the effect of the cannabinoid agonist WIN 55,212–2 on the activity of SNR neurons after subthalamic nucleus stimulation was recorded. A knife cut was performed to isolate the subthalamic nucleus from the rostral areas of the basal ganglia. The cannabinoid agonist WIN 55,212–2 reversed the increase in activity of SNR neurons induced by stimulation of the subthalamic nucleus. Furthermore, the effect of the cannabinoid agonist was reversed by the cannabinoid receptor antagonist SR 141716A. Therefore, cannabinoids might be exerting important actions in the basal ganglia through their modulation of the subthalamic input to the SNR.
Supported by NIDS33247–01A 1R01 to JMW

353.14

PUTATIVE PROPERTIES FOR A PACE MAKER FIRING MODE IN SUBTHALAMIC NUCLEUS NEURONS. A STUDY IN RAT BRAIN SLICES. C. Beurrier, J. Audin and B. Bioulac*. CNRS UMR 5543, Univ. Bordeaux 2, 33076 BORDEAUX FRANCE.

The subthalamic nucleus (STN) is part of the indirect striato-pallidal pathway of the basal ganglia (BG). STN neurons form the glutamatergic subthalamo-pallidal bundle and exert an excitatory effect upon the globus pallidus internalis, i.e. the main outpout structure of BG. A current hypothesis, concerning Parkinson's disease, infers an overactivity of this glutamatergic pathway. In order to reach a better understanding of the physiology of STN neurons and thereby their possible dysfunctions, we have analyzed their membrane properties in rat brain slices.

A first characteritic of these neurons is their spontaneous pace maker firing mode (22.8 Hz, n=15). However, the main observation is the presence, for all these neurons (n=18), of a low threshold spike (LTS or slow action potential, SAP). SAP is obtained in neurons with a membrane potential around -70 mV after the offset of an hyperpolarizing pulse (-0.1 to 0.5 nA) and leads to a burst of action potentials. The SAP is blocked by a Ca^{2+} free medium (with Co^{2+}, Cd^{2+} or Mg^{2+}) and may be linked to the activation of T type Ca^{2+} channels. Moreover, membrane responds to an hyperpolorizing pulse by a clear anomalous rectification. Finally, in some cases (n=7), a weak depolarizing pulse (0.05 to 0.1 nA) elicits a slow depolarizing potential (SDP) in neurons with a membrane potential around -60 mV. Again SDP is abolished by a Ca^{2+} free medium (with Co^{2+}, Cd^{2+} or Mg^{2+}) and could correspond to the activation of L type Ca^{2+} channels.

The interplay between these two Ca^{2+}-dependent mechanisms and the anomalous rectification could contribute to generate the pace maker firing mode. Experiments, with more specific Ca^{2+} channels blockers, are currently carried out to precise the respective role of Ca^{2+} channels in SAP and SDP. Further works will attempt to study how, after lesion of dopamine neurons as in parkinsonism, the pace maker firing mode of STN neurons becomes hyperactivated.

CNRS, FRM.

353.15

ANTICONVULSANT EFFECTS OF FOCAL INHIBITION OF THE SUBTHALAMIC NUCLEUS. D. Dybdal *and K. Gale. Deptartment of Pharmacology, Georgetown Univ. Med. Ctr., Washington DC, 20007.

The subthalamic nucleus (STN) plays a crucial role as a regulator of basal ganglia outflow by providing excitatory drive into the substantia nigra (SN) and the entopeduncular nucleus (EPN). This excitatory influence has been shown to be mediated by glutamatergic neurons projecting from STN to SN/EPN, and to be subject to GABA-ergic inhibition at the level of STN. Because focal blockade of glutamate transmission within SN or EPN has been shown to have an anticonvulsant effect, we hypothesized that focal, pharmacological inhibition of STN would also reduce seizure susceptibility. To test this hypothesis, the $GABA_A$ agonist, muscimol (200 pmol) , was infused uni- or bilaterally into the STN of awake, behaving rats prior to seizure induction.

Limbic motor seizures were evoked either focally from area tempestas, an epileptogenic trigger site in the pre-piriform cortex, or by intravenous injection of bicuculline. Unilateral pretreatment with muscimol in STN produced postural asymmetry but had no significant protective effect in either model of seizure induction. Bilateral pretreatment with muscimol in STN, 5 min prior to evoking seizures from area tempestas, resulted in a reduction in both severity and frequency of seizures compared to saline pretreatment in STN. Bilateral pretreatment with muscimol in STN also protected against seizures evoked by intravenous bicuculline. Anticonvulsant effects were site specific inasmuch as bilateral injections of muscimol into areas adjacent to STN were without effect. These results indicate that inhibition of STN is capable of reducing seizure susceptibility, and are consistent with the view that the STN is an important source of excitatory drive to SN and EPN.

Supported by NIH grants # NS20576 and F31 MH10812

CONTROL OF POSTURE AND MOVEMENT: MOTOR LEARNING

354.1

ADAPTIVE LEARNING GENERALIZES ACROSS DIFFERENT MOVEMENTS. M. A. Conditt*, F. Gandolfo, F. A. Mussa-Ivaldi. SMPP, Rehab. Inst. Of Chicago, Chicago, IL 60611 and Dept. of Brain and Cog. Sci., MIT, Cambridge, MA 02139.

It has been shown (Flash and Gurevich, 1992; Shadmehr and Mussa-Ivaldi, 1994) that the human motor control system adapts to changes in the dynamics of the environment while performing point-to-point reaching movements. Furthermore, Gandolfo *et al.* (in press) have shown that the effects of adapting to a particular force field are limited to a region surrounding the states (positions and velocities) explored during the training period. The purpose of this study was to determine whether adaptation is restricted to the particular trajectories that have been exposed to the field. The first objective was to determine whether adaptive behavior is evident in movements other than reaching movements. We found that subjects were able to adapt to a viscous field while performing more complex movements. The second objective was to determine whether adaptive learning transfers to different movements across the same region of state space. This is an important issue because the inability to transfer adaptation between different movements would contradict the hypothesis that adaptation is achieved by means of an internal model of the environmental forces. To address this issue, we asked subjects to execute reaching movements in different directions within a field of viscous perturbing forces until the reaching movements were fully adapted. Immediately following adaptation, we asked the subjects to execute circular movements that passed through many of the same positions and velocities as during the adaptation phase, albeit in a different temporal order. We found that in all our subjects, adaptation was preserved during the execution of the circular movements. Our results indicate that adaptation is a property of the visited states and does not depend upon the temporal order in which these states have been explored. This provides evidence that the internal model of the environment responsible for adaptation may be implemented in a manner functionally independent of the representation of the planned movement trajectory.

Supported by ONR (N00014-95-1-0571, N00014-88-K0372), NIH (AR26710), and the Ralph and Marian C. Falk Medical Research Trust Fund.

354.2

ESTIMATION OF PRIMITIVES FOR MOTOR ADAPTATION

F. Gandolfo* and E. Bizzi.
Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

We studied the mechanism of the adaptation to perturbations during arm movements. We asked human subjects to execute planar arm movements while grasping a manipulandum which was used both to record kinematic data and to perturb the subjects. The perturbations, normal to the movement and proportional to the movement velocity, significantly altered the trajectories. We tested the subjects after the perturbing forces were discontinued and observed aftereffects as a mirror image of the perturbed trajectories. We interpreted the aftereffects as evidence for an internal model, defined as a mapping between the limb state and the torques to compensate for the external disturbances.

Adaptation to disturbances is local in the sense that the changes affect the perturbed trajectories and a small region around them. We present evidence that the size of the affected region depends on the experienced states, and can therefore be adjusted to the smoothness of the perturbation. We investigate how far the resolution of the system can be pushed and how it can be manipulated by training.

The experiments were carried out both on human subjects and in simulation. In the simulation, the compensatory torque field is estimated using smooth local kernels modulated by the adaptation process. For each simulated movement, the model uses the current map and updates it to incorporate the new data.

We show that the procedure converges onto a smooth map which models the perturbations. We successfully simulated psychophysical experiments on humans. The model produces comparable results not only with regard to the generalization properties of adaptation, but also to the time course of learning.

Acknowledgements: ONR N00014/88/K/0372 and NIH AR26710.

354.3

FORWARD INTERFERENCE IN MOTOR LEARNING. KA Thoroughman*, and R Shadmehr. Dept. of Biomedical Engineering, Johns Hopkins Univ., Baltimore, MD 21205.

We have conducted psychophysical studies in which human subjects learn to make reaching movements in novel dynamic environments (Shadmehr and Mussa-Ivaldi, *J. Neurosci.* 1994). Here we show evidence that learning one force field can interfere with the ability to learn a second field. This forward interference occurred if subjects were exposed to a second field immediately after the first, but not if there was a five-hour delay between fields. We have trained other groups of subjects with thirty minutes or two hours between the fields, and have shown that the forward interference diminshes gradually with increased delay. We have measured EMG signals from the arms of subjects learning one field, then a second field either immediately after or six hours after the first. When subjects initially move in the first field, muscles around the shoulder and elbow cocontract. With continuous practice, however, this cocontraction decreases and muscles selectively activate to appropriately resist the force created by the robot. Subjects who are presented the second field with no delay after the first retain the same pattern of muscle activation, even though this pattern is inappropriate for the new field. Subjects with the six-hour delay, however, rapidly produce an activation pattern appropriate for the new field. These results suggest that the subjects build a model of the force field and activate muscles to balance the predicted force. When subjects are presented with a second field immediately after the first, they erroneously rely on the model of the first field. With a delay between fields, subjects gain the ability to quickly build a new model for the new environment. Funded by Whitaker Foundation and ONR.

354.4

INTERNAL MODELS OF DYNAMICS IN MOTOR LEARNING AND CONTROL. J.R. Flanagan*, A.M. Wing. Queen's University, Kingston, Canada, MRC Applied Psychology Unit, Cambridge, U.K.

We examined movements of a hand-held object under inertial, viscous, and elastic load conditions. The grip force used to hold the object, the load, and the movement trajectory were measured. Under all load conditions, we observed that grip force was modulated in phase with fluctuations in load force. This finding indicates that the motor controller is able to precisely predict acceleration-, velocity-, and position-dependent loads. This prediction is presumably based on an internal model of the dynamics of the motor apparatus and external load.

Over the initial trials with the unfamiliar viscous load, the velocity profile evolved from an irregular to a smooth pattern. However, during this period (with the exception of the very first trial) grip force was still modulated in phase with the load. This indicates that the controller was able to predict the load before learning the commands required to generate smooth movements. These results suggest that learning to move an unfamiliar load involves two processes: (1) a rapid updating of the internal model of dynamics and (2) a slower adaptation of central commands to achieve a desired trajectory.

354.5

Optimization Criteria Driving Adaptation in Manipulative Hand Movements. K.E. Novak, L.E. Miller, J.F. Baker *, and J.C. Houk.
Northwestern University Physiology Dept.; 303 E. Chicago Ave.; Chicago, IL 60611

This study aimed to understand the processes driving motor adaptation in rapid manipulative hand movements. Subjects grasped a grooved knob and made brisk turning motions to various LED targets, similar to turning a radio dial. A motor attached to the knob shaft could be configured to apply a destabilizing negative viscous load. Following a period of practice with no load, the load was applied, typically resulting in target overshoots and subsequent corrective movements. After about an hour and nearly 1000 trials, subjects again learned to move accurately and rapidly in the new dynamical environment, returning their performance near previous levels. Measures of performance included the accuracy of the primary movement, the frequency and amplitude of corrective movements, task success rate, mean squared jerk, and mean displacement from a no load condition velocity template. Subjects tended to make discrete corrective movements towards the target during the decelerative phase of the movement and/or after the primary movement in order to improve upon the end point accuracy of the primary movement. Both the amplitude and frequency of corrective movements decreased during adaptation as the subjects made fewer target overshoot errors. These results are consistent with the adjustable pattern generator array model of cerebellar motor learning (Berthier et al., 1993, J. Cog. Neuroscience 5: 56-78), in which adaptation of the motor controller is driven by the occurrence of corrective movements signalling errors in primary movement endpoint accuracy. It is believed that the smoothness of movements, which some hold as the optimization criterion in adaptation, is instead a secondhand result of the increased accuracy and decreased occurrence of corrective movements resulting from motor adaptation. A more biologically plausible optimization criterion is the minimization of the occurrence of corrective movements or the effect of applied perturbations, which are proposed to trigger climbing fiber "training signals" for adaptive change in the cerebellum.

This work was supported by NIMH Center grant #5 P50 MH 48185, and Whitaker Foundation awards to K.E. Novak and L.E. Miller.

354.6

LEARNING THE DYNAMICS OF A VIRTUAL OBJECT: PRACTICE BUILDS AN INTERNAL MODEL. G. Robles-De-La-Torre and R. Sekuler*. Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254.

We developed a novel paradigm to study how human performers acquire and use internal models of the dynamics of unfamiliar, virtual objects. A performer tries to maintain alignment between a virtual object and a static target whose position in one dimension is randomly perturbed every few seconds. By applying a series of discrete force-pulses, performers try to drive the object as quickly as possible into alignment with the target. The virtual object's responses to force pulses are governed by differential equations equivalent to an n-stage network of mechanical resistors and springs. By minimizing complexity of visual and motor components, the paradigm allows us to characterize the internal model (IM) that arises out of interactions with the virtual object. We compared the behavior of human performers to the behavior of an Ideal Performer (IP), a predictive control system that minimizes performance error. Over hundreds of trials, subjects' performance approached that of the IP. Given that the IP uses complete knowledge of the virtual object's physics (its differential equations), subjects' improvement suggests that their IMs also incorporate basic features of this physics. We discuss experiments to distinguish between an abstract IM (incorporating function-like information about the underlying physics, as the IP does) and an IM based on manipulation of exemplars, without explicit knowledge of the task's physics.

This work was supported by CONACYT (Mexican Government agency) and the M.R. Bauer Foundation.

354.7

DYNAMIC COORDINATION OF BODY PARTS DURING THROWING AT VISUAL TARGETS B.E. Gregor, T.A. Martin, W.T. Thach. Dept. of Anat. and IWJ Rehab. Res. Inst., Washington Univ. School of Med., St. Louis, MO, 63110.

In throwing at a visual target while wearing laterally displacing wedge prisms, subjects adaptively widen the gaze-throw angle. Over a short term (≈50 throws), subjects widen this angle (adaptation). The new calibration is retained for a day or longer. Over a longer term (3900 throws, 2 weeks) of alternate prism/no-prism throws, subjects acquire a wide gaze-throw prism calibration in addition to the narrow no-prism calibration (learning) (Martin et al., 1996). Having acquired the 2nd calibration subjects hit the target upon each first throw, prism or no-prism.

We asked the question: does the widened gaze-throw angle consist of a fixed relation among body parts, or instead a changing and interdependent relation among body parts? In two long term prism/no-prism trained subjects we video-recorded positions of head, shoulders, and arm while throwing with and without prisms. Knowing that eyes foveated the target we computed the angular positions of eyes-in-head, head-on-trunk, trunk-on-arm and ball-on-target. In each subject, the gaze adjustment was not confined to any one set of 2 members (e.g. eyes-in-head), but instead was distributed across all 3 sets of coupled body parts. Each subject had a different distribution of coupling across the 3 sets of body parts. The distribution of the coupling changed unpredictably both within a single throwing session and across sessions. The angular variation among the 3 sets of coupled body parts was in most cases substantially larger than the angular variation of the hits on the target. The data indicate that the coupled body parts must be changed interdependantly in order to account for the high accuracy of ball-on-target.

Previously, we created a schematic model based on the knowledge that the cerebellum plays roles both in the coordinated movement of multiple body parts and in motor learning (Thach et al., 1995). The data supports this model by showing a high degree of coupling across body parts; and by demonstrating a dynamic and generalized solution as evidenced by the distribution of the gaze-throw adjustment across body parts. (Supported by ONR N00014-92-J-1827 and NIH Grant NS12777)

354.8

ADAPTATION OF THROWING TO VERTICALLY-SHIFTING PRISMS IS NOT LIMITED TO TIMING OF RELEASE, BUT INVOLVES THE MODIFICATION OF SEVERAL PARAMETERS. T.A. Martin* and W.T. Thach. Dept. of Anatomy and Neurobiology and IWJ Rehab. Res. Inst., Washington University School of Medicine, St. Louis, MO, 63110.

Hore et al. (J.Neurophysiol., **75**, 1996) have hypothesized that the normal variance in vertical error while throwing at visual targets may be due to changes in any of three throw parameters: hand translational velocity, hand location in space, and hand orientation. From an analysis of skilled throwers, they concluded that the vertical error correlated best with the variance in hand orientation which was related to the timing of ball release.

We have previously reported that adaptation of throwing to laterally-shifting wedge prisms is an automatic and gradual process resulting in a widening of the horizontal gaze-throw angle (Martin et al., 1996; Thach et al., 1995). We demonstrated that the widened gaze-throw angle (approximately 17.5° for 30 diopter prisms) is distributed among three sets of coupled body parts -- eyes-in-head, head-on-shoulder, and shoulder-on-arm.

We were interested in the types of modifications that would occur when subjects adapted throwing while wearing vertically-shifting prisms (with the prisms placed base up or down). Presumably, the gaze-throw angle would similarly widen but in the vertical direction.

Video-based motion analysis shows that timing of release is not the only variable modified during adaptation of throwing to vertically-shifting prisms. Modifications of both position and velocity of the hand play an important role in the adaptation. While Hore et al. may be correct in their analysis of normal, over-practiced throwing, from our results, we conclude that during learning, more degrees of freedom and sources of potential error are modified to achieve the gaze-throw recalibration required to hit the target. (Supported by ONR N00014-92-J-1827 and NIH Grant NS12777)

354.9

THE TIME-COURSE OF PRISM ADAPTATION IN A MONKEY GIVEN DELAYED VISUAL INFORMATION. S. KITAZAWA*, AND T. KIMURA. Neurosci. Section, Electrotechnical Lab., PRESTO, Japan.

The ability to accurately reach toward a visual target is impaired when the visual field is displaced by prisms, but returns with practice (prism adaptation). When the prisms are removed, subjects misreach in the opposite direction (after-effect), but eventually adapt again. Previously, we reported that such adaptation in humans is dependent on the availability of visual information within 50 msec after the completion of the movement (Kitazawa et al., 1995, J Neurosci, 15:7644). The present study involves a monkey trained to reach rapidly (within 300 msec) at a target on a tangent screen (200 mm away). Vision was always blocked during the reaching movement, but was unblocked for 300 msec after the monkey's index finger touched the screen. In the experimental phase (n=112), the visual field was displaced by prisms (25 diopter) in one of eight directions (0, 45, 90, etc, to 315 deg) and vision was unblocked after the finger touch without delay. The monkey misreached in the direction of visual displacement. Errors decreased with practice by an amount proportional to the error in the preceding trial, as was observed in our human studies. After the prisms were removed, vision was available only after a delay period of 0-500 msec, while the monkey maintained its final pointing position. The monkey misreached in the opposite direction initially, but again the error decreased with practice. The rate of decrease of error was generally largest when the delay was 0 msec, diminished when the delay was 50 msec ($p<0.06$), and was significantly smaller thereafter (100-500 msec, $p<0.05$). When the contralateral arm was used after removal of the prisms, no significant after-effect was observed, even in the initial trials. The results suggest that the underlying changes occur in the part of the nervous system that controls unilateral arm movement and that these changes are primarily produced by visual information that closely correlates with the motor output in a given time domain. (Supported by grants from AIST and JRDC)

354.10

A FUNCTIONAL MRI STUDY ON INTERNAL MODELS OF DYNAMIC TRANSFORMATIONS DURING LEARNING A VISUOMOTOR TASK. H. Imamizu*[1], S. Miyauchi[2], Y. Sasaki[2], R. Takino[2], B. Pütz[2], and M. Kawato[1]
[1]ATR Human Information Processing Research Labs., Kyoto, Japan; [2]Communications Research Labs., Tokyo, Japan

Some recent computational studies have stated that the central nervous system (CNS) acquires internal models of the kinematics and/or dynamics of controlled objects and environments for rapid and smooth control (e.g. Kawato & Gomi, 1992, *TINS*, 15, pp. 445-453). However, it is still unclear as to which part of the CNS is involved in acquiring these models during the control of visually guided limb movements. Using fMRI (1.5T, Siemens VISION, echo planar imaging) we examined cortical and subcortical activity in normal human subjects learning a visuomotor task under the dynamic alteration of visual feedback of the hand position. The task for the subjects was to move a computer mouse so that the cursor follows the target moving on the screen as closely as possible (a modification of the task used in Flament et al. 1994, *Soc. Neurosci. Abst.* p. 20). The position of the cursor was altered by a dynamic transformation of the mouse movement, namely integration of the previous mouse positions, in learning periods whereas no transformation was imposed in control periods. The errors, measured by taking a distance between the cursor and the target, decreased with practice in the learning period. Images (8-10 slices, 7-10 mm thick, through the cerebellum, occipital, parietal and part of the frontal lobes) scanned in both periods were compared with cross correlation. We found that the MR signal intensity in the biventer lobule of the cerebellum and parietal areas 5 and 7 was significantly higher in the learning period. The results suggest that these areas are involved in error correction by comparing visual information and kinesthetic information and in acquiring the internal models of the imposed dynamic transformation

354.11

INCREASED KINEMATIC INFORMATION IS ENCODED BY PRIMATE CEREBELLAR THALAMIC NEURONES DURING MOTOR LEARNING

E.G.Butler*, D.W.Bourke, M.C.Harvey, D.I.Finkelstein and M.K.Horne Dept of Anatomy, Monash Uni., Clayton, Victoria, Australia, 3168.

To date, there is limited evidence of involvement of the cerebello-thalamo-cortical (CTC) pathway in the learning of voluntary movement. If learning a voluntary movement is dependent on cerebellar function, the resulting signal must pass via the CTC pathway to modify a cortically-generated movement The aim of this study was to determine whether cerebellar thalamic neurones exhibit changing discharge over time with motor learning. Chronic single cell recordings were made in 2 macaques trained to perform a visually-cued reaction time task of the wrist, in which the gain between the manipulandum and the visual display was changed (to 0.5 or 2.0) to elicit a learning response. During motor learning a number of kinematic parameters were scaled, including peak velocity and acceleration, duration of movement and positional error. Time to peak velocity was usually invariant. Recordings were made from 35 wrist-related cerebellar thalamic neurones. During the recording of 20/35 neurones, the monkeys exhibited stereotyped control movements which were compared with novel movements which the animal learnt or attempted to learn. For 10/20 cells (50%) there was scaling of the amplitude of neuronal discharge (ND) over time during the learning phase of a novel gain, compared with 4/20 cells (20%) during the stereotyped control movement. Also, there was a significant correlation between ND and one or more of the kinematic parameters listed above for 10/20 cells (50%) during motor learning, compared with 1/20 cells (5%) during the control movement. These preliminary results demonstrate that during the acquisition of a motor skill there is a significantly increased number of cerebellar thalamic neurones which exhibit changes in neuronal discharge over time, typically concurrent with learning-dependent changes in movement kinematics.

Funded by Australian NH&MRC

354.12

DIFFERENCES IN GENERALIZATION OF ADAPTATION TO ALTERED GAINS AND DISPLAY ROTATIONS IN REACHING MOVEMENTS.

J.W.Krakauer, Z.M.Pine* and C.Ghez, Dept. Neurology., Dept. Rehab. Med., Ctr. Neurobiol. & Behav., Columbia Univ , New York, NY 10032

Previous work suggests that reaching movements to visual targets are planned vectorially, as an extent and a direction . We have recently shown that learning a new scaling factor for visual to hand-space representations is more rapid than learning a new directional map. The present study further analyzes differences in learning by examining generalization, with training limited to a single movement origin and target location. Subjects made out-and-back movements of their hand from a central location to a circular array of equidistant targets on a horizontal tablet. Targets and a cursor were displayed on a vertical computer monitor. In a control condition, the gain of the path display (hand:screen) was 1:1 and the upward screen direction from the origin corresponded to straight ahead for the hand. In training , hand to screen mapping was changed either by altering the gain (1.5 or 0.67) or by rotating direction 30°CCW while subjects practiced reaching to a single target location. Adaptation was rapid in both cases. However, this learning was generalized only for the gain condition but not for the rotation. Movements made without feedback after adaptation to display rotation were only accurate for the training direction and fell to zero within about 45° on either side.

The more rapid learning and generalizability of learned scaling suggests that local training allows subjects to acquire a metric rule for scaling responses to a range of locations. The substantial failure of generalization of direction suggests that the directional maps are learned locally. Generalization may require training in multiple directions and a working memory buffer. Supported by NS22715, HD01018 and T32NS07155.

354.13

PATTERNS OF REGIONAL BRAIN ACTIVATION ASSOCIATED WITH DIFFERENT ASPECTS OF MOTOR LEARNING

C.Ghez*, M.F.Ghilardi, J.R.Moeller, V.Dhawan and D.Eidelberg Ctr.Neurob.Behav, Columbia Un, NYS PI, New York; INB-CNR, Milan; North Shore Un.Hosp, Cornell Un,Manhasset New York.

To examine the variations in regional cerebral blood flow during execution and learning of reaching movements, we employed a family of kinematically and dynamically controlled motor tasks in which cognitive, mnemonic and executive features of performance can be differentiated and characterized quantitatively.

During each 90' scan using ^{15}O-H_2O PET,12 right-handed subjects moved their dominant hand on a digitizing tablet from a central location to equidistant targets displayed with a cursor on a computer screen in synchrony with a 1/s tone. Movement conditions were: M1, movements to predictable targets; M2, learning of a new visuomotor transformation where cursor motion on the screen was rotated by 30-60°; M3, discovering target sequence of 6 to 8 elements using visuospatial working memory. Control condition, S, was observing screen displays and tones. Subtraction images were analyzed with Statistical Parametric Mapping (SPM) to identify significant foci of brain activation. SPM on M1-S subtraction images showed activation of left putamen and areas 4, 7, right cerebellar hemisphere and bilateral cerebellar vermis and SMA.. For M2-M1 contrast, SPM detected activations of right areas 6, 46/9, 7 and cerebellum. Finally for M3-M1 contrast images SPM showed significant bilateral increases in areas 6, 46/9, 7 and cerebellum. These findings indicate that while motor execution is mediated by contralateral activation of motor cortex, striatum and cerebellum, by contrast visuomotor transformations and the learning of spatial sequences is mediated by the activation of additional frontal parietal and cerebellar regions. Supported by NS 22715

354.14

BRAIN NETWORKS MEDIATING IMPLICIT AND EXPLICIT ASPECTS OF MOTOR LEARNING REVEALED BY PRINCIPAL COMPONENT ANALYSIS.

JR Moeller, C. Ghez, MF Ghilardi, V. Dhawan and D. Eldelberg. Ctr.Neurob.Behav, Columbia Un, NYS PI, New York; INB-CNR, Milan; North Shore Un.Hosp, Cornell Un,Manhasset New York.

We examined the variations in regional cerebral blood flow during execution and learning of reaching movements to visual targets using ^{15}O-H_2O PET. During 90' scan, 12 subjects moved their right hand on a digitizing tablet from a central location to equidistant targets in synchrony with a 1/s tone. Success was signaled when the correct target was reached in a time window centered on the tone. A sensory control without movement(S, scrambled visual and auditory display) and three movement conditions were examined: M1, movements to predictable targets; M2, adaptation to a rotated hand-path display; M3, discovering target sequence by trial and error using visuospatial working memory. Improvement in spatial and timing errors as well as a global performance score were computed for each test. Subtraction images were analyzed by Subprofile Scaling Model (SSM) with principal component analysis to identify regional covariance patterns. Individual scores for pattern expression in SSM were correlated with changes in errors and performance scores. M1-S, improvements in spatial accuracy were predicted by pattern-related increases in *left* striatum with covariant reductions in premotor cortex. M2-M1: adaptive changes in mean direction errors were predicted by network-related increases on the *left* in areas 7, 6, 46, 24, and SMA with covariant decreases in putamen. M3-M1: Rate of sequence learning was correlated with increased activation in *right* hippocampus and reductions in area 24. Specific aspects of motor learning are expressed by distinct brain networks which are evident using principal component analysis. Supported by NS 22715.

354.15

LIMITED IMPROVEMENT OF FAST MULTIJOINT COORDINATION WITH EXTENDED PRACTICE.

S.G. Massaquoi*, K. Irfan, A. Bryant and M. Hallett, Human Motor Control Section, Medical Neurology Branch, National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA, 20892-1430.

Three normal, healthy, male subjects (ages 29, 38, 64) performed 1740 left-to right, planar, high but sub-maximal velocity hand movements in an effort to trace accurately a visibly straight (corrected for curvature illusion) template with a felt-tipped marker. Movements were performed in blocks of 60 trials, 2-4 blocks per session, 1 session every 2 days on average. Subjects' arms had free rotation at only elbow and shoulder. To prevent visual feedback in transit, the movements themselves were made in darkness, and subjects viewed tracings after 1-3 trials on average.

Studied movements were from the first block of each session and had durations of 235 ± 20 msec (peak hand velocities in the range of 3-6 m/sec). The range chosen eliminated any trend in movement time across the training. Error measures were: Peak Path Curvature (PPC) and Root Integrated Square Error (RISE). Trends in measures were assessed using linear regression versus day of testing.

Two subjects (ages 29, 64) showed a significant decline of PPC over the 9 sessions ($p<0.048$, $p<0.0096$) typically developing flatter, S-shaped paths (having two-lobed deviations) without a decline in RISE. The third subject developed a three-lobed, wave-like path with significant decline in RISE ($p<0.004$) but without decline in PPC. None of the subjects generated any movements having PPC < 0.5 radian/meter (a small but clearly visible curvature). Of the 149 movements with criterion speed, only four achieved a RISE < 0.002 meter$^{3/2}$ (corresponding to a path which deviates by less than the thickness of the marker trace) and these had PPC > 2.0.

1740 repetitions enabled improvement of path linearity in terms of either peak path curvature or separation from template but not both, and neither measure was perfected to the limits of error visibility. There may exist limitions in the neural motor control system which practically prevent complete inversion of high-speed multijoint arm dynamics and yield different approximation strategies. Alternatively, complete dynamic inversion may require extremely long or more intensive training.

354.16

THE EFFECT OF PRACTICE ON THE MANUAL INTERCEPTION OF MOVING OBJECTS.

H. Carnahan*[1], C. Hall[2], and C. Doerksen[1]. [1]Dept. of Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada, N2L 3G1. [2]Dept. of Kinesiology, Univ. of Western Ontario, London, ON.

How does practice alter our integration of environmental information into our motor map and how is timing represented in this process? Two studies were conducted that address these issues. In the first study, 6 subjects performed 5 trials picking up a small stationary object. Then they performed 80 consecutive trials picking up a moving object. Finally, they performed 5 stationary post-test trials. Results showed that when performing reaches to stationary objects subjects sped up across trials. When subjects were intercepting moving targets the speed of their reach and the speed of hand closure around the object slowed with practice. In a second study, 10 subjects performed 120 trials of reaching to grasp a static object. After a rest they performed 10 additional stationary trials , and 10 moving object trials. A second group of 13 subjects performed 120 trials of reaching to grasp a moving object. These subjects performed the same retention tests as the static trained group. Results showed that for the static trained group, reaching speed increased with practice. However, for the group trained with moving targets, there was a trend to decrease speed with practice. For the static retention test, static trained subjects moved more slowly than those trained with the moving target. For the moving target retention test, subjects training with the moving target were much slower than the static trained subjects. These findings support a specificity of training perspective. As well, they suggest that learning a dynamic visuomotor task involves developing a perceptual model of target motion and integrating this representation into a sensory- motor map. (Supported by NSERC).

354.17

PRACTICE VARIABILITY DURING MOTOR SKILL ACQUISITION

J.B. Fineman* & A.M.Gentile, Teachers College, Columbia Univ., NY 10027.

When several variations of a motor task are learned concurrently, contextual interference studies (c.f. Magill & Hall, 1990) have typically found better retention and transfer with random practice (trial-to-trial task-variations) than blocked (successive trials on one variation before switching to next). However, contradictory results (blocked better than random) have been reported (Barth et al. 1994, Pinto-Zipp & Gentile, 1995). Blocked practice purportedly stabilized movement topology which is required in early learning. Prior studies reporting a random practice benefit simply required scaling an available movement pattern; thus, subjects were beyond an initial learning phase. We examined whether initial learning is enhanced by high or low variability during practice. The task required using chopsticks to transport Small, Medium or Large buttons to a receptacle as rapidly as possible. Three practice conditions were used: Random (R)-5 trials on each button in random order. Blocked (B)-5 successive trials with each button, Consistent (C)-15 trials with M button. During acquisition (ACQ), 24 adults were trained using a crossover design involving 4 schedules: R/B; B/R; R/C; C/R. Dependent variable was movement time. Retention was tested on M button only (10-min & 7-days later); transfer tested on button sizes outside original range. During ACQ, having random practice first produced a significant benefit when switched to blocked or consistent; whereas the reverse orders did not. Retention was best when initial practice was random (R/C; R/B); poorest when initial practice was consistent (even though C/R had more trials on M button). Transfer effects were not found. Hence, during initial learning, switching practice conditions from high to low variability facilitated motor skill acquisition extending prior findings of contextual interference effects favoring random practice.

354.18

MOTOR SKILL ACQUISITION AND RETENTION IN ELDERLY WOMEN

M. Zichettella & A. M. Gentile*, Teachers College, Columbia Univ. NY 10027; University of Scranton, PA 18510.

Learning and retention (10 days) of a novel motor task were examined in 10 young (age 18-28 yrs) and 10 elderly women who were divided into two groups: Young Elderly (Ey) 65-70 yrs; & Old Elderly (Eo) 71-80 yrs. A follow-up study examined long-term retention (20 months later) for all Ey and 7 young subjects. Task involved donning a molded ankle-foot orthotic, commonly used with stroke patients. Subjects were shown the orthotic's final position on foot/leg; movements to reach this goal were not demonstrated or described. Performance on 5 consecutive days of acquisition (ACQ) and on retention tests was video taped. Two scores were derived from time-coded video: (a) movement time to successful task-completion (MT); (b) number of strategic variations (shifts in overall strategy or lower-limb support positions). Data were analyzed using multifactor regression models. Overall, elderly had significantly longer MTs than young in ACQ ($p=.03$). MT decreased linearly for young and Ey; for Eo, however, the trend was quartric ($p=.03$). By ACQ-Day 5, all groups performed comparably. In the 10-day retention test, elderly performance declined; young did not. Elderly were not simply slower; they had significantly more strategic variations during learning. After the 20-month interval, Ey had no retention loss; whereas, young subjects' performance declined significantly ($p=.04$) and was poorer than Ey. Differences in long-term retention seem due to intrinsic variability (strategic variations) during learning. Young subjects quickly derived a movement strategy during ACQ and simply repeated it with little variation which led to efficient performance but did not promote long-term retention. In contrast, the elderly's many strategic variations during ACQ increased MT but reflected active and ongoing problem-solving that seemed to enhance retention.

354.19

A MOTOR TASK WHICH REQUIRES MOTOR LEARNING IN MONKEY ARM MOVEMENTS. EIZO MIYASHITA*, TOSHIHIRO MATSUI, BUNYA KUZE, SHIGEMI MORI, Lab. of Neurobiol., Natl. Inst. Physiol. Sci., Okazaki, 444, Japan

The aim of the study was to devise a motor task which requires motor learning in monkeys. A coil was attached on the bottom surface of a freely movable handle (within a area of about 30 cm × 30 cm on the horizontal plane) which had been connected to a pole with two arms and joints. The position of the handle was detected using a digitizer and displayed as a cursor on a computer display (17 inch), surface of which was perpendicular to the surface of the digitizer. Two visual targets (target C with a radius of 1 cm in the center and target P with a radius of 1 cm in the periphery of the display) were displayed successively on the same computer display. The position of the target P in each trial was randomly chosen from eight different positions on a concentric circle with a radius of 10 cm. Two monkeys (macaques) were trained to hold the handle to keep the cursor within the target C for 3 seconds then to move the handle to move the cursor within the target P within 5 seconds. The distance between the surface of the computer display and the monkeys eyes was about 50 cm. Positions of the handle and the joints of the elbow and shoulder during the task were captured by using a high speed video camera (250 frames/s). The accomplishment of this motor task seems to require motor learning because the decrease of the movement time and of the variability of trajectories was observed with training progression. Supported by a grant from Uehara Memorial Foundation to EM.

LIMBIC SYSTEM AND HYPOTHALAMUS III

355.1

CORRELATION BETWEEN SINGLE-UNIT ACTIVITY AND THE AUDITORY EVOKED RESPONSE TO PAIRED CLICK STIMULI IN THE RAT. K.A Flach[1]*, R. Johnson[3], G.M. Rose[2,3], G.A. Gerhardt[1,2], L.E. Adler[1] and P. Bickford[2,3], Univ. of Colorado Health Sci. Ctr., Depts. of Psychiatry[1] and Pharmacology[2] and Medical Research Services VAMC[3], Denver, CO 80262.

A prominent symptom of schizophrenia is the inability to filter or gate sensory information. One quantitative measure ofof a subject's ability to filter sensory information is the ratio of vertex recorded P50 auditory-evoked potential responses to two click stimuli spaced 500 milliseconds apart, a conditioning-testing paradigm often used to demonstrate the activity of inhibitory neuronal pathways. Normal subjects have a smaller response to the second (test) click, than to the first (conditioning) click, a phenomenon that is an example of auditory sensory gating. The test response of normal controls is often less than 15% of their conditioning response, whereas the test response of schizophrenic patients is often more than 85% of their conditioning response. In order to further understand the correlation between neuronal firing and the evoked potential response, we implanted multiwire electrodes (NB Labs, Texas) into four different regions of the rat brain thought to be involved in sensory gating; the CA3 hippocampus, the entorhinal cortex, the medial septal nucleus and the striatum. Simultaneous recordings from up to eight neurons per rat in each of the four areas were made in addition to recording the local auditory-evoked responses in each of the four areas. A clear auditory response was seen in all four areas. The auditory response was consistently gated in the CA3 region of the hippocampus and the septum. In each area, sensory responsive cells were identified. Most cells were excited by the click stimulus, although some cells were inhibited. Correlations between single-unit activity and gating, and single-unit activity and the evoked responses will be presented. Supported by BNS9110308, BIR913392, MH01121, MH50787, MH44212 and the VARMS.

355.2

TRANSNEURONAL ANALYSIS OF HIPPOCAMPAL CIRCUITRY USING PSEUDORABIES VIRUS S. D. Styren*, S.T. DeKosky, M. E. O'Malley, G. C. Styren R.Y. Moore and J.P.Card Depts. of Psychiatry, Neuroscience, Neurobiology, and Neurology Western Psych. Inst. & Clinic, Univ. of Pittsburgh and Univ. of Pittsburgh Sch. of Med. Pittsburgh, PA

The hippocampal formation is responsible for integration of information from a variety of afferent projections that arise from both cortical and subcortical regions. These projections segregate to discrete, architectonically defined sub-regions within hippocampus, where information is then disseminated to other hippocampal subfields by a complex network of intrinsic connections. Delineation of these circuits is essential to our understanding of hippocampal function and how synaptic architecture is reorganized during learning or injury. We used the retrograde transneuronal tracer pseudorabies virus (PRV) to study the origin and distribution of afferent input to hippocampal subfields and intrinsic hippocampal circuitry. We injected 1 µl (~1.410×10^9 PFU/ml) of PRV into stratum lucidum, dentate gyrus and CA fields of male Sprague Dawley rats (n=24) under stereotaxic guidance. After 36 hours, the brains were serial sectioned and routine immunohistochemistry for PRV antigen was performed. Stratum lucidum injections resulted in infection of a subpopulation of ipsilateral dentate granule cells and first order infection of numerous ipsilateral, layer two, entorhinal cortex (ERC) neurons; contralateral staining was restricted to a few layer two ERC neurons. Direct injection in the dorsal leaf of dentate gyrus resulted in extensive dorsal granule cell infection throughout the ipsilateral hippocampus. Injection into CA2/3 resulted in infection of few ipsilateral layer 2 ERC neurons while numerous layer 5 neurons were intensely stained for PRV antigen. Our data suggest that use of PRV as a retrograde tracer will not only enable confirmation and clarification of previous reports of hippocampal circuitry but will provide further insight into the synaptology of the hippocampal formation and serve as a valuable tool for characterizing anatomical rearrangements and sources of axonal sprouting following injury.

355.3

CYTOARCHITECTURE AND IMMUNOCYTOCHEMISTRY OF CALCIUM-BINDING PROTEIN-POSITIVE NEURONS IN HIPPOCAMPAL FORMATION OF WHALES AND TERRESTRIAL MAMMALS [1]P.J. Morgane*, [2]I.I.Glezer , [3]P.R. Hof, [2]C.Vavasis, [2]J. Ioannou, and [2]L. David. [1]Dept. Pharmacol. , Univ. New Engl., Biddeford, Maine 04005., [2]Dept. Cell Bio. & Anat Sci. CUNY Med. Sch. NY, NY 10031, [3]Dept. of Neurobiol., Mount Sinai Sch. Med..NY, NY 10029.

Cytoarchitectonics and distribution of calcium-binding protein (CaBP)-positive neuronal populations were studied in ventral hippocampus (VH), fascia dentata (FD), subiculum (SU) and entorhinal cortex (EC) in several species of toothed and baleen whales, and in various terrestrial mammals (marsupials, monotremes, chiropterans, carnivores and primates). In cetaceans, two types of hippocampal cytoarchitectonic patterns are found: a) linear, where FD and VH are parallel to each other (found in spotted and bottlenose dolphins) and b) interlocked, analogous to that in terrestrial mammals but where FD and VH have the shape of two interlocked arches. This type is prevalent in baleen whales and, among odontocetes, in the related narwhales and beluga whales. Generally, immunocytochemical and cyto-architectonic features of the hippocampal formation of baleen whales are essentially similar to those in terrestrial mammals than in toothed whales. In cetaceans, the absolute and relative linear dimensions of FD to VH, as well the FD cell packing density, are significantly lower ($p<0.001$) than in terrestrial mammals. In the hippocampal formation and in the entorhinal cortex of terrestrial mammals all three CaBP types of neurons are present whereas in all cetacean species only calretinin- (CR+) and calbindin-positive (CB+) neurons are found, with CR+ neurons being most prevalent. Parvalbumin-positive (PV+) neurons are totally absent in cetaceans. In this regard, the ratios between different CaBP in the hippocampal formation and entorhinal cortex in cetaceans are similar to those in other parts of the brain of these species (Glezer et al., 1996). and significantly differ from the ratios in the brains of terrestrial mammals. This may reflect the largely different evolutionary histories of these taxa. [Supported by the grants HD 22539-NIH and Research Foundation of CUNY 662232].

355.4

SELECTIVE CHANGES IN HIPPOCAMPAL NEUROPEPTIDE Y NEURONS FOLLOWING REMOVAL OF THE CHOLINERGIC SEPTAL INPUTS. T.A. Milner[1]*, R.G. Wiley[2], O.S. Kurucz[1], S.R. Prince[1] and J.P. Pierce[1] Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021[1] and Lab. Exp. Neurol., VAMC, Nashville, TN 37212 [2]

The number and distribution of hilar interneurons containing neuropeptide Y (NPY), somatostatin 28 (SOM) or γ-aminobutyric acid (GABA) were examined in the dentate gyrus following removal of the cholinergic septal inputs using 192IgG-saporin. One, 2, 4, 8, 12 & 24 wks after ICV injections of 192IgG-saporin, the rats were processed simultaneously with controls for NPY, SOM or GABA-immunolabeling. At all time-points, the number of NPY-labeled neurons was significantly less in the injected rats (65 - 80% of control value). The number of SOM-containing neurons in injected rats tended to be less than controls but was only significant at 2 wks. In contrast, the number of GABA-labeled neurons in injected rats was near control values at every time-point except 24 wks where it was significantly lower (80-90%). Fields in adjacent nissl sections were examined with the optical disector method to calculate the total number of small cells (dia. ~ 5μm) within the same portion of the hilus. Cell number was less at 8 and 12 wks post-injection and reached significance at 24 wks (82%). (Although hilar volume was markedly reduced in injected rats at 8-24 wks, cell densities did not differ.) Additional dual labeling studies found that only a small portion of the NPY- and SOM-labeled neurons in the hilus colocalized GABA-immunoreactivity (18% and 5%, respectively). These studies demonstrate that: (1) removal of the cholinergic septal inputs can cause selective decreases in NPY-immunoreactivity in hippocampal interneurons; (2) at 8-24 wks post-192IgG injections, this decrease most likely reflects cell death rather than lack of expression of NPY; and (3) the small decreases in GABAergic neurons seen at 24 wks post-injection may reflect colocalization with NPY. Supported by MH42834.

355.5

MEDIAL PERFORANT PATH EVOKED TRANSMISSION IN THE DENTATE GYRUS OF AWAKE RATS IS ENHANCED DURING DENTATE SPIKES.

C.R. Bramham* Department of Physiology, University of Bergen, Årstadveien 19, N-5009, Bergen, Norway.

Dentate spikes (DSs) are positive-going, high-voltage EEG spikes which occur intermittently in the dentate hilus during alert wakefulness and slow-wave sleep, and which have been suggested to reflect synchronous activity of perforant path afferents and hilar interneurons on granule cells (Bragin et al., J. Neurophysiol. 73: 1691-1705, 1995). As part of a functional analysis of DSs we asked whether neuronal transmission in the dentate gyrus was altered during DSs in freely moving rats. The medial perforant pathway (MPP) was selectively stimulated and a vertical recording electrode array with 6 tips spaced from the dentate hilus into the CA1 field was used to monitor evoked field potentials and spontaneous EEG events. DSs were readily identified and distinguished from hippocampal sharp waves on the basis of their characteristic depth profile, amplitude, duration, and state-dependency. Using on-line detection of DSs, the timing of MPP stimulation relative to peak DSs could be controlled. MPP responses evoked at different stimulus pulse intensities during peak DSs were compared with responses elicited by manual stimulation. All evoked potentials were collected during alert wakefulness. There was a marked enhancement in granule cell excitability during DSs as reflected by larger population spike amplitudes and, in some cases, the occurrence of double population spikes. The results suggest that transmission through the MPP is transiently enhanced during the positive peak of DSs.

Funded by the Norwegian Science Council and the Meltzer Foundation.

355.6

COMPUTER MODELING OF DENTATE GYRUS USED TO DEVISE PHYSIOLOGICAL TESTS OF CONNECTIVITY

Kevin M. Hellman, William W. Lytton, Steven E. Kornguth*, Thomas P. Sutula Dept. of Neurology, Neuroscience Training Program, and Middleton Veterans Administration Hospital, University of Wisconsin, Madison, WI

In vitro study of dentate gyrus has demonstrated the detailed firing patterns of three major constituent neuron types and given an indication of which cell types are connected. Anatomical study has revealed their approximate relative numbers. However, these studies cannot adequately assess convergence and divergence among these different populations. A computer model of dentate gyrus was developed in NEURON to determine how different patterns of stimulation could be used to infer connectivity from experimentally observable single neuron responses. Granule cell (GC), mossy cell and inhibitory aspiny interneuron (AC) model current-clamp responses and synaptic currents were matched to physiology where available. The network simulation was validated by accurately simulating response to perforant path stimulation both with and without $GABA_A$ blockade. Comparison of single pulse and paired pulse stimulation of the perforant path was assessed as a probe of the relative effect of mossy fiber sprouting (MFS, equal sprouting to GC and AC) versus AC cell death in a simulated epilepsy slice model. In both the normal and MFS network, both single and paired pulse stimulation produced 1 spike in the GC. AC cell death could be detected since paired pulse now produced a 2 spike GC response, irrespective of whether or not MFS was present. MFS could be detected by using GC depolarization, since paired pulse produced 2 spikes without MFS and 1 with MFS. The computer network model could be used to explore more complex stimulation paradigms to determine physiological experiments to probe network architecture.

[Supported by the EFA, NINDS, VA, and Klingenstein Fund.]

355.7

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF MORPHOLOGICALLY-IDENTIFIED HILAR INTERNEURONS OF THE RAT DENTATE GYRUS. H.E. Scharfman* Neurology Res. Ctr., Helen Hayes Hosp., West Haverstraw, NY 10993-1195, and Depts. of Pharmacol. & Neurol., Columbia University, NY, NY 10032

The hilus of the dentate gyrus contains neurons of diverse morphologies. Many are thought to be inhibitory interneurons using GABA as a neurotransmitter. Very little is understood about the electrophysiological properties of these interneurons and how these properties vary with morphology. Therefore, sharp microelectrode recordings were made from 85 putative hilar interneurons of adult rats in hippocampal slices. Electrodes were filled with Neurobiotin to label each cell. These cells were compared to 29 interneurons sampled from the granule cell layer.

The electrophysiological characteristics of hilar interneurons demonstrated similar heterogeneity as those described for pyramidal-shaped cells located at the granule cell layer/hilus border (H.E. Scharfman, Hippocampus 5:287-305, 1995). Intrinsic properties demonstrated a wide range of resting potentials, input resistances, time constants, and action potential durations, but dv/dt ratio was more consistent (1.3-2.1). Weak adaptation, dv/dt ratio, and fast EPSP kinetics were the best predictors of cell type when comparing interneurons to mossy cells and granule cells. There was no particular morphology (soma size, dendritic orientation, axonal plexus) that correlated with any specific electrophysiological characteristic that was tested.

Synaptic responses were heterogeneous, like intrinsic properties, and again similar to those of interneurons in the granule cell layer. Responses to stimulation of area CA3 were more robust than responses to molecular layer stimulation in many cells, emphasizing that dentate hilar interneurons receive strong input from CA3 or afferents passing through CA3. The results emphasize the previously mentioned heterogeneity of interneurons in the dentate gyrus and suggest that the interneurons of the granule cell layer overlap with those in the hilus in electrophysiological and perhaps functional roles. Supported by NIH grant NS 30831.

355.8

ULTRASTRUCTURAL LOCALIZATION OF NEUROTRANSMITTER IMMUNOREACTIVITY IN MOSSY CELL AXONS AND THEIR SYNAPTIC TARGETS IN THE RAT DENTATE GYRUS. H.J. Wenzel*, P.S. Buckmaster, N.L. Anderson, M.E. Wenzel and P.A. Schwartzkroin. Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195

In the normal brain, mossy cells have been hypothesized to function as excitatory cells which use glutamate as neurotransmitter. However, putative neurotransmitters have yet to be immunocytochemically demonstrated in identified mossy cell axon terminals or their postsynaptic targets. Electrophysiologically-identified and intracellularly biocytin-labeled mossy cells of the rat dentate hilus were studied using electron microscopy and post-embedding immunogold techniques. Immunocytochemistry for the excitatory neurotransmitters glutamate and aspartate, and for the inhibitory neurotransmitter GABA were used to provide 1) more detailed information about the nature of the mossy cell neurotransmitter and 2) to identify the transmitter(s) of their postsynaptic targets. Examination of single and double-immunolabeled preparations indicated: that 1) mossy cell axon terminals made asymmetric contacts onto postsynaptic targets in the hilus and stratum moleculare of the dentate gyrus, and showed immunoreactivity primarily for glutamate, occasionally for aspartate (light labeling), but never for GABA; 2) in the hilus, glutamate-positive mossy cell axon terminals targeted primarily GABA-positive dendritic shafts of hilar interneurons, and occasionally glutamate-positive spines; and 3) in the molecular layer, the mossy cell axon formed asymmetric synapses exclusively with dendritic spines associated with glutamate-positive (presumably granule cells) dendrites. The results of this study indicate that excitatory (glutamatergic) mossy cell terminals preferentially contact GABAergic interneurons in the hilar region and glutamate-positive granule cells in the str. moleculare. This pattern of connectivity is consistent with the view that mossy cells provide excitatory feedback to granule cells in a dentate gyrus associational network, and also activate local hilar inhibitory elements. Support: NIH grant NS 18895.

355.9

CALRETININ IMMUNOREACTIVE MOSSY CELLS IN THE HILUS OF THE MOUSE DENTATE GYRUS. NOBORU FUJISE[1], NOBUAKI HORI[2], TOSHIO KOSAKA[1]*. [1]Dept. of Anat. and Neurobiol., Fac. of Med. and [2]Dept. of Dental Pharmacol., Fac. of Dent., Kyushu Univ., Fukuoka 812-82, Japan.

In the hilus of the ventral dentate gyrus (DG) of the mouse, calretinin immunoreactive(CR-IR) large multipolar cells were clustered, whereas they were rare in the dorsal DG. These large hilar CR-IR cells bore characteristic grape-like spiny appendages on the proximal portions of their dendrites. None of them were GABA/GAD-IR or somatostatin-like-IR. These observations indicated that these CR-IR cells were mossy cells. We confirmed this identification with several methods. Mossy cells identified with the intracellular injection of Lucifer Yellow were CR-IR. Electron microscopic (EM) analysis of CR-IR elements clearly revealed structural features of mossy cells such as typical synapses on CR-IR thorny excrescences from mossy fiber terminals. At the supragranular (SPG) zone, a well-known target zone of mossy cell axons, a dense CR-IR band was seen, where numerous CR-IR punctae and fibers packed. Tracers (BDA or PHA-L) injected into the inner one-third of the molecular layer or into the hilus labeled nerve fibers in this SPG zone as well as granule cells and mossy cells; some of these labeled nerve fibers and terminals at the SPG zone were CR-IR. In lesion experiments, the isolation of the dorsal hippocampus from the ventral hippocampus resulted in a drastic reduction of the CR immunoreactivity as well as degeneration of many axon terminals at the SPG zone even when the connection from the supramammillary nucleus, one possible source of CR-IR axons at the SPG zone, was preserved. However the transection of fimbria, which was assumed to disconnect the DG from the supramammillary region, did not induce any remarkable change of the SPG CR-IR band. Furthermore EM observations showed that many CR-IR axon terminals in the SPG zone made asymmetrical synapses on putative granule cell dendrites and spines. On the basis of these findings, we concluded that large CR-IR cells in the ventral hilus of the mouse were mossy cells, the principal neurons in the dentate hilus.

Support: Grants-in-Aid for Scientific Research(Japanese Ministry of Education, Science & Culture), Uehara Memorial Foundation, Mitsubishi Foundation.

355.10

A putative GABAergic cell type in the outer molecular layer of the rat fascia dentata projecting to the subiculum, [1]J. Lübke*, [1]K. Ceranik, [2]P. Jonas and [1]M. Frotscher, [1]Anatomisches Institut, Albertstraße 17 and [2]Physiologisches Institut, Hermann-Herder-Str. 7, Universität Freiburg, D-79104 Freiburg, Germany

Dentate gyrus interneurons play an important role in inhibitory processes in the hippocampus. Here we describe morphological and physiological characteristics of a novel, putative GABAergic cell type in the outer molecular layer (OML) of the rat fascia dentata with a back-projection from the dentate gyrus to the subiculum across the hippocampal fissure. Recordings were made from 70 neurons in the OML in hippocampal slices taken from postnatal (P) 10 - P30 rats using the whole-cell patch clamp configuration. Neurons were visually identified by infrared interference contrast videomicroscopy. During recording, cells were filled with biocytin for subsequent light- and electron microscopical analysis. Neurons projecting to the subiculum were found throughout the entire OML. GAD *in situ* hybridization revealed the GABAergic nature of OML neurons. They had small somata (15 µm) and a fusiform to multipolar dendritic morphology. Neurons had two distinctive axonal domains: 1) a long range (1 mm), tangential projection within the OML, with few collaterals in the inner molecular layer, and 2) a dense vertical and tangential projection (1 mm) within layer 1 and stratum pyramidale of the subiculum. Synaptic contacts were formed on dendritic shafts of granule cells of the fascia dentata and pyramidal neurons of the subiculum as revealed by electron microscopy. Neurons were physiologically classified as GABAergic cells by their maximal action potential frequency (50 - 100 Hz) and by a strong afterhyperpolarization following the action potential. The mean input resistance was 278 ± 78 MΩ (174 - 381 MΩ) and the membrane time constant was 10.7 ± 6.7 ms (6.3 -11.7).

Our data suggest that OML-neurons are involved in feed-forward inhibition of at least two types of target cells, the dentate granule cells and pyramidal neurons of the subiculum. (Supported by the Deutsche Forschungsgemeinschaft)

355.11

AREA 30 (LE) IN THE MACAQUE MONKEY: CYTOARCHITECTURE AND CONNECTIVITY.

R. Morris*, M. Petrides and D.N. Pandya. Montreal Neurological Institute, McGill University, Montreal, CANADA and Boston University School of Medicine, Boston, MA, USA.

Recently, a study examining the sources of frontal input to the hippocampal system that might bypass the entorhinal cortex was carried out by means of autoradiography in the macaque monkey (Morris et al., 1995). This study revealed the existence of a monosynaptic projection originating in the mid-dorsolateral frontal cortex that courses, as part of the cingulum bundle, and which ends in the retrosplenial cortex. Careful architectonic analysis was carried out to decipher which portion of the retrosplenial cortex receives the frontal inputs. This analysis revealed that area 30 is the recipient of the projection from the mid-dorsolateral frontal cortex. It has been proposed that the retrosplenial cortex might be a critical relay-station along the fronto-hippocampal pathway which might subserve working memory processing. Injections of tritiated amino acids or fluorescent retrograde tracers confined to area 30 were performed in order to confirm this hypothesis. The results revealed that area 30 projects posteriorly to areas TH and TF of von Bonin and Bailey as well as to the subicular complex and, rostrally, to the mid-dorsolateral frontal cortex. The injections of retrograde tracers have confirmed that the mid-dorsolateral frontal cortex is the only portion of the frontal lobe that projects to area 30. Together, these findings confirm that area 30 is closely affiliated with the fronto-hippocampal association fiber system that might subserve certain aspects of working memory.

Funded by the McDonnell-Pew Program in Cognitive Neuroscience.

355.12

HIPPOCAMPAL OUTPUT TO PERIRHINAL CORTEX IN THE RAT ORIGINATES FROM THE DISTAL PORTION OF CA1. B. W. Leonard*, C. L. Dolorfo and T. F. Freund. Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary H-1450.

The hippocampal formation originates cortical projections from the CA1 region of Ammon's horn in addition to those that originate from the subiculum. An unresolved issue about the CA1 efferent system is the topographic and laminar organization of its terminal field in the perirhinal cortex. As part of an ongoing study on the postsynaptic targets of the CA1 region, we have accumulated anterograde tracing data that clarify some aspects of this organization and its relationship to the organization of CA1 efferents to the entorhinal cortex. Iontophoretic injections of *Phaseolus vulgaris* leucoagglutinin or biotinylated dextran amine were made into distal (toward the subiculum) or more proximal (toward CA2) portions of CA1 in male rats (n=12). Injection sites and terminal fibers were visualized using standard immunocytochemical techniques. Sections were counterstained for Nissl. Terminal fibers were observed in the perirhinal and dorsolateral entorhinal cortices only if the injection site involved the most distal portion of CA1. Such injections at septal or mid septotemporal levels resulted in terminal labeling in layers V-VI of caudal areas 35 and 36 and terminal labeling in layers V, III, II and I of mid rostrocaudal and caudal levels of dorsolateral entorhinal cortex. Injections in more proximal portions of CA1 (n=4) resulted in the previously reported terminal field organization in the subiculum (Amaral, Dolorfo & Alvarez-Royo, 1991) but did not result in terminal labeling in areas 35 and 36. To date, one of our injections involved the distal portion of ventral CA1 and resulted in a dense terminal field at mid rostrocaudal and caudal levels of entorhinal cortex only. Because direct projections to CA1 originate from superficial layers of both entorhinal and perirhinal cortices, the present results suggest that hippocampal output from CA1 may have greater direct feedback influence on its cortical input originating from entorhinal cortex than on its cortical input originating from perirhinal cortex. Supported by The Howard Hughes Medical Institute

355.13

CHARACTERIZATION OF VIP-IMMUNOREACTIVE NEURONS IN THE ENTORHINAL CORTEX OF THE RAT. C.L. Dolorfo*, J. Staiger and T.F. Freund. Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary H-1450.

Recent analyses of interneuronal networks of the hippocampus demonstrate that interneurons play an important role in the modulation of rhythmic oscillations of principal neurons and in synaptic plasticity. While hippocampal interneurons have been well characterized, little is known about the organization of interneuronal networks of the entorhinal cortex (EC), a structure that is intimately connected to the hippocampus. As part of an ongoing study characterizing local circuits of the EC, the organization of vasoactive intestinal polypeptide-immunoreactive (VIP-ir) interneurons in the EC was examined. Sections through the EC from two male rats were either immunostained for VIP or double-immunostained for VIP and the neuropeptide somatostatin or one of three calcium binding proteins (parvalbumin, calbindin, calretinin). The majority of VIP-ir cells were located in deep portions of layer I and layer II; few were found in layers III-VI. Many were bipolar and bitufted cells with radially oriented dendrites that often spanned layers I-V. While all EC layers received VIP-positive input, fibers and terminals distributed mainly to layer II. VIP-ir terminals frequently encircled unstained somata in layer II. VIP-ir terminals contacting the soma and proximal dendrites of other VIP-ir cells were common. Double-immunostaining revealed that VIP-ir interneurons innervate the soma or proximal dendrites of somatostatin-, calbindin-, and calretinin-positive cells. VIP-ir terminals rarely surrounded parvalbumin-positive somata. Post-embedding immunostaining for GABA demonstrated that postsynaptic targets of VIP-ir boutons included somata and dendrites of GABA-positive and -negative cells. The laminar organization and target specificity of VIP-ir fibers and terminals allows the possibility for modulation of principal cells, as well as other interneurons that may participate in the regulation of entorhinal intrinsic circuits and EC output to the hippocampus and other structures. Supported by The Howard Hughes Medical Institute

355.14

COMPARISON OF ELECTROPHYSIOLOGY AND MORPHOLOGY OF LAYER III AND LAYER II CELLS IN THE ENTORHINAL CORTEX.

S. v.d. Linden, M. Witter*, and F.H. Lopes da Silva. Dept. Exp. Zoology, University of Amsterdam, 1098 SM Amsterdam, The Netherlands.

Cortical input to the hippocampus is mediated by the superficial layers of the Entorhinal Cortex (EC), giving rise to the perforant path. Layer II and layer III of the EC receive different inputs, and, on their turn, project to different areas of the hippocampus (Dentate Gyrus and CA1 area, resp.). Layer II EC cells have already been described. The study presented here was done to investigate the intrinsic characteristics, the morphology, and synaptic responses of layer III cells in comparison with layer II cells. Layer III consisted mainly of pyramidal-shaped cells, while in layer II predominantly stellate cells are found. Intracellular measurements, using current clamp mode in *in vitro* slices, revealed that, in comparison with layer II EC cells, layer III cells i) had a larger Ri; ii) did neither display sag responses to hyperpolarizing current pulses, nor a rebound at the end of these pulses; iii) had a more depolarised firing threshold, and action potentials with a slower rate of decay and lacking a DAP; iv) more often had a strong adapting firing pattern upon depolarizing current pulses, and displayed no subthreshold oscillations; and v) upon deep layer stimulation responded with an EPSP that could trigger a single spike, and that was followed by a pronounced hyperpolarization. These data suggest that layer III cells are less excitable than layer II cells, but tend to display brief spike discharges to stimulation of the deep layers of the EC, which stands in contrast with the IPSP displayed in layer II cells. This may have consequences for the transfer of EC inputs (layers II and III) to the different subareas of the hippocampus. Supported by the HFSPO.

355.15

BURSTING BEHAVIOR OF DEEP LAYER PRE/PARASUBICULAR NEURONS EVOKED BY STIMULATION OF SUBICULUM AND ENTORHINAL CORTEX. M. Funahashi* and M. Stewart. Dept. of Physiology, State Univ. of New York, Health Science Center at Brooklyn., Brooklyn, NY 11203.

Recent studies suggest an important role of the retrohippocampal region in the transmission of information between neocortex and hippocampus. The connectivity of retrohippocampal neurons, including pre/parasubicular cells, has been studied anatomically. Electrophysiological and morphological data from pre/parasubicular neurons are virtually absent. To examine the properties of pre/parasubicular neurons, intracellular recordings and single-cell dye injections were performed in 300 µm horizontal slices obtained from brains of 170-280g male albino rats. Electrophysiologically, 125 recorded cells, sampled from all layers were similar to one another, regardless of cell shape. Each exhibited repetitive single spiking in response to depolarizing current injection and had similar passive membrane properties. Deep layer cells of both pre- and parasubiculum exhibited a massive burst response that often included afterdischarges in response to single stimuli (100 µs, <1 mA) delivered to subiculum, or to deep layers of medial entorhinal cortex. All deep layer cells could exhibit this bursting behavior; such behavior was never observed in superficial layer cells. The majority of deep layer cells responded to both inputs. Responses to either input were indistinguishable. Burst durations varied from 100 to 400 ms. In many cells, short-latency EPSPs were observed in response to weak single stimuli. These were clearest when stimuli were applied to subiculum. To examine subthreshold synaptic potentials which may be related to the bursting behavior, long-duration hyperpolarizing current pulses were applied 50 ms after subicular stimulation. In some cells, a subthreshold EPSP was isolated, however, burst responses in many deep layer cells were unaffected by injections of as much as 1 nA. In 2 cells, burst responses could be triggered by depolarizing current injection. These massive burst responses by deep layer pre/parasubicular neurons may reflect a population event that can be initiated from: 1) excitatory subicular or entorhinal inputs, or 2) a single pre/parasubicular neuron. In addition, the data suggest little or no deep to superficial layer connectivity in pre/parasubiculum. Supported by NIH NS33144.

355.16

RESPONSES OF PROJECTION CELLS OF THE ENTORHINAL CORTEX TO REPETITIVE SYNAPTIC STIMULATION. T. Gloveli, D. Schmitz, R. M. Empson* and U. Heinemann. *Institut für Physiology der Charité, Humboldt Universität zu Berlin, Tucholskystr. 2, 10117 Berlin*

The entorhinal cortex provides a major gateway for sensory information into the hippocampal formation. The perforant path, which originates in superficial layers of the entorhinal cortex (EC) project from layer II cells to the dentate gyrus and CA3 region and from layer III to the CA1 region and the subiculum. The mechanism by which these separate input pathways of the hippocampus are selected is not known. We studied differences between synaptic properties of the projection cells in layer II and III of the medial EC by repetitive synaptic stimulation. Trains of 30 stimuli applied at various frequencies showed that in response to low frequency stimulation (1-5 Hz) the layer II cells did not fire action potentials at all. At higher frequency stimulation of 10 or more Hz, action potentials began to appear, but this only occurred during the later part of the train and in spite of the continued hyperpolarisation of the cell. In contrast high frequency stimulus train (>10 Hz) applied to the layer III cells resulted in very little firing during the train since the cell became either extremely hyperpolarized or strongly repolarised during the train thus preventing any further firing of the cell. We propose that a high frequency switch operates to prevent output of the layer III cells whilst layer II cells are capable of firing. In particular the prolonged inhibitory postsynaptic potential following the stimulus trains in layer III projection cell could be involved in the frequency dependent switching. Pharmacological analysis shows that the slow hyperpolarisation could be divided into 3 component: i. a 1 s GABA-B, ii. a 6 s atropine and iii. a 20 s naloxone sensitive component. These mechanisms may provide a control of information flow from layer II and layer III cells to hippocampus at various frequencies.

355.17

MEDIAL VERSUS LATERAL ENTORHINAL CORTEX PATHOLOGY IN ALZHEIMER'S DISEASE. J.C. Augustinack[1], A. Solodkin[1,2], and G.W. Van Hoesen[1,2]* Departments of Anatomy[1] and Neurology[2], University of Iowa, Iowa City, IA 52242.

The entorhinal cortex of the nonhuman primate and human brain can be parcellated into several cytoarchitectural subdivisions. However, beyond cytoarchitecture, data is sparse on the individual differences among subdivisions, and several lines of evidence still support a rather simple medial versus lateral distinction. Tyrosine hydroxylase and acetylcholinesterase staining support this separation as do entorhinal efferent projections to the dentate gyrus and hippocampal formation in both monkeys and humans. Entorhinal abnormalities in temporal lobe epilepsy and schizophrenia also are skewed toward medial locations. We have studied the topography of neurofibrillary tangles and Alz-50 staining in 33 Alzheimer's disease patients and have observed that in short duration of illness cases the numbers of neurofibrillary tangles are skewed laterally in layers II and IV and only occur medially as the disease progresses. Alz-50 staining mimics this pattern, but inversely so, as the prevalent formation of extraneuronal tangles laterally reduces or diminishes immunoreactivity. In control cases, SMI-32 immunolabeling is more distinct in layers II and IV laterally and sparse to non-existent medially throughout the entire rostro-caudal extent of the entorhinal cortex. The mechanisms that underlie these topographical differences are unknown, but their frequency is sufficient to suspect that medial and lateral entorhinal areas have unique functional roles. If this is the case, their functions would subtend and cut across cytoarchitectural differences in the entorhinal cortex. Supported by NS 14944 and 19632.

355.18

ENTORHINAL CORTICAL TRANSFER FUNCTION: ANALYSIS OF LATERAL OLFACTORY TRACT IMPULSE EVOKED POTENTIALS USING ROOT LOCUS METHODS IN RAT. K. F. Ahrens* and W. J. Freeman, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

The limbic system receives olfactory input via the lateral olfactory tract (LOT), primarily at synapses on the apical dendrites of layer II stellate cells and layer III pyramidal cells in the entorhinal cortex (EC). The primary input to the hippocampus, the perforant path, originates in the superficial layers of the EC, as well. Furthermore, there are local interneurons and collateral projections which are likely to mediate inhibitory and excitatory feedback to the principal neurons of the EC, respectively. In order to describe the transformation of the olfactory neural activity as it impinges upon the EC, we recorded local field potentials in anesthetized and awake Sprague Dawley rats using bipolar wire electrodes, as well as a 16 channel silicon microelectrode. The evoked field potentials, which had the characteristic form of damped sinusoids in the awake animals were elicited by a brief (0.01-0.03ms) voltage pulse that was applied to the LOT and was just above threshold.

We blocked polysynaptic activation within the entorhinal cortex with deep pentobarbital anesthesia, effectively reducing the feedback gain. Progressively greater doses caused a reduction in the damped oscillations until only a brief (<20ms) negative deflection remained. As the effects of the drug wore off, the normal oscillatory response returned. A series of recordings taken during this period were fitted with sums of exponentials and damped sinusoids. From this data a root locus plot was made, showing the change in the poles of the transfer function as the feedback gain was manipulated. Funding: MH06686

355.19

AN ELECTROPHYSIOLOGICAL ANALYSIS OF THE PROJECTION FROM THE PERIRHINAL CORTEX TO THE SUBICULUM IN THE RAT USING CURRENT SOURCE DENSITY ANALYSIS. K.J. Canning* and L.S. Leung. Depts. of Physiology and Clinical Neurological Sciences, University of Western Ontario, London Health Sciences Centre, London, Ontario, Canada.

The inputs to the dentate gyrus (DG) and subiculum (SUB) from the perirhinal cortex (PRC) were evaluated in the urethane anaesthetized rat. Employing standard stereotaxic techniques Teflon coated steel electrodes (125 µm dia.) were placed in the PRC (angled at 24° away from midline, 6.0P, 4.0L, ~7.0V to skull surface), contralateral CA1 (4.5P, 3.5L, 2.5V) and medial perforant path (MPP) (8.0P, 4.0L, 3.0V). A glass recording micropipette filled with pontamine sky blue was lowered through the right SUB/CA1 and DG (angled at 30° toward midline, 6.3P, 6.0L). Evoked responses were recorded at 50 µm intervals deep to surface following alternating stimulation of the PRC, MPP or contralateral CA1. Stimulation of the deep cerebral white matter underlying PRC evoked short latency (~4 ms) sinks at the outer molecular layer (oml) of the dorsal DG. These sinks exhibit little paired pulse facilitation (PPF) (~5%) at an interpulse interval of 50 ms. The DG oml projection did not come from the PRC since bilateral ibotenic acid lesion of the PRC and adjacent areas did not attenuate short latency sinks at the DG oml. The latter sink may arise from stimulation of perforant path fibers originating perhaps from the anterior parts of the entorhinal cortex. In normal animals (n=8) stimulation of the superficial PRC evoked sinks at the stratum lacunosum moleculare (SLM) of SUB/CA1. The SLM sink (presumed excitation) occurred at ~8-10 ms latency without DG oml activation; and showed large PPF (~50%). In conclusion, the present study suggests that the PRC provides a direct and sizable input to the distal dendrites of the SUB/CA1 without activating the DG at the same septo-temporal location. Supported by Human Frontier Science Program and NSERC.

355.20

PRESUBICULAR AXON TERMINALS SYNAPSE WITH PARVALBUMIN - IMMUNOREACTIVE INTERNEURONS IN THE MEDIAL ENTO-RHINAL CORTEX OF THE RAT. F.G. Wouterlood*, B. Martín-Clemente, T. van Haeften, and M.P. Witter. Research Institute Neurosciences Amsterdam. Department of Anatomy and Embryology. Vrije Universiteit. 7 van der Boechorststraat, 1081 BT Amsterdam, The Netherlands.

Projections from the presubiculum selectively terminate in layers I and III of the medial entorhinal cortex. The superficial layers of the entorhinal cortex harbor neurons giving rise to the perforant pathway to the hippocampus, the so-called principal neurons. In addition, various types of GABAergic neurons are present in these layers including a specific subset that contains the calcium-binding protein parvalbumin (PV). Previously, we observed that entorhinal principal cells are among the targets of presubicular input (Caballero and Witter, 1994). This means that neurons in the presubiculum might be able to influence activity carried by way of the perforant pathway. Since the activity of entorhinal principal neurons is under strong inhibitory control by interneurons, such as PV-positive basket neurons, one interesting question is whether these entorhinal interneurons receive synaptic input from presubicular axon terminals. In the present study we set out to answer this question by injecting an neuroanatomical tracer, biotinylated dextran amine (BDA) into the presubiculum and by identifying presumed entorhinal interneurons via immunocytochemistry using antibodies against PV. In the light microscope we found a dense BDA-labeled fiber plexus in layers I and III of the ipsi-and contralateral medial entorhinal cortices, with numerous close appositions of BDA-labeled varicosities with PV-immunoreactive cell bodies and dendrites. In the electron microscope, we observed asymmetrical synapses between BDA-labeled axon terminals and PV-immunopositive dendrites. Thus, presumed inhibitory, PV-immunopositive neurons in the superficial layers of the entorhinal cortex may be influenced directly by inputs from the presubiculum.

356.1

THE CAT'S ORBITO-INSULAR CORTEX IS A HIGH-LEVEL ASSOCIATION AREA WITH WIDESPREAD SENSORY, MOTOR AND LIMBIC TRANSCORTICAL INPUTS. Alfonso Llamas*, Francisco Clascá and Fernando Reinoso-Suárez. Dept. of Morphology, Autónoma University, School of Medicine, Madrid, SPAIN 28029.

We have investigated the cortical connections of the areas recently identified in the orbito-insular region (OIR) of the cat's cerebral cortex (Clascá et al., Submitted). We used single cortical microinjections of Wheat-Germ Agglutinin-Horseradish Peroxidase in a large (n=37) series of cases. Qualitative and quantitative analysis of the retrograde cortical labeling revealed that a remarkably broad, functionally heterogeneous array of cortical inputs may interact in OIR. Dorsally located OIR areas receive widespread cortical connections. In the case of the Granular Insular area (GI) these inputs arise in somatosensory (SIV, SII, 3a, 2, 3b and SV), motor (6 and 4), lateral prefrontal and perirhinal (area 35) cortices, whereas in the case of the Anterior Sylvian area (AS) they arise in extrastriate visual, nontonotopic auditory, lateral prefrontal, cingulate and perirhinal (area 36) cortices. More ventral parts of OIR such as the Dysgranular Insular area, or the ventral part of AS receive connections mainly from adjacent GI or dorsal AS and the perirhinal cortex, as well as from few other adjacent areas. The cortex hidden in the anterior rhinal and pseudosylvian sulci (Agranular Insular and Parainsular areas) is densely connected with neighboring OIR areas as well as with the ventral prefrontal, infralimbic, and prepyriform cortices. This convergent pattern of transcortical inputs suggest that in the brain of carnivores, the orbito-insular region has evolved into a high-order, multimodal association cortex.

Support: FIS (Spain) 0197/93.

356.2

THALAMIC PULVINAR CONNECTIONS WITH THE PARIETAL CORTEX IN THE ADULT MACAQUE. Corinna Darian-Smith* and Ian Darian-Smith. Dept. of Anat. and Cell Biol., Univ. of Melbourne, Victoria, Australia 3052

We are currently investigating aspects of the neural circuitry of the thalamic pulvinar complex and associated parietal cortical areas, in the adult macaque monkey. A combination of retrogradely transported fluorescent dyes (Fast Blue and Diamidino Yellow) and anterograde markers (Biotin Dextran amine and Lucifer Yellow dextran) were injected into either the thalamus or cortex. Following dye injections into pulvinar nuclei, we were able to visualise local circuit (interneurons), thalamic reticular and corticothalamic neurons from the cortex. Similarly, thalamic relay cells and corticothalamic axons could be seen following the placement of injections into cortical locations. The dendritic morphology of neurons was visualised by injecting Lucifer Yellow into backlabeled neurons in thick fixed slices. These were immunoreacted and examined using a 3-dimensional tracing system. Following the combined injection of an anterograde and retrograde dye into the medial and lateral subdivisions of the pulvinar, two corticothalamic neuron populations could be visualised within cortical layers 5 and 6 within posterior parietal cortical areas and cingulate cortex (area 23). Corticothalamic neurons within layer 6 were invariably smaller and more numerous than those observed in upper layer 5, but these two populations were not always present together in each cortical subdivision. Two corticothalamic neuron types were also observed within the pulvinar following injections into the posterior parietal areas 7a, and 7b. These included fine calibre axons with side branches and diffuse terminal fields, and a second coarse, unbranched type with focal terminal field. Similar corticothalamic populations have been reported within the visual pulvinar (Rockland, 1994 NeuroReport 5:1865-1868, 1996 *in press*), the somatic motor nucleus, VPLo (Darian-Smith, unpublished) and the ventrobasal complex (Tan and Darian-Smith, unpublished), indicating their ubiquity within the sensorimotor thalamus. Our data, combined with recent EM studies by other groups have been used to model the circuitry of the pulvinar. (supported by NH&MRC Project Grants 950591 and 960246).

356.3

THE CONVERGENCE OF AXON TERMINALS FROM THE MEDIODORSAL THALAMIC NUCLEUS AND VENTRAL TEGMENTAL AREA ON PYRAMIDAL CELLS IN LAYER V OF THE RAT PRELIMBIC CORTEX. M. Kuroda*, K. Murakami, H. Igarashi, A. Okada. Dept. of Anatomy, Toho Univ. Sch. of Med., Tokyo 143, Japan.

We investigated the ultrastructural basis of the synaptic convergence of the afferent fibers from the mediodorsal thalamic nucleus (MD) and the ventral tegmental area (VTA) on prefrontal cortical neurons of the rat by examining the synaptic relationships between thalamocortical or tegmentocortical terminals labeled with anterograde markers, which were of lesion-induced degeneration or transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP), and unlabeled apical dendrites of layer V pyramidal cells in the prelimbic cortex which were randomly selected. WGA-HRP labeled terminals from VTA were small to large in size (0.7-2.8 μm in diameter) and established both asymmetrical and symmetrical synaptic contacts with relatively large dendritic profiles, possibly proximal segments of apical dendritic shafts and spines from layer V pyramidal cells. Symmetrical synapses, i.e., inhibitory synapses were more often seen than asymmetrical ones. Degenerating terminals from MD formed exclusively asymmetrical synapses on dendritic spines or occasionally on small dendritic shafts of apical dendrites from layer V pyramidal cells, which received tegmentocortical synapses, mostly within layer III. Accordingly, thalamocortical synapses were more distally distributed over common apical dendrites than tegmentocortical synapses although some of them overlapped. Regarding the numerical density of direct synaptic inputs from MD and VTA, identified synapses were low in number.

These results suggest that fibers from VTA exert their inhibitory effects directly on pyramidal cells in layer V via synaptic junctions with apical dendrites of these pyramidal cells, and that the tegmentocortical fibers are in an ideal anatomical position to modulate the reverberatory circuits between MD and the prelimbic cortex.

356.4

THE LIMBIC THALAMIC PROJECTIONS DISTINGUISH THE SUBICULAR CORTICES.

Th. van Groen*, I. Kadish and J.M. Wyss. Dept. of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA.

The limbic thalamic nuclei are an important link in the transfer of information between the hippocampal formation and many areas of neocortex. Previous studies demonstrated that the anterior thalamic nuclei have distinct projections to each of the subicular cortices. The present study tested the hypothesis that the projections from the subicular cortices would show a similar specificity. Sprague Dawley rats were injected with anterogradely transported tracers in the subicular cortices. Two weeks later the animals were sacrificed, and the brains were sectioned and analyzed. The postsubiculum projects predominantly to the laterodorsal nucleus of the thalamus, but a few labeled terminals are present in the anteroventral nucleus. The dorsal subiculum projects mainly to the dorsal part of the anteroventral nucleus, but the ventral subiculum projects predominantly to the parataenial and paraventricular nuclei of the thalamus, but a few terminals are present in the dorsal part of the laterodorsal nucleus. The presubiculum projects to the rostral tip of the anteroventral and anterodorsal nuclei, and to the rostral part of the laterodorsal nucleus. The parasubiculum projects to the rostral part of the anteroventral nucleus. Furthermore, all four areas of subicular cortex have collateral projections to the reticular nucleus of the thalamus. These data demonstrate that the projections from the subicular cortices to the limbic thalamic nuclei are distinctive, and that they are predominantly reciprocating the thalamic projections.

NIH: R01 AG 11958

356.5

COGNITIVE EFFECTS OF LEFT MEDIAL THALAMIC STIMULATION IN TWO PATIENTS WITH DEEP BRAIN ELECTRODES FOR RELIEF OF CHRONIC PAIN. P.C. Rinaldi*, E.S. Parker, J. Chodakiewitz, and R.L. Young, Dept. of Neurosurgery, University of California, Irvine, CA, 92717.

Two patients with stimulating electrodes in left periventricul gray (PVG) and left ventroposteriorlateral region of the thalamus (VPL) for deep brain stimulation (DBS) in relief of chronic pain were evaluated for possible cognitive effects of stimulation. A brief repeatable neuropsychological battery (oral fluency, verbal episodic memory, auditory digit span) sensitive to left fronto-limbic regions was administered with electrical stimulation (double blind) at varying levels. Patient 1, a 49 year old, right-handed woman was tested in one session under five conditions one year after DBS surgery. Highest verbal memory scores were in the two conditions when both stimulators were on and when the patient was obtaining maximal pain relief: she recalled 23 words with both PVG and VPL on, 17 with both off, 17 with both continued off, 20 words with PVG on and VPL off, and 24 words with both PVG and VPL on. No significant variation was observed on either oral fluency or digit span. Patient 2, a 43 year old right-handed woman was tested, only a few days post surgery, under four conditions. Oral fluency paralleled the stimulators with number of words generated per minute being 7 with both off, 17 with PVG on and VPL off, 11 with both off, and 15 with both on. Highest generation of words was observed in the two conditions when stimulators were on with the highest level associated with PVG stimulation. This patient became loquacious when PVG was turned on, corresponding to her fluency score. No systematic relationship was seen between electrode condition and either verbal episodic memory or auditory digit span. These findings indicate that cognitive impairment is not a consequence of analgesic levels of stimulation in PVG or VPL. Moreover, the enhanced performance on verbal episodic memory and oral fluency point to a role of medial thalamic structures and their interconnections to orbital-frontal regions.

356.6

SYNAPTIC CONNECTIONS OF CALRETININ-IMMUNOREACTIVE NEURONS IN THE HUMAN TEMPORAL NEOCORTEX. M.R. Del Río and J. DeFelipe*. Instituto Cajal (CSIC), 28002 Madrid. Spain.

Previous immunocytochemical studies in the human neocortex have shown that the calcium-binding protein calretinin (CalR) labels specific subpopulations of GABAergic and non-GABAergic aspiny interneurons (del Río and DeFelipe, J. Comp. Neurol., in press). The aim of the present study was to further characterize morphologically and chemically the microcircuitry of CalR-immunoreactive neurons in the human temporal neocortex. Combination of immunocytochemistry for CalR and GABA, CalR and the calcium-binding proteins parvalbumin (PV) and calbindin (CalB), or for CalR and non-phosphorylated neurofilament protein (identified by antibody SMI 32), showed CalR multi-terminal endings frequently innervating the distal apical dendrite or the cell body and proximal dendrites of SMI 32- or CalB-immunoreactive pyramidal cells, respectively. The cell body of interneurons immunoreactive for CalB or PV were only occasionally innervated by CalR multi-terminal endings, whereas certain GABA neurons were commonly surrounded by these endings. Furthermore, CalR-immunoreactive axon terminals form either symmetric (the majority) or asymmetric (the minority) synapses with a variety of postsynaptic elements. These results indicate that different subpopulations of CalR interneurons exist which are specialized for the selective innervation of the somatic or dendritic regions of certain pyramidal and non-pyramidal neurons.

Supported by FIS grant 94 / 0327.

356.7

DIVERGENT "FEEDBACK-TYPE" CONNECTIONS FROM AREA TF IN THE MACAQUE MONKEY. B. J. Wellman* and K. S. Rockland. Div. of Neurosurgery and Dept. of Neurology, Univ. of Iowa, Iowa City, IA 52242

Feedback cortical connections have been extensively characterized within the visual, auditory, and somatosensory pathways. Feedback terminations are, by definition, scant in layer 4, dense in layer 1, and they typically diverge over large distances (1.0 - 2.0 mm). There are also layer 1, "feedback-type" connections from higher order cortical areas, but these have been less investigated, despite their potentially important role in associational processes. For example, are these systems organized as "point-to-point" or are they spatially divergent, as has been found within early sensory areas?

We injected PHA-L into area TF of the parahippocampal gyrus in two monkeys. Layer 1 projections occurred in several posterior visual areas (Rockland and Drash, in press), as well as in several polymodal association cortices. Seven axons have been analyzed so far in serial reconstruction: three to medially adjacent area TH, two to laterally adjacent area TE, and three to insular cortex. Terminal fields in layer 1 of area TH extended over 0.75 - 1.5 mm. Those to the insula had an elongated shape, 3.5 - 4.0 mm in length, as did those to area TE (along the lower bank of the anterior middle temporal sulcus). Previous reports using other tracers indicate that these connections are reciprocal, but the present findings suggest that feedback connections from area TF to area TE and to the insula are more divergent that the feedforward. They are also more divergent than feedback connections from area V2 to V1. They are, however, comparably divergent with feedback connections from area TF to posterior visual areas, including V2, V3, and V4. This highly divergent configuration, in specific visual as well as polymodal areas, implies contacts with large numbers of spatially distributed neurons and is consistent with a non-topographic, integrative organization. (Supported by NS 19632).

356.8

POSTNATAL DEVELOPMENT OF SEROTONIN TRANSPORTER-IMMUNOREACTIVE AXONS IN MONKEY PREFRONTAL CORTEX. C.H. Kye*, T.U. Woo, and D.A. Lewis. Depts. of Psychiatry and Neurosci., Univ. of Pittsburgh, Pittsburgh, PA, 15213.

Developmental abnormalities in the serotonin innervation of the prefrontal cortex (PFC) have been implicated in the pathophysiology of both schizophrenia and depression. However, little is known about the normal postnatal development of serotonin systems in the primate PFC. In this study, we examined the the density and laminar distribution of serotonin transporter-immunoreactive (SERT-IR) axons in the PFC of 23 rhesus monkeys *(Macaca mulatta)* ranging in age from newborn to adult. Emphasis was placed on comparisons across medial PFC areas 14r, 32, and 9 because of their differences in cytoarchitecture and afferent input. The density of SERT-IR axons in these regions progressively increased from birth through the first 6 months of postnatal life, with minimal changes in older animals. At birth, SERT-IR axons in layer III appeared clustered in patches, but their distribution became more uniform with increasing age. At all ages, the density of SERT-IR axons increased from ventral (area 14r) to dorsal (area 9) regions. This ventral-dorsal gradient was most marked in layers I and II. In contrast to the protracted postnatal maturation of the dopamine innervation of primate PFC, the adult pattern of serotonin innervation is achieved much earlier, suggesting that these afferent systems may play substantially different roles in the maturation of PFC functions. In addition, the ventral-dorsal gradient in the density of SERT-IR axons in the medial PFC may be consistent with a preferential serotonin innervation of limbic-related regions. Funded by grant MH 45156.

356.9

SYNAPTIC TARGETS OF INTRINSIC AND ASSOCIATIONAL CORTICAL PROJECTIONS OF PYRAMIDAL NEURONS IN MONKEY PREFRONTAL CORTEX. D.S. Melchitzky*, S.R. Sesack, and D.A. Lewis. Depts. of Psychiatry and Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213.

In primate prefrontal cortex (PFC), the intrinsic axon collaterals of supragranular pyramidal neurons spread horizontally for considerable distances and give rise to discrete, stripe-like clusters of axon terminals in layers I-III. The principal axons of at least some of these neurons furnish associational projections within the ipsilateral PFC that arborize to form stripe-like clusters of axon terminals spanning all six cortical layers. In order to compare the synaptic targets of the intrinsic and associational projections from layer III pyramidal neurons, injections of 10% biotinylated dextran amine were placed in area 9 of macaque PFC. Intrinsic axon terminals were sampled from the same area and associational axon terminals were sampled from ipsilateral area 46. Both the anterogradely-labeled intrinsic and associational axon terminals formed exclusively asymmetric synapses. In addition, for both types of terminals, 95% of the postsynaptic structures were dendritic spines. The remaining targets were dendritic shafts, which had the characteristic features of local circuit neurons. Conversely, in a random sample of the adjacent neuropil, only 80% of asymmetric synapses were onto spines, indicating that intrinsic and associational projections of pyramidal neurons exhibit some degree of target specificity. Thus, both intrinsic and associational projections of PFC pyramidal neurons target predominantly dendritic spines of other pyramidal neurons, suggesting that their primary function is the coordinated activation of spatially-segregated populations of these neurons. Supported by MH51234.

356.10

THE INTRINSIC CONNECTIVITY OF MONKEY PREFRONTAL CORTEX: AN IN VITRO ELECTROPHYSIOLOGICAL STUDY. G.R. Gonzalez-Burgos*, G. M. DiBlasi, D.A. Henze, D.A. Lewis, and G. Barrionuevo. *Departments of Neuroscience, Psychiatry, and Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, PA 15260.*

In the primate prefrontal cortex (PFC), the axon collaterals of layer III pyramidal neurons form a stripe-like pattern of intrinsic connectivity (Levitt et al. 1993). To evaluate the electrophysiological characteristics of this pattern of excitatory connections, we have developed an *in vitro* slice preparation of monkey PFC. Coronal slices (500 μm thick, 6-8 mm long) were obtained from a tissue block removed from PFC area 9 of anesthetized cynomolgus monkeys. Action potentials (APs) were recorded extracellularly in layer III using glass micropipettes (1 M NaCl). APs were elicited by electrical stimulation of layer III, with electrodes placed at varying distances (200-1,400 μm) from the recording site. The spatial pattern of activation was assessed by determining the probability of eliciting APs in layer III as a function of the horizontal distance from the recording site. When APs were recorded from slices with intact $GABA_A$ergic inhibition, the probability of AP firing in some slices decayed monotonically with distance, reaching zero at 600-800 μm. In contrast, in other slices the probability of AP firing was bimodal. Peak values were seen at distances of 400-500 μm, and at 800-900μm, a spatial pattern predicted by the arrangement of intrinsic connections observed in anatomical studies (Levitt et al. 1993). When 0.25 μM bicuculline was added to the perfusion medium, the horizontal spread of activity increased up to 1,300 μm. In addition, APs fired in bursts of 3-8 spikes, whose latencies were longer than in control medium and increased as a function of distance. These findings indicate that the inhibitory circuitry of the PFC is preserved in the slice, and that in the presence of bicuculline, propagation of activity may occur via polysynaptic pathways. Supported by MH51234 and MH45156.

356.11

SEROTONIN INNERVATION OF NEUROCHEMICALLY IDENTIFIED INTERNEURON SUBTYPES IN THE MONKEY PREFRONTAL CORTEX R.L. Jakab* and P.S. Goldman-Rakic. Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Imbalance of the serotonin system and reduction of GABAergic interneurons in the prefrontal cortex have recently come to the forefront in the pathophysiology of schizophrenia. Evidence from this laboratory has recently shown that inhibitory interneurons are heavily innervated by serotonin axons (Smiley and Goldman-Rakic, 1996; J.Comp.Neurol.); a finding that not only provides a possible link between these two putative deficits in schizophrenia but may aid elucidating the role of serotonin in cognitive functions. These considerations prompted us to further explore the serotonin innervation of the various subtypes of (local circuit) interneurons in dorsolateral prefrontal cortical tissues of adult macaque monkeys (*Macaca mulatta*). In double label immunocytochemical experiments, antisera against serotonin and the interneuron markers calbindin, parvalbumin, calretinin, substance P receptor (SPR) and serotonin receptor (5HT2R) were used. These markers allowed us to determine interneuron subtypes not only by their neurochemical identity but also by the morphology of their dendritic arbor.

Serotonin innervation of the interneuron subtypes varied in density and layer-specificity. The somata and proximal dendrites of calretinin-stained "double bouquet" neurons in cortical layers II-III were regularly contacted by serotonergic axon varicosities and terminals while virtually all of these neurons in deeper layers were vithout such input. SPR-stained "basket" cells received a less dense innervation and these neurons were usually innervated at more distal portions of their dendritic arbors. Parvalbumin- and calbindin-containing interneurons were preferentially contacted by serotonin fibers on their perikarya. 5HT2R-containing neurons, particularly those in the white matter were seen to receive multiple contacts with serotonin fibers. Since the output of the various local circuit neuron subtypes is highly specific, these data may provide important clues in estimating the mode and range of serotonin influence in cortical circuits.

Supported by MH44866, MH38546 and The EJLB Foundation.

356.12

3-D PATTERN OF CATECHOLAMINERGIC INNERVATION ON INTRACELLULARLY LABELED LAYER III PYRAMIDAL CELLS IN THE PREFRONTAL CORTEX OF THE RHESUS MONKEY. L. S. Krimer and P.S. Goldman-Rakic*. Section of Neurobiology, Yale Univ. School of Medicine, New Haven, CT 06510.

There is now considerable anatomical and functional evidence to indicate that dopamine is an important modulator of excitatory transmission in pyramidal neurons. Dopamine axons have been shown to terminate on pyramidal cells in the prefrontal cortex of rhesus monkeys but knowledge of the density and distribution of these axons on particular neurons is not available, nor is it known whether neurons within various regions and/or different layers of the cortex are differentially innervated by dopamine axons. In order to begin to address these questions and to analyze the catecholaminergic innervation of the cerebral cortex in further detail, we have to date analyzed approximately 30 pyramidal cells that we labeled with Lucifer Yellow in paraformaldehyde fixed slices of monkey medial and dorsolateral prefrontal cortex. Each labeled cell was serially sectioned and then stained according to a double-label immunohistochemistry protocol employing anti-tyrosine hydroxylase (TH) and anti-Lucifer Yellow antisera. Dendritic tree reconstruction and synaptic contact assessment was carried out using a Neurolucida software program and Axioskop Zeiss microscope with DIC and high optical resolution (Planapo 63x,1.4 NA objective; 1.4 NA oiled condenser and 2x optovar; 1260x final magnification). With this methodology, we were able to visualize spines and TH labeled boutons and observe their direct apposition. Preliminary results indicate that 1) TH labeled axons preferentially target the distal dendrites and spines of layer III neurons while avoiding soma, first order basal dendrites and the entire main shaft of apical dendrite; 2) putative synaptic contacts were relatively evenly distributed along secondary and tertiary dendrites so that most of the dendrites bore 1-2 (and sometimes 3 or more) aminergic synapses; and 3) a given layer III neuron receives approximately 50-60 contacts . Further analysis now underway is comparing the innervation pattern of layer III neurons with neurons in other layers and the serotonergic versus TH innervation of single pyramidal cells. *Supported by MH 44866.*

356.13

THE ULTRASTRUCTURAL BASIS OF LOCAL CIRCUITS IN PREFRONTAL CORTEX AS IDENTIFIED BY COMBINING *IN VITRO* INTRACELLULAR RECORDING, BIOCYTIN FILLING AND NO SYNTHASE IMMUNOCYTOCHEMISTRY. M. Lubin*, C.S. Leonard and C. Aoki. Center For Neural Science, New York University, NY, NY 10003.

We show the successful light-(LM) and electron-microscopic (EM) labeling of neuronal nitric oxide synthase-immunoreactive (nNOS-ir) cells in sections containing single neurons first physiologically identified in coronal slices of guinea pig medial prefrontal cortex and then intracellularly filled with biocytin. Dual labeling was achieved using the silver-intensified gold (SIG) and peroxidase methods interchangeably. Examples include a layer V neuron that showed typical firing properties of a regular-spiking pyramidal cell which were modified by bath application of carbachol. LM verified the cell as pyramidal by its somatic shape and prominent basal and apical spiny dendrites that bifurcated in layer III. EM verified the cell's spines and also revealed a non-convoluted nuclear envelope. Recordings from a layer III-V neuron showed regular-spike firing but also showed a prominent after-depolarization and an initial spike doublet. LM verified that this neuron was non-pyramidal with a vertical, sparsely spiny dendritic tree. EM confirmed the presence of spines receiving unlabeled excitatory terminal contact or apposing nNOS-ir and unlabeled terminals. Another layer III neuron was confirmed as non-pyramidal without physiological recordings by its lack of spines (as seen by LM and EM) and the presence of gold-labelled terminals making inhibitory synaptic contacts. EM analysis of ~300 um of both non-pyramidal cells' dendritic perimeters revealed that <10% of the apposing terminals were synaptic. nNOS-ir soma and processes, including dendritic spines, were seen coursing near to the filled cell's processes. These observations suggest that nitric oxide's modulation of non-pyramidal neurons may occur by the paracrine mode rather than point-to-point synaptic transmission. Supported by NIMH Training Grant MH19524, NIH NS27881 & RCD 92-53750.

356.14

MODELS OF CORTICALLY INDUCED CORRELATION OBSERVED IN LGN NEURONS K. Kirkland and G.L. Gerstein*, Dept. of Neuroscience, Univ. of Pennsylvania, Philadelphia PA 19104

Two cat lateral geniculate nucleus (LGN) cells, separated by 1-4 visual degrees, and co-stimulated by a moving bar or drifting grating, show significant excess correlation of their spike trains after correction for firing rates; no such excess correlation occurs when the visual cortex is removed (Sillito et al., 1994 Nature 369:479-82). Much of the correlation occurs during short, high frequency bursts, suggesting that the low-threshold (LT) calcium channel might be involved. We have explored this and other possible mechanisms, as well as aspects of the necessary connectivity, using the simulator GENESIS. LGN cells were modeled using 2 compartments, with the soma often containing LT channels. Input to the LGN was from both retinal ganglion cells and a large area of cortex. The cortex received input from the LGN layer, had inhibitory and excitatory lateral connections, and a feedback projection to the LGN. Input to the retinal network mimicked a moving bar. Replication of the experimental results depended most strongly on the details of the corticogeniculate projection and on the presence or absence of the LT channel. The most successful matches to experiment, including the temporal details of the correlation, use the LT channel and have a center-surround corticogeniculate projection, with a direct excitatory center and a ring of indirect inhibitory connections. In this model the inhibition from the cortex leads the bar motion, thus deinactivating the LT channel and resulting in an LGN burst response to the stimulus. Other choices in model parameters either fail to produce LGN correlation or produce correlation with a time structure differing from the experimental data. In the successful model, groups of synchronized cortical cells form, disrupting the continuity of the cortical map; these groups are initially weakly formed by corticocortical activity, but are greatly strengthened, becoming distinct clusters, as the cortex influences the temporal pattern of its LGN inputs via the corticogeniculate path. Supported by NIH MH46428 and DC01249.

356.15

CALCIUM-DEPENDENT DENDRITIC RHYTHMOGENESIS IN MAMMALIAN THALAMIC NEURONS. C. Pedroarena and R. Llinás*, Dept. Physiology & Neuroscience, New York University Medical Center, 550 First Avenue, New York, NY 10016.

The synchronization of fast rhythmic activities in thalamo- cortical loops has been proposed as serving to bind sensory inputs into single cognitive events. This temporal coherence appears to relate to the intrinsic oscillatory properties of the thalamo-cortical system neurons. To characterize such oscillatory properties at the single thalamic cell level, intracellular recordings were obtained from guinea pig slices maintained at 35°C. Direct somatic depolarization of thalamic projecting neurons can generate fast membrane potential oscillation (20-50 Hz). The ionic bases for these oscillations were studied using ionic channels blockers and changing the ionic composition of the extracellular milieu. The addition of TTX (1µg/ml) to the bathing solution, or replacement of sodium by choline blocked fast spike and plateau potentials without affecting calcium dependent low threshold spikes. Surprisingly, the high frequency oscillations were not blocked by these two procedures indicating that sodium was not the charge carrier for rapid thalamic oscillations. Cadmium at 200 µM blocked the surviving high frequency oscillation, but not the low threshold spike. The amplitude of the oscillations were best observed by replacing calcium by barium. The data indicate that rhythmogenic properties of thalamic cells are in fact subserved by calcium conductances. Since HVA calcium conductances are mostly dendritic, these findings suggest that high frequency oscillation in thalamic cells originate at the dendrites. This is supported by the finding that bath applied TEA decreases the threshold for high frequency oscillations, probably by increasing the dendritic length constant. Dendritic oscillations could be a highly efficient mechanism for rhythmicity entrainment, resonance and synchronization of synaptic inputs, especially those in remote dendrites, as is the case for the cortico-thalamic input. These distal dendritic branches may in fact serve as frequency amplifiers to the thalamo-cortico thalamic resonance loop. Support: Pew Fellowship(P009SC) and NIH-NS13742.

356.16

CURRENT SOURCE DENSITY ANALYSIS OF NEOCORTICAL SPIKE-AND-WAVE PATTERNS: COMPARISON WITH SPECIFIC AND NON-SPECIFIC THALAMIC NUCLEI-EVOKED POTENTIALS A Kandel* and G Buzsáki. CMBN, Rutgers University, Newark, NJ 07102

Thalamocortical neuronal oscillations underlie various field potential patterns that are expressed in the neocortex, including sleep spindles and high voltage spike-and-wave spindles (HVS). Movable 16-site silicone probes (100 mm site intervals) and 20 mm wire electrodes were implanted medial to the barrel cortex. Neocortical field potentials and extracellular unit recordings were obtained simultaneously during HVS, and thalamically derived evoked potentials. Current source density (CSD) analysis of the spike component of HVS indicated sinks in layer IV. Ipsilateral VPL evoked potentials (EP) elicited sinks in the same position as the spike component of HVS. Another current sink was present in layer I/II. EP elicited from non-specific thalamic nuclei had similar distribution of sinks and sources. Neuronal activity in all layers was associated with the spike component of HVS. Occasionally population spikes were observed during the spike component as well as on the evoked field. THe spike component was often associated with fast field oscillations (100-200 Hz "ripples"), which in turn, correlated with rhythmic neuronal discharge. The findings indicate (1) the cooperative involvement of both specific and non-specific thalamic inputs in the generation of HVS and that (2) the major sinks, associated with these patterns, are due the activation of thalamocortical synapses with lesser involvement of intracortical connections.
(Supported by NIH, NSF, and MFSP)

356.17

THALAMIC MODULATION OF HIGH FREQUENCY (GAMMA-BAND) OSCILLATING POTENTIALS IN RAT AUDITORY CORTEX D.S. Barth* and K.D. MacDonald Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Spontaneous and evoked electrocortical oscillations in the gamma frequency band (~40 Hz) have received recent attention because of their possible association with sensory information processing. Yet, the neurogenesis of these oscillations is poorly understood. We have recently reported that both spontaneous and evoked gamma oscillations are constrained to primary and secondary auditory cortex in the rat, cortical regions that also receive dense afferent projections from the dorsal and ventral divisions of the medial geniculate nucleus (MGd and MGv). The object of the present study was to explore what role, if any, the thalamus may play in their generation.

Spontaneous and evoked potentials were recorded from 5 lightly anesthetized rats using an 8x8 electrode epipial array covering auditory cortex. The MGd, MGv and posterior intralaminar nucleus (PIL) were electrically stimulated with 500 msec trains of pulses (10 KHz). As in previous studies, bursts of spontaneous gamma oscillations were localized to areas 36 and 41. Stimulation of the MGd and MGv resulted in a large steady potential for the duration of the train, localized to area 36 and area 41 respectively. During the steady potential, spontaneous gamma was suppressed. In contrast, stimulation of PIL resulted in evoked gamma oscillations for the duration of the train and no steady potential. Both evoked and spontaneous gamma were of similar frequency and spatial distribution. These results suggest that the PIL may serve as a modality specific modulator of gamma oscillations in auditory cortex. They also demonstrate a dissociation between gamma modulation and synaptic drive provided by the specific afferent projection nuclei.

Supported by NSF Grant IBN-9119525 and NIH Grant 1-R01-NS2575

356.18

ABSENCE OF DIFFERENTIAL LAMINAR DISTRIBUTION OF IPSPS IN NEOCORTICAL ASSOCIATION CELLS OF CATS *IN VIVO*. N.Dürmüller*, D. Contreras and M. Steriade. Lab. Neurophysiol., Sch. Medicine, Laval Univ., Québec, CANADA G1K 7P4.

We studied the laminar distribution of cortical- and thalamic-elicited IPSPs in association cortex (area 5 and 7) neurons in barbiturate-anesthetized cats. Cells were classified as regular-spiking, fast-spiking, and intrinsically-bursting. Upon release from hyperpolarization or upon depolarization, a subpopulation of cells showed doublets or triplets of action potentials that had a tendency to rhythmic activity at 20-40 Hz; they were called fast-bursting cells. IPSPs were evoked by stimulating either the thalamic lateroposterior nucleus or the cortex in the vicinity of the recording micropipette. In 26 out of 40 neurons recorded with K^+-acetate filled pipettes, IPSPs (measured at -60 mV) had amplitudes between 3 and 20 mV, peaked at 30 ms, and had durations at half width of 40 to 200 ms. Cortical-elicited IPSPs were consistently of higher amplitude and longer duration than those triggered by thalamic stimulation. Only occasionally had the IPSPs a clear biphasic aspect, although a second longer-lasting component of the IPSP could be revealed by its effect on the firing frequency of cells. Recordings with KCl-filled pipettes completely reversed the IPSPs, giving rise to bursting upon stimulation, and revealed the compound nature of the synaptic response that is normally cut off by the IPSP. The amplitude and duration of IPSPs showed no correlation with cell location in different laminae or cell-type. In conclusion, no simple rules can be applied to the distribution of inhibition in association cortex in cats.

Supported by MRC of Canada (MT-3689) and the Savoy Foundation.

356.19

NEOCORTICAL ACTIVATION: MODULATION BY MULTIPLE PATHWAYS ACTING ON CHOLINERGIC AND SEROTONERGIC SYSTEMS. Hans C. Dringenberg and C.H. Vanderwolf*. Neuroscience Program & Psychology Dept., University of Western Ontario, London, Ontario, Canada, N6G 2V4.

In urethane-anesthetized rats, electrical 100 Hz stimulation of the amygdala, the dorsal raphe, the locus coeruleus area, the superior colliculus, and the orbitofrontal cortex, but not of the entorhinal or cingulate cortex, could induce electrocorticographic (ECoG) activation (amplitude < 0.5 mV, including frequencies above 10 Hz). Activation elicited from the amygdala, the locus coeruleus area, and most orbitofrontal sites was abolished by administration of the cholinergic muscarinic antagonists scopolamine (5 mg/kg, i.p.) or atropine (50 mg/kg, i.p.). The activation elicited from the raphe or superior colliculus was largely resistant to anti-muscarinic treatment but could be abolished by the serotonergic antagonists methiothepin (5 mg/kg, i.p.) or ketanserin (5 mg/kg, i.p.). Electrical single pulse stimulation of the amygdala and the locus coeruleus area enhanced firing of about 60 % of extracellularly-recorded basal forebrain (BF) neurons that increased their firing during the presence of ECoG activation (putative cholinergic cortically-projecting cells). About 80 % of BF cells that fired at higher rates during periods of ECoG deactivation were inhibited by single pulse stimulation of the amygdala or locus coeruleus.

Widely distributed neuronal systems produce ECoG activation by acting through mechanisms sensitive to anti-muscarinic or anti-serotonergic drugs, suggesting that these activating influences depend on the release of acetylcholine and/or serotonin. Neural systems that produce atropine-sensitive (i.e., putative cholinergic) LVFA, believed to be dependent on the cholinergic innervation of the cortex arising in the BF, may produce ECoG activation by a dual action to excite BF cells that may contribute to ECoG activation (putative cholinergic cells) and inhibit other BF cells that may suppress activation. (Supported by NSERC Canada).

356.20

SIMULATION OF ACTIVITY DYNAMICS IN CORTEX-LIKE STRUCTURES: PATCHY PROJECTIONS SHOW AN INTEGRATIVE ADVANTAGE OVER DIFFUSE CONNECTIVITY. B.M. Ramsden*[1], G.K. Egan[2] and R.B. Silberstein[1]. [1]Centre for Applied Neurosciences, Swinburne University of Technology, Hawthorn, Vic 3122 Australia, [2]Dept. of Computer & Electrical Engineering, Monash University, Clayton Vic 3168 Australia.

Patchy intracortical neuronal populations are pervasive structural features of the superficial layers of primate neocortex, but the functional consequence of this connectivity remains unclear. Using a CRAY-YMP supercomputer, we implemented a large-scale computational simulation that compared activity dynamics in cortex-like structures featuring patchy or diffuse local connectivity.

Cortex-like structures consisted of single or multiple layers of two-dimensional (64x64) arrays of neuronal populations or "segregates" (250 μm diameter domains). Segregate activity was modeled using equations incorporating physiologically and anatomically plausible parameters. Structures were stimulated with transient increases of synaptic drive of varying strengths.

Segregates receiving patchy local projections from beyond 5mm showed enhanced linearization of input-output signal transfer relationships above a critical response threshold. In contrast, diffusely connected configurations, and segregates receiving patchy projections from less than 5mm distance, showed predominant "level-detection" behavior, with diffuse connections yielding highest thresholds.

The superficial layers of primate prefrontal and posterior parietal cortex are reported to incorporate especially extensive patchy connectivity of up to 7mm lateral reach. These simulation results suggest that such connectivity characteristics may provide a dynamical advantage for these association regions by enhancing their ability to integrate coincident bursts in corticocortical drive from other nodes of the cortical network.

356.21

CELLULAR SUBSTRATES OF THE SLEEP K-COMPLEX AND ITS DETECTION WITH THE WAVELET TRANSFORM. E. Amzica* and M. Steriade. Lab. Neurophysiology, Sch. Medicine, Laval University, Quebec, Canada G1K 7P4.

The sleeping cortical network generates a slow (<1 Hz) oscillation (Steriade et al., J. Neurosci., 1993, 13:3252). Intracellularly, this is seen as an alternation between depolarization and hyperpolarization of membrane potential. Simultaneous intracellular and focal field potential recordings in anesthetized cats disclosed a close relationship between the onset of the cellular depolarization and depth-cortical negative field potentials. Since these field potentials are reversed at the cortical surface, where they look similar to the EEG K-complexes seen in humans, we performed multi-site extracellular and field potential recordings in cortical areas 4, 5, 7, 17 and 18 of chronically implanted, naturally sleeping cats. It appeared that K-complexes, which are present at the onset of sleep, are the same waves that characterize the slow (<1 Hz) sleep oscillation. The depth-negativity (surface-positivity) was associated with increased neuronal firing. Under these conditions, K-complexes appeared rhythmic, repeating at a mean frequency of 0.7 Hz. The laminar distribution of the K-complex shows a potential reversal at a depth of 0.25-0.5 mm and the presence of a large sink in cortical layers II-III. The presence of K-complexes and their rhythmicity are better disclosed if their shape is considered, rather than by spectral analysis with Fourier transforms. This is why we approached the detection of K-complexes with the Wavelet Transform. The detection procedure included direct transform based on Daubechies' wavelets (the most similar to the K-complex), windowing and inverse transform, thus providing a signal devoid of all components but the K-complexes. *This approach revealed that K-complexes are rhythmic at the frequency of the slow oscillation.* It also unveiled their dynamic progression through various sleep stages and their relation with other sleep oscillations such as spindles. It is shown that, at sleep onset, K-complexes are the triggering factor of spindles. As sleep deepens, the hyperpolarization of thalamocortical neurons favors the development of thalamic delta oscillations and prevents spindles.

Supported by MRC of Canada, Human Frontier Science Program and FRSQ.

356.22

ANALYSIS METHODOLOGY FOR MEG DATA USING MODERN SPECTRAL TECHNIQUES B. Pesaran[1], P.P. Mitra[1]*, E. Kronberg[2], U. Ribary[2], and R. Llinás[2]. [1]Bell Laboratories, Lucent Technologies, Murray Hill, NJ 07974 and [2]Department of Physiology and Neuroscience, NYU Medical Center, 550 First Ave., New York, NY 10016.

Due to poor signal to noise, conventional applications of magnetoencephalography to the study of brain function involve heavy averaging over trials. This is inconvenient for studying spontaneous brain activity as manifested in such data. In particular, activity at frequencies higher than 20 Hz is both transient and low in amplitude. This has given rise to controversy regarding the spectral content of the data. In part, this is due to the continued use of outdated techniques for spectral analysis that are not sufficiently refined to reveal the full structure of the signal.

We have used a combination of singular value decomposition (principal components analysis) and multiple window spectral techniques to analyze multichannel MEG data, in particular, data gathered continuously during different brain states. The recordings were obtained using two 37 channel "magnum" BTi MEG System. The spontaneous activity was recorded form normal individuals in waking conditions. The line frequency (60 Hz) and heart beats were removed by appropriate methods. A critical step is the reduction of the dynamic range of the spectra by pre-whitening using an autoregressive filter determined from a smooth estimate of the spectrum. The spectra of the autoregressive filters decayed monotonically in the frequency range of interest (10-100 Hz) by two orders of magnitude. The whitened multichannel data was processed in two different ways. Firstly, the data were projected onto the first few spatial modes obtained in a space-time SVD, and then spectrally analyzed using a moving time window and a multi-window spectral estimate. This provided a time frequency analysis of the spectral content of the data. The second method employed a space-frequency SVD, which revealed the distribution of coherence over channels in a manner localized simultaneously in time and frequency. The results are exemplified by the average spectrum of the spontaneous, awake data where well defined spectral peaks were obtained centered at 10, 20 and 45 Hz. Support: Charles A. Dana Foundation, Bell Laboratories.

BRAIN METABOLISM AND BLOOD FLOW III

357.1

IN VIVO LABELING OF MITOCHONDRIAL COMPLEX I WITH [^{3}H]DIHYDROROTENONE. Deepa J Talpade*, James G Greene, Donald S Higgins, Jr. and J Timothy Greenamyre. Department of Neurology and Program in Neuroscience, Emory University, Atlanta, GA 30322

Mitochondrial enzymes play a critical role in neuronal homeostasis, and defects in mitochondrial function have been implicated in neurodegenerative disorders. Moreover, the activity of these enzymes may be used to map functional changes in synaptic firing patterns. However there is no method available for *in vivo* assessment of mitochondrial enzymes. We previously developed an *in vitro* autoradiographic assay to study complex I using binding of [^{3}H]dihydrorotenone (DHR) to the rotenone binding site (*J. Neurochem.* 59:746,1992). We now present a technique to label complex I in living animals. Briefly, male Sprague -Dawley rats recieved a jugular infusion of [^{3}H]DHR (500 mCi/kg) and were sacrificed by decapitation 2.5 hrs after drug administration. Brains were rapidly removed and frozen. Slide-mounted cryostat sections were exposed to Hyperfilm (Amersham) for 4-12 weeks; adjacent sections were stained for 2 other mitochondrial enzymes, succinate dehydrogenase (SDH) and cytochrome oxidase (CO). Across 46 brain regions, DHR binding was highly correlated with both SDH (r = 0.95) and CO activity (r = 0.91). Specificity of DHR binding for complex I was demonstrated by local inhibition of binding by intracerebral injection of rotenone and enhancement of binding by injection of NADH. This technique represents the first method to allow *in vivo* evaluation of an electron transport enzyme, and it raises the possibility of using PET scanning for this purpose. *Supported by a Mallinckrodt Scholar Award and USPHS grant AG11755.*

357.2

COMPARISON OF THE DEVELOPMENTAL EXPRESSION OF THE NEURONAL GLUCOSE TRANSPORTER, GLUT3, WITH SYNAPTIC PROTEINS IN POSTNATAL RAT BRAIN. Rebekah R. Clark, Lisa B. Seaman and Susan J. Vannucci*. Dept. of Pediatrics, Hershey Med Ctr-Penn State Univ., Hershey, PA 17033.

Glucose is the primary fuel for the immature, as well as adult, brain and a constant supply is essential for normal cerebral development. GLUT3, the neuron-specific glucose transporter, is expressed at very low levels in perinatal (P1-P7) rat brain but increases during the period of neuronal maturation and synaptogenesis. The purpose of this developmental study was to correlate regional GLUT3 expression in postnatal rat brain with proteins representative of the energetic and functional state of the neuron, i.e. Na-K ATPase (neuron-specific α_3 subunit), and the synaptic proteins, SNAP-25 and synaptophysin. Rats were studied at 7, 10, 14, 21 and 28 days of postnatal age. Five regions: cortex, hippocampus, thalamus, brain stem and cerebellum, were rapidly dissected and snap-frozen on dry ice. Membrane samples were analyzed by Western blot, relative to brain membrane "standard" and results expressed in standard units. Other brains were frozen for *in situ* hybridization histochemistry. The results of this study indicate that in the forebrain regions of cortex, hippocampus and thalamus GLUT3 expression correlates closely with SNAP-25 and synaptophysin, whereas Na-K ATPase levels were already at 50% adult levels by P7. In the brain stem, this early expression of Na-K ATPase was matched by GLUT3; the synaptic proteins displayed a more gradual time course of expression. However, in the late-maturing cerebellum, all 4 proteins followed a similar linear pattern, increasing through P21. The results of this study indicate that in forebrain regions, the expression of GLUT3 which provides the energy for neuronal function, is most closely related to synaptic function. **Supported by Whitaker Summer Scholarship (RRC) & HD31521,NICHD.**

357.3

LOCALIZATION OF GLUCOSE TRANSPORTERS 1 AND 3 IN HUMAN POSTMORTEM BRAIN. A. McCall*, A. VanBueren, V. Nipper, R. Roberts, and G. Murdoch. Depts. of Cell & Dev. Biol., Med., Neurol. and Path., Ore. Health Sc. Univ., VA Med. Ctr., Portland, OR , 97201, and MD Psych.Res. Ctr.

Localization of the major glucose transporters, GLUT1 and GLUT3, in human brain parenchyma by immunocytochemistry (ICC) has been hampered by the modest affinity of available antibodies. We generated polyclonal antisera against the C-terminus of the human GLUT3 (ALM3H-S) and against GLUT1 (ALM1-K; JCBFM 16:69-76,1996). On immunoblot, ALMH3-S (1:2,500) identifies a 46 kDa band in human hippocampus and testis. It is also positive for COS-7 cells transfected with a hGLUT3 construct (Dr. C. Burant); reactivity is abolished with 10 μg/mL peptide. Human red cells, rat brain homogenates, and vector only transfected COS-7 cells are negative. For GLUT1, we used ALM1-K (1:10,000). ICC of human brain for GLUT3 shows discrete, laminar- and region-specific neuropil staining with no white matter staining. In hippocampus, dense GLUT3 ICC staining is found in the molecular layer, the lacunosum moleculare, strata oriens and radiatum in CA1, CA2 and CA3 subfields. Similar to rat, no staining of large perikarya is observed in the pyramidal cells or dentate granule cells. GLUT3 staining is much less in cerebellum and frontal cortex than hippocampus. In occipital cortex (area 17) laminar GLUT3 staining is found in layers 1, 4 and 6. For GLUT1 this same cortical area shows staining in layers 2 through 5 with less discrete lamination. In hippocampus, GLUT1 ICC staining is seen in molecular layers and extends to the granule cell layer. Heterogeneous, laminar- and region-specific density of GLUT1 and GLUT3 suggest the possibility of local regulation of glucose availability to brain by transport proteins. Support: from VA, AG08017, Maryland Brain Collection, and Oregon Alzheimer's Disease Center.

357.4

"AEROBIC GLYCOLYSIS" DURING & AFTER SENSORY STIMULATION: ALTERED FLUX OF GLUCOSE CARBON INTO METABOLITES SYNTHESIZED MAINLY IN NEURONS OR GLIA. G. A. Dienel*, R. Y. Wang, & N. F. Cruz. Lab. of Cerebral Metabolism, NIMH, Bethesda, MD 20892.

Brain appears to use disproportionately larger amounts of glucose compared to O_2 during and after brain work in well-oxygenated tissue, i.e., the so-called phenomenon of "aerobic glycolysis". Because the basis for aerobic glycolysis is unknown we developed a rat model in which the O_2/glucose uptake ratio into brain was reduced during and after sensory stimulation; lactate accumulation in brain and lactate efflux from brain did not account for the excess uptake of glucose (Madsen et al., *J. Cereb. Blood Flow Metab.* 15, S77, 1995). We, therefore, examined the flux of [6-^{14}C]-glucose into metabolite pools before, during, and after sensory stimulation (n = 6/group). Brain glucose levels and specific activities were stable throughout the activity cycle. Brain glycogen content was reduced 20-30% during and after stimulation, and glycogen specific activity doubled during recovery compared to rest and stimulation ($p<0.05$). Thus, both synthesis and degradation of glycogen (located mainly in glial cells) were enhanced during recovery. The level and specific activity of glutamine, known to be synthesized in glia, did not change with activity phase ($p>0.05$). In contrast, labeling of GABA, a neuronal marker, rose 25% ($p<0.05$) during activation compared to rest and recovery; GABA content did not change. During stimulation, the level of aspartate decreased by 20% whereas labeling of aspartate increased about 40% compared to rest and recovery; glutamate levels and labeling increased 6 and 16%, respectively, during activation, then normalized ($p<0.05$). Thus, flux of glucose carbon into metabolites synthesized mainly in neurons or in glia varies during the activity cycle; labeling of GABA but not glutamine (both are derived from glutamate) increased during stimulation. Depletion and filling of metabolic pools explain, in part, the excess consumption of glucose compared to O_2 during and after stimulation. (Funded by NIMH Intramural Program)

357.5

GLYCOGEN DEPOSITION IN NEURONS OF THE BRAIN STEM AND HYPOTHALAMUS IN THE OPOSSUM L.A. Cavalcante[1] and P.C. Barradas*[1,2] 1. Inst. Biofisica, UFRJ, 21941-590; 2. Inst. Biologia, UERJ, 20551-030 Rio de Janeiro, Brazil.

Neurons are heterogeneous cells with respect to size and shape, electric signals, neurotransmitter systems and enzymes related to energy metabolism. However, studies in small rodents have led to the view that glycogen deposition is low or non-existent in normal, mature neurons. We have used a modified histochemical method with alcoholic solutions and basic fuchsin (E-bF) to minimize glycogen elution and check for its storage in central neurons in the opossum. E-bF+ cell groups were found in the V mesencephalic nucleus and the somatic and special visceral efferent columns of the brainstem as previously shown in developing, small rodents. Scattered E-bF+ neurons also appear in a presumptive homologue of the primate raphe interpositus nucleus, and in sparse cells of the dorsal thalamus and basal forebrain. A striking, often Golgi-like accumulation of glycogen has been found in many neurons of all divisions of the supra-optic and paraventricular nuclei and in the infundibular/arcuate nucleus. Our results suggest that the patterns of glycogen storage are common to several vertebrates and may be a constant although not exclusive property of cells with axonal endings outside the blood-brain barrier. Apparent differences with respect to small-sized placentals may be related to specific metabolic rates.

[Support: CNPq, FINEP,CEPG/UFRJ, SR2/UERJ]

357.6

IN VIVO RELEASE OF NORADRENALINE BY IDAZOXAN ACTIVATES GLYCOGENOLYSIS IN RAT NEOCORTEX AND HIPPOCAMPUS

M. Fara-On, C.W. Harley, J.H. Evans, R.A.M. Brown, and R.E. Adamec*. Dept of Psychology, Memorial University, St. John's, Newfoundland, Canada A1B 3X9

Glycogen and its catabolic enzyme, active glycogen phosphorylase (aGP) are localized primarily in glia. Although the modulation of glycogenolysis by neuronally-secreted substances suggests a metabolic coupling between neurons and glia, the functional significance of this metabolic interaction has yet to be determined. Functional adrenergic receptors are expressed by glial cells. Noradrenaline (NA) and its agonists have previously been reported to activate glycogenolysis in mouse cerebral cortex *in vitro*.

In our study, we induced *in vivo* NA release in rats through IP administration of idazoxan (5mg/kg). Drug effects on glycogenolysis were assayed by histochemical assessment of aGP 45 minutes following injection of drug or saline. Relative optical density (ROD) measures of regions of the neocortex and hippocampus were taken using computer-assisted densitometric software (MCID). Large increases in levels of aGP were found in somatosensory cortex (layers 3, 4, 5a, 5b, and 6), in stratum lacunosum moleculare of CA1 and in the neuropil between the granule cells and CA4 in dentate gyrus. No significant increases in aGP levels were found in corresponding layers of the primary motor cortex, or in corpus callosum.

The novel finding that NA release activates glycogenolysis in two regions of hippocampus suggests activation of glycogenolysis may be involved in the effects of NA in this region. Our neocortical results are consistent with previous data showing significant increases in aGP in the somatosensory cortex following medial forebrain bundle stimulation, suggesting an important interaction between sensory processing and noradrenergic modulation of glycogenolysis.

Supported by NSERC grant A9791 to CWH.

357.7

DISSIMILARITIES IN PARENCHYMAL DISTRIBUTION OF GLUCOSE AMONG BRAIN AREAS AND BETWEEN TWO RAT STRAINS. J. D. Fenstermacher*, L. Wei, D. Bereczki, V. Acuff, K. Davies, F.-J. Hans, and C. Patlak. Henry Ford Hospital, Detroit, MI 48202 and SUNY, Stony Brook, NY 11794.

3-O-methylglucose (3OMG) is a non-metabolizable marker of glucose transport with a steady-state distribution space of 50 ml/100 g (50%) in brain, less than the water space (65-80%) but greater than interstitial space (10-20%). Glucose transporters facilitate the flux of 3OMG both across the blood-brain barrier (BBB) and into brain cells, but the rates appear to be different. We postulate and test in young adult Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats that 3OMG transport across the BBB is modest and "rate-limiting" and uptake into brain cells is very rapid. ^{14}C-labeled 3OMG was intravenously infused into WKY and SHR for 15-45 sec, plasma and tissue ^{14}C-activity determined, and the 15-45 sec space of 3OMG distribution estimated. The flux of 3OMG across the BBB was moderately rapid and nearly identical in SHR and WKY. Indicating very fast cellular uptake in support of the hypothesis, the 15-45 sec space of 3OMG distribution was essentially 50% in 30 (of 35) brain areas of SHR and 11 (of 35) areas of WKY. In 5 brain areas of SHR and 24 areas of WKY, the 15-45 sec space of distribution was 10-20%, suggestive of relatively similar rates of BBB influx and cellular uptake contrary to the hypothesis. In conclusion, glucose distribution between interstitium and cells may vary among brain areas and rat strains and be relatively slow, particularly in WKY. Supported by NIH grants NS-26004 and HL-35971.

357.8

HYPERGLYCEMIA-INDUCED LEUKOCYTE ADHERENCE IN CEREBRAL VENULES J.M. Gidday, J.W. Beetsch, E.R. Gonzales, A.R. Shah, R.G. Maceren, Y.-B. Lee, J.H. Thurston*, T.S. Park. Departments of Neurosurgery and Neurology, and St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, MO 63110

Acute hyperglycemia has been shown to affect cerebral blood flow, endothelium-dependent vascular reactivity, amino acid transport, cerebral metabolism, and ischemic outcome. We examined the possibility that hyperglycemia could initiate an inflammatory response in the cerebral circulation. We studied three groups of anesthetized newborn pigs: One group (n=5) was rendered acutely hyperglycemic (blood glucose = 250-300mg/dl [14-17μmol/ml]) by intravenous glucose, a second group (n=6) was rendered asphyxic for 9 min by discontinuing ventilation, and a third group (n=8) served as normoglycemic, non-asphyxic controls. Adherence of rhodamine 6G-labelled leukocytes to cerebral venules was monitored over time through a closed cranial window using epifluorescent videomicroscopy. The number of adherent leukocytes (above baseline) per mm^2 vessel surface during the initial 2 h following each intervention is shown for the three animal groups:

	30 min	60 min	120 min
Control	119 ± 68	156 ± 62 *	319 ± 107 *
Hyperglycemia	281 ± 118	583 ± 159 *#	959 ± 292 *#
Asphyxia	592 ± 149 *#	706 ± 128 *#	1413 ± 209 *#

[* p<0.05 versus baseline; # p<0.05 versus control group]

These results show that acute hyperglycemia causes a progressive increase in leukocyte-endothelial adherence to an extent not significantly different from that which follows a severe asphyxic insult. The potential pathophysiologic significance of this vascular inflammatory response to hyperglycemia remains to be determined. (NIH NINDS 21045 and 32568).

357.9

BLOOD-BRAIN BARRIER GLUCOSE TRANSPORT AND THE INFLUENCE OF DIABETES AND ANESTHESIA. Q.R. Smith,* M. Hokari, M. Aoyagi, N.M. Appel, S.J. Vannucci, and I.A. Simpson. NIA and NIDDK, Bethesda MD 20892; FDA, Laurel, MD 20708; and Dept. of Pediatrics, Penn. State Univ., Hershey, PA 17033.

Brain energy metabolism is based principally on the oxidation of D-glucose which is taken up into brain by a saturable transporter (GLUT1) at the blood-brain barrier. Studies differ on the kinetics of blood-brain barrier glucose transport and whether uptake is affected by anesthesia or chronic diabetic hyperglycemia. To address this, we examined the kinetics of sugar influx into brain using the *in situ* rat brain perfusion technique (Am J Physiol 247:H484, 1984). Cerebral D-[1-^{14}C]glucose uptake was sodium-independent and linear for 20 s during perfusion with physiologic saline. The concentration dependence of saturable D-glucose influx followed Michaelis-Menten kinetics with a V_{max} of 4.02 ± 0.51 μmol/min/g and a K_m of 8.2 ± 0.9 mM (mean ± SD) in the parietal cortex. Relative affinities ($1/K_m$) of 2-deoxy-D-glucose and 3-O-methyl-D-glucose were 1.56 ± 0.20 times and 0.63 ± 0.08 times, respectively, that of D-glucose. Blood-brain barrier D-glucose transport was not significantly reduced by either pentobarbital anesthesia (40-50 mg/kg, i.p.) or chronic streptozotocin diabetes (3 weeks duration; plasma [glucose] = 35 ± 2 mM). Similarly, diabetes did not decrease the level of GLUT1 protein in brain microvessels. In summary, the results provide a new kinetic analysis of glucose uptake into brain which suggest that transport is less sensitive to barbiturate anesthesia or diabetic hyperglycemia than previously reported. (Supported by NIA and NIDDK).

357.10

PROPOFOL ATTENUATES pCO2 MEDIATED INCREASE IN CEREBRAL BLOOD FLOW (CBF) IN NORMAL VOLUNTEERS. MG Byas-Smith[1], MA Frolich[1], JR Votaw[2], and JM Hoffman[2,3]* Department of Anesthesiology[1], Department of Neurology[2] and Emory University PET Center[2], Emory University School of Medicine, Atlanta, Georgia 30022.

The effect of propofol on the CNS has been reported. Propofol does not prevent pCO2 induced rises in CBF(Stephan H, et. al., 1987). This study was performed to determine the effects of propofol on CBF using positron emission tomography (PET). Ten volunteers participated. Prior to positioning in the scanner, lines were inserted. The O-15 water bolus technique was utilized. Subjects were scanned in a Siemens ECAT scanner. Nine scans were performed, 3 resting, 3 sedation and 3 anesthetic scans. Sedation was defined as no spontaneous responses. Anesthesia was defined as responsive only to deep pressure over the sternum. Subjects breathed spontaneously. The targeted tissue concentrations for sedation and anesthesia were 2 mcg/ml and 6 mcg/ml respectively. Arterial blood gases were taken after each scan. PET images were normalized by calculating absolute blood flow using the non-linear-squares method of Koeppe (JCBFM, 1985). Regression and paired *t* tests were used for data analysis. The CBF increased whenever the pCO2 was above baseline. When the pCO2 was at baseline, the CBF decreased. The elevations in CBF were less than predicted (2-4% increase in CBF for each 1 mmHg rise in pCO2) during sedation ($p<0.02$) and approached significance for anesthesia ($p<0.08$). Propofol may attenuate the pCO2 CBF effects.

Department of Anesthesiology, Emory University PET Center (drug provided by Zeneca Pharmaceuticals).

357.11

EFFECT OF PROPOFOL-INDUCED ANESTHESIA ON REGIONAL CEREBRAL BLOOD-FLOW: A POSITRON EMISSION TOMOGRAPHY (PET) STUDY.

P. Fiset, T. Paus*, T. Daloze, G. Plourde, N. Hofle, N. Hajj-Ali, A. Evans. Anaesthesia Dept., Royal Victoria Hospital and Brain Imaging Center, Montreal Neurological Institute; McGill University, Montreal, QC, Canada. H3A 1A1.

This study was designed to examine the effect of different levels of propofol-induced anesthesia on global and region-specific changes in brain activity, as indexed by cerebral blood-flow (CBF). In five healthy volunteers, propofol was administered throughout a 2-hour PET session. After two baseline (awake) 3-minute scans, a computer controlled infusion of propofol was started, targeting three plasma concentrations: 0.5, 1.5 and 2.5-3.0 μg/mL. Two scans were acquired at each level. At the highest propofol level, all volunteers were unresponsive to verbal stimuli.

Absolute values of CBF were calculated using a two-compartment model of Ohta et al. (1996). A significant decrease in global grey-matter CBF was observed at the highest propofol level *vs* baseline (37.9±10.5 vs. 48.2±11.4 ml/100 g/min, p=0.01). CBF data were then normalized for the global grey-matter CBF changes, and the relationship between propofol plasma concentration and regional CBF was evaluated with linear regression. This analysis revealed significant ($|z| > 3.5$) negative correlation between plasma propofol and CBF in the ventro-medial thalamus, orbitofrontal, and posterior and medial occipital cortex. There was a positive correlation between plasma propofol and CBF in the cerebellar vermis. In addition, a significant co-variation was observed between CBF in the thalamus and that in midbrain reticular formation, indicating a coordinated blood-flow response to the changing propofol level. In conclusion, propofol appears to induce not only global CBF decreases, but it also affects brain activity in a region-specific manner. Changes in rCBF observed in the thalamus and midbrain reticular formation support the importance of these structures in the control of consciousness. Supported by MRC SP-30 fund as a pilot project.

357.12

BLOCKADE OF NEUROPEPTIDE Y Y_1 RECEPTORS DOES NOT AFFECT VASCULAR RESPONSES TO ADRENERGIC NERVE STIMULATION. M. Adner, S.P. Duckles, L. Edvinsson* and D.N. Krause. Dept Internal Med, Lund Univ Hospital, Lund, Sweden and Dept Pharmacology, Univ of California, Irvine, CA, 92717, USA.

The 36-amino acid neuropeptide Y (NPY), an adrenergic co-transmitter, is known to potentiate vasoconstriction of the rat tail artery. The aim of this study was to characterize the postjunctional NPY receptor mediating potentiation in tail artery segments in vitro and to investigate the role of endogenous NPY in mediating contractile responses to adrenergic nerve stimulation. The Na^+-channel inhibitor tetrodotoxin (1 μM) totally abolished contractile responses to adrenergic nerve stimulation (15 V, 0.3 msec, 16 Hz). NPY (10 nM) elicited no contraction by itself but potentiated contractions to adrenergic nerve stimulation (10 pulses) at 0.5, 1 and 2 Hz (141±11%, 136±12% and 130±14% of control, respectively). Exposure to the selective NPY Y_1 receptor antagonist BIBP3226 (1 μM) did not cause any effect by itself but abolished NPY-induced potentiation at all frequencies tested (10 pulses; n=7), indicating that NPY induced-potentiation is mediated by the Y_1 receptor. To test whether endogenous NPY contributes to contractile responses, adrenergic stimulation was increased to 100 pulses (1-16 Hz). However, contractile responses to adrenergic nerve stimulation were not affected by BIBP3226 at any of the frequencies tested (n=3, 8-9 segments). This study suggests that NPY, acting on Y_1 receptors, potentiates norepinephrine-induced contraction, but endogenous release of NPY does not appear to contribute to contractile responses to adrenergic nerve stimulation in the rat tail artery.

NIH HL50775 and Swedish Medical Research Council (05958).

357.13

CEREBRAL VASODILATOR RESPONSES TO BRADYKININ AND HYDROGEN PEROXIDE ARE DEPENDENT ON ACTIVATION OF Ca^{++}-DEPENDENT K^+ CHANNELS. Frank M. Faraci,* Christopher G. Sobey, and Donald D. Heistad, University of Iowa, Iowa City, IA, 52242.

The goal of this study was to test the hypothesis that vasodilator responses of cerebral arterioles to hydrogen peroxide (H_2O_2) involves activation of Ca^{++}-dependent K^+ channels. Diameter of cerebral arterioles was measured using cranial windows in anesthetized rabbits and rats. In rabbit cerebral arterioles (baseline diameter= 102±5 μm), H_2O_2 (10^{-5} M - $3x10^{-4}$ M) produced concentration-dependent dilatation that was inhibited completely by catalase (100 U/ml). Iberiotoxin ($5x10^{-8}$ M), a selective inhibitor of Ca^{++}-dependent K^+ channels, inhibited dilatation in response to H_2O_2 by approximately 60%. In rat cerebral arterioles (baseline diameter=38±2 μm), bradykinin (10^{-8}-10^{-6} M) produced concentration-dependent dilatation that was inhibited completely by catalase (100 U/ml). Tetraethylammonium (10^{-3} M, an inhibitor of Ca^{++}-dependent K^+ channels) or iberiotoxin markedly inhibited vasodilatation in response to bradykinin. These findings suggest that dilatation of cerebral arterioles in response to exogenous H_2O_2, or H_2O_2 produced endogenously by bradykinin, is mediated by activation of Ca^{++}-dependent K^+ channels. Thus, activation of K^+ channels may be a major mechanism of cerebral vasodilatation in response to reactive oxygen species.

357.14

IMMUNOFLUORESCENT IDENTIFICATION OF OXYTOCINERGIC NERVES IN CORTICALVESSELS. C. Thore,[1]* F. Bari[1,2], M. Morris[1] and D. W. Busija[1]. [1]Bowman Gray School of Medicine, Winston-Salem, NC 27157 and [2]Albert Szent-Györgyi Medical University, Szeged, Hungary H-6720.

We have shown previously that oxytocin is a potent dilator agent in the cerebral circulation of piglets, but possible sources of this peptide are unclear. The purpose of this study was to determine whether perivascular nerves are immunopositive for oxytocin. Brain arteries were removed from the brain surface, immediately fixed in 4% formaldehyde, and the presence of oxytocin assessed using indirect immunofluorescence. Using an antibody known to cross-react with neurophysin-oxytocin (NPOT) and neurophysin-vasopression (NPVP), we observed an extensive network of immunostaining in large arteries and their branches. Additional experiments using a specific antibody against NPOT showed similar but less intense immunostaining, while use of a specific antibody against NPVP revealed little staining. Arteries stored overnight at 4°C before fixation showed no immunoreactivity. Lastly, arteries from piglets exposed to 5-10 minutes of brain anoxia showed no NPOT immunoreacivity. We conclude that cerebral arteries are innervated by oxytocinergic fibers in piglets.

Supported by HL 30260, HL 46558 & HL 50587.

357.15

HYPOXEMIA INCREASES BLOOD FLOW THROUGH THE RETINAL CIRCULATION IN CATS; A STUDY USING FLUORESCENT MICROSPHERES. J. Ahmed and R.A. Linsenmeier*. Dept. Of Biomedical Engineering, Northwestern University, Evanston, IL 60208-3107

The purpose of this study was to develop a method of measuring blood flow through the retinal circulation and to determine what effects hypoxemia had on retinal blood flow.

Experiments were done on urethane-anesthetized cats. 5 eyes from 4 cats were used. Retinal blood flow was measured using fluorescent microspheres (Molecular Probes, Eugene, OR; Yellow/Green, Scarlet, and Blue polystyrene microspheres; 10 and 15 μm diameters). Approximately 5 million microspheres were injected into the left ventricle via a cannula inserted through the femoral artery. A reference sample was taken using a withdrawal pump connected to a cannula in the other femoral artery.

Hypoxemia was induced by decreasing oxygen levels in the breathing gas to 5-8% O_2, resulting in arterial PO_2s of 24.2 ± 6.9 mmHg (n=3). Retinas were fixed in 10% formol-saline and wholemounted. Microspheres were counted using a fluorescent microscope and a ccd camera

Hypoxemia increased retinal blood flow by 286 ± 94% (n=3). This difference was statistically significant ($P< 0.01$; paired t-test). Control experiments in which no hypoxemia was presented showed an increase in the blood flow measurement of 41 ± 22% (n=2) between the first and second microsphere doses. This increase was not statistically significant.

These data show that the retinal circulation is very sensitive to tissue hypoxia and responds with a large decrease in its resistance. Future experiments will be geared towards understanding the chemical mechanism of this hypoxia-induced vasodilation as well as other aspects of the control of blood flow through the retinal circulation.
Supported by NIH grant EY-05034 and the Whitaker Foundation

357.16

GABAERGIC REGULATION OF RESTING TONE IN CEREBRAL MICROVESSELS OF THE RAT. A. Fergus* & K.S. Lee. Dept. of Neurological Surgery & The Neuroscience Graduate Program, Univ. of Virginia, Charlottesville, VA 22908.

Converging lines of evidence suggest that local neurons are participants in the regulation of cerebral blood flow. Interneurons are often closely associated with parenchymal microvessels suggesting that they may be capable of signalling microvascular responses. The present study examined the role of GABAergic signalling in the regulation of resting tone in microvessels of the rat. The effects of endogenous activation of GABA receptors on the tone of hippocampal and cortical microvessels were examined using computer-assisted videomicroscopy in conjunction with extracellular recording.

The resting diameter of the microvessels examined ranged from 9μm to 24μm in the hippocampus and from 30μm to 50μm on the surface of the cortex. Treatment with the $GABA_B$ receptor antagonist, 2-hydroxysaclofen, does not elicit a significant vascular response in either the hippocampus or the cortex. In contrast, $GABA_A$ receptor antagonists elicit dose-dependent constriction in hippocampal microvessels. The competitive $GABA_A$ receptor antagonist, bicuculline (100μM), elicits a 27.6 ±2.7% constriction. However, bicuculline (up to 200μM) does not elicit a significant response in cortical vessels. In the hippocampus, blockade of action potential propagation with tetrodotoxin does not significantly attenuate the bicuculline-induced constriction. These findings indicate that the tonic activation of $GABA_A$ receptors contributes to resting dilatory tone in hippocampal microvessels. These data implicate GABAergic interneurons in the signalling of parenchymal but not surface blood vessels under resting conditions. Supported by NIH grant # HL49396 to KSL.

357.17

GENDER DIFFERENCES IN MYOGENIC REACTIVITY IN RAT CEREBRAL ARTERIES. G.G. Geary, D.N. Krause and S.P. Duckles*. Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717, USA.

Although the myogenic reactivity of male rat cerebral arteries has been documented, response to pressure of female arteries has not been characterized. Therefore, the goal of the present study was to compare the myogenic reactivity of large and small middle cerebral arteries (MCA) from male and female rats. Pressure of cannulated, isolated cerebral arteries was maintained without flow by a pressure-servo system, and lumen diameter was monitored by a video dimension analyzer. Although a Ca^{++}-sensitive myogenic tone (10-20%) was present in both male and female MCA (>250 μm), only female arteries constricted (20-30%) to 10 mm Hg step increases in pressure (20-80 mm Hg). In contrast the diameter of male MCA increased with pressure. When BK_{Ca} channels were blocked with tetraethylammonium (TEA^+), myogenic responses were unmasked in the male MCA and enhanced in the female. Since myogenic reactivity of cerebral arteries has been shown to vary with location, we also determined if gender influences myogenic responses of small cerebral arteries (<150 μm). In contrast to larger arteries, a pressure-dependent reduction (30%) in diameter occurred in smaller male and female arteries. However, the range of myogenic responses in male arteries (20-80 mm Hg) was greater than in female (20-50 mm Hg). Thus gender and location influence myogenic reactivity of cerebral arteries of the rat. Concurrent activation of K^+ channels during changes in pressure modulates myogenic responsiveness, and these effects may be gender-related. *Supported by NIH grant R01HL50775*

357.18

ASTROCYTES ACCUMULATE [^{14}C]ACETATE BY THE MONOCARBOXYLATE TRANSPORTER. R. A. Waniewski* and D. L. Martin, Wadsworth Center, NY State Dept. Health and Dept. Env. Health and Tox., SUNY, Albany, NY 12201.

Exogenous acetate is believed to be preferentially accumulated and metabolized by astrocytes in the CNS. We are examining the biochemical basis for this. Transport studies with [^{14}C]acetate were conducted to determine the mechanism of its accumulation by rat cortical astrocytes in culture. The rapid loss of label from the cells necessitated 0.25-min incubations at low temperatures (4-6°C) with very rapid washing procedures (3 x 1ml in 5 sec). This was done to slow metabolism and reduce efflux of accumulated label. Under these conditions, acetate uptake was time-dependent for up to 1 min and consisted of a saturable, low-affinity component and a non-saturable, presumably diffusional, component. With acetate concentrations down to 0.04 mM, there was no evidence for a separate, higher-affinity component of transport. The saturable component displayed the properties of the proton-coupled monocarboxylate transporter. Uptake was inhibited up to 95% by coincubation with 5mM α-cyano-4-hydroxycinnamate (CN4HC), and was inhibited stereospecifically by lactate. At 0.2 mM acetate, the IC50 was 2.2 mM for L-lactate and 19 mM for D-lactate. A 15 min preincubation of astrocytes in a glucose- and monocarboxylate-free buffer eliminated most of the CN4HC-sensitive uptake. Preincubation with 10 mM L- but not D-lactate completely restored acetate uptake. This is similar to the trans-accelerating effect of intracellular L-lactate on pyruvate and lactate uptake by the monocarboxylate transporter observed in other cell systems. The most remarkable property of the acetate transporter in astrocytes is its very rapid kinetics. The V_{max} for uptake is 522 nmol/mg protein x min and the K_m is 14 mM. This K_m is similar to that reported for low-affinity lactate transport by astrocytes while the V_{max} for acetate is 3 times higher than that for lactate. Work is in progress to determine whether neurons transport acetate in the same manner as astrocytes.
Supported by the Wadsworth Center.

LEARNING AND MEMORY: PHYSIOLOGY I

358.1

STATISTICAL APPROACHES TO PLACE FIELD ESTIMATION AND NEURONAL ENSEMBLE DECODING. [1] E.N. Brown*, [2] L. Frank and [2] M. Wilson. [1] Dept. of Anes., MGH, Boston, MA 02114. [2] Dept. of Brain and Cogn. Sci., MIT, Boston, MA 02139.

The firing of CA1 place cells in the rat hippocampus correlates with the animal's position in its environment. The recorded shape of the field depends on the structure of the environment and the task the animal is required to perform. During random foraging the fields are nearly circular whereas in maze experiments, they are constrained by the structure of the apparatus. To develop better quantitative descriptions of place field properties, we estimated place field firing maps using formal statistical procedures. We model the firing pattern of a place cell as an inhomogeneous Poisson model whose instantaneous firing rate is a function of time and the animal's position in the cell's field. We approximate the noncircular geometry of the fields with ellipses. Using recordings from place cells which fired at least 50 times during free foraging, we show that the model parameters and their standard errors can be estimated by maximum likelihood analysis. Based on our analyses, the firing maps of many of the cells are better described by ellipsoidal rather than circular geometry. The model can be used to derive a linear position decoding algorithm that takes account of the observed firing pattern of the cells, the locations of the field centers, and the field geometries. We further refine the position estimate by incorporating theta rhythm modulation of the place cell firing into the decoding algorithm. This approach holds promise for giving a better quantitative description of place field characteristics during behavioral experiments and for improving neuronal decoding algorithms.
Funding Source: Supported by Anesthesia Dept., MGH and MIT.

358.2

DISCHARGE CORRELATES OF HIPPOCAMPAL NEURONS IN RATS PASSIVELY DISPLACED ON A MOBILE ROBOT. V. V. Gavrilov, S. I. Wiener, A. Berthoz*. CNRS-Collège de France LPPA, 15, rue de l'Ecole de Médecine, 75270 Paris CEDEX 06 FRANCE

Spatial and movement-selective discharge correlates of hippocampal neurons was studied in male Long-Evans rats. To detect contributions of inertial (and vestibular) cues to this activity, the water deprived rats were restrained in a sling mounted on a mobile robot. Single CA1 complex-spike cells were recorded extracellularly with glass micropipettes during translations and rotations of the robot in a square enclosure with black curtain walls. To maintain the attention of the animal, it received a drop of water as the robot arrived at a visually cued corner of the arena. Of 57 recordings sessions in four rats 15 were repeated in the light and in darkness. Fourteen neurons were selective for approaches to certain arena corners from two directions. Twenty seven of the hippocampal neurons significantly (t-test, $p<.05$) increased or decreased their firing rates when the rat was passively displaced in certain parts of the experimental arena. However spatial selectivity was for much broader regions than found in dorsal hippocampal cells of freely moving rats suggests that voluntary movements may be required for small firing fields. These discharges were often also associated with specific movement states like translation, rotation or immobility. In addition, 4 of 15 neurons analysed demonstrated similar spatial or behavioral discharges correlates in in light and darkness. These results confirm those from similar experimental recordings of rat hippocampal EEG[1] and monkey hippocampal neurons[2] indicating that there is a role of inertial inputs for the organization of activity of hippocampal neurons. Supported by EC/ESPRIT/BRA 3149 and 6615 MUCOM, the Human Frontiers Scientific Program, Programme Cognisciences, Fondation pour la Recherche Médicale, CNRS.
1) Gavrilov et al. Neurosci. Lett. 197:239 (1995).
2) O'Mara et al. J. Neurosci. 14:6511 (1994)

358.3

Behavioral correlates of nucleus accumbens neurons in rats performing a water search task in an open field. R. Shibata, O. Trullier, D. Raballand, A. B. Mulder, A. Berthoz, S. I. Wiener* CNRS-Collège de France LPPA, Paris, France.

In order to determine the dependence of discharge correlates upon the reference cue used for ongoing behavior in a region receiving hippocampal system outputs, we trained three male Long-Evans rats to perform two tasks on a rotatable 1.8m diameter elevated platform centered in a 3m X 3m cue-controlled chamber enclosed by black curtains. Advanceable, multiple fine wire electrodes were stereotaxically implanted in the shell region of the nucleus accumbens. Four identical alcoves were equally spaced along the edge of the platform; each contained a visual cue which could be illuminated as well as a water trough under automated control. The thirsty rats ran between diametrically opposite alcoves. In the *visual cue* task, cues were lit to indicate the reward-containing alcoves and these were alternated periodically. In the *landmark cue* task, the rewards were always located at alcoves at fixed positions relative to the room.

Of 29 neurons analysed, none were spatially selective in the reference frame of the platform. No clear changes in discharge correlates were found when the rat shifted between the two tasks. Five discharged selectively for several seconds prior to or after reward delivery. Nine other neurons had marked decreases in firing rate for 1-2 sec prior to and after reward delivery. These are comparable to task-related discharge correlates recorded in CA1 neurons of the hippocampus[1]. Thus the nucleus accumbens maintains these representations and could help mediate hippocampal influences on other brain structures. Support: Canon Fdn., CNRS, Human Frontiers, EC Human Capital and Mobility, EC ESPRIT 6615.

[1] S.I. Wiener & V.A. Korshunov, Neuroreport, 7, 183 (1995)

358.4

INTERACTION OF THE HIPPOCAMPUS AND THE BASOLATERAL AMYGDALA INPUTS TO THE NUCLEUS ACCUMBENS OF THE RAT. A.B. Mulder* and F.H. Lopes da Silva. Grad. School Neurosci., Inst. Neurobiol., Fac. Biol., Univ. Amsterdam, Amsterdam, The Netherlands.

This study was conducted to determine the termination patterns and the interaction of the hippocampal (HIP) and the basolateral amygdaloid (BLA), projections to the nucleus accumbens (Nacb). In halothane anaesthetized male Wistar rats, stimulation electrodes were placed in the BLA and the fornix/fimbria fibers (Fo/Fi: the output fibers of the HIP) and a glass recording electrode in the Nacb. In 12 rats, Nacb single unit activity was evoked by stimulation of the Fo/Fi (n=162) and of BLA (n=55). The cell discharges were on the peaks of the respective evoked field potentials (positive for Fo/Fi, negative for BLA stimulation). The mediodorsal shell only received HIP input, whereas in the ventrolateral shell and the ventral core only BLA driven activity could be found. Convergence of input (n=31) was found in the medial shell and the medial core regions of the Nacb. In the latter neurons successive activation of BLA then Fo/Fi pathways resulted in an enhancement of the firing probability, while in reverse sequence activation led to a depressed response (n=6: intervals 25-200 ms). Furthermore, tetanization of the Fo/Fi to Nacb pathway caused decremental *homosynaptic long-term potentiation* in the Nacb, accompanied by a *heterosynaptic long-term depression* of the non-tetanized BLA to Nacb pathway (n=4). This suggests that the HIP to Nacb pathway performs inhibitory gating of the BLA to Nacb pathway, whereas the BLA stimulation facilitated or even enhanced the Fo/Fi to Nacb pathway. This could play a role in behaviours like conditioned place preference in which the place learning is dependent on the presentation of reward.
Supported by NWO-MW program grant 900-550-093

358.5

TASK-DEPENDENT REPRESENTATIONS IN RAT HIPPOCAMPAL PLACE NEURONS.
Tsuneyuki Kobayashi*[1], Hisao Nishijo[1], Masaji Fukuda[2], Jan Bures[3], and Taketoshi Ono[1]. Depts. [1]Physiol. & [2]Behav. Sci., Fac. Med., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, Japan, and [3]Institute Physiol, Academ. Sci., Prague, Czech.

It is suggested that the hippocampal formation (HF) is essential to spatial representations by flexible encoding of diverse information during navigation. In the present study, we investigated how various types of information are represented in the HF, by recording HF complex-spike cells from rats that performed 3 types of place learning tasks in a circular open field using intracranial self-stimulation (ICSS) as reward. First, complex-spike cell activity was compared between the following 2 tasks: the rat (1) randomly explored the open field; (2) went back and forth between two specific places. Of 43 complex-spike cells recorded, 37 had displayed place fields under the first task. Although reward and place correlates of the place-neuron activity did not change, neuronal correlates to movement speed, direction, and turning angle increased in the second task in which the behavioral trails during navigation were more constant than in the first task. Second, 6 of 31 place neurons tested with the third task, in which the reward place was located outside the original place field, shifted place fields. The results indicated that neuronal correlates of most place neurons flexibly increased their sensitivity to relevant information in a given context and environment, and some place neurons changed the place field *per se* with place-reward association. These results suggest two strategies of HF neurons how to incorporate incredible variety of perceptions into a unified representation of the environment: flexible use of information, and creation of new representations.

358.6

THE SPATIAL ARRANGEMENT OF 3-DIMENSIONAL OBJECTS INSIDE THE ARENA INFLUENCES THEIR CONTROL OVER THE POSITIONAL FIRING OF PLACE CELLS. A. Cressant[1], B.Poucet*[1], R.U. Muller[2]. [1]Ctr. Res. in Cognitive Neuroscience, CNRS, Marseille, 13402 France and [2]Dept. of Physiology, SUNY, Brooklyn, NY 11203.

The angular positions of place cell firing fields are precisely controlled by a single white cue card attached to the wall of a cylinder. In contrast, angular positions are poorly controlled by 3 objects arranged in a right triangle inside the cylinder where each object is about 1/3 cylinder radius away from the center (Poucet et al., SFN Abstr, 1995, 21:946). Here we ask if the inability of objects to control spatial firing is due to the nature of the objects themselves or due to the arrangement of the objects. Place cell recordings were made while hungry rats chased pellets in a gray cylinder with 3 different objects on the floor. In the "objects at periphery" condition (16 cells), the objects were against the cylinder wall as a isosceles right triangle. In the "clustered objects" condition (18 cells), the 3 objects touched each other and were arranged in a line centered along a chord 10 cm from the cylinder wall. In each condition, the relative object locations and their distances from the cylinder wall were fixed. Recordings were made either with the objects in a "standard" position in the laboratory frame or with the objects rotated as a rigid set around the center of the cylinder. In either condition, the angular position of the firing field of all cells could be precisely predicted from the angular position of the object set. The results demonstrate that the lack of stimulus control when the objects are near the cylinder center is due to the object arrangement and not to their specific sensory qualities. A more complete understanding of which configurations of the object set permit a stable reference frame should reveal interesting computational properties of the rat navigational system.
Support: NIH, CNRS

358.7

HOW TWO CUES CONJOINTLY CONTROL HIPPOCAMPAL PLACE CELL FIRING FIELDS. A.A. Fenton* and R.U. Muller, SUNY Brooklyn, NY.

Previous work showed that the locations of place cell firing fields are controlled by a single white card on the wall of a gray cylinder. Specifically, rotations of the card position caused equal rotations of firing field locations in the absence of other polarizing stimuli. Here we describe how firing fields are controlled by two cue cards. These preliminary data are from 47 firing fields of 45 place cells recorded from one rat.

We recorded place cells while rats ran inside a 76 cm diameter gray cylinder with one white and one black cue card pasted on the wall. Each card occupied 45 deg of arc of the cylinder wall. In the "standard" arangement, the positions of the two cards were fixed in the labratory frame and the mid-points of the two cards were separated by 135 deg such that the black card was clockwise to the white card. Three experimental modifications of the standard arrangement were made. In the first, the two cards were rotated equally so that their separation remained 135 deg. This caused equal rotations of firing fields, indicating stimulus control by the two-cue configuration. The second manipulation was to remove one or the other of the two cards and then rotate the remaining card 45 deg. This also caused firing fields to rotate equally, demonstrating the salience of each card.

The third manipulation was to move the two cards closer together or further apart by 25 deg and then to rotate the altered configuration 45 deg. Changing the distance between the cards did not cause fields to stretch or compress; their shapes were unchanged. This is contrary to most theories of how firing fields are determined by sensory information. In addition, the position of each firing field changed as a function of its distance from the cue cards. Fields near the black card moved along with the black card, fields near the white card moved along with the white card and fields between the cards moved with the mean position of the two cards. Since the relationships between firing fields change, the place cell representation of space may not be uniform and rigid.
Support: NIH Grant NS20686

358.8

CHARACTERIZATION OF PLACE CELL ACTIVITY IN THE HIPPOCAMPUS OF THE FREELY BEHAVING MOUSE T.J. McHugh and M.A. Wilson* Departments of Brain & Cognitive Sciences, and Biology, MIT, Cambridge, MA 02139

As an initial step in our efforts to apply behavioral electrophysiological recording techniques in genetically engineered animals, we have adapted the parallel multi-electrode ensemble recording system developed for use in the freely behaving mouse. Microdrive assemblies containing four independently adjustable tetrodes were implanted on the skulls of wild-type CBL6/J mice and used to monitor the simultaneous activity of between 20-40 individual neurons per animal in the pyramidal cell layer of the CA1 region of the hippocampus. The activity of these neurons were recorded as the mice were exposed to a variety of environments, both novel and familiar. Pyramidal complex spike cells and inhibitory theta interneurons were identified and their firing patterns were examined for place-specific activities. The results of these experiments indicate that the cells in the hippocampus of the mouse behave in a manner very similar to those already characterized in the rat. Pyramidal cells demonstrated place and directional specific firing activity in a variety of environments with low overall firing rates. Upon exposure to novel environments inhibitory theta cell activity markedly decreased during initial exploration. The adaptation of the multielectrode recording system to the mouse will now allow us to examine, on a much more sensitive level, the deficits in learning and memory present in "knock-out" mice generated by gene targeting methods.
Supported by the Seaver Institute and the Sloan Foundation

358.9

EARLY VISUAL DEPRIVATION DOES NOT PREVENT HIPPOCAMPAL PLACE CELL FIRING IN THE RAT. Save, E., Cressant, A., Thinus-Blanc*, C., and Poucet, B. Centre de Recherche en Neurosciences Cognitives, CNRS, Marseille, France.

Previous studies have shown that the location-specific firing of hippocampal pyramidal cells is controlled by visual and non visual cues. Once established, firing fields can be maintained as the animal locomotes through the environment in the absence of salient visual landmarks (Quirk et al., J. Neurosci., 10:2008). Nevertheless, the use of non visual cues may depend on the pre-requisite formation of a stable visuospatial frame of reference. As early visual deprivation has been suggested to markedly alter such build up, we asked whether the hippocampus of totally blind rats would develop normal place cell activity by using non visual cues. Newborn rats were enucleated and four months later were implanted with electrodes. Recordings of hippocampal units were made while the rats were retrieving food pellets in a gray cylinder (76 cm in diameter) containing three different cue-objects placed along the wall in a triangular configuration. Whenever possible, cells were recorded for several sessions allowing verification of the stability of the firing fields (sessions 1 and 2) and manipulation (90° rotation) of the cue-object configuration (sessions 3 and 4). Twenty two cells that displayed location-specific firing were recorded from four rats. Surprisingly, most cells (17) had unstable firing patterns over successive sessions. The activity of the remaining five cells was found to be controlled by the objects. In contrast, all cells recorded in intact rats were controlled by the objects. These results show that place cell activity does not depend on a functional visual system. However, the lack of stability of the firing fields over sessions for most cells suggests that the system might not be able to maintain a stable frame of reference based on non visual cues.

358.10

DISCHARGE OF HIPPOCAMPAL NEURONS (INCLUDING PLACE CELLS) CAN BE TRIGGERED BY PERIODIC LIGHT FLASHES. R.U. Muller*, A.A. Fenton, A. Rotenberg and M. Stead. Dept. of Physiology, SUNY-Brooklyn, Brooklyn, N.Y. 11203

There is a great deal of evidence that the activity of hippocampal place cells is controlled by visual cues in the environment. For example, when place cells are recorded as a rat runs around inside a 76 cm diameter cylinder, the angular coordinate of the cells' firing fields is reliably determined by the angular coordinate of a cue card pasted to the cylinder wall. It is generally observed that rotating the card's position causes firing fields to rotate equally. Seminal work by O'Keefe and Conway (1978) indicates that non-visual stimuli also can control the location of firing fields, although the nature of the control has not been as well explored as for visual stimuli.

Given that place cell activity is so strongly tied to static (unchanging) visual and non-visual cues, we were led to ask if their activity could also be affected by brief, periodically applied stimuli. Accordingly, we recorded from place cells and other hippocampal neurons (including "non-place" pyramidal cells and interneurons) during pellet chasing while a strobe light was flashed at 1Hz or when a loudspeaker click was generated at 1 Hz. In the auditory domain, we found that a rather loud click sounded in either the light or the dark caused no alteration in discharge for any cell so far recorded. In contrast, a strobe flash (presented while the room is otherwise dark) evoked activity from some (but not all) place cells, many theta cells and some (but not all) cells that did not fit either of the other categories. When present, the latency of the response ranged between about 40 and 70 msec. We think that the ability to activate hippocampal neurons with a stimulus that does not affect reward contingencies is really neat and suggests that the hippocampal representation can be be dynamically modified by ongoing events.

Support: NIH Grant NS20686

358.11

FIRING PATTERNS OF HIPPOCAMPAL CELLS IN MICE EXPLORING A CUE-CONTROLLED ENVIRONMENT. Y.H. Cho*, H. Tanila and H. Eichenbaum. Ctr for Behav. Neurosci. SUNY, Stony Brook, NY 11794.

Hippocampal CA1 and CA3 cells were recorded from C57 bl/6 male mice as they navigate in a cue-controlled environment. The apparatus used was a cylinder equipped with a white cue card on its inner wall. To determine to what extent the cue controlled firing patterns of the (place) cells, the cue was either rotated 90° or deleted. Cue manipulation trials were intermixed with baseline trials.

A large proportion of the hippocampal cells exhibited location-specific firing properties. Place fields of some cells either rotated with the cue or remained unchanged, whereas other cells developed different spatial correlates of firing such that the place field either moved to a new place, or rotated to unpredictable angular locations or disappeared. Removing the cue card left the firing patterns unchanged relative to those of the preceding baseline trial or instead created new place fields that, in some cases, covered the zone of the maze where the cue had previously been placed. Furthermore, spatial correlation between the baseline trials decreased as animals experience changes in the environment.

The data indicate that the hippocampal cells in mice also exhibit spatial firing properties similar to those observed in rats, and that constant remapping occurs in the hippocampal spatial representation with repeated changes in the environment. Supported by the Human Frontier Science Program, and NIMH.

358.12

PLACE CELLS RECORDED FROM FREELY MOVING MICE: DIFFERENCES BETWEEN WILD-TYPE AND TRANSGENIC ANIMALS INCAPABLE OF SOLVING A NAVIGATIONAL PROBLEM. A. Rotenberg*,[1] M. Mayford,[2] R.D. Hawkins,[2] E.R. Kandel,[2] and R.U. Muller.[1] [1]Dept. Physiology, SUNY Brooklyn, NY, 11203 and [2]Center for Neurobiol. & Behav., Columbia University, NY, NY 10032.

To address the link between LTP and hippocampal place cells, we have begun to examine transgenic mice expressing a constitutively active form of CaMKII. These mice have selective loss of LTP in the theta frequency range and are unable to solve the Barnes spacial maze (Bach et al., 1995). We therefore asked if the inability to solve the Barnes maze is reflected in the properties of hippocampal place cells, since place cells are thought be essential components of the rat navigational system. Such cells discharge rapidly only when an animal is inside a cell-specific part of the environment (the firing field) and are virtually silent elswhere.

By adapting standard place cell recording methods for mice, we find that place cells in the hippocampus of wild-type mice strongly resemble those in rat: their firing fields are stable across days, and are controlled by sensory stimuli in much the same way as in rat. Preliminary results indicate that place cells in CaMKII transgenic mice have normal appearing firing fields, but that some of these fields are less stable than those in wild-type animals.

Support: NIH Grant NS20686 and HHMI

358.13

HIPPOCAMPAL PLACE FIELD PLASTICITY AND DIRECTIONALITY IN A SPATIAL MEMORY TASK. P.D. Martin and J. O'Keefe*. Department of Anatomy, University College London, London, UK.

Rats were trained in an enclosed "cue controlled environment" (CCE) containing six spatial cues which identified the goal. These cues were rotated by random multiples of 90° between trials to eliminate the relevance of the background cues. After training the rats were able to correctly select the goal platform at the end of the trial even when the six spatial cues were taken out of the CCE mid-way through the trial.

Hippocampal place fields are governed by the six spatial cues such that rotation of the six spatial cues causes concomitant rotation of the place fields (O'Keefe and Speakman, 1987). However upon cue removal the place fields persist. Thus although the place fields are governed by the six spatial cues they are not dependent on them.

In addition to the unit behavior previously recorded in this situation we report two new responses. Some hippocampal place units decease or cease place specific firing upon cue removal. Others increase or initiate place specific firing upon cue removal. Thus for these cells place fields appear to be governed by the six spatial cues in two senses. Their location is dependent on the location of the six spatial cues and their intensity is governed by the presence or absence of the six spatial cues.

Previous work has shown that place fields show directionality when a rat traversed an area in a stereotypical and invariant manner. Rats trained on the CCE spatial memory task were constrained to run in designated directions in order to insure uniform platform coverage. The place fields recorded in this situation were also found to display directionality.

O'Keefe J, Speakman, A. Exp Brain Res. 68: 1-27, 1987.

This research was supported by the MRC (UK) and NSERC (Canada).

358.14

SUBICULAR CELLS RECORDED IN FREELY MOVING RATS SHOW SIMILAR SPATIAL FIRING PATTERNS IN TWO GEOMETRICALLY AND VISUALLY DIFFERENT ENVIRONMENTS. P.E. Sharp*. Dept. of Psych., Box 208205, Yale Univ., New Haven, CT, 06520.

Cells in both the hippocampus and subiculum show location related firing patterns, so that the firing rate of a cell codes for the spatial location of the rat as it navigates (O'Keefe & Dostrovsky, Br. Res, 1971; Barnes et al., Prog. Br. Res., 1990; Sharp & Green, J. Neurosci., 1994). Since the subiculum receives a strong input from the hippocampus, it seems possible that the subicular spatial patterns are simply driven by the spatial signals from hippocampal place cells.

Data presented here, however, suggest that the two areas code space in fundamentally different ways. Spatial firing patterns of individual hippocampal and subicular cells were studied as rats navigated in two different environments: a cylinder and a square, of equal area. For some rats the two chambers had similar visual stimulus characteristics, while for others, the two were very different.

Most subicular cells showed very similar firing patterns in the two chambers, regardless of whether the chambers were visually similar or different. Interestingly, these results are similar to those from entorhinal cells (Quirk et al., J. Neurosci., 1992). In contrast, as predicted by earlier results (e.g. Thompson & Best, J.Neurosci,1989), hippocampal cells showed different patterns in any two chambers.

These results suggest that subicular cells have the ability to transfer a single, abstract spatial representation from one environment to another. This pattern is stretched to fit within the boundaries of the current environment. Thus, the subicular cells seem to provide a generic representation of the geometric relationships between different locations in an environment. This could possibly contribute to abilities such as dead reckoning, and novel route generation.

In contrast, it appears that hippocampal place cells provide a spatial representation which is unique for each environment and which is strongly influenced by the particular stimulus attributes of each environment.

Supported by Whitehall Foundation grant A94-06 to PES.

358.15

EVIDENCE FOR MOTOR COMMAND SIGNAL INFLUENCES ON ANTICIPATORY HEAD-DIRECTION CELLS. B.W. Lipscomb*, H.T. Blair & P.E. Sharp. Dept. of Psychology, Yale University, New Haven, CT 06520-8205.

Head-direction (HD) cells are thought to rely on information about angular head velocity to track directional changes during head turns (McNaughton et al., 1991). HD cells are influenced by passive velocity cues, such as optic flow and vestibular signals (Blair & Sharp, in press; Knierim et al., 1995), and may also depend on motor command (MC) signals during locomotive behavior (Taube, 1995; Wiener, 1993). A major difference between passive signals (vestibular/optic flow) and MC signals is that passive signals are not generated until after the rat begins to turn its head, whereas MC signals may reach HD cells before the rat begins to turn its head.

HD cells in anterior thalamus anticipate the rat's future head direction, and therefore, they shift their directional preference slightly during head turns (Blair & Sharp, 1995). To test for the possible influence of MC signals, we examined the activity of anterior thalamic HD cells during moments just prior to a head turn. We found that on average, cells began to shift their directional preference about 20 msec before the rat began to turn its head. This suggests that anticipatory HD cells may receive MC signals, allowing them to track a turn before it actually begins to occur.

In our accompanying abstract (Blair, Lipscomb, & Sharp), we report that individual HD cells in the anterior thalamus are tuned to anticipate the rat's future head direction by anticipatory time delays (ATDs) ranging from -10 to +50 msec. Interestingly, cells with long ATDs did not begin to signal turns earlier than cells with short ATDs ($r=.11$; p=n.s.). However, the amount by which a cell shifted its preferred direction during the turning state was directly proportional to the cell's ATD ($r=.66$; $P<.001$). We conclude that all HD cells in anterior thalamus begin to signal head turns about 20 msec before the turn begins, but they shift their preferred directions by different amounts during the turn, resulting in different ATDs.

Support: NIMH 1 F31 MH11102-01A1 to H.T.B.; Whitehall Found.A94-06 to P.E.S.

358.16

TEMPORAL TUNING OF ANTICIPATORY HEAD-DIRECTION CELLS IN THE ANTERIOR THALAMUS OF THE RAT. H.T. Blair*, B.W. Lipscomb & P.E. Sharp. Dept. of Psychology, Yale University, New Haven, CT 06520-8205.

Head-direction (HD) cells are tuned to fire action potentials only when a rat's head faces in a specific direction (Ranck, 1984). HD cells in the anterior thalamus are anticipatory, so that their firing rate is best correlated with the rat's future head direction (Blair & Sharp, 1995; Taube & Muller, 1995). Here, we report evidence that each HD cell in anterior thalamus is temporally tuned to anticipate the rat's future head direction by its own specific anticipatory time delay (ATD).

Anterior thalamic HD cells were recorded as rats navigated freely in a cylindrical chamber. The ATD value for each session was measured using an updated version of the method described by Blair & Sharp (1995). The mean ATD for all sessions was 19.5±3.7 msec, but values for single sessions varied greatly, ranging between -7.59 and +74.1 msec. Statistical analysis of 16 cells recorded over repeated sessions revealed that a large portion of this variance was due to variation between different cells, rather than within single cells ($df=15,40$; $F=8.48$; $p<.0001$). That is, the same cell usually showed similar ATDs when it was recorded over multiple sessions, but different cells tended to show different ATDs. This trend could not be accounted for by other variables such as animal, hemisphere, turning speed, turning distance, or session length. Interestingly, each cell's ATD value correlated positively with its directional tuning width ($r=+0.77$, $p<.001$) and negatively with its peak firing rate ($r=-0.43$, $p<0.02$). Thus, cells with longer ATDs had broader tuning widths and slower firing rates than cells with shorter ATDs.

The average ATD value for all anterior thalamic cells we recorded (n=33) was about +20 msec, but individual cells showed values ranging between -10 and +50 msec. This suggests that the anterior thalamus might contain a temporally distributed representation of the rat's future head trajectory over the next 50 msec.

Support: NIMH 1 F31 MH11102-01A1 to H.T.B.; Whitehall Found.A94-06 to P.E.S.

358.17

MICROSTIMULATION OF SCHAFFER COLLATERALS EVOKES SINGLE PYRAMIDAL CELLS AND INTERNEURONS IN THE CA1 REGION OF BEHAVING RATS. M. Stead*, R.U. Muller. SUNY Brooklyn, NY, 11203.

By implanting a bundle of Pt/Ir microwires into the Schaffer collaterals of CA3 and a second bundle of recording electrodes into CA1 we are able to selectively evoke single units in CA1. Stimuli are delivered at low frequencies (0.3-0.5 Hz) as a rat performs a pellet chasing task in a familiar environment while its position is tracked with LEDs mounted on its head. The stimulus intensity is set such that the probability of evoking the unit is near 0.5. This arrangement allows us to probe the excitability of the cell at precise moments in time and space, and to make inferences about the network input to the cell at those times.

Interneurons recorded from stratum oriens of CA1 are evoked at short latency by CA3 stimulation. The post stimulus time histogram (PSTH) also reveals a characteristic 20-25 ms period of inactivity immediately following the evoked activity that we suspect is due to $GABA_A$ inhibition. The type of stimulation necessary to stimulate some interneurons also appears to reset the theta rhythm. Whether this phenomenon is restricted to the vicinity of the recorded cell or is global is not known at this time. The positional excitability of some interneurons shows a high correlation with their spontaneous positional firing rate maps but others show a flat excitability profile as a function of position.

Pyramidal cells in CA1 have a higher threshold of activation than interneurons. PSTHs of these cells also reveal a characteristic 200 ms period of inactivity; we suspect this post-evocation silence is due to $GABA_B$ inhibition. All pyramidal cells investigated so far have been place cells. The positional excitability profiles of place cells have a high correlation with their spontaneous firing rate maps.

We are currently doing drug studies to test our hypotheses about the post stimulus inactivity periods and examining the state of excitability as a function of EEG state.

Support: NIH Grant NS20686

358.18

EXPERIENCE-DEPENDENT MODIFICATION OF BI-NARAL INTERACTIONS IN PIRIFORM CORTEX. D.A. Wilson* and R.M. Sullivan. Department of Zoology, University of Oklahoma, Norman, OK 73019

The anterior piriform cortex (aPCX) receives input from the ipsilateral nare via the ipsilateral olfactory bulb and lateral olfactory tract (LOT). The most direct input from the contralateral nare reaches the aPCX from the contralateral anterior olfactory nucleus (AON) via the anterior commissure. These bilateral inputs are segregated in aPCX layer 1, with ipsilateral LOT terminals on distal portions of pyramidal cell dendrites (layer Ia) and commissural terminals (along with intracortical fibers) on proximal portions of the dendrites (layer Ib). The anterior commissure develops postnatally in the rat, and therefore, might be expected to be particularly sensitive to postnatal experience. The present report examined the effects of unilateral olfactory deprivation on the relative strengths of these bi-naral inputs to the aPCX.

Wistar rats had a single nare sealed on postnatal day 1. On postnatal day 25-35, evoked potentials were recorded in layer 1 of the aPCX to stimulation of the ipsilateral olfactory bulb and the contralateral AON. Recordings were made in both the deprived hemisphere and the undeprived hemisphere of each animal, as well as in naive controls. Evoked potentials recorded in the aPCX to ipsilateral MOB and contralateral AON stimulation were similar, although AON-evoked responses were much smaller in amplitude and longer in latency than MOB-evoked responses. Reduced olfactory stimulation produced a significant depression (30%) of ipsilateral MOB-aPCX responses in deprived hemispheres. In undeprived hemispheres, which received increased airflow during the unilateral deprivation, there was a significant enhancement (>30%) of ipsilateral MOB-aPCX responses compared to controls. Crossed projections were depressed in both hemispheres compared to controls. Preliminary anatomical results suggest early deprivation also modifies the thickness of termination zones, particularly layer Ia.

This research was supported by NIH grant DC01674.

358.19

CHANGES IN THE ELECTROTONIC ARCHITECTURE OF GRANULE CELLS OF THE RAT DENTATE GYRUS WITH MATURATION. M.P. O'Boyle[1,2], N.T. Carnevale[2], and B.J. Claiborne*[1], [1]Division of Life Sciences, University of Texas, San Antonio, TX 78249, and [2]Department of Psychology and Neuroengineering and Neuroscience Center, Yale University, New Haven, CT 06520.

Previous studies have shown morphological changes in the granule cells of the rat hippocampus with maturation (Rihn and Claiborne *Dev. Brain Res.* 54:115, 1990; Blake and Claiborne *Soc. Neurosci. Abstr.* 21:1292, 1995). Here we tested the hypothesis that changes in electrotonic structure accompanied the variations in morphology. Using morphometric data from rats aged 14-17 days (n = 4 neurons), 40-58 days (n = 5), 6 months (n = 5), and 1 year (n = 5) and assuming Rm 26,900 Ω cm^2, Ri 200 Ω cm, and Cm 1 μf / cm^2, we computed *Vin* and *Vout* electrotonic transforms (Tsai et al, 1994, *Network* 5:21). These show the logarithm of voltage attenuation along each branch of a cell for signals spreading toward and away from the soma, respectively. Because of the possible relevance of theta and gamma frequencies to hippocampal function, we computed the transforms at 0, 6 and 100 Hz.

The transforms revealed a significant increase in the maximum attenuation per cell with age. At DC, the *Vout* change was from 0.14 ± 0.01 log units of attenuation (mean ± SD) for the youngest group to 0.25 ± 0.07 for the oldest animals, and the *Vin* increase was from 0.84 ± 0.11 to 1.41 ± 0.15 log units. The 6 Hz transforms were slightly larger than their DC counterparts. At 100 Hz, *Vout* attenuations rose from 0.94 ± 0.04 to 1.60 ± 0.37 log units and *Vin* attenuations increased from 3.02 ± 0.17 to 3.77 ± 0.22 log units between the ages of 14 days and one year. These results suggest that there is a progressive increase in the electrotonic length of granule cells in the dentate gyrus over the first year of life in the rat.

Supported by GM 08194 and the Yale Neuroengineering and Neuroscience Center (NNC)

358.20

SYNAPTIC PLASTICITY IN THE CHICKEN HIPPOCAMPUS.
T.W. Margrie, J.A.P. Rostas* & P. Sah. The Neuroscience Group, Fac. of Med & Health Sci., University of Newcastle, Callaghan 2308, NSW, Australia.

The avian hippocampus has been shown to be important for spatial memory and several types of spatial learning including homing and cache recovery. Since synaptic plasticity is believed to be the physiological mechanism underlying learning and memory, we have investigated excitatory synaptic transmission and synaptic plasticity in the hippocampus of the chicken. Coronal sections (500 μm thick) of adult chicken brain (>12 weeks) were cut in ice-cold Ringer and allowed to equilibrate at room temperature for at least 1 hr before *in vitro* intracellular and whole-cell recordings were made. All recordings were made in the middle 1/3 of the hippocampus at room temperature. Intracellular recordings were made with sharp electrodes filled with 3 M KCl or 3 M $KMeSO_4$ and 2% biocytin. Whole-cell recordings were made with patch pipettes filled with a Cs based internal solution. Bipolar stimulating electrodes were placed along the midline if the hippocampus, superior and inferior to the recording electrode. Cells had a resting membrane potential of -56 ± 3 mV and a input resistance of > 100 MΩ. Single stimuli elicited an excitatory post synaptic potential (EPSP) followed by an inhibitory post synaptic potential (IPSP). The IPSP was blocked by bicuculline (10 μM), indicating that it is mediated by $GABA_A$ receptors. Biocytin-filled cells were identified as multipolar neurons. At their resting membrane potential the EPSP was blocked by 10 μM CNQX indicating that the primary excitatory neurotransmitter at these synapses is glutamate. At +20 mV, the EPSP had a slow component which was blocked by 25 μM D-APV. Tetanic stimulation (2 x 100 Hz seperated by 20 s) induced a large increase in the initial slope of the EPSP (225% at 60 min) which was stable for > 60 min. This potentiation was synapse specific and was not blocked by D-APV (50-100 μM). We conclude that, like the mammalian hippocampus, this structure in birds demonstrates certain forms of plasticity which are believed to be important for learning and memory. (Supported by the National Health and Medical Research Council of Australia)

359.1

FUNCTIONAL OVERLAP AND CONNECTIVITY OF HIPPOCAMPAL NEURONS IN MULTIPLE DISCRIMINATIONS OF ELEMENTAL, CONFIGURAL AND SUCCESSIVE STIMULI IN THE RAT. Y. SAKURAI. *Dept. of Psychology., Toyama Med. & Pharm. Univ., and PRESTO, Research Development Coop. of Japan, Toyama 930-01, Japan.

This study aimed to know whether each individual hippocampal neuron was involved in one, two or all of memory-processes of different features of stimuli. The elemental discrimination task employed four compound discriminative stimuli (AX, AY, BX, BY), simultaneous combinations of two auditory (A, B) and two visual (X, Y) stimuli. The positive stimuli in which the rat had to respond were those which included tone A (AX, AY). In the configural discrimination task, only the combination of A and X (AX) was positive. In the successive discrimination task, a short interval was intervened between the auditory and the visual stimuli and only the combination of A and X (A-X) was positive. Around 30% of the recorded CA1 and CA3 neurons showed differential activation between the positive and the negative discriminative stimuli in one or two of the tasks. There were no neurons which showed such differential activation in all tasks. A cross-correlation analysis among the neurons showed that some of the functional connectivity changed among the tasks.

Supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

359.2

HIPPOCAMPAL ENSEMBLE ACTIVITY DURING DELAYED-NON-MATCH-TO-SAMPLE OLFACTORY DISCRIMINATION IN RATS. S. P. Wiebe* and U. V. Staubli. Center for Neural Science, New York University, New York, NY 10003.

The hippocampus has long been implicated in cognitive processing during learning and performance of working memory tasks. In recent years, evidence has accrued which indicates that this processing is not focused purely on sensory events but rather dependent on conjunctive multimodal input and higher-order, contextual features associated with task contingency. To investigate the nature in which spatial, olfactory and task-specific components are represented in ensemble hippocampal firing patterns during performance of a working memory task, simultaneous recordings of multiple single-units from a microwire electrode array positioned in the CA3 and CA1 fields of the dorsal hippocampus were acquired during execution of a DNMS olfactory discrimination task. Prior to surgery, hooded Long-Evans rats were trained to perform a 2-odor DNMS task within a Y-maze apparatus at delays of 0-50 sec. Sample odors were presented in the stem of the Y-maze, with the location of the match and nonmatch odors in the test phase at the remaining two arms being randomized across trials. Neural spike events were isolated by a DSP-based spike sorter and digitized along with behavioral events. Preliminary data suggest the ensemble spatiotemporal hippocampal encoding of task-relevant events is, to a degree, separable along spatial, olfactory and contextual/cognitive dimensions.

359.3

BEHAVIOR AND NEURONAL ACTIVITY IN RAT HIPPOCAMPUS AFTER LOW AND HIGH FREQUENCY ELECTRICAL STIMULATION M. FUKUDA* and T. ONO**Dept. of Behav. Sci. and Physiol.**, Fac. of Med., Toyama Med. and Pharmaceut. Univ., Sugitani, Toyama 930-01, JAPAN.

Long-term potentiation or long-term depression in the hippocampus is related to learning and memory. We studied the effects of tetanic electrical stimulation on Schaffer collateral fibers on behavior and neuronal activity of CA1 hippocampal cells. Rats motivated by ICSS were trained to explore a circular open-field. ICSS was delivered whenever the animal entered reward areas that were set randomly (RW) or fixedly (SN). In the RW task, spontaneous unit activity was suppressed by tetanic stimulation (100Hz, 100 pulses). Behavior of animals was disrupted, and trails were along the wall of the open field. After training that included 20 trials of the SN task during the course of two days, the stimulation induced a similar suppression of spontaneous unit activity and place field, and impaired movement. However, a place field might be different from the control state after recovery from task performance. The low frequency stimulation (1Hz, 100 pulses) may not induce apparent changes in behaviors and unit activity. These suggest that high frequency electrical stimulation might disrupt spatial information processing in the hippocampus and then alter behavior due to spatial learning. Supported by Japanese Minist. of Edu. Sci. Cult., Grant for Sci. Res., 07680867.

359.4

EFFECT OF INTRA- AND EXTRA-MAZE CUES ON PLACE FIELD LOCATION. D.B. Matthews* & P.J. Best. Cen. for Neuro. Res./ Dept. of Psy., Miami Univ., Oxford OH, 45056.

The hippocampus is critical for spatial cognitive processing as evidenced by the fact that lesions to the hippocampal system impair the learning of spatial tasks. Also, electrophysiological recordings of single pyramidal neurons, place cells, demonstrate that these neurons have spatially sensitive firing characteristic.

The location in which place cells maximally fire, the place field, has been thought to be determined by the constellation of distal, spatial cues. However, it was recently shown that proximal, non-spatial cues (Young Fox & Eichenbaum., 1994; Shapiro, Tanila & Eichenbaum, 1995) or a single distal cue can control the location of the place field (Shapiro *et al.*, 1995). In the former study (Y,F&E) the environment contained no distal cues and a rich array of proximal cues while in the later study (S,T&E) the environment contained a few salient distal cues and a rich array of proximal cues.

The present study investigates if place field location is primarily determined by a constellation of distal, spatial cues or if the location is by influenced by proximal, non-spatial cues in an environment where the saliency of the distal and proximal cues are similar. Preliminary results indicate that the location of the place field is controlled by the constellation of distal spatial cues and is only minimally influenced by proximal, non-spatial cues.

Supported in part with a Pre-doctoral grant to D.B.M. from NIAAA (AA05414)

359.5

DARKNESS DOES NOT PREVENT PLACE NAVIGATION OF RATS ON AN INCLINED ARENA. M. C. Miniaci[1], M. Moghaddam and J. Bures (SPON: ENA). [1]Fisiologia Umana, University of Reggio Calabria, Catanzaro, Italy and Institute of Physiology, Academy of Sciences, Prague, Czech Republic.

Cognitive maps, representing the relationships between any point of the charted environment and prominent landmarks allow the subject to find its position and to plot the shortest path to a known goal. Non-visual place navigation was examined in rats (n=9) trained to find among four equidistant feeders the baited one, located in the center of the upper left quadrant of a tilted (5.7°) arena (1m radius). With the slope direction changing randomly from trial to trial rats required twenty 8-trial sessions to reach the asymptotic level of 88% first choices correct when started from the center. After further training from starts at the lowest, highest and intermediate points at the perimeter of the arena rats continued to choose the correct feeder in 66% first choices. Since darkness did not impair navigation, rats were obviously able to recognize the start position according to the angle between the wall of the arena and the direction of the slope and to use their cognitive map of the arena to plot the course to the goal. This assumption was confirmed by starting the rats in darkness from randomly distributed points about 30 cm distant from the wall and the nearest feeder. Computer plotted start-goal tracks showed that the rats typically moved up the slope till they reached the wall and took then a more direct course to the goal. It is concluded that the vestibular orienting gradient allows path integration based navigation from the perimeter of the arena to any point of its surface. Supported by grants IGA AVCR 711401, BMFT 01VJ 920015/26-5a and Fondazione Bonino-Pulejo.

359.6

PROACTIVE INTERFERENCE EFFECTS DURING SPATIAL TASK PERFORMANCE IN RATS. N.Kurzina*, A.Kozlov, L.Bakanova, M.Druzin, E.Malinina Dept.Psychophysiology,Ukhtomsky Physiol.Inst.,St.Petersburg Univ.,Pavlov Physiol.Dept.,Inst.Exptl.Med.,St. Petersburg, Russia

Effects of the preceding conditional reaction on the present conditional act performance were studied. Rats were trained delayed and non-delayed spatial task in Y-shaped maze. Two error types were analyzed. First type errors,when rats run to the same arm of the maze as in the previous trial were termed as repetition errors (RE). Second type errors when rats run to the other arm of the maze in comparison with the previous trial were termed as errors of alternation (AE). It was shown that RE occurs more often under a short intertrial interval (II)and non-delayed choice.The AE occurs more frequently under a long II and delayed choice. The data obtained support the idea that not only the cue presentation and delay periods are important for a successful task performance, but the whole structure of the task influences the organization of complex behavioral acts.

359.7

SPATIAL LEARNING AND MEMORY IMPAIRMENT IN AGED RATS: CORRELATION WITH IMMUNOLOGICAL ANGIV BINDING WITHIN CA1 PYRAMIDAL CELLS OF THE HIPPOCAMPUS.

E. A. Kramár,*[1] E.S. Pederson,[1] A.V. Miller-Wing,[2] L. Stubley-Weatherly,[3] A.E. Ball,[1] J.W. Harding[1,2,3] and J.W. Wright[1,2,3]. Program in Neuroscience[1] and Departments of Veterinary and Comparative Anatomy, Pharmacology and Physiology[2] and Psychology[3], Washington State University, Pullman, WA 99164.

Since the initial discovery of a novel angiotensin receptor subtype (AT_4) that binds to Angiotensin IV(AngIV) in bovine adrenal tissue, autoradiographic studies have revealed binding sites for AngIV in numerous species and tissues including, guinea-pig and rat hippocampus, neocortex and cerebellum; these structures are classically associated with learning, memory and motor function. Cognitive testing in the rat has shown that intracerebroventricular(icv) AngIV treatments can enhance both memory acquisition and retrieval in a passive-avoidance paradigm. Along these lines, icv AngIV treated rats have displayed Fos-like immunoreactivity localized within the pyramidal cell layers of the hippocampus suggesting that AngIV is effective in activating specific brain neuronal pathways associated with cognitive function.

The present study utilized a circular water maze task to investigated learning and memory in aged Sprague-Dawley, Wistar-Kyoto and Spontaneously Hypertensive Rats. Our results indicate that the greatest learning and memory deficits were observed in 2 yr old rats, followed by 1.5 yr old rats as compared to 1 yr old rats. Immunoreactivey to AngIV in the CA1 region of the hippocampus indicates that significant differences exist among aged rats. Taken together, these results suggest that the cognitive impairment seen in aged rats may be due to a decrease in number and affinity of the AT_4 receptor site.

359.8

ORAL INGESTION OF LEAD IMPAIRS SPATIAL LEARNING

B.F. Petrie* Department of Psychology, New Mexico Tech, Socorro, NM, USA 87801

Long-Evans rats were given ad lib access to lead (Pb) in their drinking water (250ppm) for 90 days. These animals were then tested for acquisition and retention of a moving platform place task version in the Morris water maze, and their performance was compared to Long-Evans rats that had been given ad lib access to distilled water only. All animals were swum for 18 days (8 trials per day; 60 seconds maximum per trial), with the platform being moved from one discrete location to the next, in a systematic daily order. On 16 of the 18 days, the Pb exposed rats took longer on average to find the platform than did the control rats. Analysis of the swim paths indicated that the Pb animals selected ineffective strategies, consistent with the hypothesis that chronic lead ingestion in rodents impairs cholinergic function.

Supported by NMT seed fund.

359.9

VISUAL DISCRIMINATION LEARNING TASK INCREASES REM SLEEP.

Z. Xie,* R. A. Stickgold, E. Pace-Schott, J.A. Hobson. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, Boston, MA 02115

The relationship between REM sleep and learning was studied in nine subjects. Their sleep states were recorded for six days using the Nightcap, a two-channel recording device which distinguishes wake, REM sleep and NREM sleep. The average of Nightcap-measured sleep stage percentages recorded on the first three nights served as baseline. Subjects performed the human visual discrimination (HVD) task[1] on Day 4 between 8:00 p.m. and 10:00 p.m. and subsequent changes in sleep examined on Nights 4 through 6.

Our findings were as following: (1) There was no significant difference in % REM sleep among the first three days (ANOVA, $F[2, 12] = 0.99$, $p = 0.40$, n.s.). (2) Baseline % REM sleep was comparable to % REM sleep on Nights 5 and 6. (3) The % REM sleep on the night following HVD task training (41.3% ± 2.8, MEAN ± SEM) was significantly increased as compared to baseline (33.4% ± 2.1), Night 5 (35.3% ± 3.7) and Night 6 (31.4% ± 4.0)($F[3,24] = 3.06$, $p<0.05$, post-hoc LSD test).

Karni and Sagi[2] have shown that learning on the HVD task occurs during REM sleep on the night following training. Our results suggest that HVD task training also increases the amount of subsequent REM sleep, suggesting a bi-directional interaction between REM sleep and HVD learning. (*Supported by NIMH #MH-48,832 and MacArthur Foundation Mind Body Network).*

[1]Karni, A. & Sagi, D. (1991) *Proc. Natl. Acad. Sci. (USA)* **88**:4966-4970.
[2]Karni, A. *et al.* (1994) *Science* **265**:679-682.

359.10

SLEEP EFFECTS IN ELECTROPHYSIOLOGICAL MEASURES OF MEMORY IN INFANTS.

M.R. Letterman*, V. M. Little, and D. G. Thomas. Department of Psychology, Oklahoma State University, Stillwater, OK 74075.

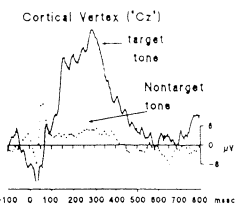
Event-related potential (ERP) data from awake 5-month-old infants showed that average ERP peak amplitude increased from Day 1 to Day 2 for a familiar stimulus but not for a novel one. Trial-to-trial latency variability decreased from Day 1 to Day 2 for the familiar stimulus but not for the novel. This pattern of results was most robust at Fz, less so at Cz, and was not found at T3. The present study used this same paradigm to investigate 24-hr memory in 5-month-old infants during a quiet sleep state. On Day 1, ERPs were recorded to 100 identical auditory stimuli (400 Hz or 700 Hz tones). On Day 2, 50 of the familiar stimuli and 50 novel stimuli were presented. The amplitude of the N2 peak of the average ERP was larger on Day 2 for the familiar stimuli compared with ERPs on Day 1 at the T3 electrode site. There was a larger average amplitude of the positive peaks (P2 and P3) on Day 2 for the familiar stimuli compared with Day 1 stimuli at the Cz electrode site. This response is similar to that found in same-age awake infants; however, the pattern is less robust. Analyses of latency variability indicated a non-significant trend for decreased variability from Day 1 to Day 2 for the familiar stimuli but not for the novel. The trend for a decrease in variability for the familiar response on Day 2 corresponds to that found with awake infants. This suggests that the organization of the neural ensemble on Day 2 is less well-defined with sleeping subjects than with awake infants. However, there is a trend towards a more stable (more "experienced") and less variable neural response. The research reported here was aided by Social and Behavioral Grants 12-0877 and 12-FY95-0025 from the March of Dimes Birth Defects Foundation.

359.11

A METHODOLOGY FOR PRODUCING A P-300 EVOKED POTENTIAL IN RATS WHICH WAS ELIMINATED BY EXTINCTION AND RECOVERED DURING RE ACQUISITION OF A DISCRIMINATED OPERANT RESPONSE.

W. D. Klipec*, S. Faux, L. Sauer, C. Corey, J. Miskimins, E. Hegel and S. Lebeda
Department of Psychology, Drake University, Des Moines, IA 50311

While an extensive literature documents the P-300 evoked potential in humans, only a few studies have demonstrated P-300 like evoked potentials in rats. In the present experiment, stainless steel electrodes, insulated except for 2-mm at the tip, were implanted 1mm into the brain, 1mm (Pz) posterior to Lambda, and 3-mm (Cz) and 7-mm (Fz) anterior to Lambda. Two additional electrodes implanted 8-mm anterior to Fz on each side of the midline served as an indifferent (Nz) and an isoground. Rats were shaped to press a lever for food reinforcement where a 4.4 -KHz target tone cued the insertion of the bar which retracted following a single reinforced response. The target tone was presented on a variable time (VT) 30-sec schedule, while a 2-KHz nontarget tone was presented on a truly random VT 15-sec schedule. Following extensive training, a strong P-300 response was observed at Fz, Cz (see insert) and Pz. Following additional training, a single session was conducted in which a baseline P-300, recorded at all three sites, was substantially diminished when the target tone failed to predict lever insertion (extinction), and reinstated when the lever was reactivated (reacquisition). The methodology necessary to produce a robust P-300 response in rats, presented in detail in the poster, makes possible pharmacological manipulation of the P-300 response.



359.12

A LONGITUDINAL STUDY OF INFANT AUDITORY EVENT-RELATED POTENTIALS.

V. M. Little*, M. R. Letterman, and D. G. Thomas. Dept. of Psychology, Oklahoma State University, Stillwater, OK 74078.

Recently, investigation into the neurophysiological aspects of memory has extended to recognition memory in human infants. Previous studies suggest that auditory ERP peak latencies and latency variability show a general decrease while peak amplitudes show a general increase during development. In this study, event-related potentials (ERPs) were obtained from 24 infants at 5, 8, 11, 14, and 17 weeks of age. Auditory ERPs were recorded from frontal (Fz) and central (Cz) electrodes. Changes in the average ERP amplitude (component N2) were assessed in relation to latency variability and single-trial amplitude. Results indicate significant developmental trends in the average amplitude, average latency, latency variability, and single-trial amplitude. Changes in the average amplitude were primarily accounted for by single-trial amplitude and secondarily, but also significantly, by latency variability. This suggests the magnitude of the response and temporal consistency, to a lesser degree, explain most of the change in the ERP average amplitude. The research reported here was aided by Social and Behavioral Sciences Research Grants 12-0877 and 12-FY95-0025 from the March of Dimes Birth Defects Foundation and by Oklahoma Center for the Advancement of Science and Technology Contract 1690.

359.13

NEURONS IN HUMAN TEMPORAL CORTEX THAT DIFFERENTIATE BETWEEN WORD PAIR ASSOCIATIONS THAT WILL OR WILL NOT BE SUBSEQUENTLY LEARNED BY AN INDIVIDUAL SUBJECT G. Ojemann*, J. Schoenfeld-McNeil. Dept of Neurol Surg, Univ of Washington, Seattle WA 98195

In our previous study of neuronal activity in human temporal neocortex during paired-associate learning (NeuroReport 6:685, 1995), neurons that changed activity with overt word reading showed overall activity when word pair associations were learned that differentiated subjects who learned pairs rapidly from those who learned pairs poorly, with high levels of activity characterizing rapid learners. This effect was also evident on the initial presentation of the word pairs, though few pairs were actually learned then (unpublished). In recordings from the same area in a new series of subjects during the same paired associate test, we have identified a subset of neurons inhibited by word reading compared to a visual perception control, that increased activity during paired associate learning with significantly greater levels of overall activity on the initial presentation of those pairs that this subject will learn rapidly and reliably compared to pairs that the same subject will learn unreliably or not at all. Neurons of this type have been recorded from both temporal lobes in separate subjects; they did not all show significant changes in activity during recent memory for the same words. These neurons are part of the pool of neurons establishing verbal associations in humans.

Supported by NIH Grant NS 21724

359.14

Reduced Orienting Response after Human Hippocampal Damage R.T. Knight*, Department of Neurology and Center for Neuroscience, University of California, Davis, Martinez Veterans Medical Center, 150 Muir Rd., Martinez, CA, 94553

Lesion and intracranial data have shown that the hippocampal formation is part of a cortico-limbic network subserving the orienting response. Focal hippocampal damage alters central markers of the orienting response as revealed by reductions in the fronto-central scalp P300 response to unexpected visual, auditory and somatosensory stimuli. Intracranial recordings from hippocampal electrodes report large, habituating responses to novel somatosensory stimuli. In the current experiment, sympathetic skin potentials (SSR) to irrelevant wrist shocks were recorded in normals and patients (n=6) with unilateral damage in the posterior hippocampus due to infarction of the artery of Uchimura. Electrical shocks set to elicit an opponens pollicis twitch were delivered randomly within an interstimulus interval of 12-20 seconds while subjects viewed a silent movie. Normals generated a large (7 mV) skin potential to the shock stimuli. The SSR was severely reduced in the hippocampal patients (1 mV; $P< 0.02$). The results suggest the hippocampal formation is part of a system that maintains a template of recent sensory events. Sufficient deviations from this code initiate a peripheral and central orienting response to facilitate processing of unexpected environmental events. Supported by NINDS grant NS21135.

359.15

SEMANTIC PRIMING IMPAIRMENT IN HIV L Nielsen-Bohlman*, D Boyle, C Biggins, F Ezekiel & G Fein Psychiatry Dept., UCSF & Veterans Administration Medical Center, San Francisco

HIV+ subjects have shown impairment on tests of automatic attention and verbal processing. This verbal impairment may be associated with disruption of automatic semantic activation. We examined behavioral and event-related potential (ERP) measures of priming in a lexical decision task in which all words had an obvious antonym ('deep') and two-thirds were presented as sequential antonym pairs ('enter' 'exit'). The 39 HIV+ and 22 HIV- subjects had no history of substance abuse, neurologic or psychiatric disorder unrelated to HIV. HIV+ subjects were divided into cognitively normal and cognitively impaired subgroups on the basis of a neuropsychological battery of 15 tests. Unlike control and cognitively normal HIV+ subjects, cognitively impaired HIV+ subjects showed no evidence of reaction time priming (unrelated versus primed words, control $t_{20}=3$, $p<0.005$, normal HIV+ $t_{15}=5$, $p<0.001$, impaired HIV+ $t_{22}=1$; group by stimulus interaction; impaired HIV+ vs control, $F_{1,40}=4$, $p<0.05$, vs. normal HIV+ $F_{1,37}=9$, $p<0.01$). Cognitively impaired HIV+ subjects also showed a reduction in N4 amplitudes at frontal sites (at Fz, unprimed-primed difference wave, 375-425 msec, control -2.4 μV, normal HIV+ -1.8 μV, impaired HIV+ -0.6 mV, control vs. impaired, $F_{1,40}=4$, $p<0.05$, normal vs. impaired $F_{1,37}=3$, $p=0.09$). The associated reduction of reaction time priming and N4 amplitude in cognitively impaired HIV+ subjects suggests that the processing of linguistic stimuli in these patients may involve reduced activation semantic networks.

Support: NIMH award MH45680, Dept. of Veterans Affairs General Medical Research Fund & Career Research Scientist Award

359.16

AN ON-LINE NEUROPHYSIOLOGICAL AND BEHAVIORAL DATABASE FOR THE NEUROSCIENTIST, M.A. Arbib*, J.S. Grethe, G.L. Wehrer, J.R. Mureika, J. Tracy, X. Xie, R.F. Thompson, T.W. Berger.
Neurosciences Program, Univ. of Southern California, Los Angeles, CA 90089-2520.

In order to address the problems associated with managing the increasingly large datasets of neurophysiological data collected in laboratories around the world, a neurophysiological database has been developed by the University of Southern California Brain Project (USCBP). The USCBP neurophysiological database consists of two primary components: 1) The *database component*, a novel extendible object-relational database schema implemented in Illustra (J.S. Grethe et al. 1996). A time series datatype has been constructed, implemented in Illustra as a datablade which defines a new base type and associated functionality, that allows neurophysiological data to be stored and manipulated in the database. 2) An *on-line notebook* that provides a laboratory independent "standard" for viewing, storing and retrieving data across the internet. The notebook interface was developed using Java and HTML 3.0 specifications. Currently, two different neurophysiological experiments, from two laboratories involved in the USCBP, have been brought on-line.

The first experimental dataset concerns single unit recordings from neurons in the deep cerebellar nuclei. This data is used to construct a single unit map of the response patterns of these cells following eyeblink conditioning in the rabbit (J. Tracy 1995). The second experimental dataset concerns electrophysiological recordings at the perforant path-dentate granule cell synapses in hippocampal slices of adult rabbits (X. Xie 1996). Field EPSPs are recorded in the molecular layer of the DG and are evoked by stimulating perforant path axons. Electrical and pharmacological manipulations are added to the preparation to investigate the induction of LTP and LTD.

On-line information can be found at http://www-hbp.usc.edu:8376/HBP/TSDB/

Supported in part by the Human Brain Project Consortium grant NIMH (5P01MD/DA52194), NSF (IBN-9215069) to RFT, NIMH (MH00343) to TWB and an NSF Graduate Research Fellowship to JSG

359.17

AN EXTENDIBLE OBJECT-RELATIONAL DATABASE SCHEMA FOR NEUROPHYSIOLOGICAL AND BEHAVIORAL DATA, J.S. Grethe*, G.L. Wehrer, R.F. Thompson, T.W. Berger, M.A. Arbib.
Neurosciences Program, Univ. of Southern California, Los Angeles, CA 90089-2520.

To address the problems associated with managing the increasingly large and diverse datasets collected throughout the neuroscience community, a novel extendible object-relational database schema has been developed. Since one can not hope to describe a priori all the experimental protocols and research data that neuroscientists will want to incorporate in a neurophysiological database the core database design needs to be easily modified to meet a specific laboratory's needs without having to make major modifications in the database's overall structure. By exploiting the object-oriented properties of new database technologies one is able to create a database schema that is completely modifiable while maintaining a standard core structure. To achieve this, the core database schema contains various super-tables that allow user defined sub-tables to be attached to the core database schema in an inheritance hierarchy. Researchers can use this method to extend their database to contain relevant information concerning the research subjects used in their laboratory, the experimental data collected, the experimental protocols used and any annotations or statistics (metadata) normally included in their experiments.

Another important issue that must be addressed is that there will not be a single monolithic database which will store all neuroscience data. Rather, there will be a federation of databases throughout the neuroscience community. In order to be able to connect and access other databases, certain "hooks" have been included in the core database schema to foster such communication.

On-line information can be found at http://www-hbp.usc.edu:8376/HBP/TSDB/

Supported in part by the Human Brain Project Consortium grant NIMH (5P01MD/DA52194), NSF (IBN-9215069) to RFT, NIMH (MH00343) to TWB and an NSF Graduate Research Fellowship to JSG

360.1

CENTRAL EFFECTS OF GONADOTROPIN-RELEASING HORMONES (I AND II) AND BETA-ENDORPHIN ON COURTSHIP BEHAVIOR IN FEMALE WHITE-CROWNED SPARROWS. D. Maney*[1], R. D. Richardson[2], and J. C. Wingfield[1], Program in Behavioral Neuroscience and Department of Zoology[1], and Department of Psychology[2], University of Washington, Seattle, WA

Like most vertebrates, birds have two forms of gonadotropin-releasing hormone (GnRH). Chicken GnRH-I is released at the median eminence to elicit gonadotropin release. Chicken GnRH-II, though its function is unknown, is hypothesized to act as a neurotransmitter controlling reproductive behavior. In the present study, we chronically cannulated the third ventricle of female white-crowned sparrows to test the effects of both forms of GnRH on courtship behavior. Female sparrows give a copulation solicitation display characterized by fluttering the wings while raising the tail and head. This behavior can be elicited in captive, estrogen-primed white-crowned sparrows by playing a recording of male song. We quantified the behavioral response to recorded song 30 minutes after i.c.v. infusion of GnRH-I, -II, and saline. GnRH-II, but not GnRH-I, increased solicitation behavior compared with saline. This suggests a central role for GnRH-II in reproductive behavior that may be independent of GnRH-I.

In addition, solicitation was suppressed by infusion of beta-endorphin, an endogenous opioid known to inhibit reproductive function and behavior in other vertebrates. This suppression was reversed by infusion of the opiate antagonist naloxone. Naloxone alone caused an increase in solicitation, suggesting that endogenous opioids may tonically inhibit the behavior. We discuss a multisynaptic model for the neuroendocrine control of solicitation display and its regulation during stress.

Supported by NSF IBN9408013

360.2

MEASUREMENT OF HYPOTHALAMIC SEROTONIN OVERFLOW IN STEROID-PRIMED RATS DURING LORDOSIS BEHAVIOR. S. Voorhis, T. Westin, D. Liu and K. Renner*, Department of Biology, University of South Dakota, Vermillion, SD 57069.

In an earlier study we demonstrated that progesterone (P) treatment decreased extracellular serotonin in the hypothalamus of anesthetized rats primed with estrogen (Farmer et al., Brain Res, 711:8-4, 1996). To further examine the effects of P-influenced changes in hypothalamic 5HT function, we monitored 5HT overflow in steroid-primed females during lordosis behavior using microdialysis. In addition, we simultaneously monitored behavior and extracellular hypothalamic 5HT after pharmacological manipulations.

Two to six days after implantation of a guide cannula directed towards the ventromedial hypothalamus, rats were primed with 5 µg E. The rats were implanted with a dialysis probe 21 hrs later and injected with 0.5 mg P. In the first experiment, lordosis behavior was tested after the 5HT baseline was stable for 3 consecutive samples. Hypothalamic 5HT overflow was not significantly altered during or following the behavioral test. In the second experiment, steroid-primed females were pretested for behavior 4 hrs following implantation of the dialysis probe. These rats were treated with ip saline or 3 mg/kg of 1-(m-trifluoromethylphenyl)piperazine (TFMPP), a concentration of the 5HT receptor agonist which elevates hypothalamic 5HT in males (Auerbach et al., Neuropharm., 30:307, 1991). TFMPP treatment completely blocked lordosis behavior and increased hypothalamic 5HT approximately 600 % in the first post-treatment sample. The drug effects on both hypothalamic 5HT and behavior were reversed after 3 hrs. Additional doses of TFMPP are being evaluated. These results suggest that the proposed facilitatory role of 5HT is not mediated as a result of increased 5HT release in the hypothalamus during mating. The results obtained with TFMPP are consistent with an inhibitory role of hypothalamic 5HT in the regulation of lordosis but evaluation of the effects of lower drug doses is required. (supported by NSF grant IBN-9596009 and HHIM 71195-539501)

360.3

D_1 DOPAMINE RECEPTOR AGONIST (SKF38393) INDUCTION OF FOS-IR IN PROGESTIN RECEPTOR CONTAINING AREAS IN FEMALE RAT BRAIN. J.M. Meredith*, A.P. Auger, J.D. Blaustein. Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Intracerebroventricular (i.c.v.) and intrahypothalamic injections of dopamine and dopamine receptor subtype agonists facilitate lordosis in estrogen-primed female rats. The D_1 receptor specific agonist, SKF38393, appears to facilitate lordosis in estradiol-primed female rats *via* a progestin receptor (PR)-mediated pathway. The current experiment was designed to examine whether SKF38393 induces Fos-immunoreactivity (Fos-Ir) in PR containing areas of the female rat brain associated with lordosis. Rats were ovariectomized, and guide cannulas were inserted immediately above the third ventricle. Ten to 14 days following surgery, animals were injected with 5 µg estradiol benzoate. Forty eight hours later animals were injected i.c.v. with 100 ng of SKF38393 (a dose previously reported to facilitate sex behavior in estradiol primed rats) or saline. One hour following injection animals were perfused and brain sections immunostained for Fos and Fos/PR. Sections were analyzed using computer-assisted image analysis for total number of Fos-Ir cells and for changes in optical density (OD) of Fos-Ir cells. SKF38393 increased Fos-Ir cell number in the mid-ventromedial hypothalamic nucleus/ventrolateral aspect (mVMHVL), the caudal VMHVL, the paraventricular nucleus and the caudate putamen. SKF38393 also increased OD of Fos-Ir cells in the medial preoptic area, the rostral VMHVL and the arcuate nucleus (Arc). SKF38393 did not increase Fos-Ir cell number or OD in the medial preoptic nucleus (MPO), the dorsomedial hypothalamic nucleus, the medial amygdala or the ovarian receptor containing area associated with the rVMHVL that extends dorsolaterally towards the fornix (rVMHVL-CRA). SKF38393 increased Fos-Ir in a population of PR-Ir cells in the cVMHVL but not in the rVMHVL-ORA, MPO or the Arc. (MH10650, NS19327 and RSA MH00850).

360.4

C-*FOS* EXPRESSION IN BRAINS OF MATED AND UNMATED FEMALE RATS AND MACAQUES. R. P. Michael*, A. N. Clancy and D. Zumpe. Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, GMHI, 1256 Briarcliff Rd., Atlanta, GA 30306.

Immunoreactive c-*fos* expression was examined in brains of sexually experienced, ovariectomized rats treated with estradiol and progesterone and intact rhesus monkeys. Comparisons of the labeling distributions were made between animals that were either mated or unmated. Stimulus males were allowed to ejaculate with sexually receptive females; females were then killed by an overdose of sodium pentobarbital within an hour after ejaculation and perfused transcardially. Brains were dissected for immunocytochemical processing. Unmated females were not paired with stimulus males but were killed, perfused and processed as controls. Adjacent sections were incubated with three different anti-c-*fos* antibodies and sections from mated and unmated monkeys and rats were processed concurrently by the ABC immunoperoxidase method using identical immunoreagents. In both species, c-*fos* was observed in the nuclei of neurons in several brain areas including the medial preoptic area, several hypothalamic regions such as the paraventricular, ventromedial and arcuate nuclei, and the medial amygdala. In rats, with each antibody, c-*fos* expression occurred in recently-mated but not in unmated animals. Preliminary analysis of monkey sections also showed that mated monkeys expressed more c-*fos* labeling than unmated animals, but two of the anti-c-*fos* antibodies produced some labeling in the brains of unmated animals. A polyclonal sheep anti-c-*fos* antibody obtained from Cambridge Research Biochemicals produced the largest difference between mated and unmated monkeys. Results suggest that in primates with complex neocortical development and cognitive skills, c-*fos* in brain regions implicated in sexual behavior may also be expressed in unmated females when they are maintained in rooms together with other conspecifics. (Supported by USPHS Grant MH 19506.)

360.5

EFFECTS OF PELVIC AND PUDENDAL NERVE TRANSECTION ON FOS INDUCTION IN FEMALE RAT BRAIN FOLLOWING VAGINOCERVICAL STIMULATION. C.B. Coopersmith*, C. Manitt, & J.G. Pfaus. CSBN, Department of Psychology, Concordia University, Montréal, QC, Canada H3G 1M8.

In the female rat, the genito-somatosensory input received during sexual stimulation is conveyed to the central nervous system primarily through the pelvic and pudendal nerves. The present study examined the relative contribution of both nerves in the expression of Fos immunoreactivity (IR) within brain regions previously shown by us and others to induce Fos IR after mating or manual vaginocervical stimulation (VCS). Bilateral transections of either the pelvic or pudendal nerves, in addition to sham neurectomies, were performed on ovariectomized, sexually-experienced rats. After recovery, the females were primed with estradiol benzoate and progesterone and given either 50 manual VCSs with a lubricated glass rod over the course of 1 hr or allowed to mate in a bilevel chamber for 1 hr with a sexually vigorous male. Females were sacrificed 75 min after the first VCS or intromission and their brains processed for Fos immunochemistry. Overall, in sham-neurectomized females allowed to mate or given VCS, Fos IR was increased in different brain regions compared to unstimulated controls. Bilateral transection of the pelvic, but not pudendal, nerve reduced Fos IR in many of these areas, including the mPOA, lateral septum, BNST, VMH, and MEA. All females given VCS, except those with pelvic neurectomy, displayed a characteristic neck hunching and immobility during VCS. These data confirm that the pelvic nerve is largely responsible for the neural and behavioral effects of VCS. *Supported by the Medical Research Council of Canada (MT-13125).*

360.6

VAGINOCERVICAL STIMULATION INDUCES FOS WITHIN GLUTAMATE-CONTAINING NEURONS OF THE RAT VMH. J.G. Pfaus* and C. Sabongui. CSBN, Department of Psychology, Concordia University, Montréal, QC, Canada H3G 1M8

Fos is induced within the ventrolateral aspect of the ventromedial hypothalamus (VMH) in female rats following copulation with intromission or manual vaginocervical stimulation (VCS). Although the VMH is considered facilitatory for lordosis, infusion of glutamate to this region inhibits lordosis. Interestingly, moderate-to-large amounts of VCS facilitate pacing at the expense of lordosis, and lead to increased rejection responses and a faster termination of estrus. Given that large amounts of VCS or glutamate infusions to the VMH both decrease lordosis, we examined whether Fos is co-localized with glutamate in the VMH following different amounts of VCS. Ovariectomized rats, primed either with estrogen and progesterone or the oil vehicle, were given 1, 10, 30, or 50 VCSs over the course of an hour with a lubricated glass rod, after which their brains processed for Fos immunoreactivity. A significant proportion of Fos cells in the ventrolateral VMH was co-localized with glutamate across all VCS conditions in the oil-treated females but far less co-localization was observed across conditions in the hormone-treated females except following 50 VCSs. These data suggest that hormone treatment blunts the ability of VCS to activate glutamate neurons in the VMH. *Supported by the Medical Research Council of Canada (MT-13125).*

360.7

IMMUNOCYTOCHEMISTRY FOR FOS AND TYROSINE HYDROXYLASE FOLLOWING MATING IN FEMALE HAMSTERS. S.M. Ramos*, J.F. DeBold. Psychology Department, Tufts University, Medford, MA 02155.

Several lines of evidence suggest that dopamine (DA) is involved in sexual receptivity. It has been shown that estradiol regulates specific DA receptor levels and DA receptor binding in the striatum of female rats. In hamsters, DA levels increase in the striatum and nucleus accumbens during lordosis. However, the role of DA in female mating behavior has not been fully elucidated. Fos, an indicator of neuronal activation, is induced in specific brain areas as a result of factors associated with mating. To determine changes in the activity of dopaminergic neurons as a result of these factors, ovariectomized female hamsters were given hormone replacement and (1) placed alone in new cage for 10 minutes, (2) allowed to receive 15 mounts only from a male, or (3) allowed to receive 15 mounts with intro-missions from a male. Double immunocytochemistry to Fos (rabbit polyclonal anti c-fos, Santa Cruz Biotechnology, Santa Cruz, CA) and tyrosine hydroxylase (TH) (mouse monoclonal anti tyrosine hydroxylase, Sigma Immuno Chemicals, St. Louis, MO) was then performed on the brain tissue. Areas assessed to date include: the ventral tegmental area, substania nigra, interfascicular nucleus, lateral habenula and paraventricular nucleus. In the LHb only cells expressing Fos were detected. Analysis of the VTA, SN, IFN and PVN is preliminary but suggests that these areas contain cells which express Fos under these conditions and those which contain TH, and that these populations of cells are, in general, distinct from each other.
Supported by NSF grant BNS 9511895 to JFD.

360.8

A POTENTIAL ROLE OF cGMP IN THE REGULATION OF LORDOSIS BEHAVIOR IN FEMALE RATS. H. -P. Chu*, J. C. Morales, and A. M. Etgen. Depts. Neurosci. & Psychiat., Albert Einstein Coll. Med., Bronx, NY 10461

Nitric oxide (NO) has been suggested to play a crucial role in the regulation of lordosis via activation of cGMP accumulation (Proc. Natl. Acad. Sci. USA. 91: 6468-6472, 1994). Whalen and Lauber (Neurosci. & Biobeh. Rev. 10: 47-53, 1986) hypothesized that many agents known to facilitate lordosis act through cGMP. 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), which has been shown to selectively inhibit NO-stimulated cGMP production (Mol. Pharmacol. 48: 184-188, 1995), was used to investigate the effects of cGMP on lordosis behavior. Female rats were ovariectomized bilaterally and implanted with cannula aimed at the lateral or third ventricles by stereotaxic surgery. Behavioral testing was performed seven days later, after animals were injected subcutaneously with 2 μg of estradiol benzoate 48 and 24 hours followed by 200 μg of progesterone 4 hours prior to the testing. Animals with lateral ventricle cannulation were given ODQ (1 μl of 80 μM) or vehicle (1 μl of 8 mM DMSO) at the time of progesterone treatment or 20 min before testing. ODQ significantly decreased lordosis quotient (LQ) and quality of lordosis (QL) at both intervals of drug infusion (LQ: $p = 0.001$; QL: $p < 0.001$; $n = 3 - 7$). Locomotor activities, measured as line crossing and rearing in an open field, were not affected by ODQ. DMSO, however, seemed to increase rearing, because animals with ODQ or vehicle administration showed higher rates of rearing in the open field than animals without any infusion. ODQ also strongly inhibited LQ and QL in the animals receiving drug in the third ventricle ($n = 2$ for both drug and control groups). We conclude that cGMP is an important modulator of female rat sexual behavior.
This study is supported by Grants MH41414 and RSDA MH00636.

360.9

SEXUAL RESPONSE OF FLINDERS LINE FEMALE RATS. G. P. Dohanich*, J. M. Daniel, A. J. Fader, S. C. Wolff, P.M. Gallogly, and D. H. Overstreet.[1] Department of Psychology, Tulane University, New Orleans, LA 70118 and [1]Skipper Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599.

The Flinders Sensitive Line of rats was bred for increased cholinergic sensitivity as indicated by enhanced physiological and behavioral responses to cholinergic agonists (Overstreet, *Neurosci. Biobehav. Rev. 17:51,* 1993). In this study, the sexual responsiveness of Flinders Sensitive Line (FSL) females was compared to that of Flinders Resistant Line (FRL) and Long Evans (LE) females. Ovariectomized FSL females exhibited significantly higher incidences of lordosis and solicitation behaviors than ovariectomized FRL and LE females over a range of estrogen doses (2, 3, 4, 5, 20 μg/kg at 48 hours before testing) in combination with progesterone (1, 2 mg/kg at 5 hours before testing). In addition, the muscarinic antagonist, scopolamine, inhibited lordosis behavior strongly in FRL females over a range of doses (0.25, 0.5, 1, 2, and 4 mg/kg), but only weakly inhibited lordosis in FSL females. Consequently, FSL females are highly sensitive to gonadal steroid effects and resistant to the effects of scopolamine. Results are consistent with previous reports of cholinergic supersensitivity of FSL rats. NSF award BNS-9021447.

360.10

DEFICITS IN REPRODUCTIVE BEHAVIOR IN DIABETIC FEMALE RATS ARE DUE TO HYPOINSULINEMIA RATHER THAN HYPERGLYCEMIA. G.B. Karkanias*, A.M. Etgen, J.C. Morales and C. Li. Dept. Neuroscience, Albert Einstein Coll. Med., Bronx, NY 10461

These studies determined whether deficits in reproductive behavior observed in streptozotocin(STZ)-induced diabetic female rats are caused by hyperglycemia or loss of insulin. Female Sprague-Dawley rats were ovariectomized and made diabetic by a single i.p. injection of STZ (75mg/kg). Reproductive behavior was measured 10 days after the onset of hyperglycemia (diabetes) following the injection of estrogen and progesterone in doses known to restore reproductive behavior in non-diabetic rats. Rats in which STZ produced diabetes showed significantly reduced receptive and proceptive sexual behaviors. Normalization of blood glucose levels either by restricting diet or by phlorizin treatment failed to restore reproductive behavior in diabetic animals. However, doses of insulin which produced normal blood glucose prevented the STZ-induced behavioral deficit. No changes in general activity were observed in any experimental group as assessed by open field activity (grid crossing and rearing). The density of the norepinephrine transporter, as measured by ^{3}H-nisoxetine binding, was reduced in the cortex but not in the brain stem, hypothalamus or hippocampus of diabetic animals. Insulin treatment prevented the loss of cortical ^{3}H-nisoxetine binding, and even partial normalization of blood glucose levels either by restricting diet or by phlorizin treatment restored the level of cortical ^{3}H-nisoxetine binding. These findings suggest that diabetes-induced reproductive deficits are due to hypoinsulinemia and cannot be corrected simply by the normalization of blood glucose whereas reductions in cortical norepinephrine transporter are the result of hyperglycemia.
Supported by ADA Grant 2376 to GBK and by HD29856, MH41414 and RSDA MH00636 to AME.

360.11

REPRODUCTIVELY-RELEVANT STIMULI ACTIVATE PROGESTIN RECEPTORS IN FEMALE RAT BRAIN. A.P. Auger*, C. A. Moffatt and J.D. Blaustein. Neuroscience & Beh. Prog., Univ. of Massachusetts, Amherst, MA 01003.

Recent studies suggest that progestin receptors may be activated *in vivo* in the absence of ligand by treatment with modulators of protein phosphorylation. Intracerebroventricular infusion of a dopamine agonist or gonadotropin-releasing hormone (GnRH) increases sexual receptivity in estradiol-primed rats, and treatment with progesterone antagonists blocks this facilitation suggesting that the facilitation occurs *via* ligand-*in*dependent activation of progestin receptors. However, it is not known if ligand-*in*dependent activation of progestin receptors occurs under natural conditions. One potential pathway by which this proposed mechanism could occur in females is *via* somatosensory information provided by a male. As both progesterone and vaginal-cervical stimulation (VCS) increase expression of the immediate early gene product, Fos, within progestin receptor-containing neurons, and both dopamine and GnRH appear to be released in the brain by mating stimuli, we hypothesized that VCS-induced Fos-IR would be reduced by a progesterone antagonist.

Ovariectomized rats were injected with estradiol followed 48 hrs later by either the progesterone antagonist RU486 or oil vehicle. One hour later, all rats received VCS or control perineum stimulation and were perfused one hour later. VCS increased Fos-IR in all areas examined. However, VCS-induced Fos-IR was reduced by the progesterone antagonist RU486 in the medial preoptic area (MPO) and ventromedial hypothalamus (VMH). In ovariectomized/adrenalectomized rats, VCS-induced Fos-IR was reduced by RU486 within the MPO, VMH, and the bed nucleus of the stria terminalis. Thus, some of the VCS-induced Fos-IR is dependent upon the availability of unoccupied progestin receptors. The results suggest that somatosensory information provided by VCS increases Fos-IR possibly *via* ligand-*in*dependent activation of progestin receptors. (supported by NS 19327 from NIH and RSDA MH 00885)

360.12

THE EFFECT OF POSTNATAL EXPOSURE OF POLYCHLORINATED BIPHENYLS (AROCLOR 1254) ON THE SEXUAL BEHAVIOR OF RATS. Y.-W. Chung and L.G. Clemens*, Department of Zoology, Michigan State University, East Lansing, MI 48824.

Polychlorinated biphenyls (PCBs) are long-lasting environmental pollutants that have been reported to reduce male fertility, as well as cause abnormal embryonic development in several species (Kholkute *et al.*, 1994; Sager *et al.*, 1987). Although there are many reports of reproductive abnormality after PCB exposure (Fein *et al.*, 1984), we know very little about the effect of PCBs on sexual behavior. We examined the effect of postnatal PCB exposure on the sexual behavior in laboratory rats. After parturition, 12 lactating rats were treated with Aroclor 1254 (Fisher Scientific), a mixture of PCBs containing 54% chlorine, by interperitoneal injection for one week. Thus, pups were exposed to Aroclor 1254 through dam's milk. Control females were given sesame oil. At 60 days of age, the female pups were ovariectomized, rehoused and allowed 7 days to recover from the surgery. The ovariectomized rats were primed with estrogen and progesterone and tested for sexual behavior. The male pups were tested after 90 days of age. When tested in a situation where the female could escape from the male after a copulatory event, PCB-treated females returned to the male with shorter latencies after intromission than did control females. In addition, the lordosis quotient of PCB-treated females was significantly lower. PCB-treated male rats, on the other hand, had longer mount latencies. The results of the behavior tests suggest that postnatal exposure of PCBs disrupts sexual behavior of both male and female rats. This research was supported by NSF grant IBN-9511971.

360.13

EFFECTS OF INTRACRANIAL ESTROGEN IMPLANTATION ON FEMALE RATS PACED COPULATORY BEHAVIOR. L. Xiao* and J. B. Becker[1,2,3], Department of Psychology[1], Reproductive Sciences Program[2], Neuroscience Program[3], The University of Michigan, Ann Arbor, MI 48104.

When the female rat is allowed free access to and can also withdraw from a compartment containing a sexually active male rat, she will actively regulate the temporal pattern of sexual contact with that male. This is called pacing behavior. As compared to nonpaced mating, paced mating by the female is more likely to initiate the neuroendocrine reflexes that induce a physiological condition necessary for pregnancy. Experimental evidence indicates that the display of pacing behavior is directly dependent upon the intensity of vaginocervical stimulation received from the male. However, the central neuroendocrine mechanism(s) mediating pacing are poorly understood. A previous study in this laboratory has demonstrated enhanced extracellular dopamine release in the striatum and the nucleus accumbens of female rats engaged in pacing. Since the striatum is involved in sensorimotor integration, we postulate that estrogen activation of the striatum plays a role in interpreting the signals relaying information about the intensity of vaginocervical stimulation, thus facilitating the appropriate motor response to the stimuli. The present study is designed to investigate the role of hormonal activation of the striatum and/or accumbens on pacing behavior using intracranial hormone implantation. Results indicate that 30% estradiol-17ß administered to the striatum for 4 hr enhances pacing behavior measured by increased percent exits from the male after intromission and delayed contact-return latency after ejaculation. When a "pure" steroidal antiestrogen, ICI 182, 780, is implanted (10%) into the striatum, the percent exits after each type of sexual contact are significantly decreased. However, the contact-return latency is not affected by the treatment. Therefore, certain components of pacing behavior are mediated by hormonal activation of the striatum. (Sponsored by IBN9514888 and NICHD 5T32HD07048)

360.14

BEHAVIORAL TESTS OF MOTIVATION BY FEMALE RAT TO WORK FOR ACCESS TO MALE RAT. T. James Matthews, M. Grigore, L.-M. Kow, L. Tang and D.W. Pfaff,* New York University and Rockefeller University, New York, N.Y.

While many neurochemical or molecular manipulations alter mechanisms for lordosis behavior directly, others may influence preceding behaviors which normally lead to reproduction. We devised formal tests of motivation in which female rats could perform arbitrary responses to gain access to a stud male rat followed by an encounter with a single intromission. Physiological variables might alter incentive value of the male or reward value of the encounter. In some tests, the female's response also immediately released male odors (cage bedding or preputial glands). We found that females will work for access to the male, whether or not the male's presence is signalled by odors. The odors themselves supported only low response rates. Both nose-poking and bar-pressing responses by the female were successfully used. Response rates were much higher and more reliable if ovariectomized females were treated with estradiol or estradiol supplemented with progesterone. Response patterns on fixed ratio schedules differed markedly from the quantitative results expected from food-reinforced responding. Preliminary results with antisense DNA reagents suggested that these motivated approach responses might vary when lordosis performance does not. In summary, we can use learned responses to assay the sensitivity of motivational processes to hormone actions in brain.

360.15

RELATION OF THE MEDIAL PREOPTIC AREA TO THE TEMPORAL PATTERN OF FEMALE SEXUAL BEHAVIOR IN RATS. L. Y. Yang* and L. G. Clemens. Neurosci. Prgm. & Dept. of Zool., Michigan State University, East Lansing, MI 48824.

MPOA lesions have been reported to increase the lordosis response in the female rats (Powers and Valenstein, 1972). Transections made anterior-dorsal to the MPOA also facilitated lordosis (Yamanouchi and Arai, 1977). Based on these results, it has been suggested that the MPOA inhibits female sexual behavior. However, MPOA lesions significantly decreased the time that the female spent with the male (Whitney, 1986) suggesting that the MPOA may play a role in facilitating female sexual behavior. The objective of this study was to investigate the relation of the MPOA to the temporal pattern of female sexual behavior in rats. Experiment 1 investigated the effect of electrolytic lesions (1.5 mA for 20 seconds) of MPOA on the pacing of copulation by the female. Females were tested in a situation where they could escape from the male following sexual contacts. Results indicated that MPOA lesions significantly increased the female's intromission return latency (IRL) and postejaculatory refractory period (PER). Experiment 2 examined the effect of ibotenic acid lesions (0.4 u L per side at a dose of 10 ug ibotenic acid/u L 0.1 M phosphate buffer, pH 7.40) of MPOA on female pacing. Results for ibotenic acid lesions were similar to electrolytic lesions suggesting that the alteration of timing in lesion females results from loss of MPOA cells and not fibers passing through MPOA. Ginton and Merari (1977) reported that MPOA lesions significantly lengthened the male's interintromission interval (III) and postejaculatory interval (PEI). Our results showed that the MPOA lesions had parallel effects in the female. Based on these results, we suggest that the MPOA plays a similar role in both males and females with regard to regulating the temporal pattern of copulation. This research was supported by NSF grant IBN-9511971.

360.16

CHANGES IN HYPOTHALAMIC PROGESTERONE RECEPTOR DENSITY OVER THE 4-DAY ESTRUS CYCLE IN THE RAT E.M. van der Beek[1]*, V.M. Wiegant[1,2] [1]Dept Human & Animal Physiol, Agricult Univ, Wageningen; [2]Dept Med Pharmacol, Rudolf Magnus Inst Neurosci, Utrecht Univeristy, the Netherlands

The timing and height of the proestrus LH surge as well as the degree and duration of sexual receptivity are determined by the feedback effects of progesterone (P) on the brain. Both the preoptic area (PO) as well as the ventromedial hypothalamus (VMH) are crucial for the regulation of these functions and contain progesterone receptors (PR) which are thought to mediate the feedback effects of P. In the present study, we determined the number of PR in the PO and VMH at different times of the estrus cycle. Female rats (LD 12:12, lights on at 5:00) with regular 4-day cycles were sacrificed at 10:00 h on the day of met- (M; n=2), di- (D; n=2), pro- (Pam; n=3) and estrus (E; n=2), and, in addition, at 16:00 h on the afternoon of proestrus (Ppm; n=3). Sections of the hypothalamus were immunocytochemically stained for PR, and the number of PR was quantified in the rostral and medial part of the PO, and in the ventrolateral and caudoventral VMH using the NIH Image analysis program. The number of PR was lowest on M and increased twofold at D in both the PO and VMH. On Pam the number of PR was not clearly different from D levels, but showed a large variation. By the afternoon of proestrus (Ppm), the number of PR was increased fourfold in the PO and VMH compared to M levels. The number of PR on the morning of E was comparable to that found on Ppm. The present results show that PR are present at all stages of the estrus cycle, and that the number of PR increases dramatically on the day of proestrus. Although P levels start to rise at proestrus, and PR are thought to be down regulated by P, the number of PR was not decreased on the morning of E compared to levels at Ppm.

360.17

FUNCTIONAL DIFFERENCES BETWEEN TWO G PPOTEIN ISOFORMS, $G\alpha_{11}$ AND $G\alpha_q$: EVIDENCE FROM THE USE OF ANTISENSE OLIGO-DEOXYNUCLEOTIDES (ODNs) IN THE STUDY OF LORDOSIS. L.-M. Kow*, D.W. Pfaff and S. Ogawa. The Rockefeller University, 1230 York Ave., New York, NY. 10021.

Based on previous findings (Kow et al., Neurosci. Biobehav. Rev. 18:251, '94), it was hypothesized that α_1-adrenergic agonists, such as methoxamine (MA), act on the hypothalamic ventromedial nucleus (VMN) to facilitate lordosis by activating the G proteins of the Gq family. To evaluate this hypothesis, ovariectomized rats were pretreated (7 intra-VMN infusions at 12-hr intervals) with three phosphorothioated 18-mer ODNs (1 µg/0.2 µL/side, bilateral). Two of the ODNs, $G\alpha_{11}$-AS and $G\alpha_q$-AS, have sequences antisense to mRNA for $G\alpha_{11}$ and $G\alpha_q$, respectively; and the third, $G\alpha_{11}$-Scr, serving as a control, has a scrambled sequence for $G\alpha_{11}$-AS. Approximately 4 hrs after the last ODN infusion, the lordosis quotients (LQs) of the rats, which were primed with estrogen 2 days earlier, were measured in pre- and post-tests with an intra-VMN infusion of MA in between. MA infusion increased the LQ (mean±SEM, pre- to post-test) significantly in rats pretreated with $G\alpha_q$-AS (44±7 to 79±8, n=9, p<.01, ANOVA), or the control $G\alpha_{11}$-Scr (55±13 to 84±10, n=5, p<.01), or saline (30 to 70, n=2). Such a MA-induced lordosis facilitation was blocked by $G\alpha_{11}$-AS (51±8 to 66±8, n=8, p>.12). Pretreatment with ODNs also reduced body weight. But, in contrast to that on lordosis, the effect on body weight was significant only in rats receiving $G\alpha_q$-AS or $G\alpha_{11}$-Scr, and not $G\alpha_{11}$-AS or saline. These results suggest that, in VMN, $G\alpha_{11}$ is involved in the mediation of lordosis facilitation, while its closely homologous isoform, $G\alpha_q$, has different functions. (Supported by PHS NS30824 from NINDS)

360.18

STEROID HORMONE AND NEUROTRANSMITTER MODULATION OF SEXUAL RECEPTIVITY IN PROGESTERONE RECEPTOR NULL MUTANT MICE. S. K. Mani*, J. D. Blaustein§ and B. W. O'Malley, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030 and §Neuroscience Behavior Program, University of Massachusetts, Amherst, MA 01003.

Estradiol followed after a suitable interval by progesterone is effective in inducing high levels of sexual receptivity in ovariectomized female mice. Preliminary studies using parental strains, C57BL/6 and the E129, indicated that repeated weekly hormonal priming and testing experience were required for the induction of maximal levels of sexual receptivity in mice. Estradiol alone was not capable of inducing lordosis response in either strains, suggesting that progesterone is essential for the induction of sexual receptivity in female mice of these strains. The female offspring from mutant mice generated by disrupting progesterone receptor (PR) gene by gene targeting (Lydon et al, 1995) were analyzed for hypothalamic cytosol PRs. *In vitro* one-point binding analyses of estradiol-induced cellular PRs in the mediobasal hypothalamus demonstrated a 70% reduction in binding in the homozygous (knockout,) females, while heterozygous females had 40% fewer as compared to the wild types. Ovariectomized mice were injected with estradiol and progesterone and tested for sexual behavior in response to mating by male mice. Both wild type and heterozygous females exhibited high levels of lordosis, while the homozygous females showed minimal response. In earlier studies, we demonstrated the necessity of PRs in mediating the effects of dopamine on female sexual behavior in rats. In order to test further this hypothesis, we have examined the facilitation of sexual behavior by a dopamine agonist in the null mutants. Intracerebroventricular (icv) administration of a D_1 receptor agonist, SKF-38393 facilitated high levels of lordosis in wild type females containing the full complement of PR, while the homozygous females having reduced cytosolic PRs showed low levels of lordosis. These studies provide further evidence that PRs are required for at least one behavioral effect of dopamine in female rodents. *(Supported by HD-07857, and NS 19327)*

360.19

THE EFFECTS OF GONADAL STEROIDS ON LORDOSIS BEHAVIOR IN THE ESTRADIOL BENZOATE (EB) PRIMED FEMALE RAT: COMPARISONS TO THE MALE. P.C. Butler, M.A. Malstrom, D.D. Saxton, S.E. Huber, J.S. Sovereign, G.J. Bloch* Dept. of Psychology, BYU,Provo, UT 84602.

In a previous study, we reported that testosterone (T) and progesterone (P), but not dihydrotestosterone (DHT) and P, acted synergistically to inhibit lordosis in EB primed male rats. In addition, we found that P did not override the inhibitory effects of DHT in the male (Bloch et al. Neurosci 21, '95). To obtain sex comparisons, the present study tested for effects in females using identical treatments as used previously in males. Adult gonadectomized female rats were given blank (BK), T, P, DHT, T+P, or DHT+P-filled Silastic capsules. Behavioral tests for lordosis were administered once a week in EB primed females for 4 consecutive weeks. Statistical analysis revealed three sex differences in the lordosis response to gonadal steroids. First, in contrast to males, T+P did not act synergistically to inhibit lordosis in females (For example, fourth week LQ means ±SEM: 73.3±36.0 vs. 36.0±10.5 for males). Second, although DHT inhibited lordosis behavior in both females and males, females were inhibited significantly more so (LQ: 22.5±6.8 vs. 52.7±10.5). Third, although the inhibition of lordosis by DHT was nullified by P in the female (LQ scores rose to 85.0±8.7), this overriding effect of P was not apparent in the male (LQ scores remained at 49.4±7.8). Thus, T+P acts synergistically to inhibit lordosis in the male but not in the female; DHT inhibits lordosis behavior in both males and females but has a greater inhibitory effect in females, and P nullifies the inhibitory effects of DHT in females but not males. Supported by HD 27334

360.20

WEIGHT LOSS DOES NOT IMPROVE REPRODUCTIVE FUNCTION IN OBESE ZUCKER FEMALE RATS. C.L.M. Bivens* & D.H. Olster. Dept. of Psychology. & Neuroscience Research Institute., Univ. of Calif., Santa Barbara, CA. 93106.

Genetically obese Zucker rats have several reproductive abnormalities, such as delayed puberty, abnormal estrous cyclicity, and decreased sexual behavior. It is possible that excessive body weight itself provokes those reproductive abnormalities. To address this fundamental issue, obese Zucker female rats were food restricted to match their body weight to those of their lean counterparts. Pubertal onset (as indicated by vaginal opening), subsequent estrous cyclicity, and sexual behavior were monitored in lean, obese, and food-restricted obese (diet obese) rats. Preventing excessive weight gain failed to accelerate vaginal opening in diet obese rats, which occurred 1.5 weeks after vaginal opening in lean rats. Immediately after vaginal opening, all lean rat displayed normal, 4 or 5 day estrous cycles while both obese and diet obese rats exhibited abnormal estrous cyclicity. After collecting estrous cyclicity data, all rats were ovariectomized, treated with estradiol benzoate (EB, 15 µg/kg sc) or EB plus progesterone (P, 2 & 20 mg/kg sc), and tested for reproductive behaviors. After EB alone, sexual receptivity was extremely low (LQ<3%) in all rats and proceptive behaviors were never observed. After treatment with EB plus P (2 mg/kg), lean rats were maximally receptive (LQ=92±4%) and proceptive (PRO=8.8±2 events/min) while both obese and diet obese rats were only marginally receptive and proceptive (LQ=30±8%, PRO=3.8±2 events/min; LQ=50±20%, PRO=4.4±2 events/min, respectively). Increasing the progesterone dose to supraphysiological levels (20 mg/kg) elicited vigorous sexual receptivity (LQ=94-99%) and robust proceptivity (PRO=16.15-20 events/min) in all rats. These data suggest that excessive body weight *per se* does not contribute to the reproductive abnormalities in obese Zucker rats. (Supported by NIH HD 28636 and American Psychological Association)

DRUGS OF ABUSE: COCAINE—DA-5-HT INTERACTIONS

361.1

SEROTONIN MEDIATION OF THE EFFECTS OF COCAINE ON EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS. C. M. Andrews* and L. Lucki
Depts. of Psychiatry and Pharmacology, Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Several studies have shown that serotonin (5-HT) facilitates dopamine (DA) release (Benloucif and Galloway, 1993). We examined the ability of 5-HT to modulate extracellular DA levels in the nucleus accumbens (NAc) following cocaine administration. *In vivo* microdialysis techniques, in awake unrestrained Sprague-Dawley rats, were used that measure both DA and 5-HT levels in dialysis samples using a HPLC with electrochemical detection. Animals were treated with 150 mg/kg p-CPA on two consecutive days, starting 3 days prior to the experiment, in order to deplete 5-HT. A microdialysis probe was implanted into the nucleus accumbens 48 h after the first p-CPA injection. The day after surgery samples were collected for 2 hours to establish baseline values. Baseline levels of 5-HT in the p-CPA treatment group were undetectable. Pretreatment with p-CPA reduced the effect of systemic administration of cocaine (25 mg/kg) from increasing NAc DA concentrations 600% in non-treated animals to only a 400% increase in p-CPA treated animals. Pretreatment with p-CPA also reduced the effect of infusion of cocaine (10 mM) through the microdialysis probe from increasing NAc DA levels by 1000% to only a 575% increase over basal in p-CPA treated animals. Systemic administration of 25 mg/kg cocaine in the presence of 10 mM cocaine infusion decreased NAc DA levels by 30%, presumably by acting at VTA autoreceptors, but decreased NAc DA levels by 55% in p-CPA treated animals. These data suggest that serotonin potentiates cocaines ability to increase dopamine in the nucleus accumbens. *Supported by NIDA DA05186.*

361.2

CONVERGENCE OF SEROTONIN AND DOPAMINE IN CAUDATE/PUTAMEN OF RAT, MOUSE, AND RABBIT. G. Villareal[1], M. Russell[1], L. Mery[1], F. Makilan[1], P. Broderick[2], and C. Phelix*[1] 1- Division of Life Sciences, Univ. of Texas at San Antonio, TX 78249; 2- Dept. Pharmacology, CUNY Medical School, New York, NY 10031

Interaction of serotonin (5HT) and dopamine (DA) in the nucleus accumbens (NAcc) of the ventral striatum has been described anatomically. However, the neurochemical interaction of 5HT and DA in the dorsal striatum is equally interesting and demonstrates regional variation in response to cocaine administration. Since we observed such dramatic correlation between anatomical and functional convergence of 5HT and DA in the NAcc, we now examine the anatomical interaction between 5HT and DA axons in the dorsal striatum of rat and mouse. We are especially interested in the rabbit because it has a distinct caudate and putamen. Formaldehyde fixed brains were stained with sensitive immunocytochemical procedures using a selective silver intensification. Antibodies used were directed against 5HT or tyrosine hydroxylase as a marker for DA. 5HT staining was most intense in the wild strain white footed mouse. There was not an evident patch/matrix arrangement of 5HT terminals in any species examined by comparison to the prominence with DA terminals. Due to greater density overall of 5HT axons in the putamen of rabbit there was more convergence with DA axons than in the caudate, although DA terminals were perfuse and very dense (i.e., greater in matrix than patch). Evaluation of rat and mouse caudate/putamen complex with comparison to rabbit indicates that only the dorsal medial neostriatum, medial to the internal capsule, of the former two correspond to the caudate and the vast remainder corresponds to the putamen. This is consistent with the greater convergence of 5HT and DA axons in the CP of rat and mouse. Support: HL02914-02.

361.3

RESPONDING FOR COCAINE-ASSOCIATED STIMULI IS MODIFIED BY PHARMACOLOGICAL MANIPULATION OF DOPAMINE AND SEROTONIN TRANSMISSION. F. Weiss*, C.S. Maldonado-Irizarry, D. Smith and G.F. Koob. Dept. Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Clinical and neuropharmacological studies suggest that drug seeking behavior for cocaine, opiates, and alcohol can be elicited by environmental stimuli associated with the rewarding effects of these drugs of abuse. The present experiments sought to examine the effects of pharmacological manipulations of dopamine and serotonin neurotransmissions on the efficacy of cocaine-associated stimuli to maintain drug-seeking behavior in the absence of the primary reinforcer. Rats were trained to respond on a [FR5:FR5] multiple schedule with alternating food and intravenous cocaine reinforced components during which food was paired with a tone, and cocaine infusions were paired with a stimulus light. After acquisition, each session began with a 5 min conditioned stimulus component (CSC) during which responding on either of two levers (FR1) resulted only in the presentation of stimuli associated with primary reinforcers. Effects of SCH-23390 (D1 antagonist; 0-10.0 µg/kg; SC), flupenthixol (D1/D2 antagonist; 0-50.0 µg/kg; IP), PD 128,907 (D3 agonist; 0-3.0 mg/kg; IP), CGS-12066 (5-HTB1 agonist; 0-10.0 mg/kg; IP) and tropisetron (5-HT3 antagonist; 0-60 µg/kg; SC) on responding during CSC and the multiple schedule were tested in separate groups. SCH-23390 suppressed behavior maintained by both conditioned stimuli while flupenthixol, PD 128,907 and tropisetron, at these doses, produced no significant behavioral changes during the CSC. CGS-12066 significantly attenuated responding for the food-associated cue and tended to enhance responding for the cocaine cue. On the multiple schedule, SCH-23390 significantly suppressed responding for both food and cocaine. PD 128,907 attenuated responding for food and cocaine at the highest dose. CGS-12066 produced a trend toward decreased responding for food but had no effects on cocaine reinforced responding. In contrast, flupenthixol and tropisetron, at these doses produced no behavioral changes. Together, these findings confirm the role for dopamine and serotonin systems in motivational processes related to cocaine seeking behavior. (Supported by NIDA DA07348).

361.4

CHRONIC CONTINUOUS ADMINISTRATION OF MONOAMINE UPTAKE INHIBITORS PRODUCES BEHAVIORAL TOLERANCE BUT NOT A COCAINE-LIKE ACTIVITY PATTERN. P.M. Kunko*, F.I. Carroll and S. Izenwasser. Psychobiology Section, NIDA, Division of Intramural Research, Addiction Research Center, Baltimore, MD 21224 and Research Triangle Institute, Research Triangle Park, NC 27709.

Four hours after cocaine pumps (50 mg/kg/day) are implanted in rats, locomotor activity levels are increased, as compared to saline controls, with an even greater effect observed 24 hours later. Partial tolerance to this effect develops over the course of five days, with activity levels diminishing, but still significantly higher than those in saline-pump rats. These behavioral effects are not due to changes in the amount of cocaine or its metabolites benzoylecgonine or ecgonine methyl ester in either the blood or the brain, since those did not vary significantly over the course of the week. Since cocaine inhibits the uptake of all of the monoamines, it is not known which of these pharmacological effects play a role in the production of this behavior. In this study, male Sprague-Dawley rats were treated for seven days with a continuous infusion of a selective inhibitor of the uptake of either dopamine [GBR 12909 (30 mg/kg/day) or RTI-117 (3.62 mg/kg/day)], norepinephrine [nisoxetine (0.1 mg/kg/day)], or serotonin [fluoxetine (1.0 mg/kg/day)]. These doses were chosen based on a ratio of known affinities for their respective transporters, as compared to cocaine. Locomotor activity levels were measured for one hour each day, on seven consecutive days, beginning 4 hours after pump implantation. Both of the dopamine uptake inhibitors significantly increased locomotor activity; however, unlike cocaine, the highest levels were observed when animals were tested four hours after the pumps were implanted. Partial tolerance to both compounds occurred, within 24 hours for GBR and over five days for RTI. Neither fluoxetine nor nisoxetine significantly altered locomotor activity at these doses, suggesting a limited role for norepinephrine or serotonin. The pattern of behavior observed with cocaine is unique and is not fully mimicked by any of the selective uptake inhibitors. The results suggest that some combination of the uptake inhibitors is required to produce a cocaine-like effect, or that kinetic differences between cocaine and the selective dopamine uptake inhibitors are responsible for the different behavior patterns, even though locomotor activity levels were not directly correlated with cocaine levels. (Supported by NIDA)

361.5

TOLERANCE AND SENSITIZATION TO COCAINE ARE MEDIATED VIA INDEPENDENT MECHANISMS. D. French and S. Izenwasser*. Psychobiology Section, NIDA Division of Intramural Research, Baltimore, MD.

Tolerance or sensitization to the locomotor activating effects of cocaine occurs depending upon the treatment regimen that is used. When cocaine is injected on a daily basis, sensitization occurs, whereas continuously infused cocaine leads to partial tolerance. Male Sprague-Dawley rats were treated with a continuous infusion of cocaine (50 mg/kg/day) via subcutaneously implanted osmotic minipumps. Locomotor activity was measured for one hour each day for eight days, after which the pumps were removed. Rats with cocaine pumps exhibited large increases in locomotor activity, compared to saline controls, 24 hours after the pumps were implanted. Partial tolerance to this effect developed over the course of five days, with activity levels diminishing to a level still significantly higher than that in saline-pump rats. Activity levels of the cocaine-pump rats dropped to saline levels within four hours after the pumps were removed, and were significantly lower than the saline-treated group during the first three days of withdrawal, after which they returned to saline levels. Some rats were challenged with an injection of cocaine (7.5, 15, or 30 mg/kg) either two or ten days after pump removal. Two days after the pumps were removed there were no differences between cocaine-pump or saline-pump rats in the amount of locomotor activity produced by the challenge injections. However, cocaine-pump rats injected 10 days after pumps were removed (withdrawal day 10) exhibited a significant tolerance, as evidenced by a shift to the right of the cocaine curve, as compared to saline controls. When the rats were injected again on the next day (withdrawal day 11), the activity levels of both groups increased, as compared to the effects observed on withdrawal day 10. Thus, although the cocaine-pump rats were still tolerant as compared to the saline-pump rats, they were sensitized as compared to their previous response to a challenge injection. These findings indicate that tolerance and sensitization can exist simultaneously, which suggests that they are mediated by separate mechanisms. (Supported by NIDA).

361.6

TROPANE ANALOGS DISSOCIATE SLOWLY FROM DOPAMINE AND SEROTONIN TRANSPORTERS IN CULTURED CELLS. J.J. Harp, R.S. Martin and C.K. Hollingsworth, H.M.L. Davies[1], J.M. Paris* and B.A. Bennett, Center for Neurobiological Investigation of Drug Abuse, Dept. Physiol/Pharmacol, Bowman Gray School of Medicine, Winston-Salem, NC; [1]SUNY Buffalo, Natural Science and Math Complex, Buffalo, NY.

Cocaine and tropane analogs share many of the same properties in their interactions with amine transporters. However, we, and others, have reported that tropanes differ from cocaine by possessing a longer duration of action. Using an *in vitro* culture system, we have examined several tropanes (synthesized by Davies et al, 1993; 1994) and cocaine to determine the time course of transporter occupancy after a brief (3 hr) exposure. Uptake experiments were performed using midbrain cultures obtained from fetal rat brains (E15) after exposure to the tropanes (WF-11, WF23, WF-31) or cocaine on day 7 *in vitro*. Separate cultures were obtained possessing either dopaminergc or serotonergic neurons. The concentration of the drugs was chosen based on their affinity for the transporters and was approximately 10-fold greater than their respective IC_{50}'s. There was a decrease in the ability of the respective cultures to transport dopamine or serotonin after the administration of the tropanes based on washout time as well as affinity. Tropanes with high affinity for either the dopamine or serotonin transporter required 1-3 days of washout before uptake values approached control values. Our data suggest that these tropanes bind to and reduce transporter function for prolonged periods of time and that compounds with the highest affinity may produce a pseudo-irreversible inhibition of transporter function. These findings may have very significant implications for drug abuse pharmacotherapy.
Supported by NIDA grants P50 DA006634 and DA06301 (HMLD).

361.7

LONG LASTING EFFECTS OF THE NOVEL TROPANE ANALOG WF23 AT DOPAMINE AND SEROTONIN TRANSPORTERS. L.J. Porrino*, J.B. Daunais, S.L. Hart, S.R. Letchworth, H.R. Smith, T. Sexton, S.R. Childers, H.M.L. Davies[1]. Dept Physiol/Pharmacol, Bowman Gray School of Medicine, Winston Salem, NC, [1]SUNY Buffalo, Natural Science and Math Complex, Buffalo, NY 14260.

Recent reports have described the synthesis of a series of novel tropane analogs that are highly potent at dopamine and/or serotonin transporters (Davies et al, 1993; 1994). Although these compounds share many of the properties of cocaine, their actions *in vivo* and *in vitro* have been reported to be of longer duration than those of cocaine itself. In order to study the time course of the behavioral effects of WF23, which is both highly potent and relatively non-selective, this analog (1mg/kg) was administered to male Sprague-Dawley rats and activity assessed 1, 4, 8, 12, 24, 48, 120, and 240 hours after injection. WF23 elicited significant increases in spontaneous locomotor behavior for up to 24 hours, as compared to cocaine- or vehicle-treated rats tested at the same time points. Assessment of the occupancy of dopamine transporters in striatal homogenates from these rats indicated that [^{125}I]RTI-55 binding was inhibited by as much as 50% 48 hrs post injection, but had returned to control levels by 240 hrs. Measured autoradiographically, WF-23 administration inhibited both [^{3}H]CFT and [^{3}H]citalopram binding to dopamine and serotonin transporters respectively, in all structures examined, following a similar time course to binding in homogenates. There is, then, a slow dissociation of WF23 from both dopamine and serotonin transporters. This persistence of effects may have important implications for this compound as a potential therapeutic agent for cocaine abuse.
Supported by NIDA grants P50 DA06634 and DA06301 (HMLD).

361.8

ALTERATIONS IN THE SEROTONIN TRANSPORTER mRNA FOLLOWING CHRONIC EXPOSURE TO COCAINE, FLUOXETINE, OR GBR 12909: GENDER DIFFERENCES. D. Dow-Edwards*, Y. Hurd and G. Frick. Lab. of Cerebral Metabolism, Department of Pharmacology, SUNY Brooklyn, NY 11203 and Department of Clinical Neuroscience, Psychiatry Section, Karolinska Hospital, Stockholm, Sweden.

Chronic exposure to cocaine, fluoxetine, the selective serotonin (5-HT) transporter blocker, or GBR 12909, the selective dopamine (DA) transporter blocker, during the preweaning period of development in the rat produces long-term alterations in behavioral responses to psychopharmacological agents which interact with the DA and 5-HT systems (e.g. Neurotoxicol. Teratol. in press, 1996). When cerebral glucose metabolism is examined at 21 days of age following injection of the transporter blockers, specific patterns of functional alterations are obtained (Dev. Brain Res. 88:158, 1995). In females, cocaine, GBR and fluoxetine stimulate regional glucose metabolism while in males cocaine and GBR stimulate metabolism and fluoxetine reduces metabolism in a small number of regions. In order to assess the effects of these treatments on function within the 5-HT system, sections from the raphe region were examined for concentrations of the 5-HT transporter using in situ hybridization histochemistry. Sections were incubated with a sequence complementary to amino acid bases 108-156 of the rat 5-HT transporter gene. Densitometry of the resulting auto-radiographs indicated that cocaine increased the transporter density by 50% in males and had only minor effects in females. Fluoxetine had no effect in males but reduced the transporter density by 50% in females. GBR 12909 had virtually no effect. Together, these data indicate that both cocaine and fluoxetine produce gender-specific effects on the 5-HT transporter but that the effects of the two transporter blockers are quite different from each other. These and other results suggest that cocaine upregulates the 5-HT system in males but this effect does not appear to be mediated by blockade of the 5-HT transporter. In females, cocaine conversely seems to down-regulate the 5-HT system, an effect apparently mediated by blockade of the 5-HT transporter.

Supported by NIDA grant DA04118

361.9

COMBINED SEROTONERGIC AND DOPAMINERGIC AGONISTS ATTENUATE ORAL INTAKE OF DRUGS OF ABUSE IN MICE: d-FENFLURAMINE/PHENTERMINE. M. Ehrlich, M. Martinez, D.M. Patterson and B.G. Hoebel*. Dept. of Psychology, Princeton University, Princeton, NJ 08544.

Combinations of monoamine agonists might be helpful in the treatment of drug abuse and withdrawal-related symptoms (Hitzig, 1993). To test this in female C57BL/6J mice, the combination of 20 mg/kg *d*-fenfluramine plus 20 mg/kg phentermine (*d*-FEN/PHEN) was injected i.p. with a 24 hour two-bottle choice of a drug solution and water. *d*-FEN/PHEN reduced ingestion of 10% ethanol solution by 50.7%, 0.02 mg/ml nicotine solution by 46.9%, 0.6 mg/ml caffeine solution by 28.6%, and 50% chocolate syrup solution by 37.1% compared to intake following i.p. saline. With *d*-FEN/PHEN treatment subjects in the ethanol, nicotine, and caffeine choice groups increased water consumption roughly corresponding to the decrease in drug solution intake, such that no significant change in total fluid intake was observed. *d*-FEN/PHEN had no effect on the choice of water and a solution containing 0.075 mg cocaine plus 0.045 mg heroin per ml. Subjects pre-exposed to either 10% ethanol or 0.02 mg/ml nicotine consumed significantly more 0.05 mg/ml cocaine than naive subjects, and the *d*-FEN/PHEN treatment significantly attenuated subsequent cocaine intake in the two-bottle choice tests for both groups. Treatment with another combination of monoamine agonists, 20 mg/kg fluoxetine plus 20 mg/kg bupropion, decreased intake of 10% ethanol solution by 50% compared to i.p. saline treatment in the two-bottle test; this was similar to the result with *d*-FEN/PHEN. The efficacy of both treatments may rest, in part, in their ability to raise serotonergic and dopaminergic activity simultaneously. Supported by PHS grant #NS30697.

Reference: Hitzig, P. (1993). *Maryland Medical Journal, 42 (2)*, 153-156.

361.10

SEROTONIN-1B RECEPTOR STIMULATION ENHANCES COCAINE REINFORCEMENT, COCAINE-INDUCED INCREASES IN NUCLEUS ACCUMBENS DOPAMINE AND COCAINE-INDUCED DECREASES IN VENTRAL TEGMENTAL AREA GABA. L.H. Parsons*, G.F. Koob and F. Weiss, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The involvement of serotonin-1B (5-HT-1B) receptors in the modulation of cocaine reinforcement and cocaine-induced alterations in mesolimbic neurotransmission was examined in male Wistar rats using the 5-HT-1B agonist RU 24969. Pretreatment with RU 24969 (0.1 - 1.0 mg/kg, SC) dose-dependently ($p<0.05$) enhanced the reinforcing effects of cocaine self-administration (0.125 mg/injection, IV; FR5) as indicated by increased intervals between drug injections. RU 24969 pretreatment (1.0 mg/kg, SC) also shifted the dose-effect function for cocaine self-administration (0.03 - 0.5 mg/injection, IV; FR5) to the left ($p<0.05$). These findings indicate that 5-HT-1B receptor stimulation enhances the reinforcing effects of cocaine. To investigate the neurochemical basis for the effects of 5-HT-1B receptor-mediated alterations in cocaine reinforcement, dual probe *in vivo* microdialysis was used to monitor changes in nucleus accumbens (NAcc) dopamine (DA) efflux and ipsilateral ventral tegmental area (VTA) GABA efflux in drug-naive rats following RU 24969 pretreatment and subsequent cocaine challenge. Pretreatment with RU 24969 (1.0 mg/kg, SC) significantly reduced VTA dialysate GABA levels by 25% ($p<0.05$), but produced no significant changes in NAcc dialysate DA levels. In the animals pretreated with RU 24969, cocaine challenge (10 mg/kg, IP) produced significantly greater increases in NAcc dialysate DA levels (382% increase; $p<0.01$), and significantly greater decreases in VTA dialysate GABA levels (59% decrease; $p<0.05$) as compared with saline pretreated control animals, which displayed a 231% increase in NAcc dialysate DA and a 37% decrease in VTA dialysate GABA. These data suggest that 5-HT-1B receptor stimulation enhances cocaine reinforcement by increasing mesolimbic DA neurotransmission, potentially via an interaction with terminal 5-HT-1B heteroreceptors on VTA GABA neurons. (support: NIDA DA05564)

361.11

THE 5-HT1B/1D ANTAGONIST GR127935 DECREASES THE EFFECTS OF COCAINE ON C-FOS EXPRESSION AND LOCOMOTION.

N. Castanon, J.J. Lucas, K. Scearce, and R. Hen*. Center for Neurobiology and Behavior, Columbia University, New York, NY 10032.

The reinforcing and locomotor activating effects of cocaine are mediated by the mesostriatal and mesolimbic dopamine pathways. However, a contribution of the 5-HT system has also been suggested. In particular, the ventral midbrain 5-HT_{1B} receptors (the rodent counterpart of the human 5-$HT_{1D\beta}$ receptors) have been postulated to modulate the activity of DA neurons. Furthermore, our previous studies indicate that mice lacking the 5-HT_{1B} receptor respond differentially to cocaine than wild type mice which suggests an involvement of 5-HT_{1B} receptors in the response to cocaine.

The 5-HT1 agonist RU24969 stimulates both c-Fos immunoreactivity (IR) in the caudate putamen and locomotor activity. Both effects are absent in mice lacking the 5-HT_{1B} receptor and are therefore probably mediated by this receptor. In order to test that hypothesis, we examined the effect of the recently developed 5-$HT_{1B/1D}$ antagonist GR127935. A 10 mg/kg i.p. dose of GR127935 was able to completely block the effect of a 5 mg/kg i.p. dose of RU24969 both on c-fos IR and locomotion, demonstrating the ability of GR127935 to antagonize 5-HT_{1B} receptors in vivo.

Since cocaine has been suggested to act as an indirect 5-HT_{1B} receptor agonist, we also tested the ability of GR127935 to antagonize the effects of cocaine. Interestingly, GR127935 produced a dramatic reduction in c-Fos IR and locomotion induced by a 30 mg/kg i.p. dose of cocaine.

These results suggest that some of the reinforcing effects of cocaine may also be antagonized by 5-$HT_{1B/1D}$ antagonists.

(Supported by NIDA R01DA09862)

361.12

CHRONIC EXPOSURE TO COCAINE OR THE SEROTONIN 5-$HT_{1A/1B}$ AGONIST RU24969 DECREASES 5-HT_{1B} RECEPTOR SENSITIVITY: LOCOMOTOR ANALYSES. A.C. McCreary* and K.A. Cunningham. Dept. Pharmacol. Univ. Texas Medical Branch, Galveston, TX, 77555-1031.

Both cocaine (COC) and the 5-$HT_{1A/1B}$ agonist RU24969 (RU) elicit hyperactivity, however, repeated treatment with COC and RU appears to result differentially in behavioral sensitization (McCreary & Marsden, *Neuropharm* 32: 387, 1993) and tolerance (Oberlander *et al. EJP* 139: 205, 1987), respectively. We assessed the cross-adaptation to COC and RU in male S.D. rats pretreated with saline (SAL), COC (15mg/kg i.p.) or RU (2mg/kg i.p.) BID for 7 days. Locomotor activity and stereotypy scores were recorded on Days 1 and 7 and on days 8, 10, and 11 upon challenge with SAL, COC, and RU, respectively. During COC treatment all activity measures, apart from rearing, showed a behavioral enhancement between days 1 and 7 ($p<0.05$). On the other hand, tolerance developed to chronic RU treatment as measured by a decrease in horizontal (-67%, $p<0.0001$), central (-71%, $p<0.0001$) and peripheral activity (-67%, $p<0.0001$), but not rearing or stereotypy. Upon COC challenge, RU-pretreated rats displayed elevated central activity (+177%, $p<0.05$) *vs.* SAL rats, other activity measures were elevated but failed to reach statistical significance. Sensitization to the COC challenge was seen in COC-pretreated rats. After RU challenge, COC-pretreated rats had diminished horizontal (-32%, $p<0.05$) and peripheral activity (-32%, $p<0.05$) *vs.* SAL rats. Thus, tolerance to the locomotor effects of RU appears to be associated with sensitization to COC. These data suggest that repeated COC and RU exposure result in a reduced sensitivity of 5-HT_{1B} receptors which may contribute to the expression of COC-induced sensitization.

Supported by NIDA 06511

361.13

SELECTIVE BLOCKADE OF COCAINE CONDITIONED BEHAVIOR WITH BUSPIRONE. Ernest N. Damianopoulos* and Robert J. Carey. Psychiatry, SUNY Health Science Center and Research and Development Service -151, VA Medical Center, Syracuse, NY 13210

Pavlovian conditioning of cocaine drug effects contributes to the development of cocaine sensitization, craving and relapse. Suggestive evidence implicates N-methyl D-aspartate (NMDA) and serotonergic (5-HT) receptors as neurosubsrate mechanisms mediating the conditioned cocaine effects. Thus, we assessed the effects of the NMDA agonist, cycloserine, and antagonist, dizocilpine (MK-801), as well as the 5-HT agonist, buspirone, and antagonist, MPPI, on the expression of conditioned cocaine behavior. A differential conditioning procedure was used to induce conditioned cocaine hyperlocomotion. Adult male rats received saline in one test chamber and saline or cocaine in a second chamber in 5 daily treatments. The animals were injected immediately prior to placement into each test environment and locomotor behavior was recorded for 10 min using a video-image analysis system. Weekly tests followed with saline or NMDA/5HT drug on 1 of 2 test days separated by a non-test day and, 2 days later, by 1 saline/cocaine reconditioning treatment. All saline and all drug tests, except for buspirone, showed equivalent levels of conditioned hyperlocomotion. Buspirone (3.0 mg/kg ip) completely blocked the conditioned hyperlocomotion response but had no effect on the locomotion of each animal in the first test (non-cocaine) environment nor did it show any effect on the saline/saline control treatment animals, thus, ruling out non-specific motoric effects as factors in the observed blocking effect. As an anxiolytic drug buspirone is primarily a 5-HT1A agonist; but it also impacts upon dopaminergic transmission as well as corticosterone stress responses. While it is unclear which mechanism is critical for the observed pharmacological blocking, the results support clinical evidence of buspirone as a useful adjunct drug in the treatment of cocaine abuse.

361.14

Cocaine Sensitization, Tolerance and 5-HT_3 Receptors. G.R. King[1], M. Mattell[2], and P. Little[3*], Departments of Psychiatry[1], Psychology[2], and Pharmacology[3], Duke University Medical Center, Durham, NC 27710

Previous research has demonstrated that 5-HT_3 receptor activation can induce dopamine release in a variety of corticolimbic areas, suggesting that 5-HT_3 receptors may be important for the development of cocaine sensitization and tolerance. The present poster presents the results from two experiments. In the first experiment, rats received continuous saline or cocaine for 14 days. On day 7 of withdrawal slices from the nucleus accumbens were obtained. The slices were stimulated with 25 mM K+ in the absence and presence of mCPBG (0-50 μM), a 5-HT_3 agonist, and DA release measured with HPLC-EC. The results indicated that continuous cocaine induced a functional down-regulation of accumbens 5-HT_3 receptors. In the second experiment, rats received either intermittent or continuous cocaine (40 mg/kg/day) for 14 days; during this 14 day pretreatment regimen, the rats also received sc injections of a 5-HT_3 antagonist (0-1.0 mg/kg/day). The results indicated that ondansetron blocked the development of both behavioral sensitization and tolerance, as assessed by a cocaine challenge injection on day 7 of withdrawal from the pretreatment regimen. The overall pattern of results therefore suggest that 5-HT_3 receptors may be critical for the development of cocaine tolerance and sensitization.

This research was supported by a NIDA FIRST Award (DA08899), G.R. King, Principal Investigator.

361.15

CONTINUOUS ON-LINE STUDIES OF SYNAPTIC MESOACCUMBENS DOPAMINE AND SEROTONIN RELEASE AFTER COCAINE IN THE BEHAVING FAWN-HOODED ANIMAL MODEL OF DEPRESSION P.A. Broderick[1,2*], T. Kunwar[1], B. Gardiner[1], O. Hope[2], C. Okonji[2], P. Jeannot[2], P.J. Baptiste[2], S.A. Green[2], H. Charleton[2], C.S. Aulakh[3]. CUNY Med. Sch. & Grad. Sch., N.Y. 10031[1]; MARC, MBRS Program, CCNY, N.Y. 10031[2] and Nat'l Inst. Mental Health, MD. 20892[3].

The Fawn-Hooded (FH) rat is suggested to be a genetic model of depression and is known to have a platelet storage deficiency of serotonin (5-HT) (Aulakh, et al., JPET: 262, 1992). Further, our laboratory has shown evidence of a deficiency of dopamine (DA) and 5-HT in the Nucleus Accumbens of the FH rat (manuscript in progress). Cocaine (10 mg/kg IP) was administered to freely moving and behaving male FH rats in a novel chamber. Using *in vivo* voltammetric (electrochemical) techniques, 5-HT was detected concurrently yet separately with DA, within a temporal resolution of seconds in one voltammogram. Voltammograms were recorded up to ten months. Detection occurs by means of a graphite stearate microelectrode (Broderick, P.A. *Brain Res.* 495: 115, 1989). Behavior, i.e., locomotor activity (ambulation), rearing, stereotypy and central ambulations were also studied concurrently with infrared photobeam detection. Cocaine's effects in the FH rat were remarkable because the usual increase in DA and 5-HT release did not occur as usually occurs in the Sprague-Dawley (SD) rat model (Broderick et al. *Pharmacol. Biochem. Behav.* 46: 715, 1993). Instead, the animals sat in one corner and exhibited rotational behavior. These striking observations further compel us to strongly support 5-HT's role as a mediator in cocaine's mechanism of action (Broderick and Phelix, *Neurosci. and Biobehav. Rev., in* press, 1996). Withdrawal data from the FH rat also showed differences *vis-a -vis* the SD rat. (Supp: PSC/CUNY RF666221, NIH NIGM 07639 & 08168)

361.16

NEUROTRANSMITTER RELEASE IN THE MESOLIMBIC SYSTEM OF RATS TRAINED IN THE CONDITIONED PLACE PREFERENCE PARADIGM WITH COCAINE. E.A. Jones*, H.L. Williams, R.D. Myers and B.A. McMillen. Dept. of Pharmacol., Sch. of Medicine, East Carolina Univ., Greenville, NC 27858.

Cocaine-HCl, 5.0 mg/kg injected i.p., produced a significant conditioned place preference (CPP) in rats trained in a three compartment chamber. The chamber consisted of a central neutral compartment and two dissimilar outer chambers. Cocaine was administered to the treatment animal immediately before placement in one chamber for 15 minutes. Saline was administered to the control animals immediately before placement in the alternate chamber. After four daily sessions, the cocaine treated rats exhibited a preference for the cocaine paired chamber. The saline treated rat did not show any preference for the saline paired chamber. In order to determine possible differences in neurotransmitter release between cocaine and saline treated animals, microdialysis of the medial prefrontal cortex, nucleus accumbens and ventral tegmental area was performed on cocaine and control animals during exposure to the respective training chamber. The effect of amperozide, a 5-HT_2 receptor antagonist, shown to block cocaine (5.0 mg/kg) CPP, on neurotransmitter release was also examined. (Suppt. by USPHS grant AA-04200-13)

361.17

DIFFERENTIAL EFFECTS OF COCAINE ON DISTINCT COMPONENTS OF 8-OH DPAT-INDUCED SEROTONIN SYNDROME IN RATS. B. Ahmad, N. Uray* and N.A. Darmani. Depts. of Pharmacology and Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

It is thought that the different components of serotonin syndrome (SS) such as lateral head-weaving (LHW), forepaw treading (FPT), hind leg abduction (HLA), straub tail (ST), tremor (T) and flat body posture (FBP) are mediated via the activation of postsynaptic 5-HT_{1A} receptors. We have previously shown that acute cocaine administration inhibits the DOI-induced 5-HT_{2A} receptor-mediated head-twitch response. The aim of the present study was to investigate the acute effects of cocaine (1.25 - 10 mg/kg, i.p.) and selective 5-HT (sertraline), NE (nisoxetine) and DA (GBR 12935) uptake inhibitors (1.25 mg/kg, i.p.) on the SS produced by the 5-HT_{1A} agonist, 8-OH DPAT (1 or 5 mg/kg, i.p.). The cited drugs were administered 10-min prior to 8-OH DPAT injection and individual rats were observed continuously for the next 20 min. Relative to controls, cocaine (5 and 10 mg/kg) significantly enhanced LHW, FPT and HLA to a greater extent in 1.25 versus 5 mg/kg 8-OH DPAT-injected rats. Sertraline, nisoxetine and GBR 12935 at 1.25 mg/kg also produced similar effects on the cited doses of 8-OH DPAT-induced symptoms. On the other hand, varying doses of cocaine had no effect on FBP induced by either dose of 8-OH DPAT but significantly increased ST and T at 10 mg/kg produced by the 5 mg/kg dose of 8-OH DPAT. Unlike cocaine, sertraline, nisoxetine and GBR 12935 potentiated FBP but had no effect on ST and T produced by 1.25 mg/kg 8-OH DPAT. Furthermore, sertraline only significantly decreased T but nisoxetine and GBR 12935 had no effect on ST and FBP produced by 5 mg/kg 8-OH DPAT. These results indicate that cocaine via its monoamine components produces different effects on the 8-OH DPAT-induced SS. This study was supported by NIDA grant DA 07627.

361.18

COCAINE BLOCKS SEROTONIN-INDUCED MOBILIZATION OF PLATELET INTRACELLULAR CALCIUM, S. M. Delisi, L. M. Konopka, J. W. Crayton*. Biological Psychiatry Section, Hines V. A. Hospital, Hines, IL 60141. USA..

The mechanisms by which cocaine increases cardiovascular morbidity and mortality, as is frequently observed in cocaine abuse patients, may involve direct effects on platelet reactivity. However results obtained to date by others have been inconclusive. Both proaggregatory and platelet activation inhibitory effects have been reported. Since mobilization of intra-platelet calcium ($[Ca^{++}]_i$), represents a critical step in platelet activation, and since serotonin is a potent activator of platelets, we explored the effects of cocaine on the serotonin-evoked rise in $[Ca^{++}]_i$. Human platelets were isolated over a Sepharose 2B gel and then loaded with the calcium-sensitive fluorophore fura-2 AM. The elevation in $[Ca^{++}]_i$ following stimulation with 10 μM 5-HT was measured in the presence of 1.0 μM to 1.0 mM cocaine HCl. In this dosage range, cocaine produced a dose-dependent attenuation (41.55% reduction at 1mM cocaine) of the serotonin-induced rise in $[Ca^{++}]_i$. In order to determine whether cocaine's effect was the result of local anesthetic effects, we examined the influence of lidocaine HCl on the 5-HT induced increase in $[Ca^{++}]_i$. Lidocaine had no effect on $[Ca^{++}]_i$ at any dose studied (1.0 μM to 1.0 mM). Cocaine's inhibitory effect does not extend to all promoters of platelet activation since it had no effect on thrombin-induced rises in $[Ca^{++}]_i$.

This study was supported by a NARSAD grant.

DRUGS OF ABUSE: COCAINE I

362.1

GENDER DIFFERENCES IN COCAINE-INDUCED HPA ACTIVATION. Cynthia M. Kuhn* and Reynold Francis. Dep't of Pharmacology,, Duke University Medical Center. Durham NC 27710

Enhanced sensitization of both amphetamine and cocaine-induced locomotion have been reported in female rats. As CRF has been implicated as a contributing factor in this sensitization, we investigated the possibility that gender differences in cocaine-induced CRF release would lead to enhanced HPA activation in female rats. To investigate this possibility, we treated male and female rats with saline or cocaine and evaluated serum ACTH levels 30 minutes after treatment. Female rats exhibited higher ACTH secretion following challenge with 5, 10, or 20 mg/kg of cocaine relative to males. To investigate the role of different monoamine systems in this gender difference, responses to varying doses of fluoxetine, desipramine and GBR 12909 were evaluated. Females showed enhanced responses to all three uptake inhibitors. In addition, females showed enhanced responses to CRF (0.1 or 1 ug s.c.). Finally, the impact of gonadectomy on ACTH responses to cocaine was evaluated. Male and female rats were treated with saline or cocaine 1 week after gonadectomy, and serum ACTH evaluated as before. Gonadectomy lowered ACTH responses in females, but had no effect in males. These results support a role for estrogen and/or progesterone in the enhanced HPA response of female rats to cocaine and other monoamine uptake inhibitors. (Supported by DA-9079)

362.2

DISSOCIATION BETWEEN BEHAVIORAL ACTIVATION AND NEUROENDOCRINE STRESS RESPONSES TO COCAINE Wilkinson,C.W., Sarkar,D.K.°, Peskind,E.R.*, Rasmussen,D.D. VA Medical Center and University of Washington, Seattle, WA 98195 and °Washington State University., Pullman, WA 99164.

In rats, i.v. administration of low-dose (e.g., 0.5 and 1 mg/kg) 1 min pulses of cocaine is reinforcing; higher i.v. dosages are aversive (Nomikos and Spyraki, 1988). Since investigations of neuroendocrine responses to cocaine have commonly used higher dosages, more non-physiological (e.g., i.p.) routes of administration, and/or naive rats for which the unfamiliar subjective response to cocaine may induce novelty stress, we reasoned that demonstrated neuroendocrine responses may be in part due to non-specific stresses. Consequently, we have developed a model in which "experienced" rats receive low dose (0, 0.3, 0.6 mg/kg/pulse) 1 min i.v. cocaine pulse infusions at signaled 10 min intervals during the dark (active) photophase to produce and sustain (for 3 h) plasma cocaine levels which have been demonstrated to be associated with reinforcement. The 0.6 mg/kg cocaine pulses produced and sustained plasma cocaine levels of approximately 300 ng/ml, and increased activity levels (videotaped; behavior scoring scale) throughout the 3 h infusion period. However, this cocaine stimulation did not significantly increase plasma adrenocorticotropin (ACTH) or corticosterone (CORT) concentrations at 30, 60, 180 or 360 min after initiation of the pulsatile infusions; both saline- and cocaine-treated rats exhibited low (e.g., non-stressed) ACTH (<30 pg/ml) and CORT (<30 ng/ml) levels at all time points. In contrast, i.p. administration of 10 mg cocaine/kg to naive rats in a parallel experiment greatly increased plasma CORT levels at 30 min (cocaine: 271±21 ng/ml *vs* saline: 85±13 ng/ml), as previously reported. These results demonstrate that acute atraumatic induction of physiologically meaningful plasma cocaine levels and associated behavioral activation does not necessarily stimulate the neuroendocrine stress axis. Ongoing analyses will further characterize potential interactions with other related endocrine systems (NIH/NIAAA Grant AA10567 and Dept of Veterans Affairs)

362.3

CONDITIONED NEUROENDOCRINE EFFECTS OF STIMULI ASSOCIATED WITH COCAINE. A.C. DeVries, S.E. Taymans and A. Pert* Biological Psychiatry Branch and Developmental Endocrinology Branch, NIMH, Bethesda, MD 20892.

Elevated levels of corticosterone may be an important factor in determining individual vulnerability to drug abuse. Stress-induced corticosterone (CORT) secretion facilitates behavioral sensitization to drug effects and may enhance the positive reinforcing effects of abused substances. The purpose of the present study was to confirm and extend a previous finding that endocrine responses to cocaine do not exhibit tolerance or sensitization following repeated cocaine treatment, and to evaluate changes in blood CORT levels in response to situational stimuli associated with cocaine. In the first study there was no significant difference in the cort response to an acute cocaine challenge (30 mg/kg) in rats treated for the previous 7 days with saline or cocaine. The CORT levels of both groups differed from the control group which received saline injections for all 8 days. The second study assessed the conditioned endocrine effects of cocaine. The rats were randomly assigned to 3 groups, the first group of rats (paired) were injected with cocaine and immediately placed into a locomotor activity chamber for 30 min. One hour after being returned to their home cages the rats were injected with saline. The second group (unpaired) was treated in a similar manner, except that the rats received an injection of saline prior to being placed in the locomotor chamber and cocaine one hour after being returned to their home cages. The third group (control) received an injection of saline prior to being placed into a locomotor activity chamber and another saline injection one hour after being returned to their home cages. This routine was repeated daily for one week. On the 8th day, the rats were placed directly into the locomotor chambers without receiving an injection. After 30 min. the rats were removed from the chambers one at a time, decapitated, and their blood collected. The CORT levels and locomotor activity were significantly higher in the paired group than those in the unpaired and control groups, indicating conditioned increases in locomotor activity, as well as CORT release, by cocaine associated cues. Understanding the role of hormones in the development and expression of addictive behaviors may provide new insights for the treatment of drug addiction. Supported by NIMH.

362.4

MAINTENANCE OF COCAINE SELF-ADMINISTRATION IS NOT AFFECTED BY PHARMACOLOGICAL BLOCKADE OF BRAIN CRF RECEPTORS, S.H. Ahmed, M.P. Epping-Jordan, P.S. Griffin, A. Markou, S.C. Heinrichs*, E. B. De Souza, J. Rivier, G.F. Koob, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California

Numerous studies have implicated the endogenous release of central corticotropin-releasing factor (CRF) in the genesis of anxiety-related behaviors in rodents exposed to challenging environmental situations. Because anxiety is regarded as an important determinant of cocaine abuse in humans, central CRF could be a potential modulator, along with other neurotransmitters, in the maintenance of cocaine self-administration (SA). The CRF antagonist, D-Phe CRF_{12-41} (5 to 10 times more potent than α-hel CRF_{9-41} in blocking stress-induced behavioral alterations, Menzaghi et al., 1994, J. Pharmacol. Exp. Therap., 269: 564-572) was tested on male Wistar rats that had acquired stable baseline cocaine SA (FR5 TO 20 sec; cocaine dose: 0.25 mg per infusion; 2-hours daily session). The results obtained show that at all doses (0.04; 0.2; 1; 5 μg), icv injection of D-Phe CRF_{12-41} has no effect on the total number of cocaine infusions per session. This finding was confirmed in a more detailed analysis in which D-Phe-CRF_{12-41} had no effect either on the latency to self-administer the first infusion or on the number of infusions during the 1st 10 min of the SA session. This lack of effect of D-Phe CRF_{12-41} on cocaine SA is not dependent on the relatively low dose of cocaine used since a similar result is also observed in rats self-administering a four-fold higher dose of cocaine (1mg per infusion). These data suggest that brain CRF receptor stimulation is not critical for the maintenance of cocaine SA. Future studies will be required to determine the role of brain CRF receptors on other aspects of cocaine SA, such as initiation and reinstatement of cocaine SA.

This work was supported by grant DA76741.

362.5

INTERACTION OF COCAINE WITH SOCIAL DEFEAT STRESS ON FOS IMMUNOHISTOCHEMISTRY IN N. RAPHE AND VENTRAL TEGMENTAL AREA OF MOUSE BRAIN. E.M. Nikulina*, J.F. Marchand, R. Kream and K.A. Miczek. Department of Anesthesiology and Department of Psychology, Tufts University, Medford, MA 02155

Stress and cocaine may have a common mechanism in the activation of the mesocorticolimbic dopamine system and development of behavioral sensitization to psychostimulants. In order to examine neuronal activation in dopamine and serotonin soma after cocaine and social stress we studied Fos immunohistochemistry in ventral tegmental area (VTA) and dorsal (DR) and medial (MR) raphe nuclei. "Social stress" was defined as defeat and display of submissive behavior after brief confrontation with an aggressive resident mouse and subsequent exposure to the resident's threats in a protective cage. Mice were injected with cocaine (20 and 40 mg/kg, i.p.) and perfused 1 hour after injections or social stress. Social stress alone in naive mice increased Fos-positive cells in DR, but not in VTA or MR, when compared to saline or untreated mice. Cocaine induced Fos immunoreactivity in DR and MR; this Fos expression was significantly attenuated in mice that were defeated. Fos-positive staining after cocaine in combination with social stress was the same as in saline control mice. In contrast, in VTA neither social stress nor cocaine injection induced pronounced Fos immunohistochemistry. Thus, acute cocaine and social stress induced large changes in Fos expression in raphe neurons, but not in VTA, and these results suggest a role for serotonin neurons in development of stress-induced sensitization to cocaine. (Supported by INVEST grant from NIDA, DA04128 and DA02632).

362.6

EFFECTS OF CHRONIC COCAINE ADMINISTRATION ON BASAL AND STRESS-INDUCED CORTICOSTERONE SECRETION AND COGNITIVE FUNCTIONS IN RATS. Z. Sarnyai[1, 2], C. D. Conrad[1], Y. Kuroda[1*], M. J. Kreek[2] and B. S. McEwen[1]. Lab. of Neuroendocrinology[1] and Lab. of Biology of Addictive Diseases[2], The Rockefeller University, New York, NY 10021

Chronic use of cocaine may lead to cognitive disturbances characterized by persistent memory deficits and impaired attention in humans. We studied restraint stress-induced corticosterone (CORT) response before and after chronic binge pattern cocaine administration (BPCA; 15 mg/kg cocaine i.p., 3 inj./day for 21 days) and hippocampus-dependent cognitive functions after BPCA by using a spatial maze (Y-Maze). BPCA produced an adrenal hypertrophy and elevated basal CORT levels compared to pre-BPCA values. Furthermore, after BPCA, rats with smaller CORT elevations during their initial stress test prior to BPCA commencement (low responders, LR) showed significantly higher post-stress recovery CORT levels compared to high-responders (HR, high CORT response to initial stress) and controls. Cocaine-treated HR and control animals showed similar maze performance by identifying the novel arm. Although, cocaine-treated LR animals also recognized the novel arm quickly, they entered the novel arm more often than the other arms throughout the Y-Maze testing period, while controls and HR cocaine-treated rats did not show arm preference after the first few min. These data show that BPCA leads to a chronic overstimulation of the hypothalamic-pituitary-adrenal (HPA) axis with a possible disturbance in negative glucocorticoid feedback in a subgroup of animals. In previous studies using the Y-Maze, curiosity for the novel arm has been shown to decrease rapidly with time in intact animals. Animals with altered HPA functions after BPCA tend to show cognitive changes suggesting that they have difficulties inhibiting this behavior. The results imply involvement of hippocampus and frontal cortex. *This work was supported by the Arthur Vining Davis Foundation and MH41256 from the NIH/NIMH to BSM.*

362.7

STRESS SUBSTITUTES FOR COCAINE IN RATS TRAINED USING A DRUG DISCRIMINATION DESIGN J.R. Mantsch*, G.F. Guerin, N.E. Goeders. Department of Pharmacology & Therapeutics, LSU Medical Center, Shreveport, LA 71130.

The ability of the interoceptive cues produced during stress to substitute for the discriminative stimulus properties of cocaine was investigated. Adult male Wistar rats were trained to discriminate cocaine (10 mg/kg i.p., n=12; or 20 mg/kg i.p., n=6) from saline using a two-choice, operant, food-reinforced, drug discrimination task. Restraint stress (15 min.), intermittent electric footshock (15 min. session, 0.6 mA, 0.5 msec duration, every 30 sec.), and conditioned fear (10 sec. tone and stimulus light paired with the delivery of electric footshock under an random-interval 5 min. schedule during daily 60 min. sessions for 2 weeks) each partially substituted for both doses of cocaine (10 and 20 mg/kg). During test sessions, substitution was defined as the percent responding on the cocaine-appropriate response lever prior to the delivery of the first reinforcer when each stressor was administered following an injection of saline (i.p.). Experiments designed to investigate the ability of increasing doses of chlordiazepoxide (3.0, 5.6, 10.0 mg/kg, i.p.) to block the substitution of stress for cocaine as well as the discrimination of the training doses of cocaine were also performed. Additionally, studies designed to determine the potential involvement of dopamine, serotonin, and corticosterone will be discussed. Similarities between the discriminative stimulus properties of stress and cocaine would support the hypothesis that these interoceptive cues are produced by the activation of common effector systems.
(Supported by USPHS grant DA06013 from NIDA)

362.8

THE EFFECTS OF DAILY CORTICOSTERONE PRETREATMENT ON THE ACQUISITION OF INTRAVENOUS COCAINE SELF-ADMINISTRATION IN RATS N.E. Goeders*, G.F. Guerin, J.R. Mantsch. Departments of Pharmacology & Therapeutics and Psychiatry, LSU Medical Center, Shreveport, LA 71130.

It has been previously demonstrated that daily exposure to electric footshock facilitates the acquisition of low-dose cocaine self-administration in rats and that this is positively correlated with plasma corticosterone (CORT). The following experiments were designed to investigate whether these effects could be mimicked by a daily CORT pretreatment regimen which produced increases in plasma CORT similar in magnitude and duration to those occurring during stress. Following the establishment of baseline responding, adult male Wistar rats were tested for the intravenous self-administration of increasing doses of cocaine (0, 0.031, 0.0625, 0.125, 0.25, 0.5, and 1.0 mg/kg/infusion) under a fixed-ratio 1 (FR1) schedule of reinforcement during daily 1 hour sessions. Rats were treated daily, 15 minutes prior to each self-administration session with a CORT suspension (2.0 mg/kg in saline, i.p., n=10) or saline (n=10). Rats were initially exposed to a low dose of cocaine (0.031 mg/kg/infusion), and this dose was doubled weekly. Rats pretreated with CORT acquired self-administration at a lower dose of cocaine versus saline-pretreated controls (0.0625 vs. 0.125 mg/kg/infusion) as defined as a significantly greater number of infusions per session compared to baseline. Experiments designed to examine the potential involvement of Type I and Type II corticosteroid receptors using daily pretreatments with aldosterone and dexamethasone will also be discussed.
(Supported by USPHS grant DA06013 from NIDA)

362.9

GLUCOCORTICOIDS INCREASE THE REINFORCING EFFECTS OF COCAINE AND INDUCE REINSTATEMENT OF COCAINE SELF-ADMINISTRATION.
V. Deroche, M. Marinelli, M. Le Moal and P.V. Piazza.* Psychobiologie des Comportements Adaptatifs. INSERM U. 259, 33077 Bordeaux Cedex, France.

Several data suggest that corticosterone (CS) could facilitate the reinforcing properties of psychostimulant drugs and be an important factor in the vulnerability to develop and relapse substance abuse. We studied the influence of CS on intravenous self-administration (SA) of cocaine: i) as a function of CS plasmatic concentration, ii) as a function of the dose of cocaine per infusion, and iii) in a reinstatement paradigm. In a first experiment, we tested, in adrenalectomized (ADX) rats, the effects of different CS substitutive treatments on SA of 0.8 mg/kg/inf. of cocaine. Once the CS substitutive treatment reversing the effects of ADX was identified, a full dose-response curve (0.025, 0.05, 0.1, 0.2, 0.4, 0.8 mg/kg/inf.) for cocaine SA was performed comparing controls, ADX rats and ADX rats receiving the CS substitutive treatment. We showed that ADX decreases cocaine SA by decreasing the maximal effect of the drug. This effect was dose-dependently reversed by CS, a complete restoration being obtained for stress-induced levels of the hormone. In a second experiment, we tested the effects of an i.v. administration of CS (0.09, 0.18, 0.37, 0.58 mg/kg) on the reinstatement of SA behavior in intact rats submitted to extinction after acquisition of cocaine SA at 0.25 mg/kg/inf. We showed that CS dose-dependently precipitated the reinstatement of cocaine SA; the maximal effect being obtained for a dose of CS producing stress levels of the hormone. In conclusion, our results suggest that glucocorticoids may be a determinant factor in the vulnerability to psychostimulants abuse and open new therapeutic strategies for drug addiction.
This work was supported by INSERM, Université de Bordeaux II and Conseil Régional d'Aquitaine.

362.10

STRESS AND COCAINE INJECTIONS REINSTATE COCAINE-SEEKING AFTER PROLONGED EXTINCTION. S. Erb, Y. Shaham, and J. Stewart*. Center for Studies in Behavioral Neurobiology, Psychology Dept., Concordia University, Montreal, Quebec, Canada

Rats were trained to self-administer cocaine and were given tests for reinstatement in which they were exposed to priming injections of cocaine and to intermittent footshock stress. Footshock stress was found to induce relapse to cocaine-taking behavior after prolonged extinction sessions and after a 4- to 6-week drug-free period; the effect was comparable to relapse induced by a priming injection of cocaine. Animals were initially allowed to self-administer cocaine HCl (1.0 mg/kg/infusion, IV) during one 3-h session/day for 12 days. Subsequently, extinction conditions were introduced by substituting saline for cocaine. Extinction conditions were maintained until animals reached a baseline level of responding of 10 responses or less over a 3-h period (5-13 days). Subsequently, animals were tested for reinstatement of cocaine-taking behavior in response to a non-contingent injection of cocaine (2.0 mg/kg, IV) and exposure to intermittent footshock (10 min, 0.5 mA, 0.5 sec on, mean off period of 40 sec). After an additional 4- to 6-week drug-free period, tests for reinstatement were repeated. Reinstatement of cocaine-taking behavior was observed in both sets of tests in response to footshock and cocaine. These results extend previous reports from this laboratory that footshock stress is an effective stimulus for reinstatement of drug-taking behavior in the rat.

362.11

EFFECTS OF CHRONIC DESIPRAMINE TREATMENT ON EXTINCTION AND REINSTATEMENT OF COCAINE SELF-ADMINISTRATION BEHAVIOR. R.A. Fuchs*, L.T.L. Tran-Nguyen, S.E. Specio, R.S. Groff, and J.L. Neisewander. Dept. Psychology, Arizona State University, Tempe, AZ 85287.

Craving is a major factor in recidivism of cocaine abuse. However, animal models of craving have not been well established. Extinction of self-administration behavior, as well as reinstatement by cocaine-associated stimuli, may provide a useful animal model of craving. The present study examined the predictive validity of the extinction/reinstatement model. Rats were trained to lever press for cocaine (0.75 mg/kg/0.1 ml infusion) or received yoked infusions of saline during daily 3-hr sessions. A stimulus light and tone were presented with the cocaine infusions. After responding stabilized on a VR 5 schedule of reinforcement, rats received an additional 14 days of training. They then underwent 11 days of withdrawal during which half of each group received either daily injections of desmethylimipramine (DMI, 10 mg/kg, IP) or saline (N=6-10). On day 12 of withdrawal, rats were allowed to respond under extinction conditions (extinction phase). After 1 hr without any responses had elapsed, the cocaine-paired stimuli were presented repeatedly (reinstatement phase). Across the cocaine groups, rats treated with DMI exhibited fewer responses relative to those treated with saline during the first 60 min of extinction. However, all groups exhibited a similar number of responses during reinstatement. Regardless of cocaine history, DMI-treated rats exhibited a lower rate of responding during extinction. During reinstatement, however, DMI-treated cocaine rats exhibited a lower rate of responding relative to cocaine controls, whereas DMI-treated saline rats did not differ from saline controls. Regardless of DMI treatment, cocaine animals exhibited similar extinction latencies during both extinction and reinstatement phases. The modest decrease in responding during extinction and the reduced response rate during reinstatement produced by DMI in cocaine rats lend support to the predictive validity of this model. (Supported by DA07730 and HHMI).

362.12

REINSTATEMENT OF COCAINE-SEEKING BEHAVIOR IN A NONHUMAN PRIMATE MODEL OF RELAPSE. R .L. Barrett-Larimore and R. D. Spealman*. NERPRC, Harvard Medical School, Southborough, MA, 01752.

Relapse to drug abuse is a distinctive feature of drug addiction and perhaps the most difficult to treat. Clinical and experimental evidence implicates drug-associated environmental stimuli and drug priming as important determinants of the relapse process. The present study investigated the role of these factors in the reinstatement of cocaine-seeking behavior using a nonhuman primate model of relapse and examined the attenuation of reinstatement by selective DA receptor antagonists. Squirrel monkeys were trained to self-administer cocaine under a second-order schedule of i.v. injection. Completion of every tenth response (FR 10) during a 10-min interval (FI 10-min) produced a brief (2 sec) visual stimulus. The first FR 10 completed after the 10-min FI elapsed produced both the brief stimulus and an injection of cocaine. High rates of responding (0.8-2.0 resp/sec) were maintained under these conditions by 0.3 mg/kg/injection cocaine. Responding was subsequently reduced or eliminated during a series of extinction sessions in which saline was substituted for cocaine and the brief stimulus was omitted. Following extinction, responding could be reinstated by administering a priming injection of cocaine before the session, by restoring the brief stimulus previously associated with cocaine injections, or maximally by both. Under the later condition, cocaine-induced reinstatement was dose- and time-dependent and could be attenuated by the D_1 receptor antagonist SCH-39166, the D_2 antagonists eticlopride and nemonapride, and the novel D_3/D_4 antagonist YM-43611. The putative dopamine autoreceptor compounds (-) 3-PPP, (+) UH-232, or (+) AJ-76 were largely ineffective in blocking cocaine-induced reinstatement. The results suggest that this model can provide an empirical means for studying pharmacological and environmental factors contributing to relapse and for evaluating potential anti-relapse medications. Supported by DA00499, MH07658, MH14275 and RR00168.

362.13

BLOCKADE OF D-1 AND D-2 DOPAMINE RECEPTORS ATTENUATES "RELAPSE" ELICITED BY COCAINE-ASSOCIATED ENVIRONMENTAL STIMULI FOLLOWING PROLONGED EXTINCTION. C. S. Maldonado-Irizarry*, D. Smith, G. F. Koob and F. Weiss. The Scripps Research Institute, Department of Neuropharmacology, La Jolla, CA 92037.

The clinical literature suggests that subjective feelings of craving contribute significantly to the perpetuation of drug abuse or to relapse after a period of abstinence. This literature also suggests that drug taking behavior can be elicited by drug-related environmental stimuli. The goal of the present experiments was to develop procedures that would permit the study of cocaine-seeking behavior elicited by environmental stimuli associated with cocaine availability. Rats were subjected to 3 daily 1-hr IV self-administration sessions in which either saline or cocaine (0.25 mg/kg) was available on a FR1TO20s schedule in a random sequence. Animals were trained to discriminate a stimulus context predictive of cocaine availability (S+) from a stimulus context predictive of saline availability (S-). Following training, rats were given daily extinction sessions in which both the discriminative stimuli and cocaine or saline infusions were withheld. These animals met extinction criterion (less than 4 responses) by 24 sessions. Re-introduction of the cocaine S+ but not the S- elicited and maintained responding in the continued absence of the primary reinforcer. Rats initially exposed to the S- during the relapse phase also showed cocaine seeking behavior when presented with the cocaine S+. Pharmacological tests during the relapse phase, conducted in different groups of rats showed that SCH-23390 (D-1 antagonist; 10, 20 µg/kg, SC) or Raclopride (D-2 antagonist; 50, 100 µg/kg, SC) decreased cocaine seeking behavior. The present findings illustrate that drug-seeking behavior elicited by the present procedures is sensitive to pharmacological manipulation. Furthermore, blockade of DA neurotransmission attenuated the motivational properties of the drug-associated environmental stimuli, thus, implicating the DA system in cocaine seeking behavior elicited by environmental stimuli. (Supported by NIDA DA07348)

362.14

DOPAMINE OVERFLOW IN THE AMYGDALA DURING WITHDRAWAL FROM SELF-ADMINISTERED COCAINE. L.T.L. Tran-Nguyen*, R.A. Fuchs, G.P. Coffey, D.A Baker, L.E. O'Dell, J.L. Neisewander. Department of Psychology, Arizona State University, Tempe, AZ 85287-1104.

Cocaine and cocaine-associated stimuli elicit cocaine craving in humans and reinstate extinguished cocaine self-administration (SA) behavior in rats. It has been suggested that craving and reinstatement of cocaine SA behavior may be mediated by enhanced dopamine (DA) neurotransmission. To test this, experimental rats were trained to lever press for cocaine (0.75 mg/kg/0.1 infusion) 3 hr daily for 14 days. Control rats received yoked infusions of saline. A stimulus light and tone were paired with cocaine infusions during training. Rats were then withdrawn from cocaine or saline for 7-8 or 30-31 days (N=4-7 per group). *In vivo* microdialysis was then used to measure DA overflow in the basolateral amygdala during 1) baseline, 2) extinction of lever presses, 3) reinstatement of lever presses by the cocaine-paired stimuli and 4) reinstatement of lever presses by cocaine (15 mg/kg, IP). Cocaine rats exhibited more lever presses relative to controls during extinction and cocaine reinstatement phases. However, there were no differences in lever presses between the two cocaine groups undergoing different periods of withdrawal. There were no differences in DA overflow between controls and the cocaine group tested on day 7-8 of withdrawal in any of the test phases. However, there was a significant increase in baseline DA overflow, as well as cocaine-elicited DA overflow, in the cocaine group tested on day 30-31 relative to the two other groups. The results suggest that there is a time-dependent increase in DA overflow in the basolateral amygdala following withdrawal from self-administered cocaine. The results also suggest that there is no correlation between lever presses and DA overflow in the basolateral amygdala. (Supported by DA07730, HHMI, and MH-19547).

362.15

DRUG-SEEKING BEHAVIOUR MEASURED USING A SECOND-ORDER SCHEDULE OF I.V. COCAINE REINFORCEMENT: EFFECTS OF WITHDRAWAL FROM FREE-ACCESS TO COCAINE

M. Arroyo, A. Markou, T.W. Robbins and B.J. Everitt.*
Dept Experimental Psychology, University of Cambridge, Cambridge CB2 3EB, UK and The Scripps Research Institute, La Jolla, California 92037, U.S.A.

Rats were trained to self-administer cocaine (0.25mg/infusion I.V.) under a second-order schedule (FI15min:FR10:S). Initially, each infusion of cocaine was made contingent on a response on one of two identical levers and was paired with a 20s light conditioned stimulus (CS). When rats acquired stable rates of self-administration, the response requirement for cocaine was increased progressively to FR10; then the response requirement for the CS+ was also increased to 10 [i.e. FR10(FR10:S)]. At this point, an overall fixed interval (FI) second-order schedule was instigated, whereby the I.V. cocaine infusion was earned following the completion of the first FR10 responses (and CS presentation) after 15 minutes had elapsed [i.e. a second-order schedule FI15min(FR10:S)]. Rats received 5 cocaine infusions per day under this schedule and were allowed to stabilise responding for several days before being allowed free-access to cocaine during a 12h self-administration session ("binge"). Their responding under the second-order schedule for a single infusion of cocaine was then measured at 6, 12, 24, 48 and 72h after this cocaine "binge". All animals showed marked reductions in responding for cocaine at 6 and 12h which recovered transiently at 24h, but then continued at significantly low levels for the next 2 days. By contrast, the same animals under control conditions (i.e. no cocaine withdrawal) showed increased responding for cocaine when the usual 5 daily infusions were reduced to 1 (as used under the withdrawal conditions described above). These results indicate that withdrawal from cocaine is associated with significant reductions in cocaine-seeking behaviour.

(Supported by MRC G9407194N to BJE and DA00231 to AM)

362.16

EFFECTS OF COCAINE AND DOPAMINE AGONISTS ON COCAINE-SEEKING BEHAVIOR IN RATS: A SECOND-ORDER SCHEDULE OF REINFORCEMENT. A. Markou*, M. Arroyo and B.J. Everitt. The Scripps Research Institute, La Jolla, California, U.S.A. and University of Cambridge, U.K.

A second-order schedule of drug reinforcement allows the assessment of: (a) the motivation to self-administer a drug (drug-seeking behavior) before any drug administration or after drug injections; and (b) the control exerted by conditioned stimuli or reinforcers (CSs) over drug-seeking behavior. Rats were trained to respond for intravenous (IV) cocaine (COC, 0.25 mg/inj) under a fixed interval 15 min schedule, during which time CSs paired with COC were presented on a fixed ratio 10. Non-contingent intraperitoneal (0, 10, 15, 20 mg/kg) or IV (0.25 mg) COC increased the latency to initiate responding and decreased the number of CSs earned, which can be interpreted as a decrease in drug-seeking behavior. By contrast, contingent IV COC (0.25 mg/kg) decreased the latency to initiate responding and increased the number of CSs earned, which may be interpreted as an increase in drug-seeking behavior and/or increased control over responding by the CR. Furthermore, non-contingent injections of quinpirole, a relatively selective dopamine D2 receptor agonist (0, 0.5, 0.1, 0.3 mg/kg, SC), had similar effects to non-contingent COC. Finally, low doses (0, 0.003, 0.01, 0.03, 0.1 mg/kg, SC) of a relatively selective D3 dopamine receptor agonist, PD-128,907 hydrochloride, had no consistent effect on drug-seeking behavior, while higher doses (0.3, 0.6 mg/kg) had non-specific effects on performance. In summary, drug-seeking behavior can be reliably measured in rats who will work for 15 min and emit hundreds of responses to gain access to COC. Furthemore, contingent COC increases, while non-contingent COC and quinpirole, a D2 agonist, decrease cocaine-seeking behavior, demonstrating the significance of contingent versus non-contingent injections. Finally, PD-128,907 hydrochloride, a D3 agonist, appeared to have no effect on cocaine-seeking behavior. (Supported by DA00213 to AM and MRC G9407194N to BJE).

362.17

ELECTROPHYSIOLOGICAL AND BEHAVIORAL CHARACTERIZATION OF AN ANIMAL MODEL OF COCAINE WITHDRAWAL. T.E. Koeltzow*, D.C. Cooper, A.J. Vartanian and F.J. White. Neuropsychopharmacology Lab, Dept. Neuroscience, Finch Univ Hlth Sciences/Chicago Med Sch, North Chicago, IL 60064.

During withdrawal, cocaine addicts may experience anergia, depression, anhedonia, anxiety and cocaine craving. Previous animal models have produced anhedonia and anxiety, but not anergia/depression. Using an escalating, binge-like regimen of cocaine administration, we describe a model of cocaine withdrawal chararcterized by nocturnal hypoactivity and decreased activity of VTA dopamine neurons. Rats received 3 daily injections (1 hr apart between 9 am and 11 am) of cocaine or saline as follows: days 1-2, 10 mg/kg; days 3-4, 20 mg/kg; days 5-7, 30 mg/kg. Prior to behavioral testing on day 8, all animals received a cocaine challenge (10 mg/kg) to verify locomotor sensitization to cocaine in cocaine treated rats. When locomotor activity was monitored continuously for 72 hrs, cocaine withdrawn rats were significantly hypoactive during the 12 hr dark cycles compared to controls. No differences in locomotor activity were observed during light cycles. To characterize possible electrophysiological alterations in the mesoaccumbens dopamine system, we conducted cells/track analysis using extracellular recording techniques in the VTA. Preliminary results obtained on day 8 (no cocaine challenge) indicate a significant decrease (~70%) in spontaneously active dopamine neurons in animals receiving cocaine compared to controls. On day 12 (5 days withdrawal), this effect was no longer present. No significant differences in basal firing rates were observed on either day. These findings contrast with earlier reports of enhanced dopamine cell activity during early withdrawal from intermittant or continuous cocaine administration. Our results suggest that this model may parallel certain symptoms of the withdrawal syndrome observed in human cocaine addicts, and that reduced dopamine neuronal activity may contribute to anergia and psychomotor depression. (Supported by DA 04093 and DA 00207 to FJW.)

362.18

ALTERED ACTIVITY OF MIDBRAIN DOPAMINE NEURONS FOLLOWING 7-DAY WITHDRAWAL FROM CONTINUOUS OR INTERMITTENT COCAINE PRETREATMENT. W.-Y. Gao, T.H. Lee and E.H. Ellinwood*. Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

Using in vivo single-unit recording, effects of continuous and intermittent cocaine pretreatment regimens on the spontaneous activity of A_9 and A_{10} dopamine (DA) neurons were compared 7 days after withdrawal. Rats were pretreated for 14 days with: (1) saline injections (1 ml/kg, s.c., qd); (2) cocaine injections (40 mg/kg, s.c., qd); or (3) continuous cocaine infusion (40 mg/kg/day, s.c., osmotic minipumps). Predetermined blocks in the midbrain were sampled by passing the recording electrode nine times; the number of spontaneously active DA neurons as well as their firing rates and bursting patterns were determined.

In the A_9 area, the continuous group exhibited a significantly reduced number of neurons without changes in the firing rates or bursting patterns, while the injection group showed no differences in any of the indices. After an acute sulpiride injection (50 mg/kg, i.p.) 1 hour before sampling, the reduction in the continuous group was no longer demonstrable, suggesting a supersensitivity of D_2 receptors (autoreceptors), rather than a depolarization block, as an underlying mechanism. Daily injections of apomorphine (100 µg/kg, s.c., qd) between withdrawal days 1 and 5 selectively prevented the reduced number of active A_9 DA neurons in the continuous group.

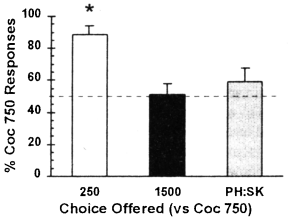
In the A_{10} area, continuous infusion reduced the bursting activity without affecting the number of active DA neurons, while the intermittent group showed an increased number of neurons without changes in the bursting pattern. Sulpiride reversed the changes in the both pretreatment groups, again suggesting involvement of D_2 receptors. Our results will be compared and contrasted to those from previous studies.
Supported by R01 DA-06519 to THL.

DRUGS OF ABUSE: COCAINE II

363.1

CHOOSING BETWEEN COCAINE AND COMBINATIONS OF DOPAMINE D1 AND D2 AGONISTS IN SELF-ADMINISTRATION TESTS. J.D. Belluzzi*, J.A. Del Rio, A. Manzardo and L. Stein. Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

We have previously reported that rats will self-administer solutions containing combinations of dopamine D_1 and D_2 agonists. Response rates and patterns induced by the combinations were nearly identical to those induced by cocaine. Here we test whether rats prefer cocaine to a D_1/D_2-agonist combination in a choice test. Daily 3-hr tests were made in a 2-lever box. During training, cocaine (750 µg/kg/inj, i.v.) was available at either the right or left lever and the active side was alternated daily. After rates stabilized (±10% for 2 days), different groups were offered cocaine (750 µg/kg/inj) at one lever and cocaine 250 µg/kg/inj, cocaine 1500 µg/kg/inj, or the dopamine D_1/D_2 agonist combination (PHNO 0.3 µg/kg/inj + SKF 82958 3 µg/kg/inj) at the other lever. Choice of Coc 750 was 89% vs Coc 250 (Fig; *$p<0.01$) and 51% vs Coc 1500, indicating the following order of preference: 750≈1500>>250. However, choice of Coc 750 was only 59% vs the D_1/D_2 agonist combination (PH:SK). Since rats did not significantly prefer cocaine to the D_1/D_2 combination, we conclude that dopamine agonist combinations can largely substitute for, and may be indistinguishable in reinforcement value from, cocaine in self-administration tests.



(Supported by NIDA grant DA07747)

363.2

THE EFFECT OF ANTAGONIST PRETREATMENT ON DOPAMINE D3 RECEPTOR MEDIATED ROTATION IN 6-OHDA LESIONED RATS. **P.M. Robinet*, C.K. Akins, C.R. Goetz, J.M. Dose & M.T. Bardo. Dept. of Psychology, University of Kentucky, Lexington, KY 40506.**

In two experiments, rats received unilateral injections of 6-OHDA into the A9/A10 cell body region. Only rats that turned in a robust manner to 1 mg/kg apomorphine were included in the experiments. Both experiments used a mixed factor design with pretreatment as a between groups measure and drug dose as a repeated measure using a pseudo-latin square such that each dose order was accounted for. In Experiment 1, the rats were pretreated with either the D2/D3 antagonist eticlopride (0.1 mg/kg) or saline. Fifteen min later they were injected with a D3 agonist, either 7-OH-DPAT (0.0, 0.001, 0.10 or 1.0 mg/kg) or PD123389 (0.0, 0.01, 0.1 or 1.0 mg/kg) and the number of rotations were recorded for 30 min. Both 7-0H-DPAT and PD123389 increased the number of rotations dose dependently and 7-OH-DPAT induced a greater magnitude of rotations than PD123389. However, pretreatment with eticlopride did not affect rotation in either the 7-OH-DPAT or PD123389 treated animals. In Experiment 2, rats were pretreated with either eticlopride (1.0 mg/kg), the D1 antagonist SCH23390 (0.3 mg/kg) or saline. Fifteen min later the rats were injected with PD123389 (0.0, 0.5, 1.0, 1.5 or 2.0 mg/kg) and the number of rotations were recorded for 30 min. PD123389 induced rotation at all doses; however, the number of rotations did not differ across drug doses. Pretreatment with eticlopride significantly decreased rotation in response to PD123389; however, pretreatment with SCH23390 had no effect on the number of rotations. Taken together, these results suggest that, at higher doses, PD123389-induced rotation is mediated by D2/D3 rather than D1 receptors.

363.3

ENVIRONMENTAL INFLUENCES ON THE PSYCHOMOTOR RESPONSE TO INTRAVENOUS COCAINE: A DOSE-EFFECT INVESTIGATION. Kaitlin E. Browman*, Aldo Badiani and Terry E. Robinson. Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48109-1109.

It was reported previously that unsignalled intravenous (IV) infusions of 1.0 mg/kg of amphetamine produced a very small acute response and no sensitization if rats were given drug treatments in their HOME cage, relative to rats that received the same treatments in a physically identical but NOVEL test cage (Crombag et al., 1996). The present experiment was designed to determine if there is a similar effect of environmental condition on the response to IV cocaine (COC), and the extent to which the effect may be dose-dependent. We compared, therefore, the response to IV administrations of COC in unilaterally DA-denervated rats, in which rotational behavior was used as an index of the psychomotor response. Rats received one IV infusion of COC a day for five days (0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, or 12.0 mg/kg), either at HOME using a remotely activated infusion pump, or following placement in a NOVEL environment. As with previous studies using i.p. drug administration (Badiani et al., 1995), there was no effect of environmental condition on the acute response to COC. Preliminary data suggest, however, that there was an effect of environment on sensitization to the psychomotor activating effects of COC.
(Grant #DA 04294)

363.4

ASSOCIATIVE FACTORS CONTROL ACCUMBENS CELL FIRING DURING COCAINE SELF-ADMINISTRATION IN RATS. R.M. Carelli* and S.A. Deadwyler. Center for the Neurobiological Investigation of Drug Abuse, Dept. of Phys./Pharm., Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27157.

We have previously reported that a subset of nucleus accumbens (NA) neurons exhibit 4 types of patterned discharges relative to the cocaine reinforced response following the onset of stable self-administration behavior in rats (JPET 277:385,1996). Preliminary findings indicate that NA phasic firing which occurred *following* the response during the time of cocaine delivery (0.33 mg/inf, 5.8 sec) paired with a tone-houselight stimulus (20 sec) may reflect a conditioned stimulus-related activation (Synapse, in press). The purpose of the present study was to examine further whether NA post response-related cell firing was controlled by stimulus presentation, drug delivery, and/or the drug-reinforced response. Of 286 cells recorded in 11 well-trained self-administering rats, 73 cells (25%) exhibited phasic changes (increases or decreases) in activity following the response. Removal of the stimulus during the session (after 10-12 self-administration trials) did not alter established NA post response-related discharges. This finding indicates that the response which produces cocaine infusion is sufficient to maintain NA post response-related activity. Response-independent random presentation of the stimulus in the absence of cocaine similarly activated NA phasic firing, however, no changes in NA cell firing were observed during response-independent cocaine delivery in the absence of the stimulus. Furthermore, self-administration behavior was extinguished when animals responded for the stimulus only in the absence of cocaine. These findings indicate that cocaine self-administration may either require or is greatly facilitated by the presence of a conditioned sensory stimulus paired with cocaine delivery. The basis for this seems to be the selective activation of NA neurons by the stimulus and not the drug.
[Supported by NIDA grants DA05535 (RMC) and DA06634, DA00119 (SAD).]

363.5

INFLUENCE OF COCAINE TRAINING DOSE ON SUBSTITUTION BY COCAINE AND (+)-AMPHETAMINE IN A 3-LEVER VS. A 2-LEVER DRUG DISCRIMINATION PROCEDURE. C.A. Amendola*, M.A.Edwards, A. Lefevour and K.M. Kantak Department of Psychology, Boston University, Boston, MA 02215.

Rats were trained to discriminate between saline, a high dose (10 mg/kg) and a low dose (3 mg/kg) of cocaine in a food reinforced, 3-lever drug discrimination procedure. After 200 to 230 daily training sessions, 2 of 4 subjects met the training criteria of >90% lever-appropriate responses, with no more than 4 incorrect responses before delivery of the first reinforcer for 10 consecutive sessions. In substitution tests with cocaine (0.1-17.8 mg/kg) and saline, 3.0 mg/kg cocaine engendered >90% low dose-appropriate responses, while higher and lower doses of cocaine engendered >90% responses on the high dose- and saline-appropriate levers, respectively. In substitution tests with (+)-amphetamine, 1.0 mg/kg engendered >90% responses on the high dose-appropriate lever, while 0.1 mg/kg engendered >90% responses on the saline-appropriate lever. A dose of 0.56 mg/kg (+)-amphetamine engendered responses primarily on the low dose-appropriate lever. These data indicate that rats are able to successively discriminate between a high and a low dose of cocaine and saline. In addition, the substitution profiles are similar to those reported using a 2-lever drug discrimination procedure where training dose of cocaine was independently varied (Kantak et al., 1995).

363.6

EFFECTS OF CONTINGENT VS NONCONTINGENT INTRAVENOUS COCAINE INFUSIONS ON THE FIRING PATTERNS OF INDIVIDUAL NEURONS IN THE NUCLEUS ACCUMBENS OF RATS. L.L. Peoples*, A.J. Uzwiak, and M.O. West Dept. of Psychology, Rutgers, The State University, New Brunswick, NJ, 08903.

Male Long Evans rats (N=20) were chronically implanted with arrays of microwires, positioned in the Nucleus Accumbens (NAcc), and with jugular catheters. During cocaine self-administration training sessions, each lever press was followed by an infusion of cocaine (0.7 mg/kg/infusion). Individual NAcc neurons showed phasic changes in firing rate time-locked to cocaine self-infusion. To determine whether these changes may be related to appetitive behavior, we conducted a within-neuron within-session yoked control test. After an initial phase in which the rat attained a stable drug level (calculated), the session was conducted according to an ABAB design in which cocaine infusions were alternately response-contingent (C) in A phases and response-noncontingent (NC) in B phases. Each phase included 5-10 infusions (i.e., 5-10 trials). The pattern of C infusions was determined by the rat, as during training sessions. The pattern of NC infusions was yoked (i.e., equal) to the pattern of preceding C infusions. Stereotypic behavior and drug level were the same during the C and NC phases. Inter-spike-interval histograms were used to identify single neurons (N=85). The most common short-duration change in firing rate time-locked to C infusions (i.e., increase during first few seconds after self-infusion) was absent or diminished during NC trials devoid of lever presses. The most common long-duration change in firing rate time-locked to C infusions (i.e., decrease during 2 minutes after infusion followed by a progressive increase that culminated at the next infusion) was blunted during NC trials. Changes in firing during occasional NC trials in which the rat made a lever press (nonreinforced) were comparable to those time-locked to C infusions. The data suggest that the most common firing patterns time-locked to cocaine self-infusion contribute to drug-directed appetitive behavior and are not determined solely by direct actions of the drug or by the cues associated with the infusion. Supported by DA 06886.

363.7

CONDITIONING OF LOCOMOTION AND REINFORCING PROPERTIES TO DISCRETE STIMULI THROUGH ASSOCIATION WITH INTRAPERITONEAL COCAINE ADMINISTRATION. L. V. Panlilio* and C. W. Schindler. Preclinical Pharmacology Laboratory, NIH/NIDA DIR, Baltimore, MD 21224.

Studies of conditioned locomotor activity have demonstrated that excitatory properties are conditioned to a distinctive environment that has been associated with psychomotor-stimulant administration. Unlike traditional studies of classical conditioning, conditioned-activity studies have used the entire environmental context as the conditioned stimulus. It is not clear whether conditioned locomotion can be elicited by specific, discrete stimuli. To test this possibility, rats (n=24) were injected ip with saline prior to daily, 30-min sessions in a locomotor activity chamber. Interspersed (1-2 times/week) with these baseline sessions, rats were injected with cocaine (20mg/kg) and placed in the chamber, where a tone or flashing light was presented. While baseline levels of activity remained stable, sensitization occurred (i.e., locomotion increased) over 6 drug-stimulus pairings. When a cocaine challenge was administered in the absence of the conditioned stimuli, locomotor activity was attenuated compared to when a conditioned stimulus was present (indicating conditioned sensitization). When the rats were presented with tone or light without receiving cocaine, locomotor activity increased significantly above baseline (indicating conditioned locomotion). To determine whether incentive-motive properties were also conditioned to tone and light, the rats were then tested in an operant chamber containing two nose-poke holes. Responding in one hole produced a 2-sec conditioned-stimulus presentation. Response rates were significantly higher in this hole than in the other, inactive hole (indicating conditioned reinforcement). Thus, locomotor activity can be conditioned to discrete stimuli, and the incentive-motive properties conditioned to these stimuli in the locomotor-activity chamber can transfer to other environments. In this respect, the drug-conditioning effects seen in rodents may be analogous to the physiological responses and self-reports of craving obtained when drug abusers are presented with discrete, drug-related stimuli.
Supported by NIDA DIR

363.8

STIMULANT AND REWARDING PROPERTIES OF COCAINE FOLLOWING INTRA-VENTRICULAR OR INTRA-AMYGDALA INFUSIONS. L.E. O'Dell*, A.N. Sussman, K.L. Meyer, and J.L. Neisewander. Department of Psychology, Box 871104, Arizona State University, Tempe, AZ 85287-1104.

The effects of intracerebroventricular (ICV) or intra-amygdala infusions of cocaine on locomotion, conditioned place preference (CPP), and conditioned activity were assessed. Five two-day conditioning trials were conducted in a 2-compartment apparatus over 10 consecutive days. On one day of the trial, rats received either ICV or intra-amygdala infusions of cocaine at doses of 0 (n=12, 11), 25 (n=3, 9), 50 (n=7, 10), and 100 (n=10) µg/0.5 µl/per side and were placed into a distinct compartment for 30 min. On the other day, rats received sham infusions and were placed into the other compartment for 30 min. Locomotion was assessed during conditioning using a photocell system. CPP was assessed 24 hr after conditioning as an increase in time spent in the cocaine-paired compartment relative to the other compartment. Conditioned activity was assessed 24 hr after CPP as an increase in locomotion in the cocaine-paired compartment in cocaine-treated rats relative to controls following sham infusions. Cocaine infusions into either region produced a dose-dependent increase in locomotion, and the magnitude of this effect was greater following ICV relative to intra-amygdala infusions. Only the ICV dose of 50 µg produced CPP. In contrast, only the intra-amygdala doses of 50 and 100 µg produced conditioned activity. (Supported by DA07730, NIMH/MNTP grant MH-19547, and HHMI).

363.9

SINGLE NEURONAL ACTIVITY IN MESOLIMBIC SYSTEM DURING COCAINE AND HEROIN SELF-ADMINISTRATIONS IN FREELY MOVING RATS.. *J.-Y. Chang*, and D. J. Woodward.* Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

An effective approach to examine the neuronal mechanism(s) underlying cocaine and heroin self-administration is to investigate and compare the neuronal activities in the mesolimbic areas during self-administration behaviors. Using a chronic, multi-channel, single unit recording technique, up to 32 neurons were simultaneous recorded from medial frontal cortex/nucleus accumbens and ventral tegmental area/amygdala of rats trained in different sessions to self administer cocaine and heroin. Sessions either started with cocaine (1mg/kg per infusion) and were followed by heroin (30µg/kg per infusion) self-administration, or started with heroin and followed by cocaine self-administration. Self-administration of mixture of cocaine and heroin (0.5mg/kg cocaine +15µg/kg heroin and 1mg/kg cocaine+30µg/kg heroin per infusion) was also tested. Neurons in these mesolimbic areas exhibited responses both before (anticipatory responses) and after cocaine and heroin self-administration. About 40 to 50 percent of neurons displayed no response in medial frontal cortex and nucleus accumbens during both cocaine and heroin self-administration. A small percent of neurons (<10%) showed the same responses either before or after both cocaine and heroin self-administrations. A majority of neurons responded differently to cocaine and heroin during the operant task of self administration. Combining cocaine and heroin in the same solution yielded the responses different than observed with cocaine and heroin self-administration alone. Some anticipatory responses associated with cocaine self-administration disappeared when cocaine and heroin in combination was employed but could be recovered by injection of naloxone (1mg/kg, i.p.). Synergistic effects on post-drug neuronal response were found with combinations of cocaine and heroin. These synergistic effects were readily blocked by naloxone. The result suggests different neuronal circuits in mesolimbic system may be involved in the behavior produced during cocaine and heroin self-administration. The interaction of these neuronal circuits may be related to "speedball effects" produced by combinations of cocaine and heroin in drugs of abuse. *(Supported by DA-2338 to DJW. http://biogfx.neuro.wfu.edu)*

363.10

PHASIC FIRING PATTERNS RECORDED FROM INDIVIDUAL NUCLEUS ACCUMBENS NEURONS DURING COCAINE APPETITIVE BEHAVIOR. A.J. Uzwiak, M.O. West* and L.L. Peoples . Dept. Psychology, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903.

Previous studies have characterized phasic firing patterns in ventral striatal neurons during cocaine self administration. This study characterized phasic firing patterns attributable to neurons (n = 110) that were either in the sub-territories of the nucleus accumbens (NAcc) or in the anterior olfactory nucleus, posterior (AOP) and that were verified to be single units by interspike interval analysis. Individual male Long Evans rats (n = 29) were implanted with a microwire array positioned in the NAcc and AOP and a jugular catheter. Following the attainment of an asymptotic drug level, patterns time-locked to within ±10 seconds of the cocaine (0.7 mg/kg/inf) reinforced lever press (Fixed Ratio 1) were characterized. Two basic types of patterns were identified--single phase and multiple phase. The single phase pattern was sub-typed depending on whether the change in firing rate occured before the lever press, after the lever press, or began before and ended after the lever press. The single phase change patterns were further characterized by the direction (i.e. increase or decrease), duration and latency of the change in firing rate. The multiple phase change pattern was characterized by the number and direction of changes in the firing rate. Although the patterns observed in this study replicated some of the patterns described in previous studies, there appears to be a differential distribution of these patterns among ventral striatal brain areas. Our findings suggest that the multiphasic firing patterns are limited to the most anterior NAcc and AOP. Research supported by DA06886

363.11

ACUTE COCAINE DOES NOT ENHANCE SENSITIVITY TO SOMATOSENSORY STIMULI IN AWAKE BEHAVING RATS. E. M. Gould*[1], D. M. Devilbiss[1], J. P. Druhan[2] and B. D. Waterhouse[1]. [1]Dept. Neurobiology and Anatomy, [2]Division of Behavioral Neurobiology, Dept. of Psychiatry, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19102.

Previous studies from this laboratory have shown that acutely administered systemic cocaine alters the central processing of afferent signals from the rat mystacial vibrissae in halothane anesthetized rats. In the present study, we examined whether acute IP cocaine administration altered sensory signal processing in awake behaving rats trained to detect whisker pad stimulation in an operant discrimination task. A bipolar electrode for delivering subcutaneous stimuli was permanently implanted under the whisker pad, and the animals were required to press one of two levers to obtain food after brief presentations of whisker pad stimulation, or on the alternative lever on trials with no stimulation. Stimulus-response functions were then obtained by varying the intensity of the stimulation and determining the tendency to respond on the stimulation appropriate lever after each stimulus. The effects of cocaine (1, 2, 4, 10 and 20 mg/kg) on the stimulus-response function was then determined. One, two, four, ten mg/kg cocaine did not alter the responses to whisker pad stimulation. Only at 20 mg/kg was there a significant change in the animals' response pattern to the stimuli. This dose decreased stimulation lever responses at all intensities, so that rats responded to higher currents as though they were lower. Response measures obtained during the inter-trial interval (ITI) indicated that this dose also increased the rate of unrewarded responding, but did not affect the distribution of un-cued responses on the two levers during this period. These data suggest that cocaine does not enhance rats' sensitivity to sensory cues, and actually decreases detection of whisker pad stimulation at high doses. Supported by NIDA DA 05117 to BDW.

363.12

ACTIONS OF COCAINE (COCA) IN SOMATOSENSORY CORTEX: CORRELATIONS WITH CELL TYPES, MONOAMINES AND CHANGES IN EXCITABILITY. F.M. Sessler*, J. Zhai, T.N. Felder, F. Chen, A.E.K. Kosobud, B.D. Waterhouse, R.C.S. Lin and J. Lehmann. Depts. of Neurobiol. & Anat. and Neurosurg., Med. Col. of Pennsylvania and Hahnemann Univ., Philadelphia, PA 19102, and Dept. of Psychol., Indiana Univ. IN 47405.

In previous studies we reported differential actions of COCA in somatosensory cortex. We further examined the impact of this drug on processing capabilities of sensory neurons, by characterizing its effects on input resistance (IR) and rheobase (Rh) of four major types of identified layer V pyramidal neurons. In addition, we used amphetamine (AMPH) and fenfluramine (FENF) to mimic COCA actions on these neurons. Intracellular recordings were conducted in slices from rat somatosensory cortex. Non-local anesthetic concentration of COCA (1uM) decreased IR and increased Rh in 54% of the cells tested. This was mimicked by AMPH (1uM) but not by FENF (1uM). In contrast, IR increased in 75% of the cells tested with local anesthetic concentration of COCA (100uM). Changes in conductance reflecting possible local anesthetic effects of COCA were not mimicked by 100uM AMPH or FENF. In comparing drug actions on different cell types, we found that Rh was increased and IR decreased by COCA (1uM) in all the intrinsic bursting (IB) and in 67% of doublet spiking (DS) cells, resulting in decreased cell excitability. 55% of non-adapting and adapting regular spiking (NA-RS & A-RS) neurons showed an increase in excitability with increase in IR and decrease in Rh. Thus, most of the excitatory influences of COCA were found on RS neurons, and little or none on DS and IB cells. These observations suggest that an understanding of the impact of drug abuse on sensory processing within cortical circuits requires a characterization of both, monoaminergic influences and drug action on different cell types. (Supported by NIDA DA08405 to FMS and DA05117 to BDW).

363.13

ACUTE SYSTEMIC COCAINE ALTERS THE PROCESSING OF SENSORY INFORMATION FROM THE RAT VIBRISSAE. J. J. Rutter,*K. L. Simpson and B. D. Waterhouse. Dept. Neurobiology and Anatomy, MCPHU, Philadelphia, PA 19102-1192.

The impact of cocaine on neural networks innervated by monoamines other than dopamine has been inadequately studied. In order to characterize the effects of cocaine on activity transmitted through noradrenergically and serotoninergically innervated pathways, we have been evaluating the drug's ability to alter the central processing of afferent signals from the rat mystacial vibrissae. This system was chosen as a well-characterized sensory pathway which receives prominent norepinephrine (NE) and serotonin (5-HT) innervation at both cortical and subcortical relay sites. In the present study, extracellular responses to graded, mechanical displacement of individual whiskers on the contralateral face were recorded from single units in the ventral posterior medial (VPM) thalamus of halothane-anesthetized rats. Previous results from this laboratory had shown that both the magnitude and latency of such stimulus-evoked discharge could be altered following acute cocaine challenge (0.75 mg/kg, iv). When an effect was observed, cocaine invariably decreased response latency; however, changes in response magnitude could occur in either direction from control. Our more recent results support the notion that low concentrations of NE may have a facilitating effect on evoked signals, but that increasing the concentration of NE can depress the same signal. We hypothesized, therefore, that this bimodal shift in response may be due, in part, to the differential efficacy of a single dose of cocaine on fluctuating basal levels of 5-HT and NE. To test this, the sensitivity of a VPM unit to a range of whisker deflections was first determined, and then a fixed level of stimulation from the middle of the curve was identified and chosen for testing. The whisker was then driven at this level of stimulation and cell responses were recorded before and after administration of iv cocaine in a cumulative dose paradigm (0.25-2 mg/kg, dose every 10 min). Drug-induced changes in cell responses were determined from quantitative analysis of post-stimulus time histograms. Preliminary results reveal that cocaine increased the magnitude of response at the lowest dose tested (0.25 mg/kg), had a variable effect at intermediate doses, and, in the majority of cases, caused a decrease in the response to whisker stimulation at the highest dose tested. In addition, cocaine produced decreases in latency to response at low doses, with a more variable effect at high cocaine doses. The present findings suggest that: 1) cocaine may differentially affect sensory signal processing in a manner similar to that observed previously for NE, and 2) cocaine can independently alter specific stimulus coding properties of neurons along the trigeminal somatosensory pathway. Supported by NIDA DA05117 to BDW.

363.14

CELLULAR ACTIVATION DURING COCAINE EXPOSURE: A FOCUS ON THE HABENULA, J.L.Petrie*and G.D.Ellison Department of Psychology, UCLA, Los Angeles, CA, 90095.

Previous studies have shown that continuous exposure to cocaine will produce degeneration of fasciculus retroflexus, the major efferent of the habenula. 2-DG autoradiography indicates that the glucose utilization of the terminals in this area is reduced by cocaine. This study focuses on the cellular activity by using c-fos to continue the mapping of metabolic activity.

Three cocaine treatments were used: 1)single acute injection (40mg/kg), 2)five day pellet + single injection on day 6, 3)five day pellet + single injection on day 19. Cocaine was shown to significantly increase c-fos expression in animals treated with cocaine versus controls. However, there was no difference in expression between the three cocaine treated groups. The increase in cellular activity paired with a known decrease in terminal activity suggests a GABA-ergic afferent pathway. The lack of either a tolerance or sensitivity to the drug indicates that this may be a piece of the circuit involved in the psychotomimetic syndrome of stimulant psychosis.

Funding (NIDA grant #DA07344)

363.15

SEX HORMONES MODULATE DOPAMINE RESPONSE TO COCAINE M. N. Cook and B. C. Jones*. Department of Biobehavioral Health, The Pennsylvania State University, University Park, PA 16802.

In our laboratory, we have observed sex differences in dopaminergic response to cocaine, particularly in the medial prefrontal cortex of C57BL/6 mice(B6). Males and females of this strain had opposing dopaminergic responses to ip injection of 15 mg kg^{-1} cocaine. Cocaine increased dopamine utilization, as evaluated by the ratio of HVA/DA, in males and decreased utilization in females. To further investigate the sex differences in C57BL/6 mice, we undertook the present study. Male and female mice, 60 days of age, from the C57BL/6 mouse strain were either sham-operated or gonadectomized. Gonadectomized animals were implanted with one of several hormone pellets: placebo, estradiol, testosterone, dihydotestosterone, progesterone or the combination of estradiol and progesterone. One week following gonadectomy, animals were given either a single injection of 0.9% sterile saline or 15 mg kg^{-1} cocaine and killed ten minutes post-injection. The medial prefrontal cortex (MPFC), nucleus accumbens (NACC), caudate-putamen (CP) and ventral midbrain (VMB).were assayed, by HPLC, for Dopamine and Serotonin and their metabolites, HVA and DOPAC and 5HIAA respectively. Sex differences in dopamine utilization in the medial prefrontal cortex were replicated in this study. We also observed that, in the MPFC cocaine-related change in dopamine utilization in B6 castrated males treated with estradiol was similar to that of intact B6 females and ovariectomized B6 females treated with testosterone displayed dopaminergic response similar to that of intact B6 males. These preliminary data suggest that sex hormones are capable of modulating dopaminergic response to cocaine. Supported by USPHS Grant DA 07171

363.16

A HISTORY OF RESPONDING MAINTAINED BY SHOCK POSTPONEMENT DOES NOT ALTER THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE. T.A. Tatham[1]*, T.A. Hasling[1], J.E. Barrett[2]. [1]Psychiatry Department, Uniformed Services University, Bethesda, MD 20814; [2]CNS Division, Wyeth-Ayerst Research, Princeton, NJ 08543.

Behavioral history can alter the effects of drugs on behavior. For example, cocaine (COC) normally decreases food-maintained responding that is punished by the presentation of mild shocks. This laboratory has demonstrated, however, that COC increases punished responding following a history of responding maintained by postponement of shock. This outcome is especially interesting, for decreases in punished responding are consistent with the effects of anxiogenic drugs, whereas increases are the hallmark of anxiolytic compounds.

The present experiment examined whether cocaine shares discriminative stimulus (DS) effects with well-established anxiolytics mediated through the benzodiazepine (BZ) receptor following a history of responding maintained by shock postponement. Four squirrel monkeys were trained to discriminate COC from saline. On alternate days COC (0.56 mg/kg, i.m.) or saline (SAL) was injected. Ten-min later, 30 presses on the left lever produced food after SAL injections, whereas 30 presses on the right lever were reinforced after COC injections. After the monkeys performed accurately, tests were conducted in which COC (.1 - .56 mg/kg), midazolam (MDZ, .1 - .56 mg/kg) or pentobarbital (PB, 1.0 – 5.6 mg/kg) was injected and responding on either lever produced food. COC dose-dependently occasioned responding on the drug lever, whereas MDZ and PB engendered SAL-appropriate responding. Discrimination training was then suspended and 10 sessions were conducted in which pulling a chain postponed mild tail shocks. Discrimination training was reinstated and dose-response curves were redetermined. Dose-response curves for COC were not affected by the shock postponement history. MDZ and PB again occasioned only SAL-appropriate responding. These results suggest that the DS effects of COC do not resemble those of anxiolytic drugs mediated by the BZ receptor following a behavioral that changes COC's effect on punished responding from anxiogenic-like to anxiolytic-like.

Supported by NIH RO1 DA06828 awarded to J.E.B and NIH R29 DA09078 awarded to T.A.T.

363.17

SECOND-ORDER SCHEDULES OF COCAINE REINFORCEMENT IN RATS
S. R. Goldberg*, E.B.Thorndike, C.W. Schindler & M.Shoaib. Preclinical Pharmacology Laboratory, National Institute on Drug Abuse, D.I.R., N.I.H., Baltimore, MD 21224.

Response rates in drug self-administration are relatively low for rats in comparison to primates. Most of the current intravenous drug-self administration experiments performed in rodents have used simple fixed ratio (FR) schedules of reinforcement; rats are required to emit a fixed number of responses which produces an injection of drug. Since, intermittent presentation of stimuli (e.g. tone or light) associated with cocaine injection has been shown to effectively increase response rates in primates under second-order schedules of drug injection, the present experiments sought to establish self-administration of cocaine under second-order schedules in rodents. Male Sprague Dawley rats were trained to nose-poke for injections of cocaine (0.66 mg/kg/inj) under two different second-order schedules. Each drug injection was associated with a 2 sec period of light oscillation (5Hz). One group was trained to respond under a FR5 (FR5:s) schedule where every 5 nose-pokes produced a 2 sec light oscillation, and every fifth FR completion produced both light oscillation and an intravenous injection of cocaine. The other schedule used was a VI 180-sec (VR 8:s) schedule, in which every variable ratio (VR) completed during the 3 min variable interval (VI) produced a 2 sec period of light oscillation; the first VR completed after the interval produced both the brief stimulus and intravenous injection of cocaine. Relatively high overall rates of responding were maintained by both schedules (0.14 res/sec and 0.18 res/sec respectively). Under tests of extinction, omission of cocaine resulted in marked decreases in behaviour within a session under both schedules. However, when both the brief stimulus and cocaine were omitted, extinction was more profound for the group of rats trained under the FR5 (FR5:s) schedule. When the brief stimulus was omitted, but cocaine was still injected, response rates did not change, although under the FR5 (FR5:s) schedule, the latency to self-administer the first cocaine injection in the 2-hr session increased. These findings demonstrate that high rates of responding can be maintained under second-order schedules of cocaine injection in rats, although clear facilitation of responding by the brief stimuli was observed under restricted conditions (e.g. FR5 (FR5:s)). (supported by N.I.D.A., D.I.R.)

363.18

COCAINE AND ETHANOL SUBSTITUTE FULLY FOR A COCAINE-ETHANOL MIXTURE.
J. Jenkins, B. Rocha and M. Emmett-Oglesby*. Department of Pharmacology, University of North Texas Health Science Center and Substance Abuse Institute of North Texas, Fort Worth, TX 76107

Drug abuse often involves the use of more than one drug, alone or in combination. The combination of ethanol and cocaine use produces a potent metabolite, cocaethylene, which could produce a unique discriminative stimulus. The goal of the present study was to determine if the discriminative stimulus produced by a mixture of cocaine and ethanol is unique or is the sum of the component drug stimuli. Male Long Evans rats were trained in a two-lever choice task to discriminate a mixture of cocaine (5.6 mg/kg) and ethanol (EtOH, 0.56 g/kg) from saline under a fixed ratio 10 (FR10) schedule of food reinforcement. The mixture substituted for itself in a dose-related manner. Either cocaine or EtOH alone also substituted fully for the mixture. These data support the hypothesis that the stimulus produced by the mixture of cocaine and EtOH is the sum of the component stimuli, not a new, unique entity. Supported by R01 AA9378 and AA10545.

363.19

COCAETHYLENE POTENTIATES MEDIAL FOREBRAIN BUNDLE INTRACRANIAL SELF-STIMULATION.
M. A. Raven *, C. R. Devinney, L. M. Gatzke and A. Ettenberg. Behavioral Pharmacology Laboratory, Department of Psychology, University of California at Santa Barbara, Santa Barbara, CA 90316.

Cocaethylene is a psychoactive metabolite of cocaine produced only in the presence of ethanol. Although its reinforcing and rewarding properties have been identified in drug self-administration and conditioned place preference studies, its effects on brain stimulation reward have not been examined. Drugs identified as "reinforcing" in other paradigms often serve to enhance the reinforcing properties of intracranial stimulation. This study was designed to test the effects of several doses of i. p. administered cocaethylene (0, 5, 10, 20, 40 mg/kg) on intracranial self-stimulation in animals pressing for medial forebrain bundle stimulation. Rats were implanted with chronic indwelling monopolar electrodes and trained to press for an ascending series (150-600 μA) of square-wave 0.5 s trains of brain stimulation. All subjects received all doses of cocaethylene in a counterbalanced order with a minimum of 2 days between successive test trials. Results indicate that cocaethylene reliably decreased the current required to maintain half maximal lever pressing indicating that cocaethylene, like other reinforcing drugs, enhances the reinforcing effects of medial forebrain bundle self-stimulation.

This research was supported by NIDA grants DA05041 & DA08042 awarded to A. E. and a NSF predoctoral fellowship awarded to M. R.

363.20

Dopaminergic and glutamatergic mechanisms mediate induction of FOS-like protein by cocaethylene.
Judith Horowitz*, Jean DiPirro, Mark Kristal and German Torres. Behavioral Neuroscience Program. Department of Psychology. State University of New York at Buffalo. Buffalo, New York 14260.

Cocaethylene is an ethyl metabolite of cocaine formed by liver carboxylesterases in the presence of ethanol. The temporal induction of FOS-like protein in rat brain was examined following IP administration of 20 mg/kg cocaethylene. Immunoreactivity for the protein was detectable at 1 hour in striatal neurons and had virtually disappeared 6 hours after drug treatment. Administration of specific dopaminergic (SCH23390) and glutamatergic (MK-801) receptor antagonists prior to cocaethylene indicated a significant role for D_1 and NMDA receptors in mediating the nuclear induction of the aforementioned transcription factor protein. In sharp contrast, no marked effects on FOS-like protein in discrete nerve cells of the caudate putamen were found when spiradoline (U-62066), a kappa opioid receptor agonist, was administered either IP or directly into the brain parenchyma. Although cocaethylene treatment resulted in behavioral activation (e.g. rearing and sniffing), this activation was significantly less robust than in rat cohorts treated with a comparable dose of cocaine. These findings indicate both common and disparate effects of cocaethylene and its parent compound, cocaine, on receptor pathways that regulate alterations in gene expression and on neural systems associated with behavior.

This research was supported in part by a Term Faculty Development Award and a Research Foundation Award of the State University of New York to GT.

DRUGS OF ABUSE: COCAINE III

364.1

DIRECT ADMINISTRATION OF κ-AGONISTS INTO THE NUCLEUS ACCUMBENS PREVENTS THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION TO COCAINE.
Ch. A. Heidbreder * & T.S. Shippenberg. Neuroscience Branch, Neuroimaging & Drug Action Section, NIH/NIDA, P.O. Box 5180, Baltimore, MD 21224, USA.

Repeated cocaine administration produces persistent adaptations within the mesolimbic dopamine (DA) system. These changes in DA neurotransmission are thought to mediate the behavioral sensitization that develops to cocaine following its repeated administration. Manipulations of endogenous κ-opioid systems by the administration of exogenous agonists prevent sensitization to both the behavioral and neurochemical effects of cocaine. At present, however, the site of action of κ-opioid receptor agonists in modifying the effects of cocaine is unknown. The present study sought to characterize the effects of the direct administration of the κ-opioid receptor agonist U-69593 (0.9 nmol/day x 5 days) or its vehicle into the nucleus accumbens (ACB) on cocaine-induced behavioral sensitization. To this end, the same animals were pretreated simultaneously with either repeated cocaine (20 mg/kg/day x 5 days) or saline (1.0 ml/kg/day x 5 days). In separate groups of animals, the κ-opioid receptor antagonist *nor*-Binaltorphimine (*nor*-BNI) was directly infused into the ACB before the one daily injection of either saline, cocaine (20 mg/kg/day), or U-69593 (0.32 mg/kg/day) for 5 days.
Cocaine-pretreated animals exhibited an enhanced motor response to a cocaine challenge 2 days after cessation of cocaine treatment. In contrast, a significant reduction in the response to cocaine occurred in animals that had received U-69593 in the ACB in combination with systemic cocaine. Whereas systemic U-69593 pretreatment blocked both the acute and sensitized locomotor effects of cocaine, infusion of *nor*-BNI into the ACB completely reversed the effects of the κ-opioid receptor agonist. These findings demonstrate that direct activation of κ-opioid receptors within the ACB may prevent alterations in mesolimbic DA transmission, which occur following repeated administration of cocaine, and that this action may underlie the prevention of behavioral sensitization to cocaine.

364.2

ENDOGENOUS GABA RELEASE FROM STRIATAL SLICES OF COCAINE SENSITIZED RATS
B.J. Jung*, R. Dawson, S.A. Sealey, P.A. Walker, and J. Peris, Dept of Pharmacodynamics, College of Pharmacy, Univ. of Florida, Gainesville, FL 32610

Repeated cocaine use results in a phenomena known as cocaine sensitization, which can be measured in rats by observing behavioral stereotypy (head bobbing, repetitive motions) and locomotion. Previously it has been demonstrated that sensitized rats show decreased $GABA_A$ receptor number and function (JPET 226: 1002-8, 1996). It is important to determine whether post-synaptic $GABA_A$ receptor changes are compensatory responses to changes in pre-synaptic GABA release. To address this possibility, we examined whether the endogenous release of GABA from striatal slices is modified in rats treated with cocaine. Male Sprague-Dawley rats were randomly assigned to two treatment groups: saline and cocaine (15mg/kg). Rats were injected daily (ip) with their assigned treatments for 14 days. Rat behavior was rated on a five point scale on days 1, 7 and 14. Animals were killed 20-26hr after their last injection and their brains were quickly removed. Striatal slices (400μm) were then stimulated with 30mM K^+ for 5 min while fractions were collected for a 25 min period. Endogenous GABA in collected fractions and brain slices was quantified by HPLC-EC.

Cocaine treated rats showed a significant increase in behavioral stereotypy from day 1 compared to day 14 ($p<0.05$) and a 25% decrease in total GABA release compared to saline treated rats. There was no significant change in total endogenous GABA caused by cocaine. When a similar experiment was performed using a non- sensitizing dose of cocaine (7.5mg/kg), there were no significant differences caused by cocaine. These data are consistent with an overall decrease in GABA transmission in the striatum of sensitized rats, both pre and post-synaptically. These changes are contradictory to the hypothesis that decreases in $GABA_A$ receptors are a compensatory response to increases in GABA release. An overall decrease in inhibitory GABA transmission in striatum could contribute to enhanced striatal output and this indicates that other mechanisms besides DA transmission may be altered during the development of sensitization. Supported by a grant from the Alcoholic Beverage Medical Research Foundation.

364.3

DIAZEPAM PRETREATMENT MASKS COCAINE SENSITIZATION IN RATS

L.J. Coughlin*, C.W. Coffman, and W.P. Jordan
Biology and Psychology, St. Mary's College of Maryland, St. Mary's City, MD 20686

After several doses of cocaine (COC), a rat's motor behavior becomes sensitized to subsequent doses. In the current study, COC (10 mg/kg, i.p.) produced significant increases in repetitive head-bobbing after 4 intermittent doses across 10 days. Because diazepam (DZP) is a well-established treatment for cocaine-induced seizures, this study explored diazepam antagonism of cocaine sensitization. Pretreatment of rats with DZP (2.5 mg/kg, i.p.) prior to COC prevented head-bobbing behavior. Head-bobbing emerged in these rats when given only COC in a post-test, suggesting GABA facilitation masks but does not prevent the development of sensitization to COC. The joint effect of DZP and COC prevented the habituation of locomotion across days, whereas DZP or COC-alone did not. Thus, DZP can both mask a COC-sensitized behavior, head bobbing, and mask the expression of a non-sensitized behavior, locomotion. These masking effects, however, disappear when animals are challenged with a COC-alone test.

364.4

BILATERAL IBOTENIC ACID LESIONS OF THE DORSAL PREFRONTAL CORTEX BLOCK THE EXPRESSION OF BEHAVIORAL SENSITIZATION TO COCAINE. R.C. Pierce*, J. Hicks, D. Reeder, Z.R. Morgan and P.W. Kalivas. Alcoholism and Drug Abuse Program, Washington State University, Pullman, WA 99164.

Recent studies indicate that excitatory amino acid (EAA) transmission in the nucleus accumbens plays an important role in the expression of cocaine-induced behavioral sensitization (see Pierce et al., J. Neurosci., 16:1550-1560, 1996). In order to assess the influences of the various EAA projections to the nucleus accumens, bilateral ibotenic acid (5 μg/μl/side) or sham lesions of the ventral or dorsal prefrontal cortex (PFC), basolateral amygdala, fimbria-fornix or periventricular thalamus were made two weeks after seven daily injections of cocaine (2 X 15 mg/kg, 5 X 30 mg/kg, ip). One week after surgery, all animals received a challenge injection of 15 mg/kg cocaine, ip. The expression of behavioral sensitization to cocaine was not influenced by any of the sham surgeries. The sensitized behavioral response was significantly attenuated only by a lesion of the dorsal PFC, which sends afferents to the core subregion of the nucleus accumbens. A separate experiment demonstrated that dorsal PFC lesions do not influence the behavioral response to an acute injection of cocaine. Taken together, these results indicate that alterations in the activity of the EAA pathway projecting from the dorsal PFC to the nucleus accumbens core contribute to the expression of behavioral sensitization. Supported by USPHS grants DA-03906, MH-40817 and DA-05589.

364.5

SENSITIZATION TO COCAINE MAY EXIST IN A NMDA DEPENDENT OR INDEPENDENT FORM. C. Gambarana*, M. G.De Montis, O. Ghiglieri, A. Tagliamonte. Institute of Pharmacology, University of Siena, Siena 53100, Italy.

The NMDA receptor system has been involved in several processes of neuronal plasticity related to learning and memory. In particular, NMDA receptor antagonists have been used to prevent the occurrence of the behavioral and neurochemical modifications which mediate the long-term effects of some psychotropic drugs. Sensitization and tolerance to central stimulants are among the phenomena prevented by the concomitant NMDA receptor inhibition along the induction phase. Moreover, we recently reported that rats previously sensitized to cocaine and then infused with dizocilpine plus a daily injection of cocaine for 7 days, after a 2 day wash-out show an almost complete extinction of sensitization, which instead appears unmodified if dizocilpine is administered alone (1). In the present study, rats exposed to a continuous infusion of dizocilpine (100μg/kg/day, s.c. x 14 days) during a cocaine sensitization protocol (40mg/kg i.p. every other day x 14 days), became sensitized to cocaine. However, such a sensitization (measured in terms of stereotipy scores) was evident only when animals were challenged with cocaine (10mg/kg, i.p.) under dizocilpine effect (100μg/kg, i.p.). In addition, rats previously sensitized to cocaine and then infused with dizocilpine for a week and daily treated with 10 mg/kg of cocaine, during the first days of treatment presented a clearly decreased degree of sensitization. On the other hand, also in these animals on days 6 and 7 of cocaine administration an intense stereotyped response to cocaine re-appeared, which was completely absent after 2 days of dizocilpine wash-out. That is, rats sensitized under continuous NMDA receptor inhibition were still hyper-responsive to cocaine only in the presence of acute dizocilpine, and those which shifted into an NMDA independent sensitization had a standard response to the challenge with cocaine alone. After a 7 day wash-out, animals of both groups maintained the same degree and specificity of sensitization. Thus, it appears that two distinct mechanisms of sensitization to cocaine exist: one dependent on and the second independent of NMDA receptor activity.

1) De Montis et al. Behav. Pharmacol., 6, 562-567, 1995.

364.6

BOTH IONOTROPIC GLUTAMATE RECEPTOR ANTAGONISTS AND MEDIAL PREFRONTAL CORTEX (mPFC) LESIONS PREVENT NUCLEUS ACCUMBENS (NAc) DOPAMINE D1 RECEPTOR SUPERSENSITIVITY AFTER REPEATED COCAINE TREATMENT. Y. Li*, M.E. Wolf and F.J. White. Dept. Neuroscience, FUHS/The Chicago Med. Sch., North Chicago, IL 60064

Behavioral sensitization after repeated cocaine treatment is mediated, in large part, by the mesocorticolimbic dopamine (DA) system. The underlying mechanisms of behavioral sensitization include both short and long-term cellular neuroadaptations within the system, such as VTA DA autoreceptor subsensitivity in the initiation of sensitization, and NAc D1 receptor supersensitivity in the expression of sensitization. Accumulating evidence also suggests a crucial role for excitatory amino acid (EAA) transmission in the development of sensitization, especially the EAA projections from mPFC to the NAc and VTA. Both EAA receptor antagonists and mPFC lesions prevent the development of sensitization. We previously demonstrated that both ionotropic EAA receptor antagonists and mPFC lesions prevent VTA DA autoreceptor subsensitivity after repeated cocaine treatment. The present study investigates the role of EAA transmission in the development of NAc D1 receptor supersensitivity following repeated cocaine treatment. Since D1 receptor supersensitivity lasts for at least one month following repeated cocaine treatment, the experiments were performed after 4 to 8 days of withdrawal. Using microiontophoresis and single unit extracellular recordings, we found that co-administration of either the NMDA receptor antagonist MK-801 or the AMPA receptor antagonist NBQX with cocaine, in a protocol that prevents sensitization, also prevents NAc D1 receptor supersensitivity. We next lesioned the mPFC bilaterally with ibotenic acid. Subsequent repeated cocaine treatment failed to induce D1 receptor supersensitivity in the NAc. These results indicate that EAA transmission originating from mPFC is critical in the development of NAc D1 receptor supersensitivity. (Supported by DA 04093 & 00207 to FJW, and DA 07735 to MEW.)

364.7

ALTERATIONS IN NUCLEUS ACCUMBENS (NAc) NEURONS AFTER REPEATED COCAINE TREATMENT: *IN VITRO* ELECTROPHYSIOLOGICAL STUDIES. X.-T. Hu* and F.J. White. Neuropsychopharmacology Lab, Dept. Neuroscience, FUHS/Chicago Medical School, North Chicago, IL 60064.

Our previous *in vivo* electrophysiolgical studies identifed a functional supersensitivity of NAc neurons to dopamine (DA), DA D_1 receptor (D_1R) selective agonists (SKF 38393) and cocaine after repeated cocaine administration which paralled the persistence of behavioral sensitization. Subsequent *in vivo* recordings also suggested reduced sensitivity to glutamate - in particular, to non-NMDA receptor stimulation - whereas *in vitro* studies indicated that NAc neurons were more hyperpolarized at rest, and were less sensitive to intracellular depolarizing current injection. The present study sought to determine whether these alterations observed in slice preparations *in vitro* are related to the previous finding of DA D_1R supersensitivity. Confirming our previous results, we observed that repeated administration of cocaine for 5 days (15 mg/kg/day, i.p., 3-day withdrawal): (1) significantly hyperpolarized resting membrane potentials of NAc neurons as compared to controls, (2) significantly reduced responses to depolarizing current injection, and (3)significantly decreased action potential amplitudes. Superfusion of DA (50 μM, 2-3 min) caused small hyperpolarizations, inhibited action potential generation, and reduced spike amplitudes in saline-pretreated, but not in cocaine-pretreated, NAc neurons. SKF38393 (50 μM) exerted effects that were similar to, but more pronounced than, those of DA on saline-pretreated neurons, but again, not on cocaine-pretreated neurons. In fact, there was no significant difference in the electrophysiological properties of control NAc neurons recorded with DA or SKF 38393 as compared to cocaine-pretreated NAc neurons *without* DA or SKF 38393. We are now determining whether the results in cocaine-pretreated NAc neurons are due to tonic release of DA stimulating supersensitive D_1Rs or to alterations in transduction events normally engaged by D_1Rs (Support: DA 04093 and DA 00207).

364.8

INVOLVEMENT OF G β1 IN ESTABLISHMENT OF COCAINE SENSITIZATION X.B. Wang@*, M. Funada@, H. Ujike@, R.S. Revay@, K. Dehghani@, J.T. You@, & G.R. Uhl@#. @Molec. Neurobiology Br., NIH/NIDA, IRP; #Dept. Neurol. & Neurosci., JHUSM, Balto., MD 21224

Repeated doses of cocaine or amphetamine lead to long-lasting behavioral manifestations that include enhanced responses termed sensitization through biochemical mechanisms that remain currently largely unknown. To seek candidate biochemical mechanisms for these drug-induced neuroplastic behavioral responses, we have employed an approach termed subtracted differential display (SDD) to identify genes whose expression is regulated by these psychostimulants. $rG\beta_1$ is one of SDD products that encodes a rat G protein beta subunit expressed at high levels in neurons in the nucleus accumbens shell region, a major center for psychostimulant effects in locomotor control and behavioral reward. Cocaine and amphetamine each change levels of brain $rG\beta_1$ expression in region-specific patterns. Antisense treatments that attenuate $rG\beta_1$ expression in regions including the nucleus accumbens abolish the development of behavioral sensitization when they are administrated during the repeated cocaine exposures that establish sensitization. These treatments fail to alter acute behavioral responses to cocaine, and they are ineffective at blocking the expression of cocaine sensitization that is established prior to oligonucleotide administrations. Full, regulated $rG\beta_1$ expression is a biochemical component essential to establishment of a long-term consequence of cocaine administration.

364.9

NUCLEUS ACCUMBENS 6-OHDA LESIONS DO NOT PREVENT THE EXPRESSION OF A SENSITIZED LOCOMOTOR RESPONSE TO COCAINE. B.M. Prasad*, L. Churchill, P. Duffy and B.A. Sorg. Dept. VCAPP, Washington State University, Pullman, WA 99164

Psychostimulant-induced sensitization has been divided into two phases: *initiation*, which occurs in the VTA, and *expression*, believed to result from increased dopamine (DA) transmission in the nucleus accumbens (NAcc). However, recent work also implicates an important role for NAcc glutamate in the expression of sensitization (Pierce et al. J. Neurosci.**16**:1550). The present study therefore examined whether NAcc DA was necessary for the expression phase of sensitization. Male Sprague-Dawley rats were given 7 days of saline (*n=5*) or cocaine (*n=5*; 15 mg/kg on days 1 and 7 in photocell cages, 30 mg/kg on days 2-6 in home cages). Two weeks after the last injection, rats were pretreated with DMI and infused with 6-OHDA into the NAcc (4 μg/μl, 3 μl over 12 min). Lesions resulted in DA depletion in the NAcc of 88±4% and no significant effect on norepinephrine (NE) levels. Medial prefrontal cortex DA was depleted by 67±5% with no effect on NE levels, while there were no effects of 6-OHDA on dorsolateral striatal DA or NE levels. Twelve to 14 days later, all rats were monitored for activity after a saline challenge followed by a cocaine challenge the next day. No differences in locomotor activity were found between daily saline and cocaine pretreated groups after saline challenge. Cocaine challenge did not elicit locomotion in 6-OHDA-lesioned rats pretreated with daily saline, in agreement with previous reports. However, lesioned rats pretreated with daily cocaine demonstrated a significant two-fold increase in activity for up to 30 min compared to saline controls, similar in magnitude and duration to the sensitized component routinely observed in non-lesioned rats. These results suggest that NAcc DA may not be required for the expression of long-term behavioral sensitization to cocaine. Alternatively, 6-OHDA lesions in the NAcc may alter the circuitry used to express cocaine sensitization. This work was supported by USPHS Grants DA-06612 and DA-08212.

364.10

MICROINJECTIONS OF SCH23390 INTO THE VENTRAL PALLIDUM BLOCK THE DEVELOPMENT OF SENSITIZATION TO CHRONIC COCAINE. P.I. Johnson* and T.C. Napier. Neurosci. & Aging Inst. Div. for Res. on Drugs of Abuse, Dept. of Pharmacology, Loyola Univ. Chicago, Sch. of Med., Maywood, IL 60153.

Chronic cocaine exposure is known to result in a sensitized motor response when animals, in withdrawal, are challenged with an acute cocaine injection. It is thought that the ventral tegmental area (VTA) is critical for the development of this sensitized response, whereas the nucleus accumbens (NAC) is important for the expression of the behavior. Given the direct projection from the NAC to the ventral pallidum (VP), the direct VTA dopaminergic projection to the VP, the importance of dopamine (DA) in psychostimulant sensitization, and the probability of generalized system alterations following chronic drug exposure, it is likely that the VP plays a role in the sensitization phenomenon. To investigate this possibility, three groups of rats were tested: a S-S group (n=4) received intra-VP microinjections of the DA D1 antagonist SCH23390 (2.5 μg/0.5 μl/side) 10 min prior to an i.p. saline injection, a S-C group (n = 3) received intra-VP microinjections of SCH23390 10 min prior to a cocaine (10 mg/kg i.p.) injection, and a V-C group (n = 5) received intra-VP microinjections of vehicle (0.3% tartaric acid/0.5 μl/side) 10 min prior to an i.p. cocaine injection. This procedure was repeated daily for 5 consecutive days. Two days after the final injection, all rats received an acute cocaine (10 mg/kg, i.p.) challenge with no intra-VP microinjection pretreatment. ANOVA analysis revealed that the rats in the V-C group displayed a sensitized motor response to the acute cocaine challenge (914% increase over baseline activity; $p < 0.01$) when compared to either the S-S group (210% increase) or the S-C group (249% increase). There was no difference between the S-C and S-S groups ($p = .81$). Thus, the data collected to date suggest that the VP is important in the sensitization phenomenon as DA D1 receptor blockade in the VP appears to inhibit the development of cocaine-induced sensitization. Work supported by USPHSGs DA05651 to PIJ and DA05255 to TCN.

364.11

THE 5-HT$_{1A}$ ANTAGONIST WAY 100635 ATTENUATES COCAINE-INDUCED LOCOMOTOR HYPERACTIVITY, BUT NOT BEHAVIORAL SENSITIZATION. R. De La Garza II* and K.A. Cunningham. Department of Pharmacology, University of Texas Medical Branch, Galveston, TX 77555-1031.

The mesolimbic dopamine (DA) system is known to mediate the behavioral effects produced by cocaine (COC), while serotonin (5-HT) systems possess modulatory actions on DA systems and thus may potently modulate the effects of COC. For example, we have previously demonstrated that the 5-HT$_{1A}$ agonist 8-OH-DPAT (DPAT) dose-dependently enhanced acute and chronic COC-induced hyperactivity localized to the periphery of photobeam activity monitors. To determine whether an attenuation of COC-induced hyperactivity would occur with an antagonist, we tested the 5-HT$_{1A}$ antagonist WAY 100635 (WAY: 0.1 mg/kg, sc; N=8 rats/group) or saline (SAL) 15 min prior to an injection of COC (15 mg/kg, ip) or SAL twice daily for 7 days. On DAY 1, acute WAY significantly attenuated COC-induced hyperactivity without effecting baseline behaviors. On DAY 7, WAY pretreatment produced a similar attenuation of COC-induced hyperactivity. During withdrawal, challenge with COC alone produced similar levels of hyperactivity in animals exposed to the chronic WAY regimen (WAY+COC) vs. SAL+COC controls. Thus, WAY did not block the *development* of COC sensitization. Therefore, a 5-HT$_{1A}$ agonist (DPAT) can enhance, while an antagonist (WAY) attenuates acute COC-induced locomotor hyperactivity, but these compounds do not modify the long-lasting changes that comprise behavioral sensitization.

Supported by NIDA DA 05638

364.12

EXTINCTION CAN ELIMINATE COCAINE CONDITIONED BEHAVIOR BUT LEAVE COCAINE SENSITIZATION INTACT. R. J. Carey* and J. Gui. Psychiatry, SUNY Health Science Center and Research and Development Service 151, VA Medical Center, Syracuse, NY 13210.

Repeated cocaine treatments in the same environment can lead to the development of a conditioned cocaine response and to context specific cocaine behavioral sensitization. The conditioned cocaine response is elicited by the environmental cues in the absence of cocaine whereas, the sensitization effects occur as an augmentation of the behavioral response evoked by the cocaine treatment. Since both phenomenon are linked to specific environmental cues, both are considered to involve association processes. A minimalist interpretation of these effects is to consider that the conditioned cocaine effects summate with the direct cocaine effects that the combination generate the behavioral sensitization effect. We directly investigated this possibility by comparing the cocaine sensitization effects in rats in which a conditioned cocaine response was maintained as compared to animals in which the cocaine response had been extinguished by six successive daily 20 min placements in the test environment following saline injections. Our findings showed that the six non-drug parings led to extinction of the cocaine conditioned response but did not modify the cocaine behavioral sensitization effects. We used five daily cocaine treatments (10 mg/kg) to induce conditioning and the control conditions included saline treated as well as unpaired cocaine (10 mg/kg) treatment groups which received the five cocaine treatments thirty min after each test session in the homecage. The behavioral measures of the cocaine response were determined with the use of a video image analysis system which tracked the location of the animal's head in a 60×60 cm open-field. Both locomotion distance and center zone entries were monitored and both measures showed conditioning and sensitization effects. The present findings support the proposition that conditioning and sensitization are distinct phenomenon and also point to the limitation of strictly behavioral treatments such as extinction to treat drug abuse problems.

364.13

COMPARISON OF SENSITIZATION TO COCAINE'S MOTOR ACTIVATING AND REINFORCING PROPERTIES PRODUCED BY COCAINE PREEXPOSURE S. Schenk* and B. Partridge Texas A&M University, Dept. Psychology, College Station, TX 77843

Repeated, intermittent administration of psychostimulants produces an enhancement of the subsequent behavioral effects of these drugs. This effect can be manifest as an increase in the magnitude of cocaine-induced hyperlocomotion or as a reduction in the latency to acquisition of cocaine self-administration. In the present study, we compared a number of preexposure conditions on the ability to sensitize rats to the motor activating or reinforcing effects of cocaine. Rats were pretreated with daily injections of cocaine (10.0 or 20.0 mg/kg) for 5 days. They received either a single injection per day or 2 injections separated by one hour. Context-dependent effects of preexposure were minimized by administering the pretreatments in a distinct environment from the testing environment. Three days following the last treatment, the acute motor activating effects of cocaine (5.0, 10.0 or 20.0 mg/kg) or the latency to acquisition of cocaine self-administration (0.25 mg/kg/infusion) were determined. Sensitization to both of the effects of cocaine was obtained. However, a less stringent preexposure regimen was required for sensitization to the motor activating effects of cocaine to be observed.

Supported by DA 06825

364.14

PHARMACOLOGICAL SENSITIZATION TO COCAINE DEPENDS ON SIMILARITY BETWEEN INJECTION AND TESTING CONDITIONS. G. N. Carmona, S. R. Goldberg and C. W. Schindler*. NIH/NIDA Division of Intramural Research, Preclinical Pharmacology Laboratory, Baltimore, MD 21224

When animals are injected repeatedly in their home cages and tested in a different environment, pharmacological sensitization is assumed to have occurred if the response to the drug is larger than for animals injected repeatedly with saline. Having a different test environment is intended to control for conditioning that may contribute to sensitization when animals are injected repeatedly in the test environment. However, subtle similarities between the home cage and the test conditions may also allow conditioning to contribute to any apparent pharmacological sensitization. To test this possibility, we injected rats in their home cages with either 20 mg/kg ip cocaine (n = 18) or saline (n = 18) for 4 days. On the fifth (test) day, all animals were injected with 20 mg/kg cocaine in a different environment (locomotor activity cages). Half the rats were given their injections by the same individual who administered their previous injections and tested in locomotor activity chambers that contained the same wood chips used for bedding in their home cages (SIMILAR groups). The other half of the rats were injected by a different individual and tested in the same activity chambers with no bedding (NOVEL groups). Clear sensitization for distance traveled was observed for the cocaine-treated SIMILAR group, but not for the NOVEL group, despite the fact that they had the same experience with cocaine. These results indicate that conditioning contributed to the apparent pharmacological sensitization, even though the sites of the repeated injections and final test environment were different. The observation of an increased locomotor response to cocaine (sensitization) was dependent on the similarity of the chronic injection and test conditions. (Supported by NIDA DIR)

364.15

BEHAVIORAL CROSS-SENSITIZATION BETWEEN QUINPIROLE AND COCAINE IN PREWEANING RATS. E. Tirelli* & R. Schoonbroodt. Pharmacopsychology Lab., University of Liège, Sart-Tilman Campus Bldg. B-32, B-4000 Liège-1, Belgium.

We have previously shown that preweaning rats are able to express behavioral sensitization to cocaine following regimens of 2 and 4 daily injections (submitted manuscripts). In this study, we examined the ability of preweaning pups to express cross-sensitization between the D2/D3 agonist quinpirole and cocaine, and conversely. In a first experiment, 7-, 14- and 21-day-old pups were injected daily with either saline or cocaine (15 mg/kg, i.p.) over 3 consecutive days and challenged 24 hours later with either saline or quinpirole (0.025, 0.05 or 0.1 mg/kg, i.p.). In a second experiment, pups received 3 daily injections of either saline or quinpirole (0.05 mg/kg, i.p.) and were tested for cocaine responsiveness (7.5, 15 or 30 mg/kg, i.p.) on the fourth day. The testing context was kept constant throughout the pharmacological treatment. On test day, pups were filmed from underneath for 45 min and the video-records scored with a time-sampling technique. Behavioral categories mainly included locomotion, jumps, rearing and head movements. It appeared that 21-day-old pups expressed cross-sensitization from cocaine to quinpirole (locomotion, jumps), and vice versa (locomotion but not jumps), whereas younger pups showed no sign of cross-sensitization. Moreover, sensitization to quinpirole alone was weak in 14- and inexistant in the 7-day-old pups. These results contrast with our previous results showing cocaine-induced sensitization in pups of the same ages. Although higher doses of quinpirole need to be tested, the results suggest a neonatal immaturity of some of the dopaminergic mechanisms subserving sensitization to dopaminergic agonists. It might be the case that these mechanisms are not indispensable for cocaine-induced sensitization to be expressed. (Supported by the FNRS, Belgium).

364.16

EFFECTS OF CHRONIC AND CROSS TREATMENTS WITH N-[1-(2-BENZO(B)THIOPHENYL)CYCLOHEXYL]PIPERIDINE (BTCP) AND COCAINE ON EXTRACELLULAR DOPAMINE CONCENTRATION IN RAT STRIATUM. R. Martin-Fardon, J.M. Kamenka, W. Kock*, A. Privat and J. Vignon. INSERM U 336, CNRS UPR 9008, INSERM U 249, 34053 Montpellier, *Centre de Recherche Pierre Fabre, 81106 Castres, France.

BTCP and cocaine (Coc) both inhibit dopamine (DA) uptake, but bind to different sites on the DA transporter (DAT). Male Wistar rats were submitted to 16 days chronic treatment (ChT: BTCP 10 mg/kg, Coc 20 mg/kg) followed by one week withdrawal or cross-treatments at the same doses (CrT: Coc 3 days/BTCP 10 days or BTCP 3 days/Coc10 days). The effects on extracellular DA concentrations ($[DA]_e$ no-net-flux method), and on the amount of DA (Q_{DA}) in the striatum were investigated by a repeated in vivo microdialysis procedure.

On the first day of the ChT, Coc and BTCP similarly increased Q_{DA} (+76 and +107% respectively). During the Coc treatment, $[DA]_e$ increased up to 250 % at day 7 and then, decreased to its initial value at day 13. In BTCP treated animals, $[DA]_e$ did not vary markedly. During both withdrawal periods, $[DA]_e$ remained stable. Q_{DA} collected after drug challenges during ChT, paralleled the evolution of $[DA]_e$. A maximum at day 7 was observed with Coc (+2500 %). The effects of BTCP during day 7 to 16 represented about 60 % of its initial value. At the end of the withdrawal periods, both drugs increased Q_{DA} similarly to their initial effects.

In cross treatment experiments, BTCP pretreatment prevented Coc induced increases of $[DA]_e$ and partially, of Q_{DA}. Conversely in Coc-pretreated animals BTCP did not maintain the Coc induced increase of $[DA]_e$. During the first day of its substitution, the effects of BTCP were increased (cross-sensitization), but up to the end of the treatment its effects were significantly lower than those of Coc.

Together, the results show that the effects on striatal DA produced by Coc exhibit sensitization, whereas those produced by BTCP exhibit tolerance. Their different binding sites on the DAT are suggested to be involved in their differential effects after chronic administration.

Supported by INSERM, MESR (Grant 94.V.0255), IRME and Ass. "Verticale".

364.17

BEHAVIORAL SENSITIZATION AND CROSS-SENSITIZATION FOLLOWING REPEATED TREATMENTS WITH COCAINE AND/OR MORPHINE. B.A.Mattingly*, J.K. Rowlett, L. Rice, T. Snyder, & C. Mossholder. Department of Psychology, Morehead State University, Morehead, KY 40351 & Department of Psychiatry & Human Behavior, University of Mississippi Medical Center, Jackson, MS, 39216.

Locomotor sensitization has been reported after repeated cocaine or morphine treatments. The present study evaluated the effects of repeated treatments of cocaine and morphine combined on the development of sensitization. In 3 experiments, male Wistar rats (250-300 g) were injected (IP) daily for 7 days with cocaine alone (0.0, 5.0, 10.0, 15.0, or 20.0 mg/kg), morphine alone (0.0, 1.25, 2.5, 5.0, or 10.0 mg/kg), or cocaine (0.0, 1.25, 2.5, or 5.0) combined with morphine (0.625 mg/kg). After each injection, locomotor behaviors (horizontal and vertical activity, and stereotypy) were measured for 60 min in automated activity boxes (Med Assoc.). On Days 8-14, all rats were given vehicle-only injections prior to testing. On days 15 & 16, all rats were tested after a challenge injection of cocaine or morphine. Cocaine produced a dose-dependent progressive increase in activity across days (i.e., sensitization). Morphine alone also increased activity over days, but only at the intermediate dose (2.50 mg/kg). Cross-sensitization was observed between cocaine and morphine on some, but not, all activity measures. The addition of morphine did not enhance the sensitizing effect of repeated cocaine treatments. Thus, the stimulating effects of cocaine and morphine do not appear to be additive.

(Supported by USPHS grant DA 09687).

364.18

PHENCYCLIDINE-INDUCED LOCOMOTOR ACTIVATION DOES NOT CROSS-SENSITIZE TO COCAINE IN THE RAT. S. M. Melnick *, J. P. Clark and A. Ettenberg. Behavioral Pharmacology Laboratory, Department of Psychology, University of California at Santa Barbara, Santa Barbara, CA 90316.

The sensitization of locomotor activity induced by the psychostimulants, amphetamine and cocaine, has been well established. Several laboratories including our own have also reported the development of a sensitized locomotor response induced by phencyclidine (PCP). In the current study, each rat received five injections at 48 hr intervals immediately prior to 90-minute locomotor activity sessions. For the first four treatments, half the subjects were administered PCP (5 mg/kg IP) while the other half received physiological saline. On test day, subjects were further divided into a total of four groups. Rats pretreated with PCP received either PCP (a PCP-PCP group) or saline (a PCP-SAL group). Subjects pretreated with saline were administered either PCP (a SAL-PCP group) or saline (a SAL-SAL group). A sensitized locomotor response was only observed in the PCP-PCP group on test day. No within-group increases in locomotion were observed in the PCP-SAL, SAL-PCP or SAL-SAL groups on test day. In a second experiment, subjects that were similarly pretreated with either PCP (5 mg/kg IP) or saline received one of 3 doses of cocaine (10, 15, or 20 mg/kg IP) on test day in order to assess whether cross-sensitization occurs between the two compounds. Activity levels induced by the three doses of cocaine were not significantly different in rats pretreated with PCP when compared to rats pretreated with saline. These data suggest that cocaine-induced locomotor activity does not rely on substrates altered by chronic PCP exposure.

This research was supported by NIDA grant DA05041 awarded to A. E. and funds from the Department of Psychology at UCSB.

DRUGS OF ABUSE: COCAINE IV

365.1

REDUCED VOLUME OF PREFRONTAL LOBE IN POLYSUBSTANCE ABUSERS: A MAGNETIC RESONANCE IMAGING STUDY. **X. Liu*, J.A. Matochik, J.L. Cadet, E.D. London. Brain Imaging Section, Intramural Research Program, National Inst. on Drug Abuse, Baltimore, MD 21224.**

The present study was conducted to test the hypothesis that structural deficits in the prefrontal cortex are associated with substance abuse disorder. Volumes of the prefrontal lobe in subjects with histories of polysubstance abuse were measured and compared with those in normal volunteers, using high-resolution magnetic resonance imaging (MRI).

Twenty men with histories of polysubstance abuse and thirteen male controls, 22-41 years old, participated in this study. Polysubstance abusers were abstinent from drugs of abuse (except nicotine) for at least 15 days before MRI scanning.

The total volumes of the prefrontal lobe (left and right hemispheres) were significantly smaller in the substance abuse group than in control group. When the prefrontal lobe was segmented for gray and white matter, subjects in the substance abuse group had significantly smaller volumes of gray but not of white matter than the normal volunteers. These results indicate that hypoplasia and/or atrophy in the prefrontal cortex accompany substance abuse, and suggest that structural deficits in the prefrontal cortex may play an essential role in the neuropathological basis of functional impairments in substance abuse disorder, as demonstrated by functional brain imaging and cognitive studies.

Supported by NIDA Intramural Research Program

365.2

PHOSPHOLIPASE A_2 ACTIVITY IS DECREASED IN THE BRAIN OF CHRONIC COCAINE ABUSERS. B.M. Ross*, A. Moszczynska, and S.J. Kish. Human Neurochemical Pathology Laboratory, Clarke Institute of Psychiatry, Toronto, Ontario, Canada. M5T1R8.

Dopaminergic transmission is most commonly associated with altered cyclic AMP levels via the modulation of adenylyl cyclase activity. However, dopamine receptors may also be linked to other signal transducing mechanisms such as the formation of prostaglandins and leukotriene second messengers via the 'arachidonic acid cascade'. The initial rate limiting step of this pathway is the hydrolysis of phospholipids to form free fatty acid, a reaction catalysed by the enzyme phospholipase A_2 (PLA_2). Thus, PLA_2 activation may be an important signal transducing mechanism for dopamine, and may contribute to the effects of drugs such as cocaine. In order to explore this hypothesis, PLA_2 activity was measured in the autopsied putamen, occipital and frontal cortices, subcortical white matter, and cerebellum of 10 subjects who had been previously been taking cocaine regularly for a period of at least 12 months, and compared to that in 10 age and sex matched control subjects. The activity of PLA_2 in the putamen was found to significantly (Student's t-test; $P<0.01$) decreased by 36% in cocaine abusers compared to control subjects, but was unchanged in the other brain areas examined ($P>0.40$ in all comparisions). Our data suggest that chronic cocaine use reduces the rate of membrane breakdown in areas of high dopamine receptor density, possibly as desensitising mechanism to the presence of the drug. Supported by NIDA grant 07182.

365.3

THE NEUROANATOMY OF DRUG CRAVING IN CRACK COCAINE ADDICTION: A PET ANALYSIS. J. Schweitzer, K. Drexler, C. Quinn, F. Muhammad, R. Gross*, T. Faber, J. Hoffman and C. Kilts Departments of Psychiatry, Neurology, and Radiology, Emory Center for PET, and the Atlanta VA, Emory University, Atlanta GA 30322

The high rate of recidivism associated with the treatment of crack cocaine dependence is plausibly related to frequent and intense drug craving. We have used a PET neuroactivation analysis to define the functional organization of the craving human brain in crack dependence. Subjects were right-handed african-american males admitted to the VA for treatment of crack cocaine dependence. Study inclusion criteria included the absence of opiate, ethanol or marijuana dependence, and above-average mental imagery ability and a positive history of drug craving. Individualized scripts were constructed from the memory content of the physiological reactions and environmental contexts of crack cocaine use or of an anger-provoking scene. Positron Emission Tomography (PET) images were acquired using the blood flow tracer $H_2{}^{15}O$. Sessions included image acquisitions during a resting condition, and during imagery of binaurally presented control (beach or forest), cocaine use, or anger scenes. Subjects reported vivid imagery for all scenes, and moderate to intense craving or anger associated with imagery of the respective scenes. Imagery-induced drug craving, compared to control scene imagery, was associated with activation of temporal, frontal and cingulate sites, predominantly lateralized to the left hemisphere. The amygdaloid complex was not activated during craving induction. Induced drug craving and anger were associated with comparable increases in heart rate. Imagery-induced anger, compared to control scene imagery, was associated with activation of the left amygdaloid complex, and orbital frontal, cingulate and temporal cortex. Common sites of activation in the orbital frontal, middle temporal and cingulate cortex were associated with induced drug craving and anger. These results support the use of script-guided mental imagery in functional neuroimaging of drug craving. Funded by the Emory Center for PET.

365.4

NEUROCHEMICAL ALTERATIONS IN THE HUMAN BRAIN FOLLOWING ACUTE COCAINE ADMINISTRATION ASSESSED BY ^{1}H MAGNETIC RESONANCE SPECTROSCOPY. S-J. LI[§], J. Pankiewicz#, Y. Wang[§], A.S.Bloom[†], L.C. Terry[¶*], and E.A. Stein[§,#,†], §Biophysics Research Institute and Departments of [†]Pharmacology, [¶]Neurology and [#]Psychiatry. Medical College of Wisconsin, Milwaukee, WI 53226.

Little is known about the acute neurochemical and metabolic consequences of cocaine administration in the human CNS. This study utilized ^{1}H MR spectroscopy to follow the effects of a single acute injection of cocaine. Generally healthy, cocaine dependent subjects were recruited from the general population (n=5). All met DSM IV criteria for cocaine abuse and gave informed consent following IRB approval. Following a urine drug screen, subjects presented for an inpatient drug run-up protocol to assure drug safety, prior to MRS scanning. MR experiments were performed on a GE 0.5 whole body imaging system. A 4" flexible surface coil was used for both image localizer and PRESSCSI spectroscopy. A 1.5 x 2.5 x 1.5 cm region of interest was defined over the left frontal lobe. Spectra were obtained every 4 min for 16 min prior to and 40 min after a 40 mg IV injection of cocaine. Absolute peak spectra intensities were used to quantify neurochemical changes using the GE Medical Systems SA/GE package. Levels of N-acetyl aspartate, creatine/phosphocreatine and choline were unchanged after drug injection, while glutamine/glutamate levels decreased at 10 min. In contrast, inositol levels progressively decreased in a time-related manner after cocaine and remained more than 12% below baseline for 40 min post drug, indicating a decline in the levels of free inositol containing compounds as opposed to membrane associated inositol containing phopholipids. These data may in part reflect a cocaine-induced alteration in the functioning of inositol based second messenger systems. Supported in part by USPHS grant DA-09465 to EAS.

365.5

BRAIN CORRELATES OF CUE-INDUCED COCAINE AND OPIATE CRAVING A.R. Childress, W. McElgin, D. Mozley, M. Reivich, and C.P. O'Brien*. Addiction Treatment Research Center, Univ. of Penn. School of Medicine, and Philadelphia VA Medical Center, Philadelphia, PA 19104

Drug-related cues (e.g., drug users, drug talk, drug locations, drug paraphernalia, etc.) can trigger profound desire in users of opiates and cocaine, but the brain correlates of this "craving" state are just beginning to be delineated. In a recently completed study, cocaine patients showed rCBF (regional cerebral blood flow) increases in amygdala, anterior cingulate and temporal pole during craving induced by exposure to a cocaine video. Systematic activation did not occur in non-limbic comparison regions, nor in response to a comparison non-drug video. Control subjects did not experience craving during the cocaine video, and did not show systematic rCBF increases to either video type. These results suggested limbic activation may be one component of cue-induced cocaine craving.

We are investigating whether limbic brain areas will be similarly activated in opiate patients during exposure to opiate vs. non-drug cues. Increased drug desire and sympathetic arousal are common to both cocaine and opiate cue reactivity, but other phenomenological features (e.g., the salience of drug-like vs. drug-opposite symptoms) can differ substantially. As in the prior study, rCBF is imaged in opiate patients during exposure to videos of both non-drug and opiate-related scenes. Imaging of rCBF is accomplished with PET (Positron Emission Tomography) scans, using radioactively-labeled (O-15) water as the flow tracer. PET scans for each subject are co-registered with an MRI (magnetic resonance image) to permit anatomical localization of radioactivity. Results from the opiate patient sample will help determine whether the limbic activation found in cocaine craving is generalizable to other drug craving states. NIDA P-50-DA-05186

365.6

MOTOR FUNCTION DEFICIT IN COCAINE ABUSE. P.B. Vrtunski* and R.A. McCormick. Cleveland VAMC and Department of Psychiatry, CWRU School of Medicine, Cleveland, OH 44141.

There has been little systematic investigation on motor deficits in cocaine addiction. Also, when dealing with other clinical groups (schizophrenics or affective disorders) where patients may have had a history of drug abuse, its confounding effect is not known. We present here two studies with a force control (FC) technique comparing normal controls and cocaine addicts. The principal question raised was whether the cocaine addicts show *any* deficit in force control function, and if so, how large the deficit is. In the first study a group of 31 control subjects were compared to 34 age-matched cocaine addicts from the VA Drug Abuse treatment program. The FC task consisted of 90 6-second long trials in which the subject was instructed to match a target light with a response light. The response was patients' index-finger pressure on a force-sensitive button. There were 10 targets of increasing force (5 to 562 centi-Newtons), each presented 9 times. The steadiness of force maintenance was the dependent variable. In the second study, 16 control and 15 cocaine addicts, matched for age, education and hand-strength, were compared with a similar test at three force ranges (9-235, 17-478 and 26-717 cN). Results indicated that addicts had a deficit in capacity for fine motor control in both studies and throughout the force output range ($F(1,63) = 4.80$, $p < 0.05$ and $F(1,29) = 5.02$, $p < 0.05$, respectively). Based on these findings we suggest that a) cocaine addiction may be accompanied by a small but discernible deficit in fine motor control, and b) that in assessment of patients from other clinical populations this confounding factor should be taken into account. (Supported by the Veterans Administration and USPHS grant MH-46630)

365.7

EFFECTS OF ACUTE PRETREATMENT WITH RISPERIDONE ON RESPONSES TO COCAINE IN COCAINE-DEPENDENT HUMANS. L.H. Price,* G.H. Pelton, C.J. McDougle, R.T. Malison, P. Jatlow, L. Carpenter, P.D. Kirwin, D.W. O'Brien, G.R. Heninger. Clinical Neuroscience Research Unit, Dept. of Psychiatry, Yale Univ. School of Med., 34 Park St., New Haven, CT. 06519.

Although antagonism of the dopamine (DA) and serotonin (5-HT) systems has been shown to attenuate the effects of cocaine (COC), conventional neuroleptics have not been useful in the clinical treatment of COC addiction because of adverse effects. However, the novel neuroleptic risperidone (RISP), a D2/5-HT2 antagonist, is better tolerated than conventional agents. To evaluate the clinical potential of this agent in humans, we administered RISP to COC addicts and assessed their response to intranasal COC. **METHOD**: 16 hospitalized subjects (11 males, 5 females) with a DSM-IV diagnosis of COC dependence received three intranasal COC challenges (2.0 mg/kg), each preceded by acute pretreatment with RISP 0, 2, or 4 mg po. The sequence of test sessions was determined in randomized, double-blind fashion. Subjective effects (e.g., intensity of "high" on visual analog scales), biochemical effects (e.g., neuroendocrine responses), physiological effects (e.g., BP and HR), and adverse effects (e.g., extrapyramidal symptoms) were assessed. **RESULTS:** When data from all 16 subjects were analyzed, RISP showed no clear effect on the subjective "high" response to COC. However, when the analysis was limited to only those subjects manifesting a robust "high" response to the COC test dose (n=8), effects of RISP were highly significant ($p<.008$). RISP 2 mg resulted in a 42% ($p<.002$) attenuation of the "high" response, whereas RISP 4 mg caused a 32% ($p<.051$) attenuation. **CONCLUSION:** In COC addicts who report a robust subjective response to intranasal COC, acute pretreatment with RISPattenuates COC-induced euphoria. This effect of RISP is suggestive of an inverted U-shaped dose-response curve, with the 2-mg dose clearly more effective than placebo and somewhat more effective than the 4-mg dose. Since RISP was well-tolerated in our subjects, further examination of RISP as a treatment for COC addiction is warranted.
Funding Source: National Institute on Drug Abuse

365.8

PHENOMENOLOGY OF INPATIENT COCAINE WITHDRAWAL, D.A. Gorelick*, R. Stauffer, J.-K. Zubieta# and J.J. Frost#. NIDA/NIH Div. of Intramural Research, Balt., MD 21224, & #Dept. of Radiology, Johns Hopkins Univ. School of Medicine

To better understand the psychological and physiological manifestations and time course of cocaine withdrawal, we prospectively evaluated 11 physically healthy, cocaine-dependent (DSM-IIIR), medication-free subjects (10 men, 10 African-American, 1 white, mean [SD] age 31 [3.7] years, lifetime cocaine use 5.5 [4.1] years, using 3.5 [1.5] g/week, last use 13.1 [16.5] hours before admission) housed on a closed research ward for 28 days. Pulse and blood pressure stabilized in the normal range over the first 2-3 days, computerized digit symbol substitution test performance and sleep duration and quality improved over the first 3-5 days, and mood scores (within normal range at admission) and cocaine craving progressively declined over the first 7-10 days. All variables remained stable over the last 2-3 weeks. These findings suggest that signs and symptoms of moderate cocaine withdrawal progressively normalize over 7-10 days without medication in an inpatient setting, and are not consistent with the previously reported triphasic model of outpatient cocaine withdrawal.
Supported by NIDA intramural research funds.

365.9

ROLE OF CENTRAL ADRENERGIC RECEPTORS AND CHOLINERGIC NEURONS IN THE PRESSOR RESPONSE TO CENTRAL INJECTION OF COCAINE. J.A. Davis, II, L.C. Shuster, G.S. Doetsch* and J.J. Buccafusco. Depts. of Pharmacology and Toxicology, and Neurosurgery, Medical College of Georgia, and Dept. Veterans Affairs Medical Center, Augusta, GA, 30912.

Activation of both central and peripheral sympathetic sites can contribute to the cardiovascular symptoms associated with cocaine administration. However, very little is known concerning the mechanism for cocaine's central sympathetic actions. Although cocaine can indirectly activate central adrenergic receptors, the drug recently has been reported to interact directly with muscarinic receptors. This latter action of cocaine is relevant because stimulation of central cholinergic (muscarinic) neurons can evoke a sympatho-excitatory response. The purpose of this study was to determine whether direct central injection of cocaine could increase mean arterial pressure (MAP) and heart rate (HR), and whether such changes were mediated through central α-adrenergic receptors or cholinergic neurons. Lateral ventricular injection of cocaine HCl (24-96 μg) produced a dose-dependent increase in MAP up to 26 mmHg in conscious, freely-moving normotensive rats. However, intracisternal (i.c.) injection of 48 μg of cocaine evoked a significantly greater increase in MAP of 41 mmHg with no significant change in HR. The increase in MAP began within 1 min after injection and lasted about 10 min. I.c. pretreatment with 20 μg of the acetylcholine depleting agent hemicholinium-3 did not significantly alter resting MAP or HR, but inhibited the expression of the pressor response to subsequent (1 hr later) injection of 48 μg of cocaine by 63%. Blockade of central α-adrenergic receptors following i.c. pretreatment with 27.8 or 55.6 μg of tolazoline did not significantly alter resting blood pressure. Moreover, tolazoline did not significantly reduce the expression of the pressor response to subsequent injection of cocaine. These results are consistent with a lower brainstem (rather than diencephalic) site of action of cocaine, and for the possibility that local cholinergic 'pressor' neurons (rather than α-adrenergic receptors) play an important role in the sympathoexcitatory action of the drug. Supported by the Dept. Veterans Affairs Medical Research Service.

365.10

D1 RECEPTOR MODULATION OF GABA TRANSMISSION IN THE VENTRAL TEGMENTAL AREA OF THE RAT. C.D. Fiorillo* and J.T. Williams. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201

In the Guinea Pig, activation of presynaptic dopamine D1 receptors enhances $GABA_B$-mediated IPSPs in dopamine cells of the ventral tegmental area. One to two weeks after cessation of chronic treatment with cocaine or morphine, D1 activation inhibits GABA transmission through simultaneous activation of presynaptic adenosine A1 receptors. In addition, endogenous levels of adenosine are elevated. Because little is known about the behavioral aspects of chronic treatment with addictive drugs in the guinea pig, we are now examining these same physiological phenomena in brain slices from Wistar rats sensitized to the locomotor stimulant effects of cocaine. The locomotor effect of cocaine (10 mg/kg) was tested one day before and 7 days after 14 daily injections of saline or cocaine (20 mg/kg). Intracellular recordings were performed 10-20 days following the cessation of daily injections. The D1 agonist SKF 82958 (1 μM) caused an 80% increase in the amplitiude of the IPSP in saline-treated rats. In cocaine-treated rats, SKF 82958 produced a 30% increase in the IPSP. The A1 antagonist DPCPX, which augmented the IPSP by 10% in saline-treated animals, had variable effects in cocaine-treated animals but caused a mean increase of 70%. It appears that the D1- and A1-mediated changes in synaptic efficacy in the VTA induced by chronic cocaine treatment are qualitatively the same in rats and guinea pigs.
This work supported by NIH DA 04523.

365.11

THE EFFECTS OF GAMMA-VINYL GABA ON COCAINE-INDUCED LOWERING OF BRAIN-STIMULATION REWARD THRESHOLDS. S.A. Kushner*, S. L. Dewey[1], and C. Kornetsky. Departments of Pharmacology and Psychiatry, Boston University School of Medicine, Boston, MA 02118 and [1]Chemistry Department, BNL, Upton, NY 11973.

Gamma-vinyl GABA (GVG), a suicide inhibitor of GABA:2-oxo-glutarate aminotransferase (GABA-T), raises levels of GABA in nerve terminals. An experiment by Chen et al. (Neuroscience Abstracts, 767.5, 1995) found that GVG inhibits striatal dopamine (DA) release and attenuates the increases in extracellular striatal DA and locomotor activity induced by cocaine administration. In order to examine potential modulatory effects of GABA on DA-mediated reward, the effects of GVG on brain stimulation reward (BSR) thresholds were determined in 6 male F-344 rats with bipolar electrodes implanted in the medial forebrain bundle (MFB). GVG dose-dependently raised BSR thresholds at doses of 200, 300, and 400 mg/kg without significant effects on motor performance. In order to determine if GVG had similar modulatory effects on cocaine-induced lowering of BSR thresholds, the effective doses of GVG were co-administered with 2.5 and 5.0 mg/kg cocaine, doses that significantly lower BSR thresholds. Only the 400 mg/kg dose of GVG significantly blocked the lowering of thresholds seen at each dose of cocaine. Cocaine in combination with 200 or 300 mg/kg GVG, doses of GVG that significantly raise BSR thresholds, resulted in thresholds not significantly different from those obtained with cocaine alone. These data suggest that GABA may play a greater role in modulating basal reward thresholds than in modulating thresholds lowered by cocaine, and thus GABA may be less effective in mediating drug-induced enhancement of the DA system than in mediating basal dopaminergic activity. (This work was supported by NIDA grants DA02326 and KO5-DA00099 to C.K.)

365.12

ROLE OF DA RECEPTOR SUBTYPES IN LOCOMOTOR STIMULANT EFFECTS OF COCAINE. D.A. Lytle, M.J. Forster, S.A. Wild, C.S. Elsken, D.E. Johnston Jr, H. Lal, D.L. Barker* and R.R. Luedtke. Dept. Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

The purpose of the present study was to investigate the relationship between affinity of DA antagonists at D1 receptors and their potency to either suppress locomotor activity or antagonize the locomotor-stimulant effects of cocaine. Competition curves for 14 DA antagonists were performed against ^{3}H SCH-23390 in caudate harvested from Swiss-Webster mice to determine their Ki values. Locomotor activity was measured in separate groups of non-habituated Swiss-Webster mice following each DA antagonist alone or in combination with a maximal stimulant dose of cocaine. The ID_{50} (dose producing 1/2 maximal depressant activity) and AD_{50} (dose attenuating cocaine-induced stimulation by 50%) were calculated for each antagonist and compared with binding data. There were significant positive relationships between the affinity at D1 receptors and potency for depression of locomotor activity (ID_{50}) and potency for antagonism of cocaine-induced stimulation (AD_{50}). The Ki values were most closely associated with potency for antagonism of cocaine-induced locomotor stimulation. [Supported by U.S.D.H.H.S.-P.H.S contract NO1-DA-2-9305.]

365.13

***IN VIVO* ELECTROCHEMICAL STUDIES OF DOPAMINE CLEARANCE IN THE CORE AND SHELL OF RAT NUCLEUS ACCUMBENS.** D.J. David*, N.R. Zahniser, and G.A. Gerhardt. Depts of Pharmacology & Psychiatry, and the Neuroscience Training Program, University of Colorado Health Sciences Center, Denver, CO 80262.

The nucleus accumbens (NAc) has been subdivided into core, which is thought to be functionally related to neostriatum, and shell, which is considered to be part of the limbic system. *In vivo* microdialysis studies have shown that psychomotor stimulants preferentially increase extracellular dopamine (DA) levels in NAc shell, as compared with core. (Pontieri *et al.* Proc. Natl. Acad. Sci. USA 92:12304, 1995). In the present study, *in vivo* electrochemical recordings were performed in the core and shell of the NAc of urethane anesthetized rats in order to investigate the effects of cocaine on DA clearance, a measure of DA transporter (DAT) activity. Measurements involved local application of DA from micropipettes positioned 300 ± 25 μm from Nafion-coated single carbon fiber electrodes, and changes in extracellular DA were recorded using rapid (5Hz) chronoamperometric measurements. DA (5-20 picomoles) was applied by pressure ejection at 5-min intervals. Once reproducible signals were obtained, the effects of cocaine (20 mg/kg; i.p.) were investigated. Preliminary results suggest that cocaine produced a more robust increase in the amplitude of the DA signal in the shell, as compared to the core. However, cocaine administration did not appear to have a differential effect on the DA clearance times in the two areas. These results support the hypothesis that the proposed preferential effects of drugs, like cocaine, on extracellular DA concentrations in shell, versus core, of NAc may be due to a differential responsiveness of the DAT in these two subregions of NAc. (Supported by USPHS grants NS-09199, DA-00174, DA-04216, and NIH training grant HD7408).

365.14

IN VIVO MICRODIALYSIS IN AWAKE SQUIRREL MONKEYS: EFFECTS OF COCAINE ON EXTRACELLULAR DOPAMINE. L. L. Howell*, P. W. Czoty, J. B. Justice and J. E. Majors. Yerkes Reg. Primate Res. Ctr., Emory Univ., Atlanta, GA 30322.

In vivo microdialysis experiments were conducted in two awake squirrel monkeys seated in a standard primate chair to characterize cocaine-induced changes in extracellular dopamine. Under general anesthesia, guide cannulae were implanted bilaterally into caudate, and subjects were allowed 2-3 weeks recovery. During test sessions, analytical probes were inserted into the cannulae and perfused with aCSF at a flow rate of 2 μl/min. Samples were collected at 10-min intervals and assayed by microbore HPLC with electrochemical detection. Dialysate dopamine concentrations stabilized during a 2-3 hr equilibration period. Several concentrations of dopamine (10-100 nM) were added to the perfusate during 30-min periods, and the point of no net gain or loss estimated basal tissue levels at 14-22 nM. Cocaine (1.0 mg/kg) administration i.m. increased extracellular dopamine approximately 3-fold, and levels returned to baseline within 30-50 min. Repeated, biweekly sampling through the same cannulae yielded consistent results during 4 determinations. Pretreatment with the serotonin uptake inhibitor, alaproclate, had no effect on cocaine-induced changes in extracellular dopamine. This study establishes a useful model to investigate the neuropharmacology of cocaine in awake, nonhuman primates. (Supported by USPHS Grants DA-01162 and RR-00165).

365.15

COCAINE EFFECTS ON DOPAMINE IN RAT NUCLEUS ACCUMBENS: MICRODIALYSIS STUDIES. G.T. Golden*, T.N. Ferraro, F.A. Grau, G.R. Cupples, W.H. Berrettini, W.H.Vogel. Department of Veterans Affairs Medical Center, Coatesville, PA 19320 and Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA 19107

Prevailing evidence suggests that the behavioral effects of cocaine are mediated by increased dopaminergic transmission in nucleus accumbens (NAC); however, direct evidence that mesocorticolimbic dopaminergic transmission correlates with voluntary cocaine consumption is lacking. In the present study male Sprague-Dawley rats were characterized by basal extracellular microdialyzate DA levels in NAC and studied with respect to the DA response in NAC to an acute injection of cocaine (20mg/kg, ip). NAC dialysates showed a wide range of baseline extracellular DA levels (range 13.2 - 74.5 pm/ml, mean 37.9 pm/ml, s.d. 16.1). There was a significant positive correlation ($r = 0.68$, $p=.006$) between baseline DA levels in NAC and increases evoked by ip cocaine injection. Baseline DA levels in NAC were usually reestablished within one hour following cocaine injection and there was high reliability ($r=+0.88$, $p<.0001$) between baseline DA measures taken prior to and following ip injection of cocaine. A paired t-test comparison of pre-cocaine baseline DA levels with post-cocaine baseline DA levels showed no significant differences. These same rats are currently in a two-bottle (0.2% saccharin-water vs. 0.2% saccharin-cocaine 2%) choice paradigm in order to determine voluntary cocaine intake. Voluntary cocaine consumption will be correlated with reference baseline DA and acute cocaine-evoked DA levels in NAC.

Supported by the Department of Veterans Affairs and NIH grant R01 DA 08848-02.

365.16

SYNERGISTIC EFFECTS OF COCAINE AND DIZOCILPINE (MK-801) ON BRAIN STIMULATION REWARD. R. Ranaldi*, P. Bauco and R.A. Wise. Dept. of Psychol., Concordia Univ., Montreal Canada H3G 1M8

The synergistic effects of dizocilpine (MK-801), a noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist, and cocaine on lateral hypothalamic brain stimulation reward were evaluated. Eight male Long-Evans rats were trained to perform a lever-press operant to deliver trains of cathodal rectangular pulses directly into the lateral hypothalamus. Rate-frequency functions were determined by logarithmically decreasing the number of pulses in the stimulation train from a value that sustained maximum responding to one that did not sustain responding (thresholds). After thresholds had stabilized each rat was tested under 4 treatment conditions; saline+saline, dizocilpine (0.05 mg/kg, ip, 30 min before test)+saline, saline+cocaine (4 mg/kg, ip, 5 min before test) and dizocilpine+cocaine. The saline+saline and dizocilpine+saline treatments did not affect brain stimulation reward thresholds or maximum responding. The saline+cocaine treatment appeared to produce a small, non-significant reduction of thresholds and no effect on maximum responding. The dizocilpine+cocaine treatment produced large reductions in thresholds to levels significantly lower than those in the saline+cocaine treatment indicating a synergism between the two drugs and a role for the NMDA receptor in brain stimulation reward. These synergistic effects of dizocilpine and cocaine stand in contrast to the putative antagonism by dizocilpine of cocaine's psychomotor-sensitizing action. Supported by the National Institute on Drug Abuse (DA01720).

365.17

DIFFERENTIAL EFFECTS OF LOCALLY APPLIED AND I.V. SELF-ADMINISTERED COCAINE ON ACETYLCHOLINE RELEASE IN THE RAT NUCLEUS ACCUMBENS. G. P. Mark* & A. S. Keys. Dept. of Behavioral Neuroscience, Oregon Health Sciences Univ. Portland, OR 97201

An intravenous cocaine (COC) self-administration paradigm and reverse-dialysis perfusion were used to study the effects of COC on acetylcholine (ACh) release by microdialysis in the nucleus accumbens (NAc). In the first study, ACh release in the NAc was measured before, during and after 15 min COC (0.01, 0.03, 0.1 & 1 mM) perfusions via the microdialysis probe. Intra-accumbal infusion of 10 or 30 μM COC did not alter ACh output. Conversely, during 100 μM COC, ACh release was mildly depressed and at 1 mM release was decreased to 53% of control levels. These effects were possibly due to the local anesthetic property of COC at these doses. For the second experiment rats were implanted with jugular catheters and accumbens guide shafts and trained to lever-press for i.v. infusions of 0.4 mg/kg COC. After training, probes were inserted through the guide shafts and NAc ACh output was monitored in 15 min intervals before, during and after 3 hr sessions of drug availability. In response to self-administered COC, ACh release steadily increased to a maximum of 200% of pre-drug levels and remained elevated for an additional 1.25 hr following drug availability. These results suggest a differential effect on NAc ACh release depending on the method of cocaine administration.

Supported by grant MRF 25956 from the Medical Research Foundation of Oregon.

365.18

ROLE OF PROTEIN KINASE C ON THE ACQUISITION AND EXPRESSION OF COCAINE CONDITIONING PLACE PREFERENCE IN RATS. L. Cervo, S. Mukherjee, A. Bertaglia, S. Consolo* and Rosario Samanin. Istituto di Ricerche Farmacologiche "M. Negri", Milano, Italy.

A balanced conditioning place preference (CPP) paradigm was used to study the role of protein kinase C (PKC) on the acquisition and expression of cocaine place conditioning. H7, a non specific inhibitor of PKC, administered intracerebroventricularly at 10 (but not 1) µg/10 µL immediately after cocaine conditioning during the training phase, significantly blocked the establishment of place conditioning. The same dose had no effect when administered before cocaine during the training phase or before testing for place preference in the absence of cocaine (expression). Chelerythrine, a specific PKC inhibitor, partially reduced the establishment of cocaine CPP when administered intracerebroventricularly immediately after cocaine conditioning at 3 (but not 1) µg/10 µL. The results suggest that PKC is not involved in the primary reinforcing properties of cocaine or in the behaviour elicited by the stimuli previously associated with the drug action (CPP expression). The PKC seems to play a selective role in some processes involved in the consolidation and storage of information. Since H7 blocks also protein kinase A, which is involved in drug addiction, experiments are in progress to examine the role of this kinase on the acquisition and expression of cocaine CPP.

The study was supported by Consiglio Nazionale delle Ricerche, Rome, Italy, Convenzione Psicofarmacologia.

ISCHEMIA: GLUTAMATE

366.1

PRIOR ETHANOL TREATMENT DECREASES GLUTAMATE RELEASE DURING ISCHEMIA AND POTENTIATES GLUTAMATE RELEASE POST-ISCHEMIA IN GERBIL HIPPOCAMPUS, S. McCrea*, T. Wishart, H. Miyashita, S. Ijaz, W. Howlett and A. Shuaib. Saskatchewan Stroke Research Centre, Univ. of Saskatchewan, Saskatoon, Sask. Canada S7N 0W8

Previous studies have found that prior ethanol consumption may aggravate stroke outcome. Gerbils were administered subcutaneous chronic ethanol injections for 21 days at dosages of 1 and 4 g/kg. Nine days after cessation of injections subjects underwent bilateral carotid occlusion while simultaneously monitoring amino acid neurotransmitter levels in the hippocampus with in-vivo microdialysis. Both the low and high dose ethanol groups demonstrated markedly decreased glutamate release as compared to the saline-injected group immediately post-ischemia. Progressive increase in the extracellular concentrations of glutamate post-ischemia in the ethanol treated groups was also noted. These results are consistent with developing ethanol-induced NMDA receptor sensitization, both pre- and post-synaptically. Apparent ethanolic protection of ischemic striatal neurons highlights the need for further study of the role that prior ethanol consumption may play in the altered neurochemistry and outcome in stroke. This research was supported by a grant from the Heart and Stroke Foundation of Saskatchewan to A. Shuaib.

366.2

SPATIAL AND TEMPORAL ALTERATIONS IN THE EXPRESSION OF THE NEURONAL GLUTAMATE TRANSPORTER IN HIPPOCAMPUS FOLLOWING TRANSIENT GLOBAL FOREBRAIN ISCHEMIA. W.R. Woodward*, M.G. Klein, N. Lessov, S.G. Amara and G.H. Murdoch. Depts. Of Neurology and Pathology and the Vollum Institute, Oregon Health Sci. Univ., Portland, OR 97201.

Following ischemic injury in the mammalian central nervous system a complex cascade of cellular changes is initiated that culminates in the death of selectively vulnerable populations of neurons. We investigated the spatial and temporal sequence of changes in the expression of one of the neuronal, high affinity transporters for glutamate ($EAAT_3$) in specific populations of hippocampal neurons following a defined, reproducible ischemic insult. Transient forebrain ischemia (15 min, 25 torr bp) was induced in male Sprague Dawley rats using the Siesjö 2-vessel occlusion model. Animals were sacrificed at times ranging from 1 hr to 14 days after ischemia by transcardial perfusion, and 40 µm frozen sections were reacted with antibodies for $EAAT_3$. Antigens were localized in the tissue using the avidin-biotin complex staining procedure.

Immunoreactivity for $EAAT_3$ is normally present throughout the neuropil of the hippocampus and stains perikaryal membranes and proximal dendritic processes of CA1-CA3 pyramidal neurons and granule cells of the dentate gyrus. $EAAT_3$ staining increased in pyramidal cells between 6 and 24 hours then remained constant for at least 14 days postischemia despite the death of CA1 pyramidal neurons after 2-days. The persistence of $EAAT_3$ staining in CA1 neurons after 2-days likely represents membranes of deceased neurons not yet cleared from the tissue. $EAAT_3$ staining faded to background in the neuropil of CA1 (strata oriens and radiatum) and in lacunosum moleculare by 2-days postischemia, reflecting the death of the CA1 neurons. These alterations in the pattern of $EAAT_3$ immunostaining appear to be the result of both changes in $EAAT_3$ expression and subcellular localization and may be an attempt by these cells to mount a protective response to the insult. Supported by NS17493, NS33273 and the Howard Hughes Medical Institute.

366.3

ANARYSIS OF DISTINCT BIPHESIC GLUTAMATE RELEASE USING A DIALYSIS ELECTRODE IN RAT ACUTE BRAIN ANOXIA. S. Asai[1]*, Y. Iribe[1], T. Kohno[2] and K. Ishikawa[1]. [1]Department of Pharmacology, Nihon University School of Medicine, Tokyo 173, Japan; [2]Frontier Technology Research Institute, Tokyo Gas Co., Yokohama 230, Japan

Glutamate plays a key role in acute brain anoxia. We report here the successful application of a real-time method for monitoring the glutamate in the extracellular space ([Glu]e). A dialysis electrode (Sycopel International, U.K.), consisting of a microdialysis probe with a built-in platinum electrode, that was continuously perfused with glutamate oxidase and ferrocene conjugated with high molecular weight molecules, BSA, to avoid diffusing out through the dialysis membrane. During the measurement, the mixture of the ferrocene conjugated BSA and glutamate oxidase was perfused at constant flow rate (0.5µl/min). This method permitted real-time measurement of a distinct biphasic [Glu]e elevation during anoxia.

When rats were subjected to anoxic insult, the flat EEG pattern changed within 10 sec. A sharp and rapid [Glu]e peak occurred approximately 2 minutes later (1st phase) and the [Glu]e elevation shifted, continuing to rise throughout the anoxic period (2nd phase). We analyzed the biphasic [Glu]e elevation by a dialysis electrode combined with focal microinjection. Focal microinjection of KCl (300 mM, 1 µl x 2) induced a sharp and rapid [Glu]e elevation. A 15 min interval after the KCl microinjection permitted the biphasic [Glu]e elevation to occur. With anoxic insult immediately after the KCl microinjection, the 1st phase of [Glu]e elevation disappeared and a gently-sloping [Glu]e increase was observed. On the other hand, this gently-sloping [Glu]e elevation was increased by focal microinjection of KCl (300 mM, 1 µl) and decreased by that of NaCl (300 mM, 1 µl). These findings suggest that the 1st phase of [Glu]e elevation was derived from "neurotransmitter pools" of neuronal cells, and the 2nd phase from their "metabotropic pools" by regarding as the so-call phenomenon of "reversed uptake". (Supported by the Science Research Promotion Fund from Japan Private School Promotion Foundation.)

366.4

GLUTAMATE RECEPTORS, NEUROFILAMENT AND CALCIUM-BINDING PROTEINS DISTRIBUTION IN THE DOG HIPPOCAMPUS AND NEOCORTEX. P.R. Hof[1], Y.E. Bogaert[2], R.E. Rosenthal[2], and G. Fiskum[2]*. [1]Dept of Neurobiol., Mount Sinai Sch. Med., New York, NY 10029, [2]Depts of Biochem. and Mol. Biol., Emerg. Med., and Neurosci. Program, George Washington Univ., Washington, DC 20037.

Neurophysiological experiments in carnivores have revealed the existence of a large number of cortical regions and an organization of sensory systems quite similar to that found in primates. However, the cyto- and chemoarchitecture of the cerebral cortex is relatively poorly known in carnivores. We analyzed the distribution and typology of classes of neurons containing glutamate receptor subunit proteins GluR2, GluR3, Glur5-7, and NMDAR1, neurofilament protein (NFP) or the calcium-binding proteins (CaBPs) parvalbumin, calbindin, and calretinin in the hippocampus and six neocortical regions of the dog brain. The GluRs were present in most of the hippocampal and neocortical pyramidal neurons, whereas NFP labeled distinct subgroups of pyramidal neurons. The three CaBPs were found in morphologically diverse inhibitory interneurons subsets. NFP and CaBP immunoreactivity exhibited substantial inter-regional and laminar differential distribution, whereas the GluRs exhibited a more homogeneous distribution. In addition, while these markers are found in morphologically comparable neuronal types in dog, monkeys, and humans, many differences exist in their regional distribution patterns between carnivores and primates. The dog may represent a highly valuable for the characterization of potential cellular changes in the distribution and expression of specific neurochemical markers in the context of biochemical and morphologic effects of global brain ischemia and reperfusion (I/R) following cardiac arrest. In this model, we observed a reduced number of GluR5-7- and GluR2-immunoreactive pyramidal neurons, and NFP-enriched neurons were selectively affected by the ischemic episode and the time of reperfusion, whereas CaBP-containing neurons were resistant to I/R. The present data represent a normative database for the study of the relative vulnerability of morphologically and biochemically identifiable neuronal subsets during brain I/R. Supported by NIH NS34152, Emerg. Med. Fndtn, and the Souers Stroke Fund.

366.5

ISCHAEMIA ACTIVATES LATENT NMDA RECEPTORS IN THE RECURRENT EXCITATORY PATHWAY BETWEEN CA1 NEURONES. T., Lozovaya*, Tsintsadze, N., Klishin, A. and Krishtal, O. Bogomoletz institute of Physiology, 252024, Bogomoletz str. 4, Kiev, Ukraine

By using *in situ* patch clamp in hippocampal CA1 mini-slices we measured excitatory postsynaptic currents (EPSC) varying the strength of the stimulus applied to the axons of CA3 neurones. The kinetics of the EPSC was initially independent on the stimulus strength. The postishaemic potentiation of the EPSC was observed in 60-80 min after the brief periods (10 min) of anoxia/aglycaemia. The potentiated EPSC got significantly slowed down in the great majority of examined neurones. In 11 cases out of 16 its kinetics acquired the dependence on stimulus strength: slower decay corresponded to the large stimulus. The latter effect was abolished under either N-methyl-D-aspartate (NMDA) or nonNMDA receptors blocker (D-2-amino-5-phosphonovaleric acid or 6-cyano7-nitroquinoxaline-2,3-dione respectively) indicating the long-term recruitment by the transient ischaemia of the latent predominantly NMDA synapses between CA1 neurones.

366.6

NMDA CHANNEL ACTIVITIES OF HIPPOCAMPAL NEURONS FOLLOWING TRANSIENT HYPOXIC-ISCHEMIC CHALLENGE. Yu Zhang, Shokrollah S. Jahromi*, Jame H Eubanks and Liang Zhang. Playfair Neuroscience Unit, Toronto Hospital Research Institute, Depts of Medicine (Neurology) and Surgery (Neurosurgery), University of Toronto, Toronto, ON, Canada M5T 2S8.

CA1 hippocampal neurons exhibit delayed death following a transient ischemic insult, whereas neurons in dentate gyrus are less vulnerable to the insult. To investigate whether the vulnerability is related to the NMDA receptor function, we recorded NMDA single channel activities from these two types of neurons in brain slices. When recorded in the cell-attached configuration and exposed to an "external solution" that contained 10µM glycine, 5 µM NMDA and 0.3-1.5 mM Ca^{2+}, CA1 neurons of control slices showed stable channel activities which lasted for up to several min. Stabel and appeared to be enhanced NMDA channel activities were also observed in CA1 neurons that experienced transient *in vivo* global ischemia or an *in vitro* hypoxic challenge. Whereas recorded in similar conditions, controlled dentate gyrus neurons often showed fast desensitization in NMDA channel activities. Removing Ca^{2+} from the recording solution induced relatively stable NMDA channel activities in dentate gyrus neurons, suggesting a Ca^{2+}-dependent desensitization. We are currently examining whether NMDA channel activities are altered in dentate gyrus neurons following the ischemic or hypoxic insult.

Supported by MRC and the Heart and Stroke Foundation of Canada.

366.7

PRETREATMENT WITH ANTISENSE OLIGONUCLEOTIDES AGAINST mRNA TO THE NMDA GLUTAMATE RECEPTOR IS NEUROPROTECTIVE IN RODENT MODEL OF NEONATAL ISCHEMIC-HYPOXIA: BEHAVIORAL AND MORPHOMETRIC ANALYSES. E.M.Jansen*, C.D.Keene and W.C.Low, Depts. of Neurosurgery and Physiology, Grad. Prog. in Neuroscience, Univ. of Minnesota, Mpls., MN 55455.

Perinatal ischemic-hypoxia (IH) in humans creates severe neurologic injury that often manifests in motor deficits. Such injury is thought to involve the NMDA receptor. We investigated whether AON directed against the NR1 subunit of the NMDA receptor could provide neuroprotection from subsequent IH. We utilized an established model of neonatal IH that creates unilateral striatal, cortical and hippocampal damage (Ann. Neurol., 9:131, 1981). On postnatal days (pnd) 5 and 6, twice a day, Wistar rat pups received intracerebroventricular injections (1µl) of either AON (15nmol) or vehicle (saline). On pnd 7, animals underwent unilateral carotid artery ligation and were then exposed to 2.5 hours of hypoxia (8% O_2, 92% N_2, 37°C). Animals were reared normally; sensorimotor and locomotor abilities were assessed using a rotating treadmill throughout development (Rota-Rod, Ugo Basile) and apomorphine-induced rotations as adults (1mg/kg). Animals that received AON before the IH injury were able to remain on the treadmill 134 ± 15.8% longer than control animals that also underwent the IH but received vehicle alone ($p<0.01$, Student's T-test). AON treated animals showed 75% less rotational asymmetry than did controls ($p<0.03$, Student's T-test). Following behavioral studies, animals were perfused, brains were vibratome sectioned, Nissl stained, and volumetric studies were performed using the software program PC3D. Right hemispheric volume was 153% greater in AON treated animals than in animals receiving vehicle alone ($p<0.05$, ANOVA). These results suggest that AON directed against mRNA of the NR-1 subunit of the NMDA receptor can confer neuroprotection prior to ischemic-hypoxia and prevent the onset of neurologic deficits. (Supported by NIH predoctoral NRSA MH10617 (EMJ), the United Cerebral Palsy Association, and PHS R01-NS-24464.)

366.8

HALOTHANE PROVIDES PARTIAL PROTECTION AGAINST NMDA EXCITOTOXICITY IN RAT MIXED CORTICAL CELL CULTURE. JP Beirne , GD Bonaros, GW Massey, RD Pearlstein, DS Warner* Depts of Anesthesiology and Surgery, Duke Univ Med Center, Durham NC 27710

Several *in vivo* studies have shown potent protection from volatile anesthetics against ischemic insults. These drugs possess a variety of pharmacologic properties. One effect is antagonism of glutamatergic neurotransmission at the NMDA receptor. This study examined the potential for halothane to reduce neuronal excitotoxic lesions caused by the competitive agonist, NMDA. Primary cultures were prepared and allowed to mature 13-16d. A dose-response curve was generated using a 30 min exposure to 0, 10, 30, 100, 300, or 1000 µM NMDA. Cellular lethality was assessed by measurement of LDH 24 hrs later. 30µM NMDA caused ≈80% of maximal LDH release. This dose was used in further studies. In Experiment 1, culture wells (n=8) were treated with 0, 0.19, 0.70. 1.76, 2.64, or 3.96 mM halothane in the presence/absence of 30µM NMDA. A maximal effect was observed at 0.70 mM (= 2.1 vol%) wherein a 32% reduction in LDH release occurred ($p<0.001$). Additional cultures were exposed to 30µM NMDA in the presence/absence of 10µM MK-801 or 1, 10, or 100µM ACEA 1021 (a glycine antagonist). Both MK-801 and ACEA 1021 were completely effective in blocking NMDA-stimulated increases in LDH release. These data confirm that halothane has modulatory effects at the NMDA receptor but potency of this drug is less than that of specific antagonists of either glutamate or glycine. These results suggest that *in vivo* halothane protection can be partially explained by anti-excitotoxic properties although other mechanisms of action are probably also important. Funded by NIH GM39771.

366.9

CERESTAT® PROTECTS AGAINST LONG-TERM BRAIN INJURY FROM HYPOXIC-ISCHEMIA (HI) IN NEONATAL RATS. D. Zhou*, S. Wang, D. Lawson, and W. F. Holt, Pharmacology Department, Cambridge NeuroScience, Inc., Cambridge, MA 02139

In a previous study we demonstrated that the noncompetitive NMDA antagonist, CERESTAT® (aptiganel hydrochloride/CNS 1102) can reduce the total infarct volume by >90% for brains evaluated at 48 hours after an HI insult in the neonatal rat. We report here the long-term effects of CERESTAT® on HI-induced brain injury in the neonatal rat. A total of seventeen rats were used in the present study. HI was induced by ligating the left common carotid artery and then placing 8-9 day-old rat pups for 1.5 hours in an incubator filled with 7.8% O_2 at 36.5°C. CERESTAT® was administered intraperitoneally immediately after HI at a dose of 6.8 mg/kg. 90 days after the HI insult, brain damage was evaluated by counting the total number of viable neurons for a particular brain region in a given microscope field. The results were expressed as the percentage of surviving ipsilateral neurons, compared to the number of neurons on the contralateral side of the brain. In the vehicle-treated control group (n=9) only 37%±13, 52%±11 and 72%±9 of the neurons survived in the regions of the rostral hippocampus, the caudal occipital cortex and the rostral striatum. In contrast, the neuronal survival rate in animals treated with CERESTAT® (n=8) was 84%±12, 94%±2, and 95%±4 in the same regions, respectively. CERESTAT®-treated rats had significantly ($p<0.05$) more surviving neurons in the hippocampus and the cortex regions examined compared with vehicle-treated control rats. These results suggest that glutamate mediates HI-induced brain damage in neonatal rats and that the NMDA ion-channel blocker CERESTAT® can provide long-term (at least 90 days) protection against such lesions in rats.

CERESTAT is a registered trademark of Cambridge NeuroScience, Inc. CERESTAT is being jointly developed by Cambridge NeuroScience, Inc. and Boehringer Ingelheim, GmBH.

366.10

SPINAL NMDA ANTAGONISM DOES NOT ALTER DORSAL HORN NEURONAL HYPERACTIVITY EVOKED BY SPINAL CORD ISCHEMIA. [1]**Jan Galik, Martin Marsala, Tony L Yaksh***, University of California, San Diego, CA-92093; [1]Institute of Neurobiology, Kosice, Slovakia.

Ischemia of nervous tissue is a devastating process which triggers a cascade of events leading to significant CNS dysfunction. In our previous studies [1] we have shown, using a spinal cord ischemic model of thoracic aortic occlusion induced by placement of 2F Fogarty balloon catheter via the femoral artery, an increase in firing frequency as well as in number of spontaneously active units in rat spinal dorsal horns after 30 minutes of transient spinal cord ischemia. To examine possible mechanisms involved in this hyperactive state, the NMDA receptor antagonist MK-801 was applied intrathecally during reperfusion. The activity of dorsal horn neurons in halothane (1.5%) anesthetized, artificially ventilated rats before, during and after 30 min of aortic occlusion was recorded. The peak of increased neuronal activity was observed typically from 60 to 90 minutes during reperfusion. The high dose (30μg in 20μl) of NMDA antagonist MK-801 was administered intrathecally after 1 hour of reperfusion. Control recordings were made after intrathecal injection of the same volume (20μl) of vehicle (saline). No significant change in spontaneous activity after MK-801 injection was observed. These results suggest that involvement of the NMDA receptor during this initial postischemic spinal hyperactivity is not significant. The origin of this spinal activity may be: i) depolarization due to an increase in extracellular potasium or excitatory transmitters released from degenerating terminals; or ii) increased afferent drive derived from peripheral ischemic terminals. (This work was supported by NIH NS32794, M.M.)

[1] Galik, Marsala, Yaksh, Neurosci.Lett., 207 (1996) 45-48

366.11

EFFECT OF CNS1102 ON ISCHAEMIC BRAIN DAMAGE AND NEUROLOGICAL AND BEHAVIOURAL DEFICITS IN RATS AFTER PERMANENT MCAO. L. A. Lione, A. Hudson, D. Nutt, P. King, C. Campbell and A. J. Hunter*. SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Harlow, Essex CM19 5AD. UK.

Histological assessment has traditionally been the most frequently used endpoint in pharmacological studies of cerebral ischaemia, now, however, the importance of behavioural assessment is recognised. This study evaluates the behavioural deficits induced by permanent left mid-cerebral artery occlusion (MCAO) and the effect of the non competitive NMDA antagonist, CNS1102 on these deficits. Male SD rats (300-350g, n=12-15 per group) were trained on grip strength and rotarod tasks 6 times daily for 6 days. MCAO was produced on day 6 as described by Longa et al (1989, Stroke, 20, 84). CNS1102 ($1.13 mgkg^{-1}$) and 0.9% saline vehicle were administered bolus i.v. 15 min post MCAO. All behavioural and neurological tests (8-point scale) were repeated and scored 22 hrs later (day 7). Animals were then perfusion fixed at 24 hrs post MCAO and brains histologically assessed. Rotarod performance was significantly reduced in the vehicle and CNS1102 MCAO groups when compared to the sham operated controls (3.5 ± 0.8s, 12.3 ± 4.9s and 52.4 ± 4.3s on day 7 respectively, $p<0.0001$ ANOVA). A significant reduction in contralateral right paw grip strength was observed in the vehicle and CNS1102 MCAO groups when compared to the sham operated controls (71.5 ± 12.3g, 99.4 ± 13.4g and 259 ± 9.7g on day 7 respectively, $p<0.0003$ ANOVA). There was also a significant effect of MCAO on the ipsilateral left paw grip strength. ($p<0.003$) in vehicle and CNS1102 groups as compared to shams (177.7 ± 15.3g, 187.7 ± 16.9 and 222.6 ± 11.9g) on day 7, respectively, $p<0.003$ ANOVA). No significant difference was observed however between the vehicle and CNS1102 MCAO groups in the neurological and behavioural tests, nor did CNS1102 reduce total infarct volume significantly compared with controls (234 ± 9mm^3 in the control group and 186 ± 27mm^3 in the CNS1102 group; $p>0.05$ ANOVA). These data suggest that 24 hour permanent left MCAO produce quantifiable neurological deficits which were not ameliorated by CNS1102.

366.12

MK 801 DECREASES TUMOR NECROSIS FACTOR LEVELS IN THE CEREBRAL CORTEX AFTER FOCAL ISCHEMIA R. Bertorelli[1], M. Adami[1], E. Di Santo[2], P. Ghezzi[2] and E. Ongini*[1] [1]Schering-Plough Research Institute, Milan 20132 [2]Mario Negri Research Institute, Milan, 20157 Italy

It has been reported that focal cerebral ischemia in rats produces elevated levels of tumor necrosis factor-α (TNF-α) mRNA and protein in ischemic neurons. The neuronal expression of the TNFα appears to facilitate the infiltration of inflammatory cells that, in turn, can further exacerbate tissue damage in cerebral ischemia and might contribute to increase sensitivity and risk in focal stroke (Liu et al., 1994; Stroke 25:1481-1488). To understand the possible role of this cytokine on ischemic processes we carried out experiments on ischemia-induced cortical infarction and TNF production in a permanent focal model in the rat. TNF was measured by the degree of cytotoxicity on L929 cells, using human recombinant TNF as standard.

Middle cerebral artery (MCA) occlusion resulted in the development of an ipsilateral cortical infarction. MK 801 (3 mg/kg, ip), a non-competitive NMDA receptor antagonist, exerted significant protection on total volume of infarction (47% of reduction; $p<0.01$ vs ischemic-control). The maximal expression of TNF was observed 12 h after MCA occlusion (2783±299 pg/g). This effect was specifically related to focal ischemia, since no detectable TNF was measured in the ipsilateral cortex of sham-operated animals (<50 pg/g) or in the contralateral cortex of both ischemic (117±24 pg/g) or sham-operated animals (<50 pg/g). MK 801 reduced the TNF production in the ipsilateral cortex of ischemic animals by 61% ($p<0.01$ vs ischemic-control). The results indicate that TNF is involved in the process underlying cerebral ischemia and its levels are modulated by NMDA receptor activity. Further studies are needed to understand whether TNF levels are directly linked to the degree of neuronal injury or depend on specific mechanisms of neurotoxicity. *Supported by SPRI.*

366.13

The glycine antagonist GV150526A protects somatosensory evoked potentials and reduces the infarct area in the MCAo model of focal ischemia in the rat.

Claudio Pietra*, Fabio Bordi, and Angelo Reggiani, Pharmacology Dept., GlaxoWellcome Medicines Ctr., Via Fleming 4 37100 Verona (Italy).

The neuroprotective activity of the novel, selective glycine antagonist GV150526A was assessed in the middle artery occlusion (MCAo) model of focal ischemia by using somatosensory evoked potential (SEP) responses recorded from the primary somatosensory cortex of rats under urethane anesthesia. The SEP functional responses were then correlated with precise anatomical evaluations. Occlusion of the middle cerebral artery (MCAo) produced experimentally 7 days prior to electrophysiological testing, induced a clear reduction in the SEP amplitude. To evaluate the neuroprotective effects of the GV150526A, two groups of animals were treated either 1 hr post- (n=9), or 6 hr post-MCAo (n=10). GV150526A (3mg/Kg, i.v.) was able in both groups to protect SEP responses recorded from the hind-paw cortical field. SEP responses recorded from the fore-paw cortical field, an area closer to the core of the ischemic damage, were significantly protected only in the group treated 1 hr post-MCAo. Histological evaluation of the rat brain regions showed a correlated decrease in the ischemic area of GV150526A-treated groups. The volumes of the ischemic brains of both GV150526A groups were statistically different from the MCAo group ($p<0.05$). These findings demonstrate that the GV150526A is able to protect both the ischemic damage assessed histologically and the functional correlates of the ischemia evaluated by the electrophysiological SEP measurements.

Supported by GlaxoWellcome S.P.A. Verona, Italy

366.14

Effects of the glycine antagonist GV150526A on learning and memory.

Fabio Bordi*, Claudio Pietra, and Angelo Reggiani, Pharmacology Dept., GlaxoWellcome Medicines Ctr. Via Fleming 4 37100 Verona (Italy).

The effects of the glycine antagonist GV150526A were studied on learning and memory. First, the effects of the GV150526A on perforant path-dentate gyrus long-term potentiation (LTP) were tested in rats. GV150526A (3mg/Kg, i.v.) injected 30 min or 150 min prior to tetanization did not block potentiation of the EPSP slope and population spike amplitude. To compare these effects to those of the N-methyl-D-aspartate (NMDA) receptor antagonist, a group of animals was treated with MK801 (1mg/Kg, i.p.) 150 min before tetanization. MK801 produced a clear block of LTP in EPSP slope and population spike amplitude. In a second set of experiments, the effects of GV150526A were examined on two behavioral measures of learning and memory formation: the Morris Water Maze in rats and the passive avoidance in mice. Rats injected with GV150526A (10 mg/Kg or 60 mg/Kg, p.o.) did not show any impairment in learning if compared to control. The NMDA antagonist MK801 (0.08 mg/Kg, i.p.), on the other hand, significantly affected the acquisition to locate the escape platform in the Water Maze. Furthermore, mice injected with GV150526A (0.3-30 mg/Kg, i.v.) 20 min before the test, performed in the passive avoidance test as well as control group. These findings show that the GV150526A is devoid of amnestic side effects even at doses well above the neuroprotective range of action. This drug has a therapeutic potential with a much greater margin of safety than NMDA channel blockers or competitive NMDA antagonists.

Supported by GlaxoWellcome S.P.A. Verona, Italy

366.15

IN VITRO CHARACTERIZATION OF A NOVEL NMDA RECEPTOR GLYCINE SITE ANTAGONIST: GV150526A. B. Bunnemann, C. Carignani, S. Palea, M. Corsi, M. Mugnaini, D.G. Trist, A. Reggiani*. Dept. of Pharmacology, GlaxoWellcome S.p.A., Medicines Research Centre, 37135 Verona, Italy.

The strychnine-insensitive glycine site on the N-methyl-D-aspartate (NMDA) receptor represents a target for the development of a variety of therapeutic agents, such as antiischemics, anticonvulsants, anxiolytics or antidepressants. In the present study, we have characterized the activity of a new glycine site antagonist GV150526A by using membrane binding assays, receptor autoradiography, electrophysiology on primary cultured cells, as well as on recombinantly expressed NMDA receptor subunit combinations. 1) Membrane binding: GV150526A completely displaced specific [^{3}H]-Glycine binding to rat cerebral cortex membranes. Displacement characteristics are in agreement with a single binding site model. A pKi value of 8.49 ± 0.02 (n=5) has been determined. On the other hand GV150526A did not specifically displace [^{3}H]-CPP binding. 2) Receptor autoradiography: highest binding densities were present in all hippocampal subfields and the cerebral cortex. Medium binding intensity could be observed in thalamic areas. Small amounts of binding sites were present in the striatum and the cerebellum. 3) Patch clamp recordings: in embryonic spinal cord and hippocampal neurons glutamate and glycine induced currents were competitively antagonized by GV150526A with similar potency. The estimated pK_B was 7.93 (7.92-7.94) and 8.08 (8.03-8.13) for spinal cord and hippocampal neurons, respectively. Correspondingly, recordings on recombinantly expressed NMDA receptor subunit combinations revealed an inhibition of glutamate/glycine induced currents. In conclusion, GV150526A represents a highly potent and selective antagonist at the glycine site of the NMDA receptor complex.

Supported by Glaxo Wellcome S.p.A., Verona, Italy

366.16

LACK OF DESENSITIZATION OF AMPA-RECEPTORS IN ISCHEMIC SPINAL CORD INJURIES. Mia von Euler*, Mo LiLi, and Erik Sundström. Dept. of Clinical Neuroscience and Family Medicine. Karolinska Institutet, Huddinge University Hospital, 141 86 Huddinge, Sweden.

We have previously shown that AMPA receptors mediate part of the secondary degeneration after an ischemic spinal cord injury. To investigate if desensitization of AMPA receptors limits neurodegeneration after experimental spinal cord injury, we studied the effect of systemic and local administration of cyclothiazide, a known inhibitor of desensitization of the AMPA-receptor. Photochemical thrombosis was induced at Th 9 in adult female Sprague-Dawley rats (B&K Universal, Sweden). Cyclothiazide (RBI) was dissolved in 99% ethanol or in 86mM NaOH and injected sc 10 mg/kg 5 minutes after ischemia. For controls, the two different vehicles or NaCl were administred. To rule out problems with drug administration, another experiment concerned intrathecal administration of cyclothiazide dissolved in 52% ethanol (200µg/kg) and given 5 minutes after ischemia. No effect could be seen of any of the cyclothiazide treatments on morphological evaluation of lesion volume in cresyl violet stained sections of spinal cord samples at 2 weeks post-ischemia. Neither were any differences seen between the different treatment groups or controls in the neurological evaluation. To certify that cyclothiazide blocks desensitization of spinal cord AMPA receptors in the same manner as has been shown in the brain, the effect of cyclothiazide on the binding of ^{3}H-AMPA to spinal cord synaptic memebranes was studied. Preliminary results showed that ^{3}H-AMPA-receptor binding to membrane preparations from brain and spinal cord was reduced similarly by 300 µM cyclothiazide in both regions. Thus, we conclude that AMPA receptors do not desensitize during the process of degeneration after spinal cord ischemia.

This study was supported by the Swedish MRC (06555), the Marianne and Marcus Wallenberg Foundation, the Magnus Bergvall Foundation, the Spinalis Foundation, the Åke Wiberg Foundation, and the research funds of the Karolinska Institute.

366.17

THE EFFECTIVENESS OF AMPA RECEPTOR ANTAGONISTS MAY BE INFLUENCED BY TRANSPORT MEDIATED EFFLUX FROM THE CNS. S. A. Borosky*, D. F. Welty, M. G. Vartanian and G. P. Schielke. Parke-Davis Pharmaceutical Research, Division Warner-Lambert Co., Ann Arbor MI, 48105

PNQX, (PD 152247) is a novel quinoxalinedione AMPA receptor antagonist which reduces ischemic brain damage and has anticonvulsant properties. Previous studies demonstrated that probenecid, a competitive inhibitor of organic anion transport, enhanced the anticonvulsant action of another quinoxalinedione, NBQX. It is unclear if this effect is due to inhibition of drug transport at the kidney (increasing plasma levels) or at the blood-brain/CSF barriers (decreasing brain efflux), or both, since probenecid blocks organic anion transport at these sites. In order to address this issue we studied 1.the effects of probenecid and nephrectomy on PNQX anticonvulsant activity and plasma levels, and 2.the effect of PNQX and NBQX on probenecid sensitive organic anion uptake in freshly isolated choroid plexus.

Following a 3 mg/kg IV bolus of PNQX, both pretreatment with 200 mg/kg probenecid and bilateral nephrectomy elevated PNQX plasma levels above controls. Probenecid increased the magnitude and duration of the protective effect of PNQX in maximal electroshock seizures while nephrectomy had no effect. In freshly isolated choroid plexus, probenecid, NBQX and PNQX inhibited uptake of the organic anion ^{14}C PAH. Additionally, uptake of ^{14}C PNQX was saturable and partially reduced by probenecid and PAH. These results suggest that the effect of probenecid is not due to the elevated plasma levels of PNQX, but rather may be a result of reduced efflux from the brain via organic anion transporters at the choroid plexus and blood-brain barrier. (Supported by Warner-Lambert)

366.18

THE NOVEL AMPA RECEPTOR ANTAGONIST, PD 152247 (PNQX), REDUCES LESION SIZE IN A DOSE DEPENDENT MANNER FOLLOWING TRANSIENT FOCAL ISCHEMIA IN THE RAT. N.C. Kupina, J.J. Kinsora and G.P. Schielke*. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI. 48105

The discovery of compounds which selectively block AMPA receptors has generated considerable study into the role of non-NMDA glutamate receptors in ischemic brain injury. PNQX is a novel quinoxalinedione with activity at the AMPA (IC_{50} = 0.063µM), kainate (0.37µM), and glycine (0.37µM) sites. We previously demonstrated PNQX to be neuroprotective when given IP in a model of focal ischemia. This study examines the IV dose response relationship for neuroprotection in transient focal ischemia . Ischemia was induced in male Sprague Dawley rats by temporary occlusion of both carotid arteries and the middle cerebral artery with micro clips. Immediately following the occlusion, drug or vehicle was infused for 5h. All clips were removed after 3h. Brains were removed 72h later for stereological analysis of the lesion. Lesion volumes (mm^3±SE) in each group were: vehicle control (259±20), 0.1mg/kg/hr (232±18), 1mg/kg/hr (170±11), 2.5mg/kg/hr (110±23) and 5mg/kg/hr (150±22). The reduction in lesion size was statistically significant in all but the lowest dose. Body temperature, measured by telemetry for 72h, did not vary between groups. These results demonstrate that the AMPA antagonist, PNQX, can reduce ischemic brain damage when administerd IV at a dose as low as 1 mg/kg/hr, and that hypothermia did not contribute to this neuroprotective effect. (Supported by Warner-Lambert)

366.19

METABOTROPIC GLUTAMATE RECEPTOR NEUROPROTECTION INVOLVES REGULATION OF INTRACELLULAR CALCIUM. I. Ahmad*, L. Kim, M. TenBroeke, J. Kue, and K. Maiese. Dept. of Neurology, Ctr. for Molecular Med., Wayne State Univ. Sch. of Med., Detroit, MI, 48201

Metabotropic glutamate receptor (mGluR) agonists are neuroprotective during anoxia and nitric oxide toxicity (Maiese, et al. *Neurosci Lett* 194: 173-176, 1995; Maiese, et al. *J Neurochem* (in press)). The mechanisms that mediate neuroprotection by this group of receptors are not well defined, but may rely on the modulation of signal transduction pathways, such as intracellular calcium. The present study examines the role of intracellular calcium (Ca_i^{2+}) concentration on the neuroprotective effects of mGluR agonism. The mGluR agonists, (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) and L(+)-2-amino-3-phosphonopropionic acid (L-AP4), and the mGluR antagonist, dibutyryladenosine 3', 5'-cyclic monophosphate (L-AP3), were administered in increasing concentrations (50µM to 1000µM) with changes in Ca_i^{2+} imaged with Indo-1. The *trans*-ACPD doses of 50µM to 500µM, which do not significantly alter survival during neuronal injury, produced 60-100% increases in Ca_i^{2+} over baseline (n=10, p<0.001). In contrast, the neuroprotective doses of *trans*-ACPD of 750µM and 1000µM yielded only an approximate 20-30% increase in Ca_i^{2+} over baseline (n=10, p<0.001). Individual application of the mGluR agonist L-AP4 and the mGluR antagonist L-AP3 did not significantly alter Ca_i^{2+}. Administration of the mGluR antagonist L-AP3 (1000µM) with *trans*-ACPD (50µM to 1000µM) was able to significantly reduce, and in some cases, prevent the release of *trans*-ACPD induced Ca_i^{2+} release. Our work suggests that neuroprotection during anoxia and NO toxicity by the *trans*-ACPD metabotropic glutamate receptor subtypes involves the precise regulation of intracellular calcium. Protection by the mGluR agonist L-AP4 does not appear to be primarily dependent upon the regulation of calcium. Identification of the protective mechanisms employed by mGluRs during neuronal injury may provide further insight into the pathways that ultimately lead to neuronal death.

Supported by the Alzheimer's Association, AHA (National), J&J Focused Giving Award, NIH, and the United Cerebral Palsy Foundation.

366.20

PROTEIN KINASE C INHIBITORS ATTENUATE THE PROTECTIVE EFFECT OF CLASS I METABOTROPIC GLUTAMATE RECEPTOR AGONISTS AGAINST HYPOXIC/HYPOGLYCEMIC INJURY IN HIPPOCAMPAL SLICES. J. Breder, U. H. Schröder, T. Jäger, T. Opitz, C. F. Sabelhaus, D. Balschun* and K. Reymann. Federal Institute for Neurobiology and Research Institute Applied Neurosciences Ltd., D-39008 Magdeburg, Germany.

During hypoxia and ischemia an excessive release of glutamate eventually leads to sustained neuronal damage. To investigate the influence of metabotropic glutamate receptors (mGluRs) on neuronal injury caused by cerebral hypoxia/ischemia, we employed an in vitro model of hypoxia/hypoglycemia.

Hippocampal slices from 7-week-old male Wistar rats were transiently exposed to an oxygen and glucose-free environment in an interface chamber. The synaptically evoked population spike in the CA1 region was taken as a measure of neuronal viability.

Under control conditions the population spike amplitude recovered incompletely (48.5 ± 3.6 % of baseline values) within one hour after termination of the hypoxic/hypoglycemic event. The specific class I mGluR agonists trans-azetidine2,4-dicarboxylic acid (trans-ADA, 100 µM) and 3,5-dihydroxyphenylglycine (DHPG, 10 µM) were highly protective (89.3 ± 3.8 % and 93.8 ± 2.1% of baseline values respectively) when applied before the event. Co-application of the protein kinase C (PKC) inhibitors staurosporine (100 nM) and chelerythrine (30 µM) reduced the recovery rate to 39.3 ± 7.9 % and 57.1 ± 11.7% of baseline values respectively.

Our data suggest that the activation of the phospholipase C pathway prior to hypoxia/hypoglycemia exhibits a pronounced protective effect which depends on protein phosphorylation. They indicate that the protective effect of class I mGluR agonists is mediated by PKC which must be activated prior to the onset of the hypoxic/hypoglycemic event.

This work was supported by BMBF grant BEO 21-0319998B

367.1

A NON-INFECTIOUS TECHNIQUE FOR THE IDENTIFICATION OF HIV BINDING SITES IN THE HUMAN BRAIN. F. J. Denaro*. Dept. of Neurology, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.

Histological studies which include immunocytochemistry and *in situ* hybridization have been useful in identifying the location of HIV in the brain. When these techniques have been coupled with methods of increased sensitivity and resolution, such as electron microscopy or confocal microscopy, it is also possible to identify the cell types which are infected. Double labeling is also very helpful in this identification process. Such approaches are believed to reflect the *in vivo* infection of the HIV virus in the brain. In the present study, purified, synthetic HIV peptides (GP140) bound to either peroxidase or colloidal gold were used to demonstrate binding areas in brain tissue sections. By incubating fresh frozen brain tissue with these peptides, it was possible to identify areas of increased viral binding. This approach in part reproduces what has been identified in the infected brain. Positive binding was identified in human postmortem brain tissue. Pre-incubation with antibodies to CD4 or binding with unlabeled peptide prevents binding of the labeled GP120 probe. Neurons and astrocytes did not present with substantial binding. Further studies are in progress to assess for binding in the neuropile, white matter, and endothelial cells. Such a technique can give insight into how HIV binds in the brain and where there may be increased viral susceptibilities. Grant NS31857.

367.2

IMMUNOCYTOCHEMICAL IDENTIFICATION OF HUMAN CD4 PROTEIN IN THE BRAIN OF THE HUMAN-CD4 TRANSGENIC MOUSE. S. Frankl*, F. J. Denaro. Dept. of Neurology, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.

The CD4 molecule has been found on the helper T lymphocyte and to a lesser degree on other lymphocytes. CD4 functions as a receptor for the entry of HIV into the cell. Understanding CD4's role in the life cycle of HIV infection is important to understanding the development of pathology in AIDS. Advances in HIV research will, in part, depend on the development of animal model systems to study HIV infection and for the development of antivirals. The development of a transgenic mouse which expresses the human CD4 molecule may help with the examination of some of the characteristics of HIV-CD4 binding. In the present study, the expression of human CD4 was mapped in the brain of the human CD4 transgenic mouse. By use of monoclonal antibodies to human CD4, it was possible to identify those cells which were positive for CD4. Lymphocytes in the brain parenchyma were identified as positive. Endothelial-like structures also appeared to be positive, but increased resolution may be needed to differentiate endothelium from lymphocytes which may be migrating through it or microglia in close association to them. The next step will be to study the effects of live virus or viral peptides in this model system. Supported by grant NS31857.

367.3

PRELIMINARY BEHAVIORAL AND TOXICITY ASSESSMENT OF THALIDOMIDE IN THE RHESUS MACAQUE. T.A. Patterson*, W. Slikker, Jr., W.L. Zielinski[1], J.C. Reepmeyer[1] and J.A. Sandberg. Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079 and [1]Division of Drug Analysis/FDA, St. Louis, MO 63101.

Thalidomide (THAL), although not yet approved for use in the United States, is currently being evaluated for its therapeutic potential in AIDS and AIDS-related conditions. THAL has been reported to produce a painful, dose-limiting peripheral neuropathy in patients after chronic administration. The present study was designed to determine if escalating doses of THAL could produce changes in home cage behavior that may indicate toxicity or the onset of peripheral neuropathy. Rhesus monkeys (N = 3) were orally dosed once a day with the same dose of THAL for seven consecutive days using a buffered corn syrup (pH = 5.0)/biscuit vehicle. The dosing began with 10 mg/kg/day and increased up to 100 mg/kg/day over eight weeks using an alternating weekly dosing/washout (vehicle only) regimen. A 10 mg/kg dose of THAL has previously been shown to produce peak plasma concentrations in the rhesus monkey that correspond to peak plasma concentrations used clinically in humans (~1 μg/mL). In the present study, oral dosing with 100 mg/kg of THAL produced peak plasma concentrations of 2-10 μg/mL after five hours. High plasma levels of THAL (> 2 μg/mL) were still present 28 hours after dosing in 2 of the 3 animals. In summary, no overt signs of toxicity, changes in body weight or alterations in home cage behavior were observed in rhesus monkeys administered doses of THAL that produced plasma levels greater than those typically encountered therapeutically. Supported by NIEHS IAG #Y01-ES-10187.

367.4

Cytokine and neurotrophic factor expression in HIV-infected human fetal brain microspheres transplanted in SCID mice striatum V.J. Sanders*, E.A. Bonaroti, G. Wang, R.J. Bodnar, P.G. Sarnacki, and C.L. Achim. Div. of Neuropathology, Univ. of Pittsburgh, PA 15213.

In vitro studies have shown that infection of microglia with HIV causes an increase in the production of various neurotrophic factors (NTF) and cytokines. Aggregates of human fetal CNS cells containing microglia and neurons may be grafted into the brain of SCID mice. We propose that HIV-infected grafts in SCID mice striatum will show altered NTF and cytokine production compared to uninfected grafts. Immunoflourescent laser confocal microscopy was used to study the expression of the cytokines: IL-6, TNF-alpha, and TGF-beta, and NTF: brain-derived neurotrophic factor (BDNF), NT-3, glial-derived neurotrophic factor (GDNF), and hepatocytic growth factor (HGF). Cellular localization was determined by double-labelling with cell markers. Relationship between NTF and cytokine expression and viral burden was determined by staining for HIV. Cellular localization and percentage of cells stained were compared with that seen in uninfected grafts at one, two, and four months after transplantation. A higher percentage of neuroglia expressed cytokines, NTF, and receptors in the HIV-infected grafts. The uninfected transplants were also used to study the role of NTF in neuronal survival in transplants. Mice with infected grafts serve as an animal model of HIV encephalitis. Production of NTF following microglial infection with HIV suggests that NTF may play a role in the neuronal damage seen in HIV encephalitis. [VJS supported by NM 18273]

367.5

EFFECT OF ZIDOVUDINE (AZT) AND SAQUINAVIR ON RETROVIRAL INFECTION OF THE CENTRAL NERVOUS SYSTEM (CNS). Bodnar RJ, and Hamilton RL*. Division of Neuropathology, Univ. of Pittsburgh, Pittsburgh, PA 15213

In HIV encephalitis, the CNS is an important reservoir of retrovirus. It is unknown whether current antiretroviral therapy is able to cross the blood brain barrier (BBB) and reduce CNS viral burden. We used a murine retroviral encephalitis model to evaluate the ability of anti-retroviral drugs to reduce retroviral levels in the CNS. Our model uses a temperature-sensitive neurotropic retrovirus (ts-1) which is inoculated into newborn mice and causes a non-inflammatory spongiform encephalopathy primarily in the subcortical areas. Mice were treated with either AZT, Saquinavir or a combination of the two drugs beginning age 4 weeks. Total RNA was extracted from subcortical tissue and cortex from mice sacrificed at 14 weeks. Semi-quantitative RT-PCR was used to measure levels of ts-1 pol and env mRNAs relative to GA3PDH mRNA. Northern blot analysis was performed to confirm the PCR assessment. Our results correlate with previous findings using immunohistochemistry and XC assay that current antiretroviral therapies may reduce but do not eliminate virus from the CNS. This model can be used to test other antiretroviral therapeutic agents for their efficacy in reducing viral burden in the CNS.

367.6

ANTISENSE OLIGONUCLEOTIDES: A POTENTIAL GENE THERAPY FOR HIV DEMENTIA. C. Power, C. Liu, J. Paterson*, M. Anazodo, J. Wright, A. Friesen. *Departments of Internal Medicine, Anatomy, and Medical Microbiology, University of Manitoba; Genesys Pharma, Winnipeg, MB.*

HIV-1 is a neurotropic retrovirus which productively infects brain cells, resulting in the clinical development of HIV dementia. Antisense oligonucleotides (ASO) targeting different HIV-1 genes have been shown to reduce replication of homologous viruses. The objective of these studies was to determine the extent to which ASO targeting the HIV-1 *gag* and reverse transcriptase encoding regions inhibit replication of nonhomologous HIV-1 strains derived from brain (JRFL), cerebrospinal fluid (SF162) and blood (LAV) and whether ASO cross the blood-brain barrier. Replication of HIV-1 in peripheral blood mononuclear cells (PBMC) was determined by reverse transcriptase activity and immunoblot. ^{35}S-labeled thioated ASO parenterally injected into mice were detected by autoradiography and radioactivity counts in tissues at 24 and 72 hour postinjection. ASO treatment (1.0 μM) of infected PBMC resulted in reduced replication of all viruses (JRFL-65%; SF162-40%; and NL43-100%) compared to control and inverted oligonucleotide-treated PBMC 7 and 10 days following infection. Pharmacokinetic studies indicated that radiolabeled ASO were detectable in brain and other tissues. Autoradiography revealed foci of radioactivity above background, localized in the neuropil and perikarya. These studies suggest that ASO can markedly diminish replication of diverse HIV-1 strains and are able to enter the brain; thus ASO may be potential therapeutic agents for the treatment of HIV dementia. Supported by MMSF, Manitoba, Canada.

367.7

CEREBELLAR DYSFUNCTION IN PATIENTS WITH AIDS. V. Das[1,4], A. Das[4], S. Suneja[1,3], C.O. Trouth[2], and R.M. Millis[2]*. [1]Depts. of Family Practice & Comm. Hlth; [2]Physiology & Biophysics; [3]Radiology; [4]HUH - CARES, Coll. of Med., Howard Univ., Washington, D.C. 20059.

The first reported case of AIDS by CDC was in 1983 and subsequent findings have implicated HIV in the etiology of the disease; however, no cure has yet been found. Antiretroviral agents and prophylactic therapy may prolong life. With the progress of the disease there is deterioration of cerebellar function. Neurological testing revealed ataxia, horizontal nystagmus, dysmetria, scanning speech, dysdiadochokinesia, wide-based gait and significant decrements in voluntary/involuntary activities and two-point discrimination. These findings suggest an AIDS-related increase in susceptibility to accidents and injuries. The pathophysiology of this AIDS-related cerebellar dysfunction remains unknown; however, it is postulated that glial cells may express CD_4 antigens that play a permissive role in HIV infection, the HIV envelope being homologous to neuroleukin which may inhibit production of nerve growth factor(s).

Support: HUH - CARES, Washington, D.C.

367.8

MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF HIV-1 NEURAL CELL INTERACTIONS. Y. Mizrachi*, [@]N. Smalheiser, J. Alejandro, & A. Rubinstein. Albert Einstein College of Medicine, Bronx NY 10461, [@] University of Chicago, Chicago IL, 60637.

Although neural cells do not carry the CD4 receptor, HIV-1 plays an important role in a broad spectrum of nervous system disorders. Our studies have shown that soluble CD4 does not inhibit gp120 binding to, HIV-1 fusion with or HIV-1 infection of CD4 negative neural cells. This suggests the existence of non CD4 gp120 binding molecule(s) on the surface of neural cells. We have previously identified by immunoprecipitation a 69KDa gp120 binding protein on the surface of neural cells. These findings have lately been confirmed and expanded using a more sensitive western blot technology. Membrane preparations from neuroblastoma and mammalian brains were solubilized, separated by gel electrophoresis at 10% gel and reducing conditions, and blotted onto a nylon membrane. Using gp120 and its antibodies we have identified six distinct gp120 binding proteins with molecular weights of 52, 63, 69, 79, 89 and 93 kDa. Gp120 binding was Ca^{++} dependent. These gp120 binding proteins were removed from the membranes by 6M urea extractions and were retained on WGA lectin column. The gp120 binding proteins in neural cells are also being evaluated by transfection of Rat2 cells with neural cells DNA. These Rat2-DNA transfected cells do not express the CD4 receptor, however, they became susceptible to HIV-1 infection. The long term goal of this study is to develop methods to interfere with HIV-1 adverse effects on neural cells.

Support: Pediatric AIDS foundation50094-9-PG; 50168-11-PGR; to YM
ABCOM Peds dpt award to Yaffa Mizrachi
CFAR: P30AI27741

367.9

CONDITIONED MEDIA FROM HIV-INFECTED MACROPHAGES DECREASES SODIUM-DEPENDENT GLUTAMATE UPTAKE IN SK-N-MC NEUROBLASTOMA CELLS. T.C. Pappas*, S. Alagarsamy[†], R.B. Pollard and M. Nokta. Dept. of Internal Medicine, [†]Dept. of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston TX 77555-0882.

HIV envelope proteins and cytokines released from HIV-infected brain macrophages (Mϕ) have been implicated in neurotoxicity associated with HIV infection via their interaction with receptors on neuronal and glial cells. We have found that products from HIV-infected Mϕ act on the Na^+-dependent glutamate (GLU) transport as well. SK-N-MC (neuroblastoma) cells express electrogenic GLU transporters that are pharmacologically similar to EAAT1 and EAAT3. Conditioned media from HIV-infected PBMC-derived Mϕ (HIV-Mϕ CM) decreased total GLU uptake in SK-N-MC to 80.6% (± 6.8%) of control levels in as rapidly as 4 hr. Na^+-dependent GLU uptake was decreased to 70.5% (± 7.8%) of controls at this time point. GLU uptake saturation kinetics revealed that HIV-Mϕ CM decreased transporter V_{max} without a significant change in the magnitude of the K_m. This reduction in V_{max} was evident with HIV-Mϕ CM treatment from 4-120 hr, and in as little as 2.5% CM. HIV_{SF162}, $HIV_{JR\text{-}CSF}$ and a Mϕ-tropic patient viral isolate produced HIV-Mϕ CM that could reduce GLU uptake. CM from uninfected Mϕ or Mϕ infected with heat-inactivated HIV had no effect on GLU uptake. HIV-Mϕ CM were not effective at suppressing GLU transport until 7 d post-infection, suggesting that virus production may have a role in the reduction of GLU transport. Consistent with this notion, SK-N-MC treated for 4 hr with gp120 (10 nM) showed reduced GLU transport similar to HIV-Mϕ CM-treated cells. Supported by the James W. McLaughlin Fellowship Fund.

367.10

HUMAN ASTROCYTES ARE RESISTANT TO APOPTOSIS BY HIV-1 INDUCED NEUROTOXINS. R. A. Angel, G. Dbaibo[†], S.W. Perry, L.G. Epstein, S. Dewhurst[§], Y. Hannun[†], and H.A. Gelbard*, Depts. of Neurology and [§]Microbiology and Immunology, Univ. of Rochester, Rochester, N.Y. 14642, [†]Duke University Medical Center, Durham, NC 27710.

We have previously demonstrated that astrocytes in brain tissue of patients with HIV-1 encephalitis do not exhibit TUNEL-stained nuclei (Gelbard et al., 1995). In contrast, numerous adjacent neurons in this brain tissue demonstrate evidence of apoptosis. To investigate the molecular basis for this, we examined the effects of the HIV-1 gene products gp120 and Tat, and the HIV-1-induced neurotoxins tumor necrosis factor alpha (TNFα) and platelet activating factor (PAF) on cultures of astrocytes prepared from HIV-1 negative human fetal cerebral cortex. All these substances are capable of inducing neuronal apoptosis. In contrast, gp120 (1 nM) induced <5% of the total astrocytes to undergo apoptosis, while Tat (500 nM) failed to induce apoptosis. TNFα (10 ng/ml) and carbamyl PAF (125 ng/ml, resistant to metabolism by brain acetylhydrolases) also failed to induce apoptosis. 2-chloro adenosine (100 μM, positive control) induced apoptosis in ~25% of total astrocytes. Because we have demonstrated that TNFα and PAF, at doses used in these studies, increase reactive oxygen species (ROS) and that TNFα increases ceramide production in neurons, we further examined if TNFα and PAF also increase ROS production and ceramide in astrocytes. Both TNFα and PAF failed to increase ROS as measured by oxidation of dichlorofluorescin using inverted fluorescence microscopy and FACS. Neither substrate affected ceramide production in astrocytes, but we did see an increased production of the second messenger diacylglycerol. Taken together, these results suggest that two intracellular events (generation of ROS and ceramide) that may mediate apoptotic signals after stimulation with TNFα and PAF do not occur in human astrocytes. Current studies are focusing on whether the astrocyte's ability to undergo proliferation or increases in GFAP (neuropathologic features of HIV-1 encephalitis) may play a role in inhibiting generation of these pro-apoptotic signals. (Supported in part by NIH grants and the Dana Foundation).

367.11

HIV MODULATES D-ASPARTATE UPTAKE IN U251 HUMAN ASTROGLIOMA CELLS. J.J. Kort* Department of Medicine, Albany Medical College, Albany, New York 12208.

In HIV-associated disease of the central nervous system uptake of excitatory amino acids into astrocytes may be impaired by neurotoxic factors secreted by activated macrophages and microglial cells or by HIV infection, leading to excitotoxicity mediated by NMDA-receptor stimulation in neurons. D-[^{3}H]Aspartate (D-Asp) uptake in U251 human astroglioma cells was measured in the presence or absence of candidate neurotoxins, including tumor necrosis factor alpha (TNF-α), arachidonic acid , and recombinant HIV_{SF2} gp120 envelope glycoprotein. In addition, D-Asp uptake was studied in HIV_{SF162}-infected U251 cells. Arachidonic acid (20 μM, -39%), TNF-α (100 ng/ml, -25%), and gp120 (10^{-7} M, -15%) dose-dependently reduced D-Asp uptake in U251 cells ($p<0.05$, student t test). Interestingly, uptake of D-Asp was increased (+50%) in HIV_{SF162}-infected U251 cells. [^{3}H]ouabain binding studies showed a twofold increase in the number of functional Na^+/K^+-pumps in HIV_{SF162}-infected U251 cells. However, intracellular Na^+ concentrations were not elevated in HIV-infected cells. Since uptake of glutamate and D-Asp into astrocytes is primarily via Na^+-dependent amino acid carrier systems, increased D-Asp uptake in HIV-infected U251 cells may be due to the increased transport capacity for Na^+ by the Na^+/K^+-pumps. The mechanism for upregulation of functional Na^+/K^+-pumps in HIV-infected astroglioma. cells is currently being investigated.

Supported by NIAID, NIH R29AI36668.

367.12

EXPRESSION OF THE HIV-1 COAT PROTEIN GP120 IN BRAINS OF TRANSGENIC MICE CAUSES OPPOSITE CHANGES IN SHORT- AND LONG-TERM POTENTIATION IN CA1 HIPPOCAMPUS. T. Krucker*, S.M. Toggas, L. Mucke[#], G.R. Siggins. AIDS Research Center & Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037. [#]Gladstone Molecular Neurobiology Program & Dept. of Neurology, Univ. of California, San Francisco, CA 94141.

The HIV-1 envelope glycoprotein gp120 is shed from infected cells and thus can diffuse and interact with uninfected neurons. Transgenic animals constitutively expressing gp120 from astrocytes in the brain (gp120 mice) display neuronal and glial changes resembling abnormalities in HIV-infected human brains (Toggas et al., Nature 367, 1994). To assess the physiology of gp120 mice and determine whether gp120 expression impairs synaptic plasticity, we examined CA1 population excitatory postsynaptic potentials (pEPSPs) in hippocampal slices from these animals. Compared with slices from non-transgenic littermate controls, slices from gp120 mice showed: (1) a strong tendency for larger normalized pEPSP amplitudes; (2) slightly larger paired pulse facilitation (PPF) before and after induction of long-term potentiation (LTP) at 20 and 50 ms interpulse intervals (statistically significant only at 50 ms after LTP induction); (3) a tendency for pEPSPs to be smaller, whereas after LTP induction, mean peak PPF at 50 ms interpulse intervals was bigger; (4) markedly elevated short-term potentiation (STP) after 10 and 20 shocks at 100 Hz; and (5) a significant reduction in the magnitude of CA1 LTP. In slices from transgenic animals expressing lacZ from the same promoter (GFAP-lacZ mice), PPF and LTP were normal. These results indicate that brain slice preparations from gp120 mice can be used to assess pathophysiological effects of gp120 on neuronal networks. Because STP involves presynaptic mechanisms, our results suggest that gp120 expression in these mice enhances either presynaptic glutamate release or postsynaptic glutamate receptor function, or both; these changes could lead to increased Ca^{++} influx, contributing to neuronal dysfunction and injury. As LTP is a cellular model of learning and memory, our LTP results may relate to memory impairments seen in patients with AIDS.

Supported by the Swiss NSF and NIH grants MH47680 and NS34602.

367.13

INDUCTION OF MATRIX METALLOPROTEINASES IN MICE TRANSGENIC FOR HIV-1 gp120 AND IN gp120-TRANSFECTED GLIAL CELLS D.C.L. Marshall, M.F. Pietropaolo, T. Wyss-Coray, L. Mucke, M. Oscar-Berman*, and C.R. Abraham. Boston Univ. Sch. Med., Boston, MA 02118, Gladstone Molecular Neurobiology Program and Univ. Calif. San Fransisco, CA 94141.

Significant neurodegeneration has been observed in the CNS of patients with HIV encephalitis. Similar changes have been identified in brains of transgenic mice expressing the HIV-1 envelope protein gp120 (Nature 365:188). We are interested in the mediators of HIV-induced brain damage. Host factors that could contribute to neurodegeneration include proteases which degrade the extracellular matrix and may thereby impair the functioning of neurons and other CNS cells. Here, we demonstrate that C6 glioma cells stably transfected to express gp120 show prominent increases in the activity (by gelatin zymography) of matrix metalloproteinase-2 (MMP-2) and MMP-9 compared to mock-transfected controls. In contrast, the activities of tissue inhibitor of metalloproteinase-1 (TIMP-1) in gp120 and mock-transfected cells were similar (by reverse zymography). Brains of gp120 transgenic mice, which show neuropathology similar to that in AIDS dementia, show increased levels of MMP-2 activity in comparison to non-transgenic controls. These results indicate that the expression of viral products such as HIV-1 gp120 within the CNS can result in increased activity of MMPs. This process may play an important role in the development of HIV associated neurodegeneration. This work was supported by the NIH.

367.14

Changes in NMDA receptor complex binding characteristics in a murine model of AIDS.

J. A. English[1], G. Nowak[1,2]*, and I. A. Paul[1]. [1]Dept. of Psychiatry and Human Behavior, U. of MS Med. Center, 2500 N. State St, Jackson, MS, 39216,USA, and [2]Institute of Pharmacology, Polish Acad. of Science, Smetna St. #12, Krakow 31-343, Poland.

Infection of C57/Bl6 mice with the LPBM-5 murine leukemia virus causes an acquired immunodeficiency syndrome (MAIDS), leading to immunosuppression, neurological and behavioral disturbances, and eventual death. Previous studies have shown that LP-BM5 causes learning deficits and increases CNS quinolinic acid levels. These studies suggest that effects of LPBM-5 infection on EAA neurotransmission may contribute to the learning deficits observed in infected mice. Here we report that LPBM-5 infection altered NMDA receptor binding characteristics in mouse frontal cortical homogenates. C57/Bl6 mice were inoculated with virus, then sacrificed at 0, 3, 6, 9, and 12 weeks post-inoculation. LPBM-5 infection was followed by time-dependent increases in binding of [^{3}H]5,7 DCKA and [^{3}H]CGP 39653 (competitive antagonists at the glycine and glutamate sites, respectively, of the NMDA receptor). LPBM-5 also produced a time-dependent decrease in the potency of glycine to displace [^{3}H]5,7 DCKA from the glycine site. These results are consistent with the hypothesis that behavioral and learning deficits after LPBM-5 infection are mediated by changes in the binding characteristics of the NMDA receptor complex.

Supported by NIH grant MH53228 to I.A.P.

367.15

DEFICITS IN COGNITIVE AND MOTOR PERFORMANCE IN SIV INFECTED RHESUS MACAQUES. J.K. Marcario*, L.A.M. Raymond, B.J. McKiernan, S.V. Joag, L. Foresman, O. Narayan, P.D. Cheney. Dept. Physiol. & MRRC; Microbiol., Molec. Gene. & Immunol., Univ. Kansas Med. Cntr., Kansas City, KS 66160, USA.

It is well established that HIV infection can lead to neurocognitive disorder and dementia. The purpose of this study was to detect motor and cognitive impairments in SIV infected monkeys using five different behavioral tasks; progressive ratio (PR), simple reaction time (SRT), choice reaction time (CRT), turntable (TT) and working memory (WM). In addition, home cage behavior was videotaped each week and scored for activity level, general health and abnormal behavior. Of the 9 Rhesus macaques trained, 4 served as controls. The remaining 5 were infected with strains of $SIV_{mac}239$ (R71 and 17E) by bone marrow injection. Of the 5 infected monkeys, one remains asymptomatic with no performance deficits. The other 4 monkeys developed end-stage simian AIDS and were euthanized. Two monkeys did not show appreciable performance decreases. Two other monkeys exhibited performance deficits when their motivation, as measured by the PR task, was normal. One monkey showed loss of accuracy in ballistic movements as reflected by a decline in TT performance. Another monkey showed loss of motor skill as reflected by a decline in TT, and increased error rates in WM, SRT and CRT. The results of this study show that behavioral deficits associated with SIV infection vary greatly with the individual, as in HIV infection. In addition, these results support the use of this test battery for detecting cognitive and motor impairments in SIV infected monkeys. (Supported by NINDS grant #552232 , NICHD grant #HD02528)

367.16

MULTIMODAL SENSORY EVOKED POTENTIALS AS INDICATORS OF SIV DISEASE PROGRESSION. L. A. M. Raymond*[1], J.K. Marcario[1], S.V. Joag[2], L.Foresman [2], O. Narayan[2], N.E.J. Berman[3] and P. D. Cheney[1]., Depts. of Physiol. and Men. Ret. Res. Cntr.[1], Microbiol., Mol. Genet. & Immunol[2]., and Anat.& Cell Biol.[3], Univ. of Kansas Med. Cntr., Kansas City, KS 66160. USA

The Human Immunodeficiency Virus (HIV) infects the central nervous system (CNS) early in its progression and as many as 20% of HIV+ patients later develop neurological signs that include impaired congnitive ability and psychomotor slowing. The studies of evoked potentials from HIV infected patients show many abnormalities in both asymptomatic as well as symptomic subjects with and without neurological deficits (Somma-Mauvais, *Neurophysiol-Clin.* Nov.22 (5): 369-84, 1992.) In an attempt to further establish an animal model of neuroAIDS, we recorded multimodal sensory evoked potentials (EPs) from five behavorially trained rhesus macaques infected with R71/E17, passaged strains of $SIV_{MAC}239$, as well as from four age and sex-matched controls prior to and at one month increments following infection (PI). The latencies and amplitudes of somatosensory (SEP) potentials from fore and hind limbs, flash visual (VEP), brainstem (ABR) and cortical (AEP) auditory potentials were measured. No changes were seen in the EPs from the control animals. All infected monkeys exhibited abnormal evoked potentials. Combining data from all five infected monkeys , decreases in amplitude and increases in latency were observed for each EP type, further supporting the SIV model of neuroAIDS. Supported by NIH grants NS552232 & HD02528.

367.17

MOTOR EVOKED POTENTIALS IN A RHESUS MACAQUE MODEL OF NEUROAIDS. P.D. Cheney*, L. Raymond, J. Marcario, O. Narayan, N.E. Berman S.V. Joag, L. Foresman, Smith MRRC. and Depts. of Physiology, Anatomy and Cell Biol. and Microbiol., Immunol. and Mol. Genetics, Univ Kansas Medical Center, Kansas City, KS 66160

Motor system dysfunction is among the many neurologic abnormalities associated with HIV disease in humans. Damage to the corticospinal system is a common finding, particularly in pediatric AIDS. The purpose of this study was to investigate the integrity of the cortical motor system in SIV infected rhesus macaques. Five monkeys were inoculated by bone marrow injection with a combination of two strains of $SIV_{mac}239$ (R71 & E17) known to be neurovirulent. Four uninfected monkeys served as controls. Transcranial electrical stimulation of motor cortex was used to evoke EMG potentials (MEPs) in lateral gastrocnemius and abductor hallicis of the hindlimb and extensor digitorum communis and abductor pollicis brevis of the forelimb. MEPs from stimulation of the cervical and lumbar spinal cord provided measures of peripheral conduction time. Central conduction time was estimated by subtracting the latency of spinal cord MEPs from those evoked by stimulation of motor cortex. Within 13 weeks of inoculation, 4 of 5 monkeys had developed end stage simian AIDS. Fifteen weeks after inoculation, one monkey remains asymptomatic. Of the four monkeys that progressed to AIDS, two showed abnormal motor evoked potentials in association with end stage disease. Both peripheral and central conduction times were increased. In one monkey, the threshold for cortical MEPs increased two-fold. This monkey also exhibited other motor signs including ataxia, tremors and clonus. These results further establish the R71 and E17 strains of $SIV_{mac}239$ as a model of neuroAIDS. (Supported by NIH grants NS552232 and HD02528)

367.18

PREFERENTIAL AND PROGRESSIVE FRONTAL CORTICAL CHANGES IN QUANTITATIVE EEG ACTIVITY AFTER FIV INFECTION IN THE ADULT CAT. M. Podell*, D. Ruehlmann, E. Chen, L. Mathes. Dept. Vet. Clin. Sciences and Vet. Biosciences, The Ohio State Univ., College of Veterinary Medicine, Columbus, OH 43210

Quantitative EEG (QEEG) was performed on 5 feline immunodeficiency virus (FIV-MD) infected adult cats and 5 control cats 10 and 16 mo post-infection (PI). All cats were anesthetized with isoflurane gas anesthesia and controlled for changes in end-tidal CO_2 and O_2 saturation levels. Subdermal needle electrodes were placed in a modified 10-20 recording system. A minimum of 4 minutes of artifact free EEG activity was analyzed from each cat.

Power spectral analysis (PSA) group comparisons between FIV and control cats demonstrated a significantly slower median frequency of activity in the left frontal cortex only at 10 mo PI. At 16 mo PI, FIV cats had significantly slower median frequencies in all frontal cortical recording sites. The difference in the PSA between individual FIV cats and group control data was calculated at specific frequencies. Comparison of these individual differences showed a significant shift toward slower frontal cortex activity in the 16 mo recordings in 4 of 5 FIV cats. No change in coherence was found at 10 mo PI. At 16 mo PI, FIV cats exhibited significantly lower frontal interhemispheric and left and right intrahemispheric coherence compared to control cats considering all recording frequencies. The greatest difference in coherence was found for delta activity between the frontal cortices. These results show that FIV infection in the cat induces a frontally dominant slowing of EEG activity due to possible disruption of both intracortical and subcortical pathways. These changes appear analogous to QEEG changes seen in HIV-1 infected people. Supported by NIH grant: R29 MH5439-01.

368.1

ANTI-DEPRESSANT LIKE ACTIVITY OF HU-211, A NOVEL NMDA ANTAGONIST. A. Bar-Joseph, Y. Berckovitch and A. Biegon*. Pharmos LTD, Kiryat Weizman 76326, Rehovot, Israel.

NMDA antagonists have demonstrated anti-depressant like activity in animal models. HU-211 is a novel NMDA antagonist devoid of the severe side effects characteristic of many NMDA antagonists. In the present study we examined its anti-depressant activity, in three in vivo models. Male BALB/c mice were used in all studies (n=6-10 per group). HU-211 5, 10, 20, 30 and 40 mg/kg was administered IP, and compared to amytriptiline 15 mg/kg, MK-801 0.5 mg/kg and the appropriate vehicles in all three models. In the open field test, the high dose levels of HU-211 reduced the area traveled by treated mice by 40% ($p<0.05$), similar to that seen with Amtriptiline (55%) and in contrast to the hyperactivity seen with MK-801 (70%, $p<0.05$). A similar effect was also evident in other motor parameters (crossing and rearing). No other effects were detected with HU-211, while a significant hypothermia of 3°c ($p<0.05$), was seen with amytriptiline. A dose related effect of decreased immobility time was evident with HU-211 treated mice in the forced swim test (FST). These effects, induced by HU-211 20-40 mg/kg (40%) and amytriptiline (70%) were statistically significant ($p<0.05$). MK-801 increased immobility time by 30% ($p<0.05$ vs. vehicle). HU-211 treated mice performed in a similar manner in the tail suspension test (TST), 35% decrease in immobility time in the 20 mg/kg dose ($p<0.05$). This effect disappeared with the highest dose, 40 mg/kg. Amytriptiline and MK-801 reduced the immobility time by 75% ($p<0.05$).In conclusion, the present work demonstrated the ability of HU-211 to induce anti-depressant like activity.

368.2

[^{3}H]Ro41-1049 BINDING TO MONOAMINE OXIDASE-A IN THE LOCUS COERULEUS IS NOT ALTERED IN MAJOR DEPRESSION. G.A. Ordway*, V. Klimek, J.G. Richards, J.C. Overholser, H.Y.Meltzer, G.Dilley and C.A. Stockmeier. Dept. Psychiatry & Human Behavior, Univ. of Mississippi Med. Ctr., Jackson, MS; Depts. of Psychiatry & Psychology, Case Western Reserve Univ., Cleveland, OH; Pharma Division, F. Hoffmann-La Roche Ltd., Basel, Switzerland.

Recent studies have revealed an abnormal expression of certain noradrenergic proteins (e.g. tyrosine hydroxylase, norepinephrine transporters) in the locus coeruleus of subjects with major depression and/or victims of suicide. Monoamine oxidase-A (MAO-A), another key enzyme in the metabolism of norepinephrine, is also expressed in noradrenergic neurons of the locus coeruleus and inhibitors of MAO-A are effective in the pharmacological management of major depression. In this study, the binding of [^{3}H]Ro41-1049 to MAO-A was measured at multiple levels along the rostral-caudal axis of the locus coeruleus from 10 subjects with major depression and 10 age- and post-mortem interval-matched, control subjects who were psychiatrically normal. Post-mortem toxicology revealed no antidepressants in the blood or bile of any subjects. The binding of [^{3}H]Ro41-1049 to MAO-A was unevenly distributed along the rostral-caudal axis of the locus coeruleus, parallelling uneven numbers of noradrenergic neurons throughout the nucleus. Accordingly, there was a significant correlation between the number of noradrenergic cells per section and the specific binding of [^{3}H]Ro41-1049 at any particular level of the LC from control subjects ($r^2=0.28$; $p<0.0001$) and from major depressives ($r^2=0.23$; $p<0.0001$). There was no correlation between the age and the amount of [^{3}H]Ro41-1049 binding to MAO-A in control subjects or in major depressives. Unlike tyrosine hydroxylase and norepinephrine transporters, MAO-A in the LC appears to remain unchanged in major depressives as compared to psychiatrically normal control subjects. (Supported by MH46692 and MH45488.)

368.3

MOTOR ACTIVITY AND VARIATION OF MOOD IN DEPRESSION. M.R. Lemke*, A. Broderick, M. Zeitelberger, W. Hartmann. Psychiatric Clinic, General Hospital, D-85049 Ingolstadt, and Dept. Psychiatry, University of Kiel, Germany

Objective: Diurnal variation of mood in depressed patients represent a diagnostic criteria (DSM-IV) for the melancholic type of depressive disorder. Whether circadian variation of symptom intensity include changes in spontaneous motor activity, remains unclear. Therefore, the relation of motor activity and mood was studied at different times of the day in patients with major depression.

Methods: Inpatients (n=21) were included if they fulfilled DSM-IV criteria for major depression, melancholic type, and experienced diurnal variations of mood. Motor activity was measured by actigraph (activity-based monitoring) (ZAK, Germany) from 7 to 9 a.m. and p.m.. Patients recorded their mood on a visual analogue scale (VAS) during this time.

Results: VAS scores were significantly lower in the morning ($p<0,01$). Counts of activity units were significant greater in the morning ($p<0,01$) and showed an invers correlation with VAS scores ($p<0,01$).

Conclusions: Activity measured by actigraphy may not cover all aspects of psychomotor activity. However, increased motor activity may represent an observable, psychobiological behavioral equivalent of self experienced depressed mood and may serve as a biological function to elevate depressed mood via modification of neuronal serotonergic activity. (Supported by General Hospital, Ingolstadt, Germany)

368.4

NEUROPSYCHOLOGICAL IMPAIRMENTS IN UNIPOLAR DEPRESSION: THE ROLE OF PERCEIVED FAILURE, B.J. Sahakian*, R. Elliott, A.P.McKay, J.J. Herrod, T.W. Robbins and E.S. Paykel Departments of Psychiatry and Experimental Psychology, University of Cambridge, Cambridge, CB2 2QQ, U.K.

Depression has been associated with various neuropsychological impairments but no consensus explanation of these deficits has emerged. In an attempt to characterise the neuropsychological profile of depression more fully, this study used a highly sensitive test battery with strong neurological validation to assess the cognitive deficits associated with depression.

The CANTAB battery of neuropsychological tests was used to compare the performance of 28 medicated patients with unipolar depression to that of 22 matched controls. These tests assessed visuo-spatial memory, attentional set-shifting working memory and planning. The patients were impaired on almost all tests studied with 92% of patients showed at least some impairment. Both accuracy and latency of performance were compromised. Intact performance on the test of attentional set-shifting suggests that these deficits were not purely motivational. Deficits in the tests of visual memory correlated with severity of depression.

An important finding was the detrimental effect of failure on subsequent performance; having solved one problem incorrectly, patients were significantly more likely than controls to fail the subsequent problem, even after controlling for overall failure rates. Comparisons with other patient groups showing similar levels of impairment indicated that this effect was specific to depression. Patients with frontal lobe damage, temporal lobe damage, schizophrenia or Parkinson's disease were no more likely than controls to respond to one failure with another. Also the impairment did not normalise on clinical recovery suggesting that it may represent a trait factor for depression.

Oversensitivity to negative feedback may therefore be an important determinant of performance in depressed patients, interacting with general neuropsychological impairments. This demonstartion of a possible interface between mood and cognition has important implications for our understanding of the disorder.

This research was funded by a Programme Grant from the Wellcome Trust.

368.5

VALPROIC ACID TREATMENT FOR MAJOR DEPRESSIVE DISORDER:GABA AND MOOD DISORDERS. L. L. Davis*, F. Petty, VAMC and Univ. Texas Southwestern Med. School, Dallas, TX 75216

Valproic acid is a GABA agonist anticonvulsant which is also an effective treatment for mania. We studied valproic acid as a treatment for non-bipolar major depressive disorder (MDD), since a deficit in GABA is well established in MDD. 28 outpatients who met research criteria for MDD, with no history of mania or hypomania were enrolled in an open trial with valproic acid for 4 weeks with a 4 week extension. 23 patients completed 4 weeks of treatment with valproic acid, and 19 completed the 4 week extension. At week 4, 13 of the 23 completers demonstrated a clinical response, and mean Hamilton depression (HD) scores for all patients decreased from baseline 22 ± 5 to 11 ± 6 ($p < 0.0001$). At week eight, 17 of the 19 (89%) completers demonstrated clinical improvement, and HD scores decreased 7 ± 4 ($p < 0.0001$). For all subjects (intent to treat analysis at week eight), 61% were responders and HD scores decreased 52 %). Patients with melancholia were as likely to respond to valproic acid as non-melancholic depressives. These data suggest that valproic acid may be an effective and well-tolerated treatment for unipolar major depressive disorder. Double-blind, placebo-controlled trials are needed to further document the efficacy of valproic acid in the treatment of MDD. A role for GABA in depression is supported.

Department of Veterans Affairs.

368.6

VENLAFAXINE: SAFE AND EFFECTIVE ANTIDEPRESSANT THERAPY IN PATIENTS ON CONCOMITANT MEDICATIONS, **B. Zimmer, R. Kant, D. Zeiler, M. Brilmyer and L.H. Schneider*, Dept. Psychiatry and Neuropsychiatric Institute, Allegheny General Hospital, Pittsburgh, PA 15212 and Innova Biomed, INC., Irvington, NY 10533**

Venlafaxine is a novel antidepressant agent which inhibits the reuptake of both serotonin and norepinephrine. Venlafaxine has been shown to be safe and effective monotherapy in a substantial number of clinical studies with depressed patients. Compared with traditional antidepressants (TCAs and MAOIs) and the selective serotonin reuptake inhibitors (SSRIs), venlafaxine has lower protein binding and less impact on cytochrome P450 isoenzyme systems (Richelson, 1994). Thus, venlafaxine should reduce the risk of drug-drug interactions in depressed patients requiring concomitant medications.

Our study sought to assess the safety and efficacy of venlafaxine in a population of depressed patients with medical comorbidities which required continuation of concomitant medications. Twenty-six patients completed the study. Baseline evaluations included medical and blood pressure measures, as well as psychiatric evaluation. Blood pressure was monitored and clinical global improvement (CGI score) was rated when venlafaxine therapy ended. Data were analyzed by appropriate nonparametric statistical tests which classified patients into young (13-55; n=11) and old (65-82; n=15) groups.

Antidepressant efficacy was found in 64% of young and 73% of old patients, whose total daily dosages of venlafaxine [mg/day; mean (sem)] were 185.2 (24.7) and 131.7 (15.7), respectively. No interactions between venlafaxine and concomitant medications were apparent. Systolic (SBP) and diastolic (DBP) blood pressure changes over treatment were [mm Hg; mean (sem)]: Young 0.82 (5.09) SBP and 2.72 (1.97) DBP vs. old 0.73 (3.45) SBP and 4.53 (1.72) DBP. These results suggest that venlafaxine is a safe and effective first-line antidepressant for vulnerable depressed patients on concomitant medications. **[Supported in part by Innova Biomed, Inc.]**

368.7

FLUOXETINE AND DULOXETINE SIMULTANEOUSLY RELEASE SEROTONIN (5-HT), DOPAMINE (DA) AND NORADRENALINE (NAD) IN THE FRONTAL CORTEX OF FREELY-MOVING RATS: WAY 100,635 SELECTIVELY POTENTIATES THE INDUCTION OF 5-HT RELEASE. A. Gobert*, J-M. Rivet, M. Spedding, L. Cistarelli, S. Girardon and M.J. Millan, I.D.R.S., 125 Chemin de Ronde, 78290 Croissy, France.

While the 5-HT_{1A} receptor antagonist, (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclo-hexanecarboxamide) (WAY 100,635) potentiates 5-HT release provoked by fluoxetine (FLX), its influence upon DA and NAD release remains unexplored. We examined this question employing FLX, a further uptake inhibitor, duloxetine (DLX), and dialysis in freely-moving rats implanted with a cannula in frontal cortex (FCX). Doses (base) are in mg/kg, s.c. Samples were taken every 20 min and analysed by HPLC/ coulometric detection. Three basal values were taken, WAY (0.16) injected, and, 20 min later, FLX (10.0) or DLX (5.0) administered. Levels (Means±S.E.M.) were determined at 60-120 min.

	VEH + VEH	VEH + FLX	VEH + DLX	WAY + VEH	WAY + FLX	WAY + DLX
5-HT(0.9)	103 ± 6	186 ± 10#	209 ± 13#	113 ± 11	306 ± 24*	423 ± 27*
DA (1.1)	98 ± 3	148 ± 5#	155 ± 9#	95 ± 3	130 ± 4	146 ± 10
NAD (1.0)	108 ± 5	187 ± 7#	441 ± 20#	104 ± 9	141 ± 8	453 ± 38

Levels are % basal (= 100%). Absolute, basal levels (pg/20 min) are in parentheses. VEH = vehicle; # P < 0.05 vs VEH/VEH; *P < 0.05 vs VEH/FLX or VEH/DLX.

WAY selectively and markedly potentiated the FLX- and DLX-induced increase in 5-HT, but not DA and NAD. We conclude that, under these conditions, FLX releases 5-HT as well as DA and NAD in FCX. However, the 5-HT_{1A} antagonist, WAY, likely via an action at 5-HT_{1A} autoreceptors, *selectively* enhances the FLX- and DLX-induced release of 5-HT as compared to DA and NAD in FCX.

This study was supported by Servier Pharmaceuticals.

368.8

PAROXETINE CHALLENGES DO NOT AFFECT PLASMA PROLACTIN LEVELS. P. Hicks*, S. Peterson, J. Richter AND J. Browning. Dept. of Psychiatry and Behavioral Science, Scott and White Clinic, Temple 76508

A variety of pharmacological agents (e.g. clomipramine and fenfluramine) have been used to evaluate the neurochemical state of depressed patients using neuroendocrine responses. The role of serotonin in the neuroendocrine response has been studied using the serotonin precursor L-tryptophan and m-CPP (nonspecific 5-HT-2A/2C agonist). L-tryptophan enhances prolactin release which is reversed by the 5-HT-1 antagonist pindolol indicating that prolactin release is mediated by 5-HT-1 receptors (Smith et al., 1991). M-CPP enhances prolactin release which is reversed by ritanserin (5-HT-2A/2C antagonist) (Seibyl et al., 1991). Thus, there are multiple 5-HT receptor subtypes that modulate the release of prolactin. We have assessed the use of a very selective 5-HT uptake inhibitor, paroxetine, in the release of prolactin in normal volunteers.

Normal volunteers ages 20-50 years were given blinded oral challenges with either paroxetine (0, 30 or 60 mg) or clomipramine (0, 50 or 100 mg) and their prolactin levels monitored for up to 6 hours after drug exposure.

The results indicate that paroxetine did not induce prolactin release in men or women normal volunteers ($p > .86$; $F=0.14$; df 2,32). There was a prolactin response to clomipramine, however ($p < .05$ using Dunnet's Method to compare with the control group).

Paroxetine is known to be a potent and selective inhibitor of serotonin uptake. The fact that paroxetine did not induce prolactin release suggests that other neurotransmitter systems may be important in the release of prolactin induced by the serotonin uptake inhibitor clomipramine or the serotonin releasing agent fenfluramine. Precaution should be taken in the interpretation of pharmacological challenges evaluating the central serotonergic state with such agents.

368.9

EFFECTS OF CHRONIC ANTIDEPRESSANT TREATMENT ON PLASMA CORTICOSTERONE AND PROGESTERONE AFTER SWIM STRESS AND ACUTE CHALLENGE WITH FLUOXETINE. G.E. Duncan*, D.J. Knapp and G.R. Breese. Univ. of North Carolina Neuroscience Ctr, Univ. of North Carolina, Chapel Hill, NC 27599.

Although the acute pharmacological actions of antidepressant drugs are well characterized, the adaptive changes induced by chronic treatment with antidepressant drugs that are responsible for therapeutic mechanisms of action are unknown. The well documented association between stress and depression suggests that investigation of the effects of chronic antidepressant administration on stress responses could help to elucidate the mechanism of antidepressant action. In the present study the effects of chronic treatment (3 weeks) with saline, imipramine (20 mg/kg), or fluoxetine (5 mg/kg) were investigated on corticosterone and progesterone secretion induced by swim stress (Porsolt et al., 1978) in male rats. The final drug injection was given 24 hrs before the test swim. Swim stress increased plasma corticosterone (14 fold) and progesterone (22 fold) and the time course of response for the two hormones was similar. Chronic treatment with imipramine reduced stress-induced progesterone levels by 50-60% at the different times but did not alter stress-induced levels of corticosterone. By contrast, chronic treatment with fluoxetine did not alter the stress-induced changes in either hormone. Effects of chronic fluoxetine treatment were also investigated on the hormonal responses to acute challenge with fluoxetine. Injection of fluoxetine (5 mg/kg) after chronic saline robustly elevated corticosterone and progesterone to an extent comparable to that observed for swim stress. After 3 weeks treatment with fluoxetine acute administration of the drug did not alter plasma levels of either hormone. These data suggest that an adaptive responses occurred in serotonergic pathways related to the regulation of adrenal cortical secretion after chronic fluoxetine treatment. The failure of chronic fluoxetine to alter the hormonal responses to acute swim stress suggest that different neural mechanisms are responsible the elevation of corticosterone and progesterone after acute stress compared to acute challenge with fluoxetine. Supported by MH-39144, MH-33127, HD-07201, and NARSAD.

368.10

DESMETHYLIMIPRAMINE REVERSES REWARD IMPAIRMENTS OBSERVED DURING AMPHETAMINE WITHDRAWAL: VALIDATION OF A MODEL OF DEPRESSION. D. Y. Lin, M. P. Epping-Jordan*, G. F. Koob, A. Markou, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Major affective disorder in humans is accompanied by significant deficits in reward and motivation. Animal models of psychostimulant withdrawal have demonstrated similar robust and quantifiable impairments in reward processes as assessed by changes in intracranial self-stimulation (ICSS) thresholds. These models may, therefore, provide insights into the neurobiology of depression that may, in turn, lead to pharmacological therapies that would alleviate reward and motivational impairments. A previous study that used ICSS thresholds to assess changes in reward state showed that repeated administration of desmethylimipramine (DMI), a tricyclic antidepressant, shortened the duration of elevations in ICSS thresholds in cocaine-withdrawing animals. In the present study, the effects of repeated DMI administration in naive animals and animals undergoing amphetamine withdrawal (systemic amphetamine administration: 1-5 mg/kg i.p., 3 times daily for 4 days) were explored. Following the amphetamine exposure regimen, DMI was administered twice daily at either of four doses (0, 2.5, 5, 10 mg/kg i.p.) and estimates of ICSS thresholds were obtained on successive days of withdrawal. In amphetamine-exposed animals, chronic DMI treatment reduced the duration of ICSS threshold elevations in a dose-dependent manner. Interestingly, acute administration of DMI did not reduce ICSS thresholds in amphetamine-withdrawing rats at any dose. In fact, acute DMI actually elevated thresholds in naive rats (non-withdrawing) at the 5 and 10 mg/kg doses. The reversal of amphetamine withdrawal effects on ICSS reward following repeated DMI administration provides validation for the use of this model in the development of novel pharmacotherapies to treat the reward and motivational dysfunctions associated with depression. (Supported by a Sandoz grant and DA00213 grant to A. M.).

368.11

EFFECTS OF IMIPRAMINE ON 5-HT2CR mRNA AND A NOVEL DEPRESSION RELATED GENE (DRF24) EXPRESSION.

M. Tohda[1]*, Y. Nomura [1,2] & H. Watanabe[1], [1] Res. Inst. for Wakan-yaku (Traditional Sino-Japanese Medicines), Toyama Med. & Pharm. Univ., Toyama 930-01 and [2] Fac. of Pharmaceutical Sci., Hokkaido Univ., Sapporo 060, Japan.

We previously reported that some antidepressants act as serotonin 2C subtype receptor (5-HT2C) antagonists. Here, we examined the influences of antidepressants on 5-HT2C mRNA and novel type of gene expression in the rat brain. Imipramine was orally administered to male Wistar rats (8 w, 220 - 250g) once daily for 4 days. The 5-HT2C mRNA expression in the brain was detected by in situ hybridization methods using the digoxigenin-labeled antisense cRNA probe coding the third intracellular loop. Imipramine treatment produced more intense hybridization signals in nearly all regions stained, such as the hippocampus, choroid plexus, habenular nucleus and dorsomedial hypothalamic nucleus, than the control in a dose- (2 - 20 mg/kg) and a time- (1 - 14 days) dependent manner. Mianserin (20 mg/kg) and desipramine (20 mg/kg) also extended the 5-HT2C mRNA expression but nomifensine (20 mg/kg) was ineffective. These findings corresponded with the antagonistic effects of antidepressants to 5-HT2C. Since the 5-HT2C gene has been suggested to act as a protooncogene, we also searched for genes newly expressed after imipramine treatment by the differential display methods. We found a novel gene (DRF24) and read the sequence: DRF24 is 160 bp which may be a partial sequence. DRF24's mRNA existed in the basolateral amygdaloid nucleus of the imipramine-treated rat brain. We are now studying the whole sequence of the gene and its functions.

368.12

BIOCHEMICAL STUDIES OF YM992: A NOVEL ANTIDEPRESSANT AS A SELECTIVE SEROTONIN RE-UPTAKE INHIBITOR AND SEROTONIN-2A ANTAGONIST. Y. Tasaki, K. Hatanaka, T. Nomura, H. Takeuchi, S. Yatsugi and T. Yamaguchi.* Neuroscience and Gastrointestinal Research Lab. Institute for Drug Discovery Research, Yamonouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba 305 Japan.

Our biochemical studies have confirmed that YM992, (S)-2-[[7-fluoro-4-indanyl)oxy]methyl]morpholine monohydrochloride, has a novel selective serotonin re-uptake inhibition (SSRI) with 5-HT_{2A} antagonistic activity in vitro and in vivo. In the present study, we investigated 5-HT release in the frontal cortex of the rats using microdialytic teuniques. The rats were chronically treated with an antidepressant, such as citaroplam, fluoxetine, or YM992. The 5-HT release induced by YM992 was more than that induced by the other antidepressnts. In addition, the effect of YM992 on endocrine changes was examined. Many studies reported that corticotropin-releasing factor(CRF) and adrenal glucocorticoid-hormones were increased in depressive patients. Therefore, we first examined one of the adrenal glucocorticoid-hormones, corticosterone, in immobilized rats treated with the antidepressants. In a single injection of these drugs, there were no significant changes in serum corticosterone concentration of all groups. In chronic treatment of these drugs(14 days), however, only YM992 showed significant decrease of serum corticosterone concentration compared with the other antidepresants. These results suggested that the antidepressive effect of YM992 appear earlier than that of other SSRIs.

368.13

ROLE OF 5-HT1A AND 5-HT2A RECEPTORS IN THE ANTIDEPRESSANT ACTIVITY OF YM992 IN THE MOUSE TAIL SUSPENSION TEST.

S. Yatsugi, H. Takeuchi, S. Hayashibe, S. Tsukamoto, K. Koshiya, S. Usuda, A. Iwai* and T. Yamaguchi. Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co.,Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan.

YM992 ((S)-2-[[(7-fluoro-4-indanyl)oxy]methyl]morpholine monohydrochloride) has selective affinities for serotonin (5-HT) uptake sites (Ki = 21 nM) and 5-HT2A receptors (Ki = 86 nM). YM992 shows more robust antidepressant activity in the mouse tail suspension test than selective 5-HT reuptake inhibitors (SSRI), such as citalopram or fluoxetine. A likely explanation for this characteristics of YM992 could be a synergistic effect of its dual action. Here we investigated the possible involvement of 5-HT2A antagonism of YM992 in its antidepressant activity in the mouse tail suspension test by comparing the efficacy of a typical SSRI citalopram, a selective 5-HT2A antagonist MDL100907, and their combined treatment. In addition, we examined the effect of selective 5-HT1A antagonist, WAY 100, 635, on the antidepressant activity of YM992. Mice were intraperitoneally or subcutaneously dosed with drugs 30 min before test. They were suspended with the tail for 6 min, and the duration of immobility was measured. MDL100907 alone did not affect the immobility time in a dose range of 0.01 to 0.3 mg/kg (ip). Citalopram neither showed significant effect at a dose of 10 mg/kg (ip). However the coadministration with ineffective doses of the both compounds exerted a marked reduction in the immobility time. This effect was almost equipotent to that of 10 mg/kg (ip) of YM992. Although WAY 100,635 (0.1 and 0.3 mg/kg, sc) alone did not affect the immobility time, it dose-dependently antagonized the effect of YM992. These results suggest that the robust effect of YM992 in this model is due to its 5-HT2A antagonistic activity in addition to 5-HT re-uptake inhibition activity, and the stimulation of 5-HT1A receptors with 5-HT, which is potentiated by 5-HT2A receptors blockade, plays an important role in the antidepressant activity of YM992.

368.14

COMBINED ADMINISTRATION OF THE 5-HT_{1D} ANTAGONIST GR127935 AND SERTRALINE SYNERGISTICALLY INCREASES EXTRACELLULAR 5-HT LEVELS IN GUINEA PIG HYPOTHALAMUS

T. Clarke, J. Sprouse, D.W. Schulz and H. Rollema*
Department of Neuroscience, Pfizer Central Research, Groton CT 06340

The 5-$HT_{1B/1D}$ terminal autoreceptor is a potential target for drugs aimed at enhancing serotonergic transmission, since blockade or activation of this receptor is expected to increase or decrease 5-HT release, respectively. We studied the effects of the 5-HT_{1D} antagonist, GR127935, on 5-HT release by microdialysis in guinea pig hypothalamus. Experiments were performed under basal conditions and following pretreatment with the SSRI sertraline.
At 0.3 mg/kg, GR127935 had no effect on basal 5-HT release, but a dose of 5 mg/kg produced a small increase (to 135% of basal levels) in 5-HT release in guinea pig hypothalamus. Low doses of sertraline (2 mg/kg sc) increased extracellular 5-HT to the same extent (to 130% of basal levels). The combined administration of this dose of sertraline with 0.3 mg/kg sc of GR127935 had marked synergistic effects: the maximal 5-HT increase was much higher (200% of basal) than the sum of the effects of either drug given alone. In addition, the 5-HT levels remained elevated for more than 5 hours, resulting in at least 4 times greater AUC values for the combination than for the additive AUC's of each individual drug. When 2 mg/kg sertraline was combined with 5 mg/kg GR127935, the maximal 5-HT increase was somewhat higher (to 250% of basal) and longer lasting than with 0.3 mg/kg GR127935.
These data demonstrate that activation of 5-$HT_{1B/1D}$ terminal autoreceptors reduces the net effect of SSRI's on extracellular 5-HT levels and that the combination of a SSRI with a 5-HT_{1D} antagonist could be useful in augmenting and/or hastening the onset of antidepressant effects.

368.15

LITHIUM-INDUCED ENHANCEMENT OF 5-HT_{2A} RECEPTOR FUNCTION IS NOT ASSOCIATED WITH ALTERATIONS IN RECEPTOR NUMBERS.

J.M. Moorman and R.A. Leslie* Oxford University SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, OX2 6HE, England.

Lithium is used in the treatment of bipolar affective disorder, yet its mechanism of action is poorly understood. We investigated the effects in rats of chronic treatment with lithium on 5-HT_{2A} receptor-mediated behavioural responses, Fos expression, and the density of this receptor subtype in the brain. Rats received either control or lithium-containing (0.1% $LiCO_3$) chow for 3 weeks prior to challenge with the 5-$HT_{2A/2C}$ agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). DOI-induced head shakes were then counted and locomotor activity was monitored for 30mins. Animals were killed 120mins later and their brains removed for immunohistochemical localisation of Fos protein. Another group of rats, also receiving either control or lithium-containing chow, had their brains analysed for the distribution and density of 5-HT_{2A} receptor binding sites by quantitative [^{3}H]ketanserin (2nM) autoradiography (± 500nM unlabelled spiperone). Treatment with lithium was found to have no effect on DOI-induced head shakes, but it significantly enhanced both DOI-induced locomotor activity and DOI-induced Fos expression throughout the cerebral cortex. In contrast, such treatment had no effect on the density of [^{3}H]ketanserin binding to 5-HT_{2A} receptors in any brain region examined. Throughout, plasma lithium levels, measured by atomic absorption spectroscopy, were between 0.4 and 0.6mEq/l. Since the enhancing effect of lithium on DOI-induced locomotor activity and Fos expression are not mediated by a change in the density of 5-HT_{2A} receptor binding sites, another mechanism, such as an alteration in second messenger function (eg phosphatidyl inositol turnover) must be considered.

368.16

ELEVATED INTRACELLULAR CALCIUM IN IMMORTALIZED B-LYMPHOBLASTS IN BIPOLAR-I DISORDER. M. Emamghoreishi, L. Schlicter, P.P. Li, S. Parikh, J. Sen, A. Kamble and J.J. Warsh*. Clarke Institute of Psychiatry and Playfair Neuroscience Institute, University of Toronto, Toronto, ON, Canada

Increasing evidence implicates altered calcium (Ca^{+2}) signaling mechanisms in the pathophysiology of bipolar affective disorder (BD). Higher resting ($[Ca^{+2}]_B$) and agonist-stimulated ($[Ca^{+2}]_S$) intracellular calcium concentrations have been found in platelets and $[Ca^{+2}]_B$ in lymphocytes from untreated manic and depressed BD patients compared with unipolar depressed patients and heathy controls (Dubovsky et al., Am. J. Psychiatry 149:118, 1992; Eur. Arch. Psychiatry Clin. Neurosci. 243:229,1994). To clarify whether such changes are trait-related, we determined $[Ca^{+2}]_B$ in Epstein-Barr virus-immortalized B-lymphoblasts obtained from physically healthy subjects with DSM-IV diagnoses of BD (bipolar I [BP-I], N=28; bipolar II [BP-II], N=10), major depression (MDD, N=13), non-mood psychiatric disorder (NMD, N=14) , and no psychiatric disorder (N=20). $[Ca^{+2}]_i$ was measured by ratiometric fluorescence assay using fura-2. $[Ca^{+2}]_B$ and phytohaemagglutinin-(PHA, 10µg/ml) stimulated $[Ca^{+2}]_i$ were also determined in freshly isolated T-lymphocytes from these same subjects. $[Ca^{+2}]_B$ was significantly higher (13%) in transformed lymphoblasts from BP-I (63.3 ± 9.4, mean ± SD, nM), but not BP-II (58.8 ± 3.9), MDD (57.5 ± 7.3) or NMD (54.8 ± 5.3) patients compared to healthy subjects (55.9 ± 8.25) (ANOVA, F=4.01, *df*=4,79; *p*=0.005). $[Ca^{+2}]_B$ values did not correlate with age or blood pressure, nor was there any significant interaction between gender and diagnosis on $[Ca^{+2}]_B$ in lymphoblasts. $[Ca^{+2}]_B$ was also significantly higher (30%) in T-cells from BP-I, but not BP-II or MDD subjects compared with healthy controls (ANOVA, F=2.34, *df*=4,66; p=0.06). $[Ca^{+2}]_S$ did not differ between subject groups but there was a significant reduction in the % response relative to $[Ca^{+2}]_B$ in BP-I compared with healthy subjects, most likely a result of the elevated $[Ca^{+2}]_B$ values in the former group. These findings provide additional evidence suggesting that trait-dependent factors account, at least in part, for elevations in $[Ca^{+2}]_B$ in lymphocytes from BP-I subjects.(Supported by Medical Research Council of Canada grant MT 12851).

368.17

CHANGES IN NA^+,K^+-ATPase ALPHA ISOFORM SUBTYPES IN LYMPHOBLASTOID CELL LINES FROM BIPOLAR PATIENTS.

R. Li, T. Buss and R.S. El-Mallakh*. Department of Psychiatry and Behavioral Sciences, University of Louisville School of Medicine, Louisville, KY 40292.

There are many lines of evidence suggesting that cation distribution and regulation may be abnormal in patients with manic-depression. For example, fresh lymphocytes derived from bipolar individuals fail to show the normal increase of Na^+,K^+-ATPase activity seen when cells are exposed to ethacrynic acid *in vitro*. We have investigated the response of immortalized lymphoblastoid cells to ethacrynate. Lymphoblastoid cells from Old Order Amish bipolar patients (n=9) and normal controls (n=7) were treated with ethacrynic acid (10 µM) or vehicle for 72 hours. [^{3}H]-Ouabain binding, and alpha subunit protein expression were analyzed. Ethacrynic acid induced an increase (134%) in [^{3}H]-Ouabain binding in lymphoblasts derived from normal individuals, however, lymphoblasts from bipolar patients exhibited a slight decrease (89%). A similar pattern was observed by Western blot in which there was a decrease in the α1 isoform of the Na^+,K^+-ATPase in ethacrynic acid-treated cells from patients compared to normal controls. (The α2 and α3 isoforms were never expressed in either normal or bipolar lymphoblasts.) We conclude that the absence of adaptive responses of Na^+,K^+-ATPase to ethacrynic acid that occurs in fresh lymphocytes also occurs in lymphoblastoid cell line from bipolar patients, and it is related to variations in α1 Na^+,K^+-ATPase protein expression.

368.18

AN ANIMAL MODEL FOR MANIA: PRELIMINARY EVIDENCE FOR SENSITIZATION TO OUABAIN

R. S. Levy,* R. Li, R. S. El-Mallakh, L. T. Harrison, D. G. Changaris, and G.Bao. Depts. of Psychiatry, Biochemistry, Neurology, and Laboratory of Biological Psychiatry, University of Louisville School of Medicine, Louisville, KY 40292.

Mood-state-related reduction of erythrocyte Na,K-ATPase pump activity is a consistent trait seen in manic or depressed bipolar patients. We have previously demonstrated that chronic intracerebroventricular (ICV) infusion of the Na,K-ATPase inhibitor, ouabain, in male Sprague-Dawley rats can produce either hyperactivity or hypoactivity. These ouabain-induced changes in behavior can be prevented by lithium preadministration, and ameliorated when lithium is given after induction of the behavioral change. Effects of other antimanic agents such as valproic acid are currently under investigation. We recently have found preliminary evidence that this technique may model yet another characteristic of human mania -- sensitization. In human mania this manifests as a worsening course of illness as a function of prior manic episodes. ICV cannulae were placed stereotactically under anesthesia. Four days later the animals were given a single ICV injection of 5 µL ouabain 10^{-3}M over 30 seconds. Three weeks later the rats were rechallenged with a chronic infusion of ouabain 10^{-3}M or artificial cerebrospinal fluid (aCSF) at 5 µL/hr (via an ALZET osmotic mini pump). Locomotor activity was quantified using an infrared beam activity monitor before chronic ouabain infusion (baseline) and again at days 3, 10, 14, 21, 28 and 35. Baseline behavior was not different between the animals. After 10 days of ouabain infusion, activity was increased to 424% of baseline, while animals receiving aCSF experienced an increase in activity of only 115%. Without prior sensitization with ouabain, activity typically increases 140% of baseline.

Supported by grants from the University of Louisville School of Medicine

368.19

MOOD STABILIZERS AND ENDOGENOUS ADP-RIBOSYLATION. L. T. Young*, C. M. Woods, V. Asghari and I. Patelis-Siotif. Departments of Psychiatry and Biomedical Sciences, McMaster University, Hamilton, Ontario,Canada, L8N 3Z5.

Bipolar disorder (BD) is associated with increased levels and function of the G-protein, $G\alpha_s$, which may be normalized by treatment with mood stabilizing medications (i.e. lithium, valproic acid and carbamazepine). Since ADP-ribosylation of $G\alpha_s$ has been shown to increase turnover of this protein, we examined whether mood stabilizers affected this process in cultured cells, or in platelets from patients with BD, before and after treatment. In C6 glioma cells, endogenous ADP-ribosylation of a 50 kDa protein was markedly increased by lithium chloride (+83%, $P<0.005$) and decreased by valproic acid (-48%, $P=0.07$) whereas carbamazepine had no effect. In contrast, levels of endogenous ADP ribosylation of a 50 kDa protein were significantly higher ($p<0.05$) in platelet membranes from drug free BD patients (1.92±0.41 densitometric units, N=13, Mean Age 33±2.7yr), compared with controls (1.17±0.17, N=13, Mean Age 27±2.0) and lithium treated euthymic patients with BD (1.21±0.20, N=13, Mean Age 33±2.5). These results suggest a mechanism of action for lithium distinct from the anticonvulsants which may be important to G-protein function and which occurs in brain but not in peripheral tissues. (Supported by a MRC grant (L.T.Y)).

NEURO-ONCOLOGY: TUMOR BIOLOGY

369.1

BEHAB, THE GENE FOR A CNS SPECIFIC HYALURONAN-BINDING PROTEIN, MEDIATES INVASION OF GLIOMA CELL LINES *IN VITRO* AND *IN VIVO*. H. Zhang, G. Kelly, D. Jaworski and S. Hockfield*. Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Malignant astrocytomas (gliomas) aggressively invade the surrounding normal brain, a feature not shared by brain metastases of non-glial tumors, which grow as circumscribed masses with well demarcated borders. Tissue- or tumor-specific extracellular proteins are prime candidates for mediating the distinctive invasive behavior of primary brain tumor. The gene for a brain-specific extracellular protein, BEHAB, is expressed during periods of gliogenesis, but is not detectable in normal adult human cortex. BEHAB mRNA is expressed in surgical samples of astrocytoma, and has not been detected in any tumor of non-glial origin. Glioma cell lines maintained *in vitro* do not express BEHAB, however, BEHAB expression can be induced in glioma cell lines when grown as intracranial grafts. Only glioma cell lines that grow invasively express BEHAB; BEHAB is not induced in intracranial grafts of cell lines that do not show invasive properties (Jaworski et al., Cancer Res. 56:2293). Together these results demonstrated that BEHAB is a unique and selective marker for astrocytoma and suggested that it may be an important element in brain tumor invasion.

A possible role for BEHAB in tumor invasion has been tested by transfecting 9L gliosarcoma cells with BEHAB. 9L cells do not express BEHAB *in vitro*, and, when grown as intracranial grafts, form non-infiltrating masses that do not express BEHAB. 9L cells transfected to express BEHAB show increased invasion both *in vitro* and *in vivo*. In a matrigel *in vitro* invasion assay, transfected 9L cells show an increased ability to migrate through an artificial matrix, and this ability is potentiated by the presence of hyaluronan. Intracranial grafts of transfected 9L cells develop small cell clusters distant to the site of implantation. These data provide strong support for an important role for BEHAB in glioma invasion and suggest possible therapeutic strategies.
(Supported by EY06511 and NS35228 [to SH] and EYO6451 [to DJ])

369.2

OLIGODENDROCYTE mRNAs CAN BE USED TO PREDICT THE EXTENT OF NEOPLASTIC INFILTRATION IN GLIAL TUMORS. Landry, C.F.*, Cherman, L., Verity, M. A., Yates, A.[1] and Campagnoni, A.T., MRRC, UCLA School of Medicine, Los Angeles, California 90024 and [1]Div. Neuropath., Ohio State, Columbus, Ohio.

The identification of biochemical markers specific for glial neoplasms would contribute greatly to an understanding of the cell biology of these tumors. We have applied non-radioactive *in situ* hybridization histochemistry to an analysis of the expression of glial and neuronal mRNAs in a range of glial tumors. We found that low grade tumors contained a high number of proteolipid protein (PLP) positive cells and that the number of PLP stained cells decreased markedly with increasing tumor grade. In addition, the ratio of PLP to MBP stained cells in low grade astrocytoma was 2:1 but approached 1:1 with increasing tumor grade. Interestingly, gemistocytic astrocytomas and post-radiation gliomas that are generally considered to be highly invasive contained a high number of PLP positive cells and a PLP to MBP ratio close to 2:1 similar to normal tissue and low grade tumors. In addition, gemistocytes were consistently found to be strongly vimentin positive, to contain little or no GFAP mRNA and not to stain for PLP or MBP mRNA. These results highlight the selective expression of glial mRNAs within astrocytic tumors and suggest that these molecular markers can be used to predict the extent of tumor infiltration in specific astrocytomas. (Supported by NIH grants NS23022, NS23322, HD25831 and NMSS grant RG2233A1.)

369.3

OLIGODEOXYNUCLEOTIDE-MODULATED EXPRESSION OF METALLOTHIONEIN IN NEUROBLASTOMA IMR32 CELL. H. El Refaey, P.L. Iversen, R. Bashir, M.L. Heidrick, F.M. Hamada, J. Mata, A.M. Earle,* and M. Ebadi. Depts. of Pharmacology, Neurology, Biochemistry, Cell Biology and Anatomy, Univ. of Neb. Coll. of Med., Omaha, NE 68198-6260

Human neuroblastoma (NB) is a malignant tumor of neural crest origin, and is the most common extracranial solid neoplasm in children. Cytogenetic studies have revealed that the most common abnormalities in neuroblastoma are homogeneously staining regions which corresponds to genomic N-myc amplification, and rapid tumor progression. In addition, it has been shown that proto-oncogenes such as Ha-ras and Trk, which encodes a receptor for nerve growth factor are associated with a favorable prognosis; whereas the expression of Bcl2-proto-oncogene is strongly associated with an unfavorable histology. The overexpression of metallothionein (MT) represents one mechanism of resistance to a subset of clinically important anticancer drugs and oligonucleotide-mediated alteration of MT gene exhibits specificity in inhibiting MT synthesis. Therefore, we delineated the effects of antisense oligonucleotide probes on viability, proliferation, and cell cycling, of a number of tumor cell lines, including MCF-7WT and MCF-7/Adr; KB and KBV; Colo 320, Hct116, Lovo, and HT29; IMR32; and U373MG. Studies using immunohistochemistry and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay along with apoptotic data revealed that antisense oligonucleotide of MT inhibited the viability and survival of neuroblastoma, glioma and colon cancer cell lines. In view of the fact that MT possesses a protective role against oxygen, nitrogen, and carbon centered free radicals, we conclude that MT may have a wide-spread but selective importance in cell survival. (Supported in part by a grant from USPHS-NS34566).

369.4

ONCOSTATIN M (OSM) INHIBITS PROLIFERATION AND INDUCES DIFFERENTIATION IN HUMAN GLIOMA CELLS. F. Stögbauer*, P. Young, E.B. Ringelstein, R. Westermann and H. Halfter. Dept. Neurology, University of Münster, D-48129 Münster, Germany

OSM is a member of a family of pleiotropic cytokines named neuropoietic cytokines exerting various effects on certain cell types in vitro and in vivo. This includes growth inhibition and differentiation as well as proliferation of tumor cell lines. We have recently described three new established cell lines of human glioma origin in terms of expression of receptors and ligands of members of the neuropoietic cytokine family. All cell lines express receptor components for ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF) and OSM as well as the ligands CNTF and LIF.

In order to investigate potential effects of OSM on tumor cells we performed cell growth and differentiation assays. Incubation with OSM (10 ng/ml) induces remarkable inhibition of cell proliferation as determined by reduced DNA synthesis in BrdU-labeling assays compared to control cultures. Morphological signs of apoptosis could not been detected. However, after four to five days of treatment with OSM a morphological change of the glioma cells could be observed. Cells switched from a unique polygonal morphology to a cell type characterized by two to three outgrowing processes resembling an astrocytic phenotype indicating differentiation of the malignant cells. Treatment with OSM induces remarkable expression of *c-fos* within fifteen to thirty minutes demonstrating a specific effect of the protein on cell proliferation and differentiation. This is supported by the finding that these cells express glycoprotein-130 the known binding molecule of OSM.

Future studies will include the putative effects of OSM in animal models of glioma as well as the analysis of the intracellular signal transduction induced by the cytokine.

369.5

CHARACTERIZATION OF A PUTATIVE NEUREGULIN/ERBB RECEPTOR AUTOCRINE LOOP IN SCHWANN CELL NEOPLASIA. P. W. Frohnert*, S. L. Carroll. Div. of Neuropathology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Schwannomas frequently share common genetic alterations, whether they arise sporadically or as part of the hereditary neoplastic syndromes neurofibromatosis types 1 (NF1) and 2 (NF2). Ablation of the NF1 tumor suppressor gene, however, does not produce neurofibromatosis in transgenic mice and it is believed that other, as yet unidentified, mitogenic factors drive proliferation in both sporadic and inherited schwannomas. The neuregulins (NRGs), a recently described family of growth and differentiation factors, may be among the unknown agents promoting neoplastic Schwann cell proliferation as NRGs are potent Schwann cell mitogens *in vitro* and high levels of a NRG-like activity are present in human schwannomas. We have examined the expression of the NRGs and their receptors, the erbB membrane tyrosine kinases, in the rat JS1 schwannoma cell line. We have found that JS1 cells express extremely high levels of NRG mRNAs, representing a mixture of GGF (glial growth factor) and SMDF (sensory and motor neuron-derived factor) splice variants. Of the four known erbB receptors, JS1 cells express only erbB2 and erbB3. Immunoblots of JS1 cell lysates probed with an antiphosphotyrosine antibody demonstrate a 185kD band, suggesting that the erbB2 and/or erbB3 receptors are constitutively activated. Full-length cDNAs of the Schwann cell erbB2 and erbB3 receptors have been isolated, characterized and used to construct "dominant negative" mutations which are being stably transfected into JS1 cells. Based on our observation that JS1 cells express both NRGs and NRG receptors, we propose that these schwannoma cells stimulate their own proliferation via an autocrine NRG/erbB loop and predict that erbB receptor "dominant negative" mutants will disrupt this putative autocrine loop, thereby diminishing JS1 proliferation.

Supported by the American Cancer Society.

369.6

EPIDERMAL GROWTH FACTOR RECEPTOR GENE TRUNCATION IN HUMAN GLIOMA CELL LINES: AN UPSTREAM MECHANISM THAT NON-MUTATIONALLY INACTIVATE WILD-TYPE p53. Anil Nanda*[1], Yuchun Han[1], Cornelio G. Caday[1], Webster K. Cavenee[2], H.-J. Su Huang[2]. [1]Neurosurgery, Louisiana State Univ. Med. Center, 1501 Kings Highway, Shreveport, LA 71130; [2]Medicine, Univ. of California at San Diego, La Jolla, CA 92093.

Epidermal growth factor receptor (EGFR) gene truncation and p53 gene mutation are intimately involved in the pathogenesis of many human cancers. In the present study, their relationship with respect to human glioma tumorigenesis was investigated using U87MG human glioma cells. We have, for the first time, demonstrated immunocytochemically that U87MG cells overexpressed wild-type p53 which involved both cytoplasm and nucleus. Transfecting U87MG cells with truncated EGFR resulted in significantly decreased levels of wild-type p53. After treatment with tyrphostin AG 1478, a preferential inhibitor of the truncated EGFR, protein levels of wild-type p53 increased in a dose-dependent manner. These results suggested a non-mutational inactivation of wild-type p53 mediated by the truncated EGFR. It was further shown that following treatment with AG 1478, p53-dependent apoptosis was induced, thus representing a novel mechanism different from DNA-damaging agents. Cell cycle analysis revealed that EGFR gene truncation resulted in a significant increase in the S phase as compared with the parental cells. This increase in S phase was shifted into G1 phase in response to AG 1478 treatment. Since EGFR gene truncation results in constitutive autophosphorylation of the 140-155 kD truncated EGFR that lead to enhanced cell proliferation and aggressiveness, combined with current data, it was concluded that EGFR gene truncation functions as an upstream mechanism that, on one hand, enhances glioma cell proliferation; on the other hand, inactivates function of wild-type p53, the suppressor of tumor cell growth, thereby making glioma cells more aggressive.

369.7

DIFFERENTIAL EFFECTS OF TYRPHOSTIN AG 1478 ON HUMAN GLIOMA CELLS EXPRESSING TRUNCATED OR WILD-TYPE EGFR Yuchun Han*[1], Anil Nanda[1], Webster K. Cavenee[2], H.-J. Su Huang[2], Cornelio G. Caday[1]. [1]Neurosurgery, Louisiana State Univ. Med. Center, 1501 Kings Highway, Shreveport, LA 71130; [2]Medicine, Univ. of California at San Diego, La Jolla, CA 92093.

Epidermal growth factor receptor (EGFR) gene truncation and amplification have been frequently demonstrated in and are closely linked to the pathogenesis of human gliomas. Screening anti-glioma inhibitors specific for mutated EGFR gene targets offers a greater therapeutic advantage over those general inhibitors that may also target the normal EGFR. In this study, we tested the effects of a new EGFR tyrosine kinase inhibitor, tyrphostin AG 1478, on three glioma cell lines: human U87MG expressing endogenous levels of wild-type EGFR, U87MG transfected and overexpressing either amplified wild-type (U87MG.wtEGFR) or truncated (U87MG.ΔEGFR) EGFR. Our results revealed that transfecting U87MG cells with truncated EGFR resulted in enhanced DNA synthesis and constitutive autophosphorylation of 140 and 155 kD truncated EGFR. Following treatment with tyrphostin AG 1478, cell growth (Alamar Blue assay), DNA synthesis ([^{3}H]-incorporation assay), EGFR tyrosine kinase activity (ELISA assay) and its autophosphorylation (Western Blot) of all cell lines were inhibited in a dose-dependent manner. In addition, the most significant inhibitory effects of AG 1478 were noted on U87MG.ΔEGFR cells. The increased sensitivity of cells with truncated EGFR to AG 1478 is due to more selective inhibition of the constitutive autophosphorylation of the 140-155 kD truncated EGFR. Our results indicated that tyrphostin AG 1478 represents the first inhibitor that preferentially inhibits truncated EGFR. This finding has significant implication for chemotherapy since the truncated EGFR is frequently expressed in glioblastomas as well as in breast and lung cancers.

369.8

CELL CYCLE REGULATION OF INTERLEUKIN-6 SECRETION FROM HUMAN GLIOBLASTOMA CELLS. Garret L. Yount* and Mark A. Israel. The Preuss Laboratory for Molecular Neuro-Oncology, The Brain Tumor Research Center, University of California San Francisco, CA 94143-0520.

Astrocytes and microglia are known to synthesize interleukin-6 (IL-6) when stimulated by other cytokines such as IL-1 and TNF-α. IL-6 is synthesized and released from nearly all glioblastoma cell lines and high levels of IL-6 have been found in the cerebral spinal fluid of patients with gliomas. *In vitro* studies of the IL-6 gene promoter have demonstrated that p53 and pRB may function as transcriptional repressors to diminish IL-6 mRNA expression. We examine whether IL-6 secretion was regulated during cell cycle progression of U-87 MG glioblastoma cells. Performing ELISA analysis on cells synchronized in the cell cycle by mitotic selection, we found that IL-6 secretion increases 5-fold at the G1/S phase transition. Competitive RT-PCR analysis confirms that IL-6 mRNA levels were elevated at this point in the cell cycle. Subsequent analyses using a panel of U-87 derived cell lines transfected with vector DNA or DNA encoding a dominant negative mutant p53 revealed that wild-type p53 function was required for the regulation of IL-6 secretion during cell cycle progression. Clones in which wild-type p53 function was disrupted show constitutively high levels of IL-6 secretion throughout G1 phase and the G1/S phase transition. This work is supported by The Robert Steel Foundation for Pediatric Oncology Research and the Brain Tumor Research Center Program Project Grant.

369.9

INTERFERON REGULATORY FACTOR 2 ELEVATION IN ASTROCYTOMAS C. B. Soults, C. Wu and P. T. Massa* Depts. of Neurosurgery and Neurology, SUNY HSC, Syracuse, NY, 13210

The system of interferon regulated genes is increasingly being recognized as an important part of normal growth and tumorgenesis. Interferon Regulatory Factor 1 (IRF-1) activates genes that in general function as growth and tumor suppressors while the structurally similar Interferon Regulatory Factor 2 (IRF-2) antagonizes the function of IRF-1. Fresh human astrocytomas removed in the course of normal treatment were examined for the expression of IRF-1 and IRF-2. As controls, human brain tissue removed in the course of various non-tumorous neurosurgical procedures was used. Cytoplasmic and nuclear proteins, as well as RNA, were extracted. Gel mobility shift assay demontstrated a specific increase in IRF-2 binding activity in high grade astrocytomas compared to control brain or lower grade tumors. Western blot and PCR data confirmed an increase of IRF-2 protein and mRNA in high grade astrocytomas. These data demonstrate that in some astrocytomas there is an alteration of the interferon regulatory system with an elevation of IRF-2. The increase in IRF-2 may offset the normal IRF-1/IRF-2 balance and be permissive for tumor progression.

Source of funding: Dept. of Neurosurgery, SUNY HSC, Syracuse, NY, 13210

369.10

GROWTH OF MENINGIOMA CELLS IN CULTURE AND AS TRANSPLANTS. K. Yoo*[1,2], A.F. Krisht[2], Q. Al-Mefty[2] and D.L. Davies[1]. Depts. of Anatomy[1] and Neurosurgery[2], Univ. of Arkansas for Med. Sci., Little Rock, AR 72205.

Meningiomas are common intracranial tumors; due to the invariable abundance of high affinity somatostatin receptors on meningioma cells, these tumors have been successfully imaged using a radiolabled somatostatin analog, octeotide. In an endeavor to assess the potential vulnerability of meningioma cells to octreotide toxicity, cell cultures prepared from surgically excised meningiomas (n=4) were administered octreotide (50 nM - 50 μM) for 7 days. The cultures consisted predominately of cells immuno-positive for vimentin and fibronectin. The astrocyte marker GFAP and endothelial marker Factor VIII were not detected. To date, epithelial membrane antigens and desmoplakins Types I & II have not been found. In octeotide exposed cultures, cell counts and MTT assays revealed a dose- and time-related suppression in culture growth. Similar responses were obtained from cultures of normal meninges. Cultures derived from the same surgical specimens have also been transplanted subcutaneously in a Matrigel® matrix into nude mice. Preliminary findings indicate that the resultant tumors exhibit the cytological features of *in situ* meningiomas. The results suggest that meningioma cells retain key morphological features in cell culture and animal based models which would permit further investigation of adjunctive therapy targeted to the somatostatin receptors on meningioma cells. Supported by: Arkansas Sci. & Technol. Authority Basic Res. Prog. 96-B-01 (A.F.K.) and Am. Cancer Soc. Instit. grant IRG-187 (A.F.K.).

369.11

GANGLIOSIDE BIOSYNTHETIC GENE EXPRESSION IN MURINE BRAIN TUMORS. J.A. Ecsedy*, M.G. Manfredi, M.M. El Abbadi, and T.N. Seyfried. Department of Biology, Boston College, Chestnut Hill, MA 02167-3811.

Alterations in ganglioside biosynthesis occur in brain tumors. β-1,4-N-acetylgalactosaminyl transferase (GalNAc-T) and cytidine monophospho-N-acetylneuraminic acid hydroxylase (NeuAc-H) function in ganglioside biosynthesis. GalNAc-T adds a β-linked N-acetylgalactosamine (GalNAc) to the oligosaccharide chain of gangliosides GM3 and GD3 for the synthesis of GM2 and GD2, respectively. NeuAc-H synthesizes the sialic acid N-glycolylneuraminic acid (NeuGc) by hydroxylation of cytidine monophospho-N-acetylneuraminic acid (CMP-NeuAc). NeuGc-containing gangliosides are not present in neurons or glia, but are abundant in mouse non-neural cells. Using RT-PCR, we show that the GalNAc-T and NeuAc-H genes are expressed in two experimental mouse brain tumors, EPEN and CT-2A, when grown *in vivo* as solid tumors. In contrast, the GalNAc-T gene is not expressed in cultured EPEN cells, but is expressed in cultured CT-2A cells. The NeuAc-H gene is not expressed in either cultured tumor cell line. These findings are consistent with previous work on the distribution of GM2 and NeuGc-containing gangliosides in the solid tumors and cultured cell lines (Seyfried et al., J. Neurochem. *66*: 2026-2033, 1996).

Macrophages and other host immune cells are known to infiltrate brain tumors and many of these cells synthesize GM2 and NeuGc-containing gangliosides. These infiltrating cells are eliminated during formation of the tumor cell lines, consequently, gangliosides and gene expression specific to host cells would not be present in the cultured tumor cells. We suggest that the difference in ganglioside biosynthetic gene expression between brain tumors grown *in vivo* and *in vitro* results from the presence or absence, respectively, of non-neoplastic host infiltrating cells. Supported by the NIH grant 1R01NS/CA33640 and a grant from the Boston College Research Expense Fund.

369.12

TUMOR-MEDIATED SUPPRESSION OF NITRIC OXIDE PRODUCTION BY RAT MICROGLIA IN VITRO. S.A.Walter, and W.J. Streit*. Dept. of Neuroscience, Univ. of Florida Brain Institute, Gainesville, FL 32610.

Our laboratory has previously shown that microglia respond to brain tumors by infiltrating them and assuming an activated morphology associated with tumor cytotoxicity *in vitro*. However, there is little evidence for tumor necrosis associated with microglia *in vivo*, suggesting that tumor cells may suppress the production of cytotoxic molecules by microglia. Nitric oxide (NO) production represents one of the major mechanisms of tumor cytotoxicity by microglia *in vitro*. Using NADPH-diaphorase histochemistry as an indicator of nitric oxide synthase (NOS) activity combined with lectin histochemistry to label microglia, we examined NOS activity in activated microglia associated with the rat RG2 glioma. While a select population of cortical neurons and all tumor cells exhibited NOS activity, the tumor-associated microglia did not. *In vitro* tests were carried out to examine the effect of tumor-derived soluble molecules on NO production by microglia. The Griess reaction was used to measure nitrite, the stable metabolite of NO. Microglial production of NO increased over time and in response to increasing levels of bacterial lipopolysaccharide (LPS) and interferon-gamma (IFN-γ). In preliminary experiments, conditioned medium from cultured RG2 cells prevented the induction of NO production by stimulated microglia in a dose-dependent manner. These results suggest that tumors can suppress the production of cytotoxic NO by microglia through the secretion of soluble factors. Supported by : The American Cancer Society Florida Division, Grant No. F95UF-3.

369.13

CYTOKINE ANALYSIS BY HIGH RESOLUTION FT-ICR MASS SPECTROMETRY. Mark R. Emmett*†, Charles A. Conrad‡, Touradj Solouki† and Alan G. Marshall†. †National High Magnetic Field Laboratory-FSU, Tallahassee, FL 32310 and Trinity Lutheran Hospital , Kansas City, MO 64108.

Cytokines play an important role in cell differentiation and tumor cell proliferation. The actual contribution of an individual growth factor to the growth of a specific neoplastic cell type is unknown. In fact, the most active form of many cytokines is unknown. Recombinant cytokine activity is determined by the cytokine's effect on the growth of specific cells in tissue culture (activity assay) and antibodies are used to quantitate cytokines from biological samples (immunoassay). Neither of these two assays provide mass or structural information of the active cytokine fragment.

In this study, the analytical technique of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR/MS) will be used to analyze several cytokines that affect the proliferation of neuronal cell lines. These cytokines include TGF-alpha, EGF, FGF-basic and VEGF. FT-ICR/MS is unique in its ability to perform high mass resolution analysis of high molecular weight compounds and can also provide substantial sequence information as well. The goal of this work is to demonstrate the versatility of the FT-ICR/MS in the analysis of cytokines produced by specific tumors. It is anticipated that FT-ICR/MS will be a valuable tool in the elucidation of cytokine function in the proliferation of neuronal tumors.

This work was supported by NSF (CHE-94-13008), NIH (GM-31683) and the National High Magnetic Field Laboratory (NHMFL) at Florida State University (FSU).

369.14

NORTHERN ANALYSIS OF PROTO-ONCOGENE EXPRESSION IN HUMAN GLIOMAS. Michael J. Keherly[1] ,William W. Maggio[1], Benjamin B. Gelman[2*], and Dwayne A. Wolf[2]. [1]Division of Neurosurgery/Department of Surgery, [2] Division of Neuropathology/Department of Pathology. University of Texas Medical Branch at Galveston Texas 77555-0517.

Gliomas are the most common primary brain tumor, and present as or progress to highly malignant tumors. The malignant progression of gliomas is associated with an increasing number of alterations in both recessive and dominant proto-oncogene expression. These alterations include the loss of retinoblastoma (RB) expression, the loss of wild-type p53, overexpression of mutated p53, and an increase in c-fos expression. Previous studies have primarily focused on the loss or overexpression of these proto-oncogenes at the protein level. Therefore, we studied 27 gliomas of various type and grade at the RNA level using Northern analysis. Although previous studies found a loss of RB protein in higher grade gliomas, we found RB expression, at the transcript level, comparable to or greater than normal cortex RNA in a majority of the gliomas independent of type or grade. As with other studies, we observed aberrantly high expression of the p53 message predominantly in the higher grade gliomas. Analysis for c-fos expression showed unusually high expression in certain gliomas, especially those with oligodendroglial components. There was also an inverse relationship between c-fos and p53 expression, although not absolute. Further analysis of the expression of other proto-oncogenes is planned. The identification of specific proto-oncogene phenotypes in various glioma types and grades may provide clues to the aberrant cell cycle regulatory processes underlying glioma tumor progression.

NEURO-ONCOLOGY: TREATMENT AND DIAGNOSIS

370.1

BRAIN TUMOR CHEMOSENSITIVITY USING THE FLUORESCENT CYTOPRINT ASSAY. M.H. Friedberg*, M.J. Glantz, P. Meitner and L. Recht. Department of Neurosurgery (MHF) Tufts University School of Medicine, New England Medical Center, Boston, MA. 02111, Dept. of Clinical Neurosciences (MJG), Brown University School of Medicne, Prov. RI, Analytical Biosystems (PM), Warwick, RI. and Dept. of Neurology (LR), Univ. MA, Worcester, MA.

To individually tailor chemotherapy for patients with malignant gliomas according to tumor chemosensitivity, a rapid assay system which can be performed with high success rate is needed. The fluorescent cytoprint assay (FCA) can assess multiple chemotherpeutic agents using small (about 500 cells) tumor aggregates very quickly (about 1 wk). Tissue samples from 51 patients with malignant gliomas obtained either at the time of initial diagnosis (n=34) or at recurrence were assayed using this method. The assay success rate approached 90% in those culture samples which were histologically verified as tumor. A meaningful number of agents could be tested both on samples obtained by stereotactic biopsy (median,5) and on specimens from more extensive resections (median,6). One hundred ninty-three FCAs were performed on samples obtained from 36 patients. In only twenty six assays (14%) was an agent deemed sensitive (>90% cell kill) to a chemotherapeutic agent. 62% of sensitive FCAs were observed in tumors tested against the activated analog of cyclophosphamide, 4-hydroxyperoxycyclophosphamide (4HC), where a sensitivity rate (# samples sensitive/total tested against agent) of 64% (95% CI, 36.6-77.9%) was noted. This rate was significantly higher than with any other agent tested (p=0.012, two sided McNemar's test) and was not affected by age, histology, or disease status. We conclude that: (1) the FCA represents a feasible method for quickly assaying tumors for sensitivity to multiple chemotherapeutic agens; and (2) malignant gliomas may be particularly sensitive to 4 HC.

370.2

RAPID BRAIN UPTAKE OF L-META-SARCOLYSIN FOR THE TREATMENT OF BRAIN TUMORS. M. Hokari,* P.A. Crooks, and Q.R. Smith. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda MD 20892 and Div. of Med. Chem., University of Kentucky, Lexington, KY 40536.

The blood-brain barrier restricts the brain uptake of most chemotherapeutic drugs and limits their efficacy in the treatment of brain tumors. One way to overcome this problem is to design drugs that are shuttled into brain by the saturable nutrient transporters of the brain capillaries. Previously we showed that melphalan, the para-substituted nitrogen mustard derivative of L-phenylalanine, is taken up into brain by the large neutral amino acid transporter (System L1) of the blood-brain barrier. However, melphalan's affinity for the transporter is low (K_m = 100 ± 5 μM) and it fails to reach therapeutic concentrations in brain except at toxic doses. With computer modeling, we predicted that the meta-isomer of melphalan would exhibit improved affinity and transport into brain via the System L1 transporter. To test this, we synthesized L-meta-[^{3}H]sarcolysin and studied its brain uptake in rats using the *in situ* brain perfusion technique (*Am J Physiol* 247:H484, 1984). With saline perfusion (T=10-30 s), brain uptake of L-meta-[^{3}H]sarcolysin was rapid with a cerebrovascular permeability-area (PA) product of 5.80 ± 0.13 x 10^{-2} ml/s/g at tracer concentration. Influx was saturable and mediated by the cerebrovascular large neutral amino acid transporter with a K_m of 1.4 ± 0.2 μM. The results demonstrate that L-meta-sarcolysin is taken up into brain ~100 times more rapidly than melphalan and may be of use for the treatment of brain tumors. (Supported by NIA).

370.3

Potential Radiotherapy of Human Gliomas with [^{18}F]fluorodeoxyglucose. M.A. Meyer, C.G. Caday*, Y. Han, B. Vickers and A. Nanda. Biomedical Research Institute, Louisiana State University Medical Center, Shreveport, LA 71130.

Survival of patients diagnosed with high grade gliomas is very poor. These patients eventually die, even after chemotherapy, radiotherapy or radiosurgery, due to recurrence of the tumor. The preferential *in vivo* uptake of [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) in solid tumors is a basis for monitoring tumor progression and its potential regression following radiation therapy or chemotherapy. We have shown that intra-arterial injection improved the tumor to normal tissue ratio of [^{18}F]FDG uptake in the human brain. This led us to consider whether [^{18}F]FDG can be developed as a potentially novel radiotherapy to deliver therapeutic doses of [^{18}F]FDG and selectively destroy cancer cells. This study showed that high doses of [^{18}F]FDG inhibited DNA synthesis, arrested cell growth and subsequently caused cell death in the glioma cell lines tested in cell culture. Active uptake of [^{18}F]FDG caused cell death while [^{18}F$^-$] was less effective in killing glioma cells. The IC_{50} for inhibition of DNA synthesis was 0.1-0.3 mCi/ml [^{18}F]FDG while >90% of DNA synthesis was inhibited at ≥ 2 mCi/ml [^{18}F]FDG. Studies using human glioma cells xenograft in nude mouse showed that [^{18}F]FDG dramatically reduced the tumor volume without any apparent deleterious effect on the mouse. Taken together, these findings suggest a great potential of using high doses of [^{18}F]FDG to be developed as a novel radiotherapy against solid tumors, including gliomas.

370.4

Inhibition of glioma invasiveness in vitro following treatment with interferon and anti-CD44. Wiranowska, M., Saporta, S. and Phelps, C.* Department of Anatomy, College of Medicine, University of South Florida, Tampa, FL.

The treatment of malignant gliomas of the central nervous system is difficult in part due to their invasive character. Recently CD44, a transmembrane adhesion molecule found on glioma cells was implicated in glioma invasion. Interferon (IFN), which has antiproliferative glioma activity in vitro, was used in clinical trials of glioma patients with only modest responses. In this study we evaluated the effect of mouse MuIFNα/ß and anti-CD44 on the invasiveness of mouse glioma G-26 in vitro. Non-treated G-26 cells or cells treated for 2 or 3 days with MuIFNα/ß at $8x10^2$ or $8x10^3$ IU/ml were plated at 5 $x^4$10 cells in Matrigel Invasion chambers and in control cell culture chambers in the presence or absence of MuIFNα/ß and/or anti-CD44 and incubated for 1 day. Filters from control and invasion chambers were fixed, stained with hematoxylin and eosin and invading cells counted in 10-40 microscopic fields. Invasiveness of G-26 was significantly inhibited (p<0.05) following IFN treatment at $8x10^3$ IU/ml (60%-70%) or anti-CD44 (60%). Treatment with a combination of IFN and anti-CD44 was synergistic, resulting in approximately 80% inhibition of invasiveness of (p<0.05).

This project was supported by USF Research and Creative Scholarship Award.

370.5

HYPOXIA POTENTIATES SURAMIN NEUROTOXICITY IN RAT DORSAL ROOT GANGLIA *IN VITRO*. X. Sun* and A. J. Windebank. Molecular Neuroscience Program, Mayo Clinic, Rochester, MN 55905.

Injury to neurons can be initiated by a variety of insults but the cellular events may be mediated by a final common pathway leading to cell death. Initiating events which converge on this pathway may interact with each other. If correct, these events may be antagonistic, additive, or synergistic. The mechanisms of hypoxia- and antineoplastic agent suramin-induced nerve injury in the peripheral nervous system have not been fully defined and the effect of double insults has been difficult to study. Our hypothesis was that both insults cause cell injury by a final common pathway resulting in apoptosis. Modified isobolographic analysis has been used to study the interaction between hypoxia and suramin neurotoxicity. Using primary dorsal root ganglion neuronal culture as an *in vitro* model system, we have studied the mechanism of interaction of the two insults. Bis-benzamide fluorescence dye was used to label nuclei. Phase-contrast and fluorescence micrographs were taken and cell numbers were counted. All data were converted into percentage of apoptotic cell number versus total cell numbers of the same treated group. The results showed that in the control group the average number of cells undergoing apoptosis was 0.6%. In the suramin-treated (300 µM) group, 1% cells were undergoing apoptosis. Hypoxia (95% N_2 plus 5% CO_2) for 2 h, caused 0.6% cells to show sign of apoptosis. Combination of 300 µM suramin and 2 h hypoxia caused 3.5% cells to undergo apoptosis. The present observation indicates that the combined insults potentiate each other by activating the same intracellular pathways triggering apoptosis (NIH, NS 29769).

370.6

DIFFERENT METABOLIC PATHWAY OF A THYMIDINE ANALOG IN RG2 RAT GLIOMA CELLS VS. RAT BRAIN MEASURED WITH ^{19}F NMR SPECTROSCOPY. K.D. Bahk[1,2], R. Pouremad[1], A.M. Wyrwicz[1,2], L.H. Pinto*[1,2]. [1]Center for MR Research, Evanston Hospital, [2]Neurobiology and Physiology Dept., Northwestern University, Evanston, IL 60201

Trifluridine (trifluorothymidine, TFT) is a fluorinated thymidine analog with antiviral and antitumor properties that is incorporated into DNA and has a greater activity than fluorodeoxyuridine against adenocarcinoma 755 and L1210 Leukemia in mice. We have investigated the delivery of this compound to the brain and its metabolic fate in that tissue as well as in the RG2 rat glioma cells using fluorine-19 (^{19}F) NMR spectroscopy. RG2 is a chemically induced rapidly proliferating cell line with the S-phase approaching 42%. Rats were given an IV injection of 150mg/kg TFT and euthanized 30 min. - 8 hrs after injection. Brain tissue was separated and extracted with a chloroform/methanol (2:1 v/v) solvent. Cells were incubated in DMEM media containing 100µM TFT for 12-72 hours, washed with saline, and extracted the same way. The aqueous/methanol layers of the extracts were dried and used for NMR spectroscopy. The primary metabolite detected in the ^{19}F NMR spectra of brain extracts is trifluoromethyl uracil (TFMU), a product of TFT hydrolysis by thymidine phosphorylase. 5,6-dihydroxy-TFMU(TFMUG), a catabolite generated further along the oxidative pathway, as well as a small amount of residual TFT and its oxidation product (TFT-glycol) are also detected in the ^{19}F spectra of the extracts. The product of TFT hydrolysis by thymidine phosphorylase (i.e. TFMU) is not detected in the spectra of the RG2 cell extracts. TFT is metabolized through both the oxidative (5,6-dihydroxy compounds) and the reductive (5,6-dihydro compounds) pathways in these cells. Phosphorylated products of the anabolic pathway were observed only in the cell extracts. The different metabolic products of TFT observed in rat brain and RG2 cells reflect differences in enzyme levels or activity between the two systems.

370.7

IGF-1 R ANTISENSE AND NONSENSE OLIGONUCLEOTIDE THERAPY FOR 9L GLIOBLASTOMA RESULTS IN AN IMMUNE RESPONSE AGAINST AN UNRELATED SYNGENEIC TUMOR IN FISCHER 344 RATS. M.A. Wallenfriedman*, G. Lee, P.C. Graupman, J.A. Conrad, L. Chiang, D.K.Y. Wen, W.A. Hall, W.C. Low. Depts. of Neurosurgery and Physiology, Graduate Prog. in Neuroscience and the Cancer Center, Univ. of Minnesota, Mpls., MN 55455.

Previous studies have demonstrated that tumor cells transfected or simply incubated with antisense oligonucleotides to the IGF-1 receptor (IGF-1 R) are rejected following transplantation into immunocompetent hosts. Subcutaneous injections of these antisense-modified cells can activate the immune system of rats to recognize both transfected and non-transfected glioblastoma cells, whether implanted peripherally or centrally. In the present study we have examined the immunological specificity of this antisense-induced response. IGF-1 R antisense or nonsense oligonucleotides were injected directly into peripherally established glioblastomas on days 11, 13, 15. Control animals were either uninjected or injected with buffer. Tumor volumes were measured daily by an observer blinded to the treatment group. In contrast to controls, oligonucleotide-injected tumors regressed and were ultimately eliminated. These animals were resistant to further peripheral or central glioblastoma challenges. We then tested the specificity of this oligonucleotide-induced anti-tumor response with a peripheral injection of Mat B III breast carcinoma. Naive controls (n=5) reached average tumor volumes of 48,000 mm^3 on day 23 before death. This is consistent with identically challenged rats (n=60) from previous experiments. In contrast, rats that had been previously cured of glioblastoma by intratumor injections of oligonucleotides had an enhanced immune response to Mat B III challenge. Their average tumor volume on day 23 was 22,000 mm^3. 50% (n=10) were alive after the death of all controls and 20% were living on day 50. This is unlikely to result from recognition of shared tumor antigens between glioblastoma and breast carcinoma. Rodents surviving glioblastoma after treatments other than intratumor injection of oligonucleotides do not demonstrate immunological protection against breast carcinoma (n=20). These data suggest that direct injections of either IGF-1 R antisense or nonsense oligonucleotides may promote a long-lasting immune response that can be generalized beyond the initial tissue type of tumor treated. Supported by grants from the Minnesota Medical Foundation and The American Cancer Society.

370.8

WAF1/CIP1 INCREASES THE SUSCEPTIBILITY OF p53 NON-FUNCTIONAL MALIGNANT GLIOMA CELLS TO CISPLATIN-INDUCED APOPTOSIS. S. Kondo*, B.P. Barna, Y. Kondo, J.W. Peterson and G.H. Barnett. The Cleveland Clinic Foundation, Cleveland, OH 44195.

Induction of apoptosis in tumor cells is an important determinant in the outcome of therapy. Molecular details of the apoptosis pathway, however, are still poorly defined. The recently discovered WAF1/CIP1 gene is a potent inhibitor of cyclin-dependent kinases and a mediator of tumor-suppressor p53-dependent apoptosis by DNA damage. In addition, WAF1/CIP1 expression is shown to be triggered through p53-independent pathway. The relationship between WAF1/CIP1 and p53-independent apoptosis by DNA damage, however, remains unclear. In this study, we show that WAF1/CIP1 was induced in p53-dependent apoptosis of U87-MG glioma cells by cis-diamminedichloroplatinum (cisplatin), and overexpression of WAF1/CIP1 induced apoptosis in U87-MG cells without cisplatin treatment. In contrast, GL26 glioma cells did not express WAF1/CIP1 in p53-independent apoptosis by cisplatin. Overexpression of WAF1/CIP1 inhibited the DNA synthesis of GL26 cells, but did not induce apoptosis. Interestingly, WAF1/CIP1 increased the susceptibility of GL26 cells to cisplatin-induced apoptosis. These results suggest that overexpression of WAF1/CIP1 may have potential for the treatment of tumors with non-functional p53.

370.9

A 'SAFE' HERPES SIMPLEX VIRUS (HSV) VECTOR FOR TUMOR THERAPY OR GENE DELIVERY IN THE BRAIN.

S. D. Rabkin[1,2]*, W. D. Hunter[2], K. C. New[1], M. Toda[2], R. L. Martuza[2]
Departments of Microbiology & Immunology[1] and Neurosurgery[2], Georgetown Univ. Med. Ctr., Washington, DC 20007.

We have created a new, multi-gene mutant of HSV, designated G207. It is deleted at both γ34.5 loci and is ICP6 (large subunit of ribonucleotide reductase) deficient because of an insertion of the *E. coli* lacZ gene, yet is still replication-competent. Both of these HSV genes contribute to neurovirulence. G207 is able to replicate in tissue culture cells, including tumor cells and to function as a helper virus for the generation of defective HSV vectors. G207 inoculation of intracerebral human malignant gliomas or subdural meningiomas in nude mice causes prolonged survival and some apparent cures.

Defective HSV vectors with G207 as a helper virus were considerably less toxic to primary neurons than were other helper viruses. G207 was 'safe' when injected (10^7 pfu) intracerebrally, intravenously or intraventricularly into young BALB/c mice. Intracerebral injection (10^7 pfu) of HSV-sensitive non-human primates resulted in no long-term CNS pathology, whereas 10^3 pfu of the wild-type parental HSV was lethal within a week. MRI evaluation of G207 inoculated primates revealed no abnormalities. The lack of pathology observed with G207 suggests that it should be considered for clinical evaluation.

Supported by grants from NINDS and NCI

370.10

PRE-EXISTING IMMUNITY AGAINST HERPES SIMPLEX VIRUS (HSV) MUTANT hrR3 DECREASES, BUT DOES NOT ABOLISH INFECTION OF D74 GLIOMA CELLS IN RAT BRAIN TUMORS. U Herrlinger, KS Aboody-Guterman, CM Kramm, K Johnston, P Pechan*, M Chase, J Burwick, EA Chiocca, DN Louis, XO Breakefield. Mol. Neurogenetics Unit, Mass. Gen. Hospital, Boston, MA 02129

We determined the impact of pre-existing anti-HSV immunity on the efficacy of HSV-based gene therapy. CD Fisher rats were vaccinated intraperitoneally (IP) with 10^8 pfu of a HSV-1 mutant (hrR3), or sham-vaccinated with control media. Three to 4 weeks later $4x10^4$ syngeneic D74 rat glioma cells were injected intracranially into all rats. Four days later, rats were treated intratumorally (IT) with either 10^8 pfu hrR3, or control media. Eleven vaccinated and treated rats (+/+), 11 non-vaccinated, but treated rats (-/+), and 11 non-vaccinated, non-treated rats (-/-) were assessed for survival. Histology was obtained from 2 rats per group on days 2 and 5 after virus. Median survival time was 20 days both in (+/+) (p=0.017, rank sum test) and (-/+) animals (p=0.008) as opposed to 18 days in (-/-) animals. Immunohistochemistry with anti-HSV-thymidine kinase (tk) Ab on day 2 after virus revealed numerous tk+ tumor and non-tumor cells in (-/+) rats, but very few tk+ cells in (+/+) rats. Neutralizing anti-HSV antibodies (Ab) in blood samples pooled from two rats per group were assessed in a complement dependent plaque inhibition assay. In (+/+) rats a titer of 1:1024 persisted from day 9 to day 25 after vaccination. IT virus challenge raised the titer to 1:4096 in (+/+) rats, while (-/+) rats showed a rise from >1:8 to 1:1024 on day 10 after IT virus inoculation. Our results demonstrate a robust immune response to hrR3 after IP and IT application. As the life-prolonging effect of IT hrR3 application was not affected by vaccination, it appears that pre-existing immunity to hrR3 does not completely curtail on site infection and virus replication. An immune reaction which markedly decreases the possible infection of neurons could be a benefit for gene therapy of brain tumors using HSV vectors.

Supported by Deutsche Forschungsgemeinschaft (UH), Deutsche Krebshilfe (CMK), and NINDS grant NS24279 (KSA, PP, EAC, DNL, XOB).

370.11

GENE TRANSFER TO HUMAN BRAIN TUMOR CELLS USING A NOVEL ADENO-ASSOCIATED/ HERPES VIRUS HYBRID VECTOR, Karen M. Johnston*, D. Jacoby, D. Schuback, P. Borghesani, P. Pechan, R. Dunn , F. Smith, Xandra O. Breakefield. Neurology Service Massachusetts General Hospital, Harvard Medical School, Boston, MA02114 and McGill Univ, Montreal (RD).

AAV is a nonpathogenic DNA parvovirus demonstrated to stably integrate into human chromosome 19. Advantages of AAV for gene transfer include its ability to infect both nondividing and dividing cells. Using either adenovirus or herpes virus as helper, AAV is also able to undergo replication in a productive phase. AAV vectors have been used to deliver foreign genes to lung and brain. Gene transfer has been limited by both titres and the small size capacity of the vector. Herpes virus, on the other hand, can be grown to high titres and has a larger gene capacity. However, toxicity and lack of stability of expression have presented ongoing problems.We therefore constructed an AAV/herpes amplicon hybrid vector with the purpose of combining the advantages of both systems to increase efficiency and stability of gene transfer. Constructs used in this study include HSV amplicon sequences in combination with a lac Z reporter gene flanked by the AAV ITR sequences. Constructs were generated with and without the rep gene to assess its importance in the expression/ integration system. Control construct contains the herpes amplicon sequences and reporter gene but not AAV sequences. Optimal packaging and transfection conditions were determined prior to infection of U87, a human glioma cell line. A time course for comparison of stability of expression between constructs was determined. The amplicon without AAV sequences was associated with reporter gene expression for less than 1 week, expression for at least 3 weeks could be achieved with the AAV/amplicon infections. Interestingly, expression was higher for the rep+ as compared to the rep - construct. Ongoing studies will determine the integration status of these constructs as well as packaging efficiency . These experiments show that a hybrid vector such as AAV/herpes amplicon has potential use for gene transfer. These constructs will be tested for delivery of therapeutic genes to brain tumors in experimental animals.

supported by Montreal General Hospital Research Institute and NINDS NS24279.

370.12

ARA-C INDUCED APOPTOSIS IN C_6 GLIOMA CELLS TRANSFECTED WITH WILD-TYPE *p53*

J.X. Tong*, K.M. Rich, M.Giordano, and D.H. Gutmann, Department of Neurological Surgery and Neurology, Washington University School of Medicine, St. Louis, MO 63110

Human gliomas display a variable response to commonly used DNA damaging agents. Mutations in the *p53* gene are one mechanism by which these tumors develop resistance to treatments that normally induce cell death via apoptosis. The rat glioma cell line, C_6, contains a mutant *p53* and is resistant to many DNA damaging agents. We created a C_6 glioma cell line transfected with a zinc-inducible wild-type *p53* gene to test the hypothesis that an apoptotic response to DNA damaging agents could be restored to these cells. We characterized the growth properties of the clone, C_6-20-1, and examined its ability to undergo apoptosis after exposure to the DNA damaging agent, cytosine arabinoside (Ara-C).

Wild-type p53 was induced with zinc added to the culture media(100 μM). Growth curves were performed using C_6 and C_6-20-1 cells in the presence and absence of zinc. C_6-20-1 cells grown in the presence of zinc showed a 41% decrease in growth rate when compared to C_6-20-1 grown in zinc-free media. In contrast, C_6 cells grown with and without zinc had similar growth rates. Cells were exposed to 5 μM cytosine arabinoside and examined for the presence of apoptosis. The DNA-binding fluorescent dye Hoechst 33258 was used to assess the amount of DNA condensation present 72 hours following treatment with Ara-C. We found that C_6-20-1 cells maintained in media containing zinc underwent apoptosis (10% DNA condensation) following exposure to Ara-C. A much lower magnitude of apoptosis was observed in C_6-20-1 cells maintained in zinc-free media and in C_6 cells following exposure to Ara-C (1.8% and 1.9%, respectively, $p < 0.01$). Untreated C_6-20-1 and C_6 cells showed only 0.5% and 0.4% DNA condensation, respectively.

In summary, we have constructed a C_6 clone with plasmid that carries a zinc-inducible wild-type *p53* gene. Activation of the transfected gene results in a decreased growth rate and an enhanced apoptotic response to cytosine arabinoside.

(Supported by NIH Grant NS 29477)

370.13

VISUALIZATION OF BRAIN TUMOR GROWTH AND NEURAL CELL MIGRATION IN VIVO USING RETROVIRAL VECTORS EXPRESSING GFP

[1]KS Aboody-Guterman, [1]M Sena-Esteves, [1]U Herrlinger, [2]EY Snyder, [1]XO Breakefield*
[1]Molecular Neurogenetics Unit, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, [2]Depts. of Neurology and Pediatrics, Children's Hospital, Harvard Medical School, Boston, MA 02115

A retroviral vector was constructed expressing green fluorescent protein (GFP) under the control of the mammalian phosphoglycerate kinase promoter (PGK), including an internal ribosome entry site (IRES) and a neomycin selection gene (NeoR), [PGK-GFP-IRES-NeoR], in order to track cell movements, on site gene product delivery, and stability of transgene expression *in vivo*. Plasmid DNA was transfected into the BOSC packaging cell line with approx. 50% efficiency as visualized by a strong fluorescent signal in living cells. Rat gliomas (9L, D74, CNS1), mouse glioma (GL261) and a multipotent mouse neural cell line (C17-2) were subsequently infected with retroviral supernatant. Human gliomas (U87, HGL21) were transfected directly. After G418 (neomycin) selection, the brightest clones were isolated by visualization of fluorescence and expanded. Duration and strength of GFP transgene expression in cell culture is being followed. Selected cells will be grafted into a tumor model in living mice. This will enable evaluation of the sensitivity and stability of GFP expression and allow visualization of tumor growth *in vivo*. The C17-2 cell line already contains the lac Z gene, and from previous experiments involving C17-2 intratumoral injection, we have determined that these cells migrate extensively throughout the tumor bed, without themselves becoming tumorigenic. By assessing their *in situ* GFP expression, we can evaluate the potential use for these cells as an on site gene product delivery system. The GFP labelled C17-2 cells will be grafted into a non-lablelled tumor bed and migration followed *in vivo*. Data on the full extent of migration will be obtained by post-sacrifice staining of the brain tissue for lac Z. These studies may enable us to optimize gene delivery to tumor cells *in vivo*.

Supported by NINDS NS24279 (KSAG, XOB), Deutsche Forschungsgemeinschaft (UH), Junta Nacional de Investigageao Cietifica e Tecnologica Programa Praxix XXI/BD/3248/94 -Portugal (MSE), APA, PVA, NS34247 (EYS)

370.14

ENCAPSULATED ADENOVIRUS: A NOVEL METHOD FOR THE INTRACEREBRAL DELIVERY OF ADENOVIRUS IN GLIOMA TREATMENT

S.J. Beer, C.S. Stein, J.M. Hilfinger, Y. Tsume, V.C. Traynelis*, B.D. Davidson.
The University of Iowa, Iowa City, IA and TSRL, Inc., Ann Arbor, MI

Current protocols for the treatment of intracerebral glioma using gene therapy rely on repeated intracerebral administrations of viral vectors. To circumvent the need for repeated injections, we have developed a method to encapsulate adenovirus (Ad) in a polymer which results in extended viral release.

Poly (lactic-glycolic) acid (PLGA) microspheres containing Ad were prepared using a double emulsion technique. [^{35}S]-metabolically radiolabeled Ad was encapsulated and quantitative release of virus from microspheres was measured. Sixty percent of the encapsulated virus was released over 3 days and another 30 % was detected over the next 12 days. Viability of released virus was measured following encapsulation of AdRSVntLacZ, exposure to cells and X-gal staining. Again, viral release peaked within three days and was detected over 15 days. To test the ability of this formulation to deliver therapeutically relevant concentrations of Ad, virus containing the herpes simplex thymidine kinase gene (AdRSVTk) was encapsulated and cytotoxic effects on 9L gliosarcoma cells were measured. Encapsulated AdRSVTk was exposed to 9L cells in culture for 10 days. Ganciclovir (1.0 μM) was added and cell survival assessed 5 days later. Five percent of the encapsulated preparation resulted in 16 % cell kill. This is equivalent to the cytotoxicity seen when 10^6 unencapsulated particles of AdRSVTk are exposed to 9L cells. This dose of virus is achievable *in vivo*.

To summarize, we have demonstrated extended release of viable virus from PLGA microspheres. Such formulations may obviate the need for repeated intracerebral administrations in glioma gene therapy.

Supported by the National Institutes of Health and the Carver Research Foundation.

370.15

VASCULAR ENDOTHELIAL GROWTH FACTOR IN CEREBROSPINAL FLUID OF PATIENTS WITH BRAIN TUMORS. S. C. Cortez*, V. Kuo-LeBlanc, P. Calabresi, M. H. Epstein , P. C. Song, L. G. Yee, W.M. Taylor, E. G. Stopa. Brown Univ. School of Medicine / RIH, Providence, RI.

Vascular endothelial growth factor (VEGF) has been shown to be a potent and highly specific endothelial cell activator that induces angiogenesis both in vivo and in vitro. VEGF mRNA is unregulated in primary brain neoplasms, particularly in malignant gliomas. Expression of VEGF is also seen in metastatic brain tumors. Since VEGF is normally present in conditioned tissue culture media, the present study was designed to assess whether VEGF could also be found in the cerebrospinal fluid (CSF) of patients with cerebral neoplasms. A solid phase ELISA using the Quantikine VEGF Immunoassay (R&D systems) was performed to determine the levels of VEGF. 30 cerebrospinal fluid samples from patients with high grade astrocytoma (anaplastic astrocytoma and glioblastoma multiforme), 37 samples from patients with metastatic and non-astrocytic brain tumors including lymphomas and 14 non-tumor control samples were studied. 83.3% of samples with malignant astrocytoma and 27.0% of non-astrocytoma samples had detectable levels of VEGF. VEGF was not detectable in normal CSF samples. The levels of VEGF were generally higher in high grade astrocytomas than in non-astrocytic tumors These results suggest that VEGF is detectable in cerebrospinal fluid and may be a potential marker for differentiating astrocytic from non-astrocytic tumors. Furthermore, this may be a potential target for tumor treatments.

370.16

PROTON MAGNETIC RESONANCE SPECTROSCOPIC IMAGING OF BRAIN OF CHILDREN WITH CNS TUMORS J..A.Lazareff, Ch. E. Olmstead, K. O'Hanlon, J. Abrajano, K.H.J.Bockhorst, J.R.Alger, R.S.Fisher, W.J.Peacock. Neurological Surgery, Radiological Sciences,Neurobiology. UCLA Sch. of Med., Los Angeles, CA 90095

We are interested in the effects of localized lesions on the global metabolism of the developing brain. In this study we have used H-MRSI as previously described (Child's Nerv Syst.1996,12:130-135) to evaluate the spatial distribution of choline compounds (Cho), creatine (Cre) and N-acetyl aspartate (NAA) signal amplitudes in every voxel (7.5 x 7.5 x 15mm) of normal brain from 12 patients (18 months to 10 years of age) with pediatric supratentorial low-grade astrocytomas. The tumors were either hypothalamic (N=6) or hemispheric (N=6). We observed nonrandom distributions of all three measured compounds in each of the patients. (All ANOVA's $p<.001$). Cho and Cre were highest in those structures either of high density or with high energy requirements such as the hippocampus, superior colliculi, cerebellar vermis and thalamus, in that order. NAA was highest in the cerebellar hemispheres and cerebral cortex. The lowest values of all three compounds were seen in white matter, primarily internal capsule and cerebral peduncles.

370.17

PERMEABILITY AND BLOOD VOLUME MEASUREMENTS IN INTRACRANIAL 9L RAT GLIOMA USING DYNAMIC CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING (MRI) S. Ostrowitzki[1], T.P.L. Roberts[1], J. Fick[2], D. M. Shames[1], K. Aldape[3], Z. Vexler[1]*, M. A. Israel[2], R. C. Brasch[1]. [1]Dept. of Radiology, [2]Dept. of Neurosurgery, [3]Dept of Pathology, Univ. of California San Francisco, SF, CA 94143.

Hypervascularity and hyperpermeability are characteristic features of malignant brain tumors. We have established a method to non-invasively quantify blood volume and microvascular permeability in an animal model of intracranial malignant glioma by dynamic contrast-enhanced MRI. Eleven Fischer 344 rats were inoculated intracranially with 9L glioma cells. Dynamic MRI using Gd-DTPA was performed between day 13 and 16 post implantation. Blood volume and apparent permeability were derived from the enhancement data using a bidirectional two-compartment [plasma-interstitium] kinetic model. Histologic sections were stained with standard hematoxylin-eosin and for factor VIII to evaluate the tumor vascularity. Tumors were well visualised on post-contrast images. Gd-DTPA leaked significantly (1105±151 µl/hr/cc of tissue, mean±S.E.M.) across the blood tumor barrier from the plasma space to the interstitium ($p<0.0001$ when compared to contralateral brain which was not involved with tumor). Blood volume as determined by MRI was significantly higher in tumors than in contralateral brain ($p<0.0003$). Immunohistochemistry revealed greater vascularity in the tumors compared to contralateral brain. We conclude that Gd-DTPA-enhanced dynamic MRI can be used to study microvasculature non-invasively in this glioma model because it provides good tumor visualisation on post-contrast images, quantitative estimations of tumor blood volume and a high dynamic range in apparent permeability between tumor and brain vessels.

Non-invasive characterisation of glioma microvasculature by MRI might be useful for tumor grading, chemotherapy planning and for investigation of newly developed therapies designed to inhibit angiogenesis.

(Supported in part by a grant from the German Academic Exchange Service)

372

SYMPOSIUM: CHEMICAL CODING AT CENTRAL AND PERIPHERAL SYNAPSES--THE SYNAPTIC VESICLE NEUROTRANSMITTER TRANSPORTERS. L. E. Eiden, NIH-NIMH (Chairperson); J. B. Rand, Okla. Med. Res. Found.; J. D. Erickson, NIH-NIMH; R. H. Edwards, Univ. California San Francisco; R. Jahn, Yale Univ.

The symposium focuses on what has been learned about the basis and evolution of chemically diversified neurotransmission from the molecular characterization of the vesicular monoamine and acetylcholine transporters, and from the neurogenetics and bioenergetics of several vesicular neurotransmitter transport systems.

Neurotransmitter accumulation in vesicles as a fundamental mechanism of chemical coding at the synapse will be emphasized by summarizing recent and ongoing structural, developmental, mechanistic and neurogenetic studies of vesicular transporters from worm to human.

Lee Eiden will briefly discuss how knowledge about vesicular neurotransmitter transporter structure has uncovered new aspects of chemical coding and the morphological basis of transmitter-specific neurosecretion. James Rand will discuss the neurogenetics of vesicular transporters in C. elegans, focusing on the nematode vesicular ACh transporter, required for synaptic efficacy in cholinergic motor neurons and part of an operon containing the acetylcholine biosynthetic enzyme choline acetyltransferase. Jeffrey Erickson will discuss structural specialization of the twelve transmembrane-domain ACh and monoamine transporters underlying recognition of amine neurotransmitter substrates and neuropharmacological agents. Robert Edwards will discuss how vesicular neurotransmitter transporters are partitioned among vesicle subtypes specialized for synaptic function and intracellular localization in central and peripheral neurons and neuroendocrine cells. Reinhard Jahn will compare mechanisms of neurotransmitter accumulation in storage vesicles from amino acid- versus amine-containing neurons and discuss the dynamics of exocytosis at chemically defined synapses.

373

SYMPOSIUM. MOLECULAR BIOLOGY OF PERCEPTION. G.M. Shepherd, Yale (Chairperson); D.A. Baylor, Stanford; G.M. Shepherd, Yale; S.C. Kinnamon, Colorado State; P. Dallos, Northwestern.

Analysis of sensory transduction at the molecular level is proceeding rapidly, but there is as yet little appreciation of the implications of these advances for concepts of sensory perception. The development of our understanding of the neural basis of perception will be briefly summarized. D. Baylor will describe mechanisms that mediate our perception of very dim light. Classic psychophysical experiments first suggested that single photons are registered. Recent physiological experiments reveal how the G-protein cascade in retinal rods minimizes noise and allows for accurate photon counting. G. Shepherd will discuss the interaction of odor molecules with olfactory receptors. Functional studies indicate that the odor molecules are encoded as odor images, in which individual molecular determinants are the basis for discrimination of smells. S. Kinnamon will discuss the diversity of mechanisms involved in taste transduction. Recent studies show that these mechanisms are expressed in different subsets of taste cells, which may provide a basis for discrimination of different taste qualities. P. Dallos will discuss molecular mechanisms of hair cell forward transduction and outer hair cell motor function and its modification by efferent transmitters. These processes will be placed in the context of auditory performance and its limitations.

376.1

SPATIO-TEMPORAL FREQUENCY COLUMNS IN CAT VISUAL CORTEX: A CONTINUOUS REPRESENTATION OR TWO STREAMS? D. Shoham*[1], M. Hübener[2], T. Bonhoeffer[2] and A. Grinvald[1]. [1]The Weizmann Institute of Science, Rehovot 76100, Israel, and [2]Max-Planck-Institut für Psychiatrie, Am Klopferspitz 18a, 82152 Martinsried, Germany.

We have recently used optical imaging to study the organization of spatial frequency columns across the cortical surface and their relationship to other columnar systems in cat visual cortex (Hübener et al, NS Abstr. 1995). Here we answer two unresolved questions regarding the nature of stimulus representation by this columnar system.

The first question is whether the spatial frequency maps, which were obtained by comparing the cortical response to two different spatial frequencies, are part of a continuous representation of the spatial frequency domain, or whether they represent mixtures of only two neuronal populations, preferring low and high frequencies. To answer this question we used a wider stimulus set, containing gratings of several spatial frequencies spanning a range of more than 3 octaves. The resulting optical maps suggested a parcellation of the cortex into two sets of columns, preferring either low or high spatial frequency. Each of the stimuli we used activated preferentially one of the two sets. No stimulus was found which activated preferentially a different set of columns.

The second question is whether these two populations differ also in their velocity preference, in addition to their spatial frequency preference. To test this we used stimuli of the same spatial frequency but moving at two different speeds. The resulting maps were weaker in amplitude but very similar in shape to those obtained with the high and low spatial frequencies. The low spatial frequency patches were preferentially activated by the faster moving stimuli, and the high frequency areas were activated more by the slow stimuli. This result indicates that the two populations are also differentially tuned to velocity (or temporal frequency).

Since both of the above results show that there are separate regions preferentially responding to high spatial frequency/low speed and to low spatial frequency/high speed stimuli, we hypothesize that these spatio-temporal frequency columns are the cortical manifestation of the retino-thalamic parallel streams. Recent reports support this hypothesis (Boyd & Matsubara, JCN 365: p.659, 1996; Hübener et al, this volume).

Supported by the ISF / Israel Academy and by the Human Frontier Science program.

376.2

CYTOCHROME-OXIDASE BLOBS IN CAT VISUAL CORTEX COINCIDE WITH LOW SPATIAL FREQUENCY COLUMNS. M. Hübener*, S. Schulze, and T. Bonhoeffer. Max-Planck-Institut für Psychiatrie, Am Klopferspitz 18a, 82152 München-Martinsried, FRG.

In the visual cortex of primates neurons located in the cytochrome-oxidase blobs are endowed with a set of specific receptive field properties: they are color selective, unoriented, and respond preferentially to low spatial frequencies. Recently cytochrome-oxidase blobs were also detected in the visual cortex of cats and ferrets. It is not known whether the blobs in these non-primate species bear a specific relationship to any of the columnar systems. To address this question we visualized the functional architecture of the visual cortex with optical imaging of intrinsic signals and compared the cortical maps with the blob-pattern.

Cats were stimulated with four different orientations at two spatial frequencies presented separately to the left and right eye to obtain the orientation, spatial frequency, and ocular dominance maps. The visual cortex was cut horizontally and reacted for cytochrome-oxidase. Small amounts of fluorescent dyes injected at the end of the optical imaging experiment served as landmarks to align the functional maps with the cytochrome-oxidase stained sections.

We found a clear correlation between the spatial frequency map and the blob-pattern: low spatial frequency domains were in register with the blobs, while regions of the cortex preferring high spatial frequencies coincided with the lighter staining interblob-region.

We also performed injections of retrograde tracers into the cortical spatial frequency columns. Preliminary results of these experiments indicate that LGN-cells projecting into the low spatial frequency columns tend to have larger somata than LGN-cells terminating in the high spatial frequency columns. In line with Boyd and Matsubara (J.Comp.Neurol., 365: 659, 1996) this indicates that the low spatial frequency columns respectively blobs receive a stronger input from geniculate Y-cells.

Taken together these observations suggest the presence of two parallel pathways in cat visual cortex, with the blobs devoted to low spatial frequency and perhaps motion processing and the interblob region dealing with high spatial frequency information.

Supported by the Max-Planck-Gesellschaft.

376.3

SPATIO-TEMPORAL LUMINANCE AND CHROMATIC RECEPTIVE FIELD PROFILES OF MACAQUE STRIATE CORTEX SIMPLE CELLS. N. P. Cottaris*[,1], S. D. Elfar[2] and R. L. De Valois[1,2] Group in Vision Science[1] and Dept. of Psychology[2], Univ. of Calif., Berkeley, CA 94720.

The spatiotemporal (*x-t*) receptive field (RF) maps of monkey striate cortex simple cells have been mapped with luminance stimuli (Jacobson, Gaska, Chen, and Pollen, 1993). However, to characterize fully the RF of a simple cell, a cone input *x-t* RF map is necessary to predict responses to both color and luminance stimuli.

Toward this end, we recorded extracellularly the responses of simple cells from striate cortex of anesthetized, paralyzed macaque monkeys. RF maps were obtained using two mapping techniques. A reverse correlation technique (Jones and Palmer, 1987), with small bars of the optimal orientation that activated one or all three cone types in phase or in antiphase, allowed us to measure simultaneously the luminance, cone, and color RFs of a cell. Individual cone input RF maps were also measured using the m-sequence method (Sutter, 1987). A 1- or 2-D Gabor function was fitted to the measured *x* or *x-y* profiles at different values of *t* and the following parameters were extracted: RF center, width and length, optimal spatial frequency, orientation, number of subregions and spatial phase. The variation of these parameters as a function of time was studied and compared for the luminance and the chromatic RF profiles. In addition, Fourier transforms of the *x-t* RF maps were calculated, and compared to the spatiotemporal tuning functions measured with sinewave gratings.

Preliminary data indicate that most cells exhibit similar spatial and temporal structure in their luminance and chromatic RF maps. However, a small number of cells have a more complex cone input RF map.

Supported by NIH EY00014

376.4

COLOR & LUMINANCE PROCESSING IN MACAQUE V1: SPATIAL STRUCTURE REVEALED BY INTRINSIC OPTICAL IMAGING

D. Orbach[1]*, R. Everson[2], and E. Kaplan[3], [1]Biophysics Lab, Rockefeller Univ., NY, NY 10021, [3]Ophthalmol. & [2]Biomath, Mt. Sinai Med. Ctr., NY, NY 10029

We examined the spatial organization of color and luminance processing in macaque primary visual cortex by measuring visually-evoked intrinsic reflectance responses. Our extensions of principal components analysis allow more sensitive, robust, and quantifiable detection of spatial patterns than is otherwise possible.

The questions addressed were: (i) Is there a spatial structure in V1 that differentiates among isoluminant color stimuli of various hues, and luminance-varying stimuli? (ii) Is there a spatial structure that discriminates between different stimulus contrasts, where contrast is defined both in luminance and in color? (iii) Are there privileged axes in color space (e.g. RG vs. BY)? (iv) How does spatial frequency tuning in V1 compare between luminance-varying stimuli and isoluminant hue-varying stimuli? (v) How do color- and luminance-sensitive structures relate to the already-familiar columnar organization, such as ocular dominance and orientation? (vi) How does the spatial organization of color processing relate to the CO blobs and interblobs? In each case, the issue was addressed both by attempting to generate spatial maps of the cortical response to a given stimulus set, and by quantifying the strength of the cortical response across a range of stimulus intensities.

The results show that there is a spatial structure that is activated in response to different isoluminant stimuli, as well as a structure responsive to strong luminance contrast. Additionally, the responses to various isoluminant *axes* in color space (e.g. the red-green axis vs. the red-blue vs. the blue-yellow) can be differentiated from one another. However, there do not seem to be distinct structures involved in the detection of varying contrasts along a given isoluminant or luminance axis.

Support: EY4888, EY01867, MH50166, RPB, ONR N0014-93-12079

376.5

THE CODING OF EXTENDED COLORED FIGURES IN MONKEY VISUAL CORTEX. Rüdiger von der Heydt*, Hong Zhou, and Howard Friedman. Krieger Mind/Brain Institute and Depts. of Neuroscience and Biomed. Eng., Johns Hopkins University, Baltimore, MD 21205

We have studied the neural coding of extended colored figures. Single cells were recorded from parafoveal regions of V1 and V2 in alert, fixating monkeys. In V1 we have recorded mainly from cells in the upper layers. After determining the orientation and color selectivity of a cell, a static, square figure of uniform color on a gray background was placed at various positions relative to the cell's receptive field. The figure was much larger than the response field (typically 4°).

The vast majority of cells (75/89 in V1; 43/47 in V2) were activated only when an edge was located in the response field ("edge-only"), and few cells were activated when the receptive field was inside the figure ("surface"). Most of the latter also showed some edge enhancement. The relative frequency of color selective cells, judged by the differential responses to colors of similar luminance, was found to be as high in the "edge-only" group as in the "surface" group (26/69 vs 6/14 in V1; 12/37 vs 2/3 in V2). Also the distribution of a color selectivity index that estimates the volume in 3-d color space over which a cell responds was similar for both groups. Clearly, there are many more color selective "edge-only" cells than color selective "surface" cells. Most "edge-only" cells were selective for orientation, and also many for edge polarity.

Thus, V1 and V2 seem to encode the color of extended figures by edge signals rather than isomorphic representation. Color filling-in might result from the decay of color specific edge signals rather than a spreading of color specific surround activity into the figure.

Supported by HFSP RG-31, NIH EY02966, and Whitaker Foundation.

376.6

CORTICAL REPRESENTATION OF SURFACE BRIGHTNESS: INFLUENCES FROM BEYOND THE CLASSICAL RECEPTIVE FIELD. M. A. Paradiso*, W. Kim, and S. Nayak. Department of Neuroscience, Brown University, Providence, RI 02912.

The brightness and color that a surface is perceived to have are based on light reflected from surfaces throughout the visual field. We explored the possible relationship between activity in striate cortex and perceived brightness by examining the temporal response patterns of striate neurons to stimuli within the classical receptive field (CRF) with and without the presence of surrounding stimuli. A neuron's CRF was stimulated with a single uniformly luminous patch of light of different sizes, while annular stimuli of various dimensions were presented at distances up to 15 deg from the receptive field. These experiments revealed extensive interactions from outside the CRF. In most neurons, stimuli beyond the CRF led to changes in both the magnitude and the timecourse of responses to stimuli within the CRF. The largest effects were seen in later phases of the response (e.g. 100 msec after the initial transient) suggesting that network interactions were involved. Over 50% of the neurons were significantly affected by stimuli outside the CRF, with the interactions falling into several categories. Most commonly, light outside the CRF had either a purely facilitatory or inhibitory effect. Often these effects progressively decreased as the luminance contrast edges of the surrounding stimulus moved further from the CRF. However, there were also neurons in which the surround effects increased in magnitude with distance. The spectrum of interaction types indicates that single neurons in striate cortex do not always respond in a manner consistent with perception. The larger unanswered question is whether integration of these responses can account for the diverse perceptual interactions seen in assimilation, induction, and brightness constancy.

Supported by NIH grant EY09050 and a grant from the Whitehall Foundation

376.7

SPATIAL RELATIONSHIPS BETWEEN CLASSICAL RECEPTIVE FIELD AND PROCESSES MEDIATING ORIENTATION AND DIRECTION CONTRAST. A.M. Sillito* and H.E. Jones. Dept. of Visual Science, Inst. of Ophthalmology, London EC1V 9EL, UK.

Cells signalling orientation and direction contrast effects have been described in primate visual cortex (Knierim and Van Essen, J. Neurophysiol 67: 961-980 1992, Sillito et al, Nature **378**: 492-496, 1995). We previously reported that the spatial scale of such effects can be strongly constrained to a circumscribed zone of visual space, which may be asymmetrically distributed around the receptive field and that different outer stimulus parameters can elicit modulatory effects from different locations (Jones et al, Soc. Neurosci. Abs. 21, 650.4, 1995). Here we have dissected the links between the classical receptive field (CRF) and context dependent mechanisms. We quantitatively documented the responses of 70 primate V1 cells using a range of stimuli (including concentric annular and circular patches of drifting grating and discrete grating patches positioned at various XY coordinates) to map CRF and surround mechanisms. Individual cells were studied for 6-12 hours. All effects were observed with simultaneous stimulation of CRF and surrounding areas at the same contrast. Sensitivity to orientation context was seen in oriented and non-oriented cells. Both orientation and direction context dependent effects were observed within, on the edge of, and significantly beyond the apparent confines of the CRF. The modulatory effect of a 1-2° second stimulus patch on the response to a stimulus overlying the CRF could be observed up to 3° or more away from the CRF. As this applied to receptive fields within 5° of the fovea with classical field dimensions in the range of 0.2-0.5°, it reflects a very significant horizontal convergence. We also observed strong directional influences on orientation contrast, even in non-directional cells, suggesting that the influence of stimulus direction pervades more of primate V1 than the response to single stimuli indicates.

The pattern seen varied but the data suggest that the mechanisms detecting stimulus context could often overlie as well as extend beyond the classical receptive field and for at least some cells, should possibly be considered part of its organisation.

Supported by the MRC

376.8

CENTER-SURROUND CONTRAST EFFECTS IN MACAQUE CORTEX

James R. Müller[1], Benjamin Singer[1], John Krauskopf[2], Peter Lennie*[1] Center for Visual Science, University of Rochester[1]; Center for Neural Science, New York University[2]

A V1 or V4 neuron often shows inhibitory and excitatory interactions when stimuli of contrasting structure fall in its classical receptive field and in an enclosing region. We quantify these effects, and explore the size and spatial summation of the region outside the classical receptive field. From anesthetized *m. fascicularis* we recorded responses of V1 and V4 neurons to drifting sinusoidal gratings of optimal position, size, orientation, spatial frequency, and color. We then added enclosing or nearby gratings that varied in orientation, spatial frequency, position, or size. Excitatory interactions varied regularly with the orientation of the enclosing grating; for other dimensions of stimulus structure, the contrast effects were less clear. The smallest responses were usually caused by an enclosing stimulus that matched the one in the classical receptive field. Excitatory and inhibitory interactions grew smaller as the size of the enclosing grating was decreased, or as its distance from the receptive field increased.

Supported by NIH EY 0440, EY07125, EY01319, and EY06638.

376.9

CROSS-ORIENTATION INHIBITION IN HUMAN V1. G.M. Boynton,* J.B. Demb, and D.J.Heeger, Dept. of Psychology, Stanford University, Stanford CA

Purpose: To measure and characterize cross-orientation inhibition in human V1 using functional MRI.
Background: Neurons in primary visual cortex of cat and monkey respond selectively to grating stimuli of a particular orientation and are suppressed by superimposing orthogonal gratings (cross-orientation inhibition). The suppression is strongest when the orthogonal grating is at the neuron's preferred spatial frequency. Heeger (Vis. Neurosci., 9:181-197, 1992) proposed a formal model, known as the *normalization model*, to explain cross-orientation inhibition in single cells.
Methods: Stimuli were contrast-reversing sine-wave gratings (8Hz, 0.8 or 3.2 c/deg) and plaids (superimposed orthogonal gratings) of various contrasts, that alternated every 40 seconds with uniform fields. fMRI response was quantified as the amplitude of the (40 sec period) sinusoid that best fit each pixel's time-series.
Results: For plaids with different spatial frequency components there is little cross-orientation inhibition; the responses to the plaids are about equal to the sum of the gratings responses for all contrasts. Suppression is apparent, however, when the grating components have the same spatial frequency; for all contrasts, responses to these plaids are clearly less than the sum of the grating responses. The normalization model fits the data well.
Conclusions: The normalization model is quantitatively consistent with fMRI measurements of human V1. Neurons in human V1 exhibit cross-orientation inhibition.

Supported by NIH grants MH50228 and MH10897, by a Stanford University OTL grant, and by an Alfred P. Sloan fellowship to DJH.

376.10

PRINCIPLES UNDERLYING THE MAPPING OF DIRECTION PREFERENCE IN PRIMARY VISUAL CORTEX. M. Weliky*, W.H. Bosking, L.C. Katz, D. Fitzpatrick, Dept. of Neurobiology, Duke Univ. Med. Cntr., Durham, NC 27710.

Optical imaging of intrinsic signals demonstrates that direction preference is systematically mapped within primary visual cortex (Weliky et al., *Nature* **379**). Direction preference shifts slowly and continuously within numerous patches. These patches are separated by winding boundaries across which direction preference abruptly shifts by up to 180 degs. Direction fractures tend to intersect orientation pinwheel centers, and subdivide iso-orientation domains into patches selective for opposite directions of motion. Direction preference is typically orthogonal, or within 45 degs, to preferred orientation. Modeling was used to investigate the principles underlying the formation of direction preference maps. A patch of visual cortex was modeled as having initially weak and randomly distributed directional preferences, represented as vector variables. The directional preference at cortical sites was then modified through local excitatory/lateral inhibitory interactions. The resulting maps produced by these conditions consisted primarily of singularities or 360 deg. direction pinwheels, not line discontinuities or fractures as experimentally observed. In order to reproduce the experimental observations, the model was modified so that an orientation map was formed prior to the emergence of direction preference. At each site, direction preference was randomly chosen to be in one of two opposite directions orthogonal, or within 45 degs., to orientation preference. This orthogonality was maintained throughout the simulation. An organized direction map gradually emerged, consisting of long, winding fractures that intersected orientation pinwheels as experimentally observed. The time course of map development reveals that early fluctuations occur with a roughly fixed spatial scale, forming initially weak direction domains that expand and meet. Fractures form at the boundaries between these expanding domains. Changing the balance of excitatory and inhibitory cortical interactions systematically alters the pattern of direction fractures. Supported by NIH grant EY07690 (L.C.K.), EY11488 (D.F.).

376.11

PREDICTING SYNAPTIC RESPONSES OF V1 NEURONS TO ARBITRARY TEMPORAL PATTERNS OF STIMULATION. S.B. Nelson, J.A.Varela, J. Gibson*, K. Sen & L.F. Abbott Dept. Biology & Center for Complex Systems, Brandeis Univ., Waltham, MA 02254.

Little is known about the cause of temporal filtering in V1. We apply a "synaptic decoding" method to electrically-evoked EPSCs and field potentials in vitro. This allows the prediction of postsynaptic responses to arbitrary temporal patterns of input, and elucidates the role of synaptic depression in shaping V1 response properties.

Field potentials (N=14) and monosynaptic EPSCs (N=9) were obtained from layer 2/3 pyramidal neurons in rat visual cortical slices. Responses to random stimulus trains (delivered to layer 4) were used to calculate temporal weighting functions that predict the amplitude of successive responses as a function of interval. Response amplitudes within a train varied 2-5 fold due to cumulative synaptic depression. Fits, obtained by gradient descent, closely predicted (within a few percent) data from additional random or constant frequency trains not used in the fitting process. Results from field potentials and EPSCs were similar. The observed depression decays bi-exponentially (τ_1=200-500 ms,τ_2= 3-10 s). It is presynaptic in origin, since it is reduced by modulators that decrease transmitter release (e.g. adenosine 5-50 μM), but not by a similar degree of postsynaptic response reduction (CNQX 0.3-1.0 μM).

These measurements allow us to estimate the contribution of synaptic depression to the temporal properties of V1 responses. The amplitude and time course of the depression are sufficient to account for the greater transience and lower temporal frequency tuning of cortical neurons (relative to LGN neurons) and for the kinetics of contrast adaptation. Supported by NSF and Sloan Foundation.

376.12

FUNCTIONAL CONSEQUENCES OF SYNAPTIC DEPRESSION FOR RESPONSE PROPERTIES IN V1. L.F. Abbott*, K. Sen, J.A.Varela, J. Gibson & S.B. Nelson Department of Biology & Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Excitatory synapses in V1 depress during repeated stimulation and recover over two timescales. To study the functional significance of this, we incorporated an accurate mathematical description of synaptic depression into a model of a V1 simple cell. Responses to sinusoidal contrast modulation deviated from linearity in two important respects. Over short timescales (<1 s), the cell responded best to rapidly rising input signals, but responded poorly to high temporal frequencies. Responses to low frequency (1-4 Hz) modulation were distorted and phase advanced, accurately matching intracellular recordings of responses to contrast modulation measured in vivo (Jagadeesh et al Science 1993). If the phase-shifted, non sinusoidal synaptic current arising from adapting synapses is combined with an unshifted, linear contribution from non-adapting synapses, the response of the model neuron becomes directionally and velocity selective. This mechanism could allow cells not receiving lagged LGN input to nevertheless achieve direction selectivity.

Over longer time scales (1-30 s), the slow component of recovery from depression causes responses to adapt. The strength and speed of the adaptation increase with temporal frequency as observed experimentally. Also as observed, the adaptation can occur locally within the RF.

More generally, synaptic depression enhances the information carried by neuronal firing. The firing of a neuron with adapting synapses is sensitive to the temporal pattern of synaptic input in a synapse-specific manner, making it sensitive to both spatial and temporal features of the pattern of synaptic input. Supported by NSF and Sloan Foundation.

376.13

SPATIALLY SELECTIVE ATTENTION GATES NEURONAL RESPONSES IN MACAQUE V1. T.R.Vidyasagar and G.H.Henry*. Centre for Visual Sciences and Div. of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT2601, Australia.

To study whether attentional mechanisms influence neuronal responses in the primary visual cortex, two macaques (M. nemestrina) were trained on a conditional visual discrimination task. While maintaining fixation on a small spot, the monkey had to discriminate the orientation of the bars of a grating flashed briefly (60-80 ms) in a cued part of the visual field while ignoring the grating orientation in other simultaneously presented patches. In each trial, a different part of the visual field was cued and the monkey had to discriminate between vertical and horizontal orientations by making an appropriate motor response. A chamber was then implanted over the visual cortex under aseptic conditions and general anaesthesia. In subsequent electrophysiological recordings, one set of cells in V1 (CS or cue-specific neurons) responded vigorously to a presentation of the grating over the receptive field only when that grating was cued; when other positions were cued (non-cued trials), response to the same grating was significantly less. These cells usually had latencies of at least 70 ms. Another set of cells in V1 (CR or cue-related neurons) had more classical latencies of 40 to 80 ms; their early responses were similar for both cued and non-cued trials, but the response component beyond 100 ms was more marked for cued trials. It is proposed that an attentional feedback reaches V1 about 70 to 110 ms after the presentation of the grating stimuli in this paradigm and it facilitates the responses in the cued part of the visual field and/or inhibits responses in all non-cued regions ("attentional spotlighting"). Such feedback may underlie the more sustained response of CR cells in the attended region. The CR cells in turn may excite the CS cells which may provide the final output to the relevant higher areas for behaviorally significant visual discrimination.

(Funded by the John Curtin School of Medical Research).

CARDIOVASCULAR REGULATION: VENTRAL MEDULLA II

377.1

CARDIAC SYMPATHETIC PREMOTOR NEURONS. R.M. McAllen* and R.R. Campos. Howard Florey Institute, University of Melbourne, Vic 3052, Australia.

Premotor neurons in the rostral ventrolateral medulla (RVLM) control the sympathetic supply to the heart as well as to blood vessels, but the distribution and actions of the cardiac neurons are ill-defined. We mapped cardiac sympathetic premotor neurons in 7 chloralose-anesthetized cats (70mg/kg, i.v.), by microinjecting glutamate (0.5 nmol) into 133 RVLM sites whilst recording activity from the ipsilateral inferior cardiac nerve. For comparison, muscle vasoconstrictor (MVC) activity was recorded simultaneously from the peroneal nerve. Baroreceptors were denervated. RVLM glutamate injections increased cardiac nerve activity by up to 395% and MVC activity by up to 487%. Most injections excited both nerves, but selective MVC responses could be obtained from the caudal end, and cardiac nerve responses from the rostral end, of the subretrofacial nucleus.

Nine chloralose-anesthetized cats were adrenalectomized, vagotomized and given prazosin (1mg/kg, i.v.) to minimize blood pressure changes. The peak rate of pressure rise (dP/dt_{max}) was measured from a left ventricular cannula. Glutamate injections into 130 RVLM sites increased heart rate by up to 65% and dP/dt_{max} by up to ten-fold, indicating strong chronotropic and inotropic drives. Diastolic ventricular pressure usually fell, and the tachycardia *per se* (reproduced by atrial pacing) caused only a small increase in dP/dt_{max}. RVLM injections on the right caused stronger tachycardias than those on the left (36.8±19.5 vs. 14.9±14.2 beats/min), but inotropic responses to left and right stimuli were similar. The same pattern was seen in response to electrical stimulation of the left and right cardiac sympathetic nerves (3 animals).

We conclude that both chronotropic and inotropic sympathetic actions are driven by RVLM premotor neurons, whose distribution is partly distinct from vasomotor pathways. Their projections to the heart are heavily ipsilateral.

Supported by NH&MRC. RRC is a FAPESP (Brazil) postdoctoral fellow.

377.2

SYMPATHO-EXCITATORY NEURONS OF THE ROSTRAL VENTROLATERAL MEDULLA OF RABBITS ARE ORGANIZED TOPOGRAPHICALLY Y. Ootsuka and N. Terui*. Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Our present experiments were undertaken to examine whether sympatho-excitatory reticulospinal neurons of the rostral ventrolateral medulla (RVLM) are organized topographically or not. Micro-injections of a neuro-inhibitory agent, γ-amino-n-butyric acid (GABA, 50 mM 10-30 nl), into the RVLM were done during simultaneous measurements of arterial pressure and blood flow of the kidney (with an ultrasonic-Doppler cuff), the ear skin and muscles of fore- and hind-limbs (with laser-Doppler probes) in urethane-anesthetized and immobilized rabbits. Conductance of each vascular bed was calculated by mean arterial pressure (MAP) and each blood flow. Injection sites where the maximal changes in conductance of the vascular beds of fore- and hind-limb muscles were evoked were very close to the site where the maximal changes in MAP (MAP site) were evoked. More rostral sites to the MAP site were found to evoke the maximal changes in renal conductance. Neurons located in the medial sites to the MAP site affected conductance of the ear skin vessels. Because the preganglionic neurons that control vessels of the ear skin and the fore-limb muscles are located at the upper thoracic segments of the spinal cord and those that control the vessels of the hind-limbs are located at the lumber segments, these observations indicates that the sympatho-excitatory reticulospinal neurons in the RVLM are divided into subgroups and they are localized in the RVLM according to their function but not their anatomical characteristics.

377.3

CONVERGENT INPUTS FROM THE AORTIC AND VAGAL NERVES TO NEURONES OF THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) IN THE RAT. K.Ishizuka[1], A.Zagon*, I. Rocha, and K.M.Spyer Dept. of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, U.K., [1]Dept. of Oral Physiology, The Nippon Dental University, School of Dentistry at Niigata, 1-8 Hamaura-cho Niigata 951, Japan.

Previous physiological studies have shown that the RVLM plays an important role in the integration of autonomic reflexes. In the present study we examined the existence of convergence between aortic (AN) and vagal (VN) afferent inputs using *in vivo* intracellular recordings. Data were obtained in anaesthetised (pentobarbitone sodium 60 mg/kg i.p., followed by α-chloralose, 30 mg/kg/h i.v.) and artificially ventilated rats.

Electrical stimulation of the ipsilateral cervical vagus (4-6 V, 0.1 ms, 1 Hz) evoked EPSP, IPSP or multiphasic responses in RVLM neurons. Convergent inputs were observed in about two-third of the neurones where stimulation of the aortic nerve (parameters as above) evoked either an IPSP or EPSP. A tendency to receive common excitation or inhibition was evident. The onset latency of AN and VN inhibition was similar (20-47 and 24-51 ms) while the onset latency of VN excitation (19-57ms) was regularly longer than that of the AN response (14-41 ms). The neurones that received both aortic and vagal inputs were concentrated in the medial aspect of the RVLM.

A possible network of mediating VN and AN responses in the RVLM is suggested.

Supported by the British Heart Foundation.

377.4

CARDIORESPIRATORY NEURONS IN THE VENTROLATERAL MEDULLA RESPOND TO MULTIPLE INPUTS. C. Wu, P.C. Nolan and T.G. Waldrop*. Depts. of Molecular & Integrative Physiology, Neuroscience Program and College of Medicine, Univ. of Illinois, Urbana, IL 61801.

The ventrolateral medulla (VLM) is an important integrative site for cardiorespiratory reflexes; neurons in this region are known to play a role in central chemoreception and are involved in baroreceptor and somatosympathetic reflexes. Previous studies from this and other laboratories have shown that many VLM neurons are excited by hypoxia and project to the intermediolateral cell column. The purpose of the present study was to determine if individual VLM neurons respond to multiple inputs or only one type of sensory modality. Extracellular, single unit activity of VLM neurons was recorded in anesthetized, male rats. VLM neurons were tested for responses to three somatosensory stimuli (activation of hair, skin and skeletal muscle receptors) applied to the hindlimbs. In addition, VLM neuronal responses to inhalation of hypoxic (10% O_2/90% N_2) and hypercapnic (5% CO_2/95% O_2) gases and to baroreceptor activation (phenylephrine-induced increases in arterial pressure) were examined. Over ninety percent of VLM neurons responded to hypoxia; the discharge frequency of a smaller proportion was altered during hypercapnia or baroreceptor stimulation. All of the hypoxia-sensitive neurons responded to at least one of the somatosensory stimuli including input from both ipsilateral and contralateral limbs. The majority of these neurons had basal discharge patterns related to cardiac and/or respiratory rhythms as revealed by computer signal averaging. Thus, cardiorespiratory neurons in the ventrolateral medulla integrate input from a variety of stimuli in order to produce appropriate cardiorespiratory responses during conditions such as exercise and hypoxic stress. (Supported by NIH HL06296).

377.5

Different Regions of the Rostral Ventrolateral Medulla (RVL) Regulate Sympathetic Nerve Activity or Regional Cerebral Blood Flow (rCBF) and EEG. D.J. Reis*, D.A. Ruggiero, and E.V. Golanov. Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., NY, NY 10021.

While stimulation of the RVL electrically, chemically or by hypoxia elevates arterial pressure (AP) and rCBF and synchronizes the EEG (Golanov and Reis, *Am. J. Physiol.*, 1994) the region maximally elevating rCBF is caudal to that exciting sympathetic neurons. We investigated whether the RVL was organized into functionally distinctive regions for regulation of AP, rCBF and EEG by stimulation of RVL, the nucleus tractus solitarius (NTS) or by brainstem hypoxia (NaCN). Rats (n=32) were anesthetized, spinalized, ventilated, and instrumented to record AP, cortical rCBF (laser-Doppler flowmetry) and EEG. The medulla was systematically explored by microstimulation with 5 sec trains (100 μA, 50 Hz). Stimulation within the sympathoexcitatory (C1) region of the rostral RVL (rRVL) increased rCBF within 2 sec by 20±3% and increased the power of the 4-6 Hz EEG rhythm. However, the rCBF responses were significantly larger (by 34±8%, $p<0.05$) from a caudal vasopressor region (RVLc). Microinjections of L-glutamate (5 nM, 40 nL) produced greater increases in rCBF from RVLc. Inactivation of the RVLc by procaine or electrolytic lesions reduced the increase in rCBF (by 70%, $p<0.05$) and EEG synchronization evoked from RVLr **and** NTS while inactivation of RVLr did not alter evoked changes in rCBF or EEG evoked from RVLc nor NTS. Tracing efferent projections from RVLr or RVLc with biotinylated dextran-amine (n=4) demonstrated RVLr projections terminating within the caudal paraambigual region corresponding to the RVLc and, reciprocally, RVLc projections terminating in RVLr. We conclude: (a) Neurons of the RVLc mediate effects elicited from RVLr and NTS on rCBF and EEG; (b) neurons of the RVLr are the principal oxygen sensors in RVL which, when excited, elevate rCBF and synchronize the EEG over an intramedullary projection to RVLc and increase sympathetic activity by a spinal sympathetic pathway; (c) regions of RVL exciting spinal sympathetic neurons are anatomically distinct from those regulating rCBF and EEG.

377.6

VENTROLATERAL MEDULLA MEDIATES DEPRESSOR RESPONSES ELICITED BY ACTIVATION OF SODIUM-SENSITIVE NEURONS IN NUCLEUS TRACTUS SOLITARIUS. S. L. Hochstenbach* and J. Ciriello. Department of Physiology, University of Western Ontario, London, ON, Canada, N6A 5C1.

We have previously shown that activation of sodium-sensitive sites in nucleus of the solitary tract (NTS) elicits depressor and bradycardic responses. However, the pathways mediating these effects are unknown. Experiments were done in male Wistar rats to identify anatomically and functionally the brainstem nuclei that may mediate the depressor responses elicited by activation of sodium-sensitive neurons in NTS. In the first series, the anterograde tract-tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) was iontophoresed unilaterally into NTS sites at which microinjection (20 nl) of a 150-175 mM NaCl solution elicited depressor responses. Injection sites were found to be localized to the caudal medial subnucleus of NTS (Sm). PHA-L labelled fibers and presumptive terminal boutons were found throughout the rostrocaudal extent of NTS, dorsal motor nucleus of the vagus, area postrema, ventrolateral medulla (VLM), and nucleus ambiguus bilaterally, with an ipsilateral predominance. In the second series of experiments, the effect of blocking synaptic transmission in VLM with cobalt chloride ($CoCl_2$; 5 mM, 100 nl) on the cardiovascular response elicited by microinjection (20 nl) of hypertonic saline (150-175 mM) into the ipsilateral Sm was investigated in the alpha-chloralose anesthetized, paralyzed and artificially ventilated rat. Microinjection of $CoCl_2$ into VLM, at sites shown in the previous experiment to receive efferent projections from Sm, significantly attenuated the depressor (60%) and bradycardic (80%) responses to stimulation of Sm. These data indicate that the sodium-sensitive region of the caudal Sm innervates VLM neurons and suggest that these VLM neurons are involved in mediating the depressor and bradycardia responses elicited by changes in the extracellular concentration of sodium. (Supported by the Heart and Stroke Foundation of Ontario.)

377.7

THE 10-HZ AND CARDIAC-RELATED (C-R) SYMPATHETIC RHYTHM GENERATORS CONVERGE ON BULBOSPINAL NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) AND CAUDAL MEDULLARY RAPHE (CMR) OF CATS. S.M. Barman* and G.L. Gebber. Dept. Pharmacology & Toxicology, Michigan State Univ., East Lansing, MI 48824.

Studies from this laboratory support the view that the generators of the 10-Hz and C-R rhythms in sympathetic nerve discharge (SND) are comprised of different pools of neurons. The current study was designed to test the hypothesis that the outputs of the two generators converge at the level of bulbospinal neurons. One would expect that only brainstem neurons that project to the spinal cord would have activity correlated to both rhythms in SND if this hypothesis is correct. Thus we determined if RVLM and CMR neurons with activity correlated to both the 10-Hz and C-R rhythms in inferior cardiac postganglionic SND could be antidromically activated (AA) by electrical stimulation of the first segment of the thoracic spinal cord (T1SC) of urethane-anesthetized, vagotomized cats. The axons of 16 of 18 RVLM neurons with activity correlated to both rhythms projected to the T1SC. Also, 31 of 34 CMR neurons with activity correlated to both the 10-Hz and C-R rhythms in SND were AA by T1SC stimulation. These data support the hypothesis that the 10-Hz and C-R rhythm generators converge at the level of bulbospinal neurons. We also found 13 RVLM and 4 CMR neurons with activity correlated to only the 10-Hz or to only the C-R rhythm in SND. Only one of these RVLM and none of these CMR neurons could be AA by T1SC stimulation. These data reveal that a substantial proportion of RVLM neurons and a small group of CMR neurons with activity correlated to SND are not bulbospinal. The axonal trajectories and functions of these neurons remain to be determined. (Supported by NIH grant HL33266.)

377.8

DISTINCT PATHWAYS WITHIN THE ROSTRAL VENTROLATERAL MEDULLA (RVL) MEDIATE PRESSOR AND BLOOD FLOW RESPONSES TO SCIATIC NERVE STIMULATION (SNS). Cravo, S.L.*; Possas, O.S.; Lopes, O.U. Dept. of Physiology, Univ. Federal de São Paulo, São Paulo, Brazil.

Sciatic nerve stimulation (SNS) produces hypertension, tachycardia and muscle vasodilation. Previous studies demonstrated that the RVL mediates heart rate and pressor responses to SNS, however, its role in the vasodilation remains unclear. Male adult Wistar rats were anesthetized (urethane, 1.2 g/kg, iv), paralyzed and artificially ventilated. The right carotid artery and jugular vein were catheterized for mean arterial blood pressure (MAP) and heart rate (HR) recordings and drug administration, respectively. Hindlimb blood flow (HBF) was recorded by Doppler flowmetry and expressed as percentage of the baseline. Hindlimb relative vascular conductance (HVC) was calculated by dividing Doppler shift (kHz) by the MAP (mmHg). Results obtained demonstrate that: 1) SNS (square pulses, 1 ms duration, 20-50 Hz, 800-1200 µA, 10 s) produced hypertension (+28 ± 4.3 mmHg, N=5), tachycardia (+26 ± 4.4 bpm) and increased HBF (134 ± 7.7%) and HVC (105 ± 2.2%); 2) after unilateral microinjection of kynurenic acid (2 nmol/100 nL) into the RVL contralateral to the sciatic nerve stimulated, MAP and HR responses to SNS were reduced to -1 ± 4.0 mmHg and 8 ± 5.5 bpm ($p<0.05$) whereas HBF response was not modified (120 ± 4.6%), therefore the HVC response increased (122 ± 5.7%, $p<0.05$); 3) when bicuculine (400 pmol/100 nL) was microinjected into the contralateral RVL, pressor response was not modified (+23 ± 3.4 vs. 31 ± 2.6 mmHg, N=7), tachycardia was reversed to bradycardia (+28 ± 5.4 vs. -33 ± 13.4 bpm, $p<0.05$), and increase HBF was abolished (134 ± 8 vs. 94 ± 5.2%, $p<0.05$), hence HVC decreased (106 ± 6.8 vs. 73 ± 3.3 %, $p<0.05$). These results suggest that glutamatergic afferents within RVL mediates pressor responses while GABAergic afferents modulates vasodilatatory responses to SNS.
Supported by FAPESP, Brazil.

377.9

HETEROGENEITY OF METABOTROPIC GLUTAMATE RECEPTOR EXPRESSION IN CARDIOVASCULAR REGULATORY REGIONS OF THE MEDULLA. M. Hay*, P. J. Conn‡, H. McKenzie, K. Lindsley and E. M. Hasser. Dalton Cardiovascular Research Center, Dept. of Vet. Biomedical Sciences, University of Missouri, Columbia MO 65211 and ‡Dept. of Pharmacology, Emory University School of Medicine, Atlanta GA, 30322.

The expression of mGluR1α, mGluR2/3, mGluR4α and mGluR7 was examined with immunocytochemistry and in situ hybridization in rat medullary regions known to be involved in cardiovascular control. Within the rostral and caudal extent of the nucleus tractus solitarius (NTS) studied in this report, there was no evidence for either mGluR1a protein or mRNA. Likewise, there was also an absence of mGluR2/3 immunoreactivity within the NTS. mGluR4α was identified within both cell bodies and fibers within the NTS while mGluR7 expression appeared to be limited to fibers and processes throughout the rostral and caudal extent of the NTS. The present study also revealed intense mGluR1α immunoreactivity within the n. ambiguus and an absence of any detectable mGluR2/3, mGluR4α or mGluR7 immunoreactivity within the n. ambiguus. Within the dorsal motor nucleus of the vagus (DMN), (+) immunoreactivity for mGluR1α, mGluR2/3, and mGluR4α was observed. Expression of mGluR2/3, while present in cell bodies and fibers in the caudal regions of the DMN, could be detected only in the neuropil and fibers in the rostral regions of the DMN. Of the mGluR(s) studied in this report, only mGluR2/3 and mGluR4α were detectable within the area postrema. Expression of mGluR2/3 was clearly evident in cell bodies and fibers within the area postrema. In some cases, (+) mGluR2/3 processes were observed to transverse across the border of the area postrema into the adjacent NTS. Expression of mGluR4α was very dense and punctate within small perikarya of the area postrema. Within the caudal ventral lateral medulla (CVLM), (+) mGluR1α and mGluR4α immunoreactivity was observed within both cell bodies and processes. mGluR4α (+) labeling was also observed within cell bodies and processes of the rostral ventral lateral medulla (RVLM). These results suggest a complex and subtype specific role for mGluR(s) within cardiovascular regulatory regions of the medulla. Supported by NIHL50304 to M.H. and NIHL43600 to E.M.H.

377.10

A STUDY OF THE NATURE OF THE PROJECTIONS FROM NUCLEUS RAPHE OBSCURUS TO THE VENTROLATERAL MEDULLA. H. A. Futuro Neto, M. A. Dantas , M. P. Gilbey* and J. G. P. Pires. Dept. Ciências Fisiológicas, CBM, UFES, Vitória, ES 29040, Brazil.

Pressor responses induced by stimulation of nucleus raphe obscurus (NRO) are dependent on the functional integrity of the rostral ventrolateral medulla (RVLM; Campos Jr. *et al.*, Braz. J. Med. Biol. Res. 26: 623-631, 1993). The present experiments were designed to investigate the participation of 5-HT receptors in this pathway. Adult male rats anesthetized with urethane (1.2 g kg^{-1}, *iv*) after ether induction were instrumented for BP, HR, respiratory movements and renal nerve activity (RN) recordings. Stainless steel electrodes were positioned within NRO for stimulation (100 Hz, 1 ms, 40-70 µA for 10 s). Test drugs or artificial CSF were microinjected bilaterally in the RVLM by means of microcannulas introduced through the dorsal surface of the medulla. Stimulation of NRO induced hypertension, bradycardia, sympathetic excitation and apnea. Microinjections in the RVLM of the 5-HT_1/5-HT_2 antagonist methiothepin (MT; 200 ng, 200 nl) did not alter basal BP, HR or RN and did not change the responses to NRO stimulation (control ΔBP +42.9 ± 3.4 mmHg; ΔHR - 113 ± 45 bpm; after MT ΔBP +43.0 ± 4.3 mmHg; ΔHR - 128 ± 33 bpm, n=6). Microinjections in the RVLM of the 5-HT_3 antagonist granisetron (GR; 10 ng, 200 nl) did not alter basal BP, HR or RN and did not change the responses to NRO stimulation(control ΔBP +36 ± 3 mmHg; ΔHR - 41 ± 24 bpm; after MT ΔBP +34 ± 2.6 mmHg; ΔHR - 45 ± 26 bpm, n=6). Glycine (5 µg, 200 nl) markedly reduced BP and HR responses to NRO stimulation. These results indicate that the pressor responses induced by NRO stimulation, that are dependent on the functional integrity of RVLM, are not mediated by 5-HT_1, 5-HT_2 and 5-HT_3 receptors. The nature of this pathway is presently under investigation.
Supported by: CNPq, FCAA, FINEP and The British Council.

377.11

BAROSENSITIVE NEURONS IN ROSTRAL VENTROLATERAL MEDULLA (RVLM) ARE INHIBITED DURING HEMORRHAGE-INDUCED SYMPATHOINHIBITION. A.M. Schreihofer*, D. Huangfu, R.L. Stornetta, P.G. Guyenet. Dept. of Pharmacology, University of Virginia, Charlottesville, VA 22908.

Hemorrhage initially stimulates sympathetic outflow by unloading arterial baroreceptor afferents. However, a further reduction of blood volume produces a vagally-mediated inhibition of most sympathetic nerves (renal splanchnic and lumbar, but not adrenal nerve). The present study examined whether this inhibition is correlated with a decrease in activity of RVLM neurons that provide the tonic drive to sympathetic vasomotor nerves. Chloralose-anesthetized rats were hemorrhaged to 50 mmHg (10 ml/kg) through a femoral catheter while AP, splanchnic nerve discharge (SND), and RVLM unit activity were recorded. Hemorrhage initially increased SND (25%, n=10), and then significantly decreased SND (-65% from baseline, $p<0.01$). The firing of 8 of 10 barosensitive RVLM units (1 unit/rat) mimicked the SND response pattern (baseline firing= 8.8/sec, initial increase of 35%, followed by a decrease of 50% from baseline, $p<0.05$.) In contrast, in 2 rats, RVLM unit activity progressively increased despite hemorrhage-induced inhibition of SND (SND decreased 76%, RVLM units increased 126%). To determine whether a suppression of central respiratory drive accompanies hemorrhage-induced inhibition of SND, phrenic nerve discharge (PND) was measured. In 4 rats PND was depressed, but in 3 rats PND was stimulated. These results suggest that hemorrhage-induced inhibition of SND may occur via an inhibition of RVLM vasomotor neurons. However, a population of RVLM barosensitive neurons exists that is only excited by hemorrhage. These cells may contribute to the stimulation of adrenal nerve activity during hemorrhage. In addition, although a suppression of central respiratory drive may occur with hemorrhage, it is not required for the production of sympathoinhibition. Supported by NIH R01-HL 28785, NIH training grant 5T32HL07284-19 and NIDA DA-07353.

377.12

FUNCTIONAL MAGNETIC RESONANCE IMAGING DURING BLOOD PRESSURE MANIPULATION. R.M. Harper*, R. Bandler, J. Alger, K.H.J. Bockhorst, J. Mintorovitch and D. Spriggs. Depts. of Neurobiology and Radiology, UCLA, Los Angeles, CA 90095; Dept. of Anatomy and Histology, University of Sydney, NSW, 2006.

In man, the brain structures involved in mediation of cardiovascular regulation are not well described. We examined activity changes in brain areas following challenges which altered blood pressure and heart rate by >15%. Magnetic resonance imaging, using echo-planar sequences designed to rapidly assess changes in brain tissue microcirculation, was performed in 6 healthy volunteers (22-52 yrs) using a Siemens 1.5 Tesla scanner (time of relaxation (TR): 3.5 sec; time of excitation (TE): 44.5 msec; slice thickness: 6 mm; flip angle: 90°; field of view (FOV): 31 cm). Twenty slices were collected 18 times under each experimental condition, with a total acquisition time of 60 sec. Images were collected during unilateral hand immersion in cold water, following a cold compress to the forehead, and during intervening baseline conditions. Images were averaged, values from experimental conditions were subtracted from baseline, and pixel-by-pixel elements were subjected to t-tests with Bonferroni correction. Significant changes in image signal intensity consistently occurred in the posterior diencephalon, in regions near the bed nucleus of the stria terminalis, and in the ventral medulla. The findings confirm structural roles determined by other means in animal studies, and demonstrate a prominent role for rostral brain structures in mediating cardiovascular challenges. *(Supported by HD-22695 and NHMRC 950143).*

GENE STRUCTURE AND FUNCTION II

378.1

LONG-LASTING EXPRESSION OF FOREIGN GENES IN RAT CORTICAL CULTURES AFTER LIPOSOME-MEDIATED TRANSFECTION. **F.A. Boeckman*, R.M. Whelan, K.A. Hartnett and E. Aizenman.** Dept. of Neurobiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Introduction of foreign genes into cultured neurons has previously been achieved by viral infection with high efficiency. The inherent problem with this technique is that viral infection is usually associated with severe cytotoxicity in which cells typically die 6 to 9 days post-infection. Alternatively, cationic lipid-mediated transfection has been useful in transformation of mammalian cell lines with relatively little associated cell death, but with moderate efficiency. Here, we compare the efficiency of three cationic lipid formulations (LipofectAMINE, Tfx-50, Tfx-20) to one another in the transfection of rat embryonic (E16) cortical cells in culture. Optimized conditions for the three lipid formulations were developed using a luciferase reporter assay; the Tfx lipids proved to be substantially more effective than LipofectAMINE. Transfection efficiency was assessed by counting β-galactosidase positive cells and was found to be 1.5% for neurons (11 DIV) in adherent cultures, as assessed 2 days post-transfection (at 13 DIV). Two weeks following transfection of these cultures, 0.4% of neurons was still positive for β-gal activity (25 DIV). An alternative protocol for transfection in suspension immediately following cell dissociation resulted in at least a two fold increase in transfection efficiencies in neurons. In addition, with this method nearly 1% of neurons were β-gal positive after four weeks in culture (28 DIV). This efficiency was not unique to β-gal as luciferase activity showed a similar pattern of expression. The use of this technique with viable markers such as green fluorescent protein may prove useful in the study of the electrophysiological properties of neurons transfected with various genes of interest such as ion channels and receptors. Supported by NIH grant NS 29365.

378.2

DIFFERENTIAL LOCALIZATION OF DEFECTIVE PARTICLE AND HELPER VIRUS GENE EXPRESSION FOLLOWING INJECTION OF DEFECTIVE HERPES SIMPLEX VIRUS (HSV) VECTORS IN THE BRAIN.

J.-O. Hahm[1]*, K.C. New[2], R.L. Martuza[1] and S.D. Rabkin[1,2]. Departments of Neurosurgery[1] and Microbiology & Immunology[2], Georgetown Univ. Med. Ctr., Washington, DC 20007.

Defective HSV vectors are being used for gene delivery in the CNS. A defective HSV vector stock consists of a mixed population of defective particles (containing genes of interest) and helper HSV particles. We have examined the pattern of reporter gene expression, from defective particles and helper virus, after inoculation into discrete regions of the brain.

Defective HSV vectors were generated containing the genes for human placental alkaline phosphatase (AP) or LacZ driven by the CMV_{IE} promoter. Helper virus was detected histochemically for expression of β-galactosidase (insertion of the LacZ gene driven by the HSV ICP6 promoter) or immunohistochemically for the HSV immediate-early gene product ICP4. A 1μl volume of vector stock was injected over a 10 minute period into either the substantia nigra or somatosensory cortex of adult rats. 2-7 days post-inoculation, animals were sacrificed and their brains processed for detection of β-galactosidase, AP or ICP4.

Preliminary data indicates that lacZ (helper virus) and AP (defective) are expressed in different sites throughout the brain, as well as in overlapping sites. In the nigral injections, helper virus was only detected at the site of injection, whereas defective vector was taken up and retrogradely transported to multiple sites in the thalamus.

Supported by a grant from NINDS.

378.3

SELECTIVE GENE TRANSFER IN VIVO INTO HIPPOCAMPAL GRANULE CELLS: LOCALIZATION AND SPECIFICITY. P.A. Serrano*, I. Cantallops, R.L. Neve[1] and A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern University Institute for Neuroscience, Evanston, IL 60208; [1]McLean Hospital and Harvard Medical School, Belmont, MA 02178.

The possibility of developmental compensatory mechanisms in null mutants and transgenic mice complicates the interpretation of results derived from this technique. Here we have begun to explore the use of viral vectors for gene transfer as an alternative strategy since it allows for gain of function or gene knockout in adult animals. The HSV-mediated gene transfer may also be advantageous if target cell uptake selectivity is achieved. Using electrophysiological guidance we observed selectivity of the mutated herpes virus uptake into a particular cell type in the hippocampus. Under electrophysiological control, the viral vector was injected into the dentate hilus, which is rich in presynaptic terminals, the preferred route of entry of the viral vector. The vector had 1 of 2 cDNA inserts: a *lacZ* reporter or the neuron-specific protein, F1/GAP-43. With both vectors there was a similar selectivity of uptake into granule cells though terminals of pyramidal and hilar cells are present in this area. We considered two candidate mechanisms for the selective uptake: (1) the viral promoter entered all cells but only granule cells contained the transcription factors necessary for cDNA insert activation; (2) the receptors on presynaptic terminals necessary to allow uptake of this viral strain are only present on mossy fiber axons. Recent evidence indeed indicates that the herpes receptor is part of the TNF/NGF receptor superfamily.

We wished to determine the initial site of infection and subsequent "viral load" borne by the granule cells. Using an antibody specific for viral envelope glycoprotein C (gC; gift of P. Spear, NU), immunoreactivity was observed in the granule cell body and dendrites at 3d demonstrating a preferred uptake in granule cells. The presence of gC at 3 days indicates that the infected cells synthesize and transport HSV-1. Supported by NSF postdoc grant to PAS., "la Caixa" grant to IC., HD24236 to RLN, MERIT Award MH25281-21 to AR.

378.4

RECOMBINANT ADENO-ASSOCIATED VIRUS (RAAV) PRODUCES SUSTAINED TRANSDUCTION OF MURINE CNS NEURAL CELLS.

W.D. Lo *, R. Chen, G. Qu, T. Sferra, R. Clark, P. Johnson. Dept Pediatrics, Ohio State University, Columbus, OH.

rAAV has promise as a viral vector for gene transfer, but most studies have shown only transient transduction of CNS cells. We used a rAAV vector that expresses the E. coli lacZ gene under the control of the IE CMV promoter (rAAV/b-gal) to determine the temporal pattern of viral-mediated transgene expression. A CsCl purified rAAV/b-gal viral stock was generated from adenovirus infected D6 "producer" cells (HeLa derived). This cell line contains all AAV helper genetic elements (rep, cap, and rAAV vector sequences) required for rAAV production. The right caudate nucleus of adult BALB/C mice was stereotactically injected with 10^8 IU rAAV/b-gal (2 μl injection volume) or sterile diluent as a control. Three mice at each time point (5 days, 2, 4, and 8 wk after inoculation) were sacrificed and serial frozen brain sections were analyzed for b-galactosidase activity (X-gal stain). Five random sections containing positive cells were selected from each animal and the total number of positive cells per section was tabulated. Cells/section at 5 days were 76 (S.D. +/- 39), steadily increasing to 339 (S.D. +/- 38) at eight weeks ($p = 0.06$ by ANOVA, repeated measure).

We have demonstrated persistent (or increasing) transgene expression in CNS cells following rAAV/b-gal inoculation. This finding has implications for long-term gene transfer to cells of the CNS using high titer rAAV vectors.

Supported by CHRF, NIAID, and Alkermes

378.5

Adenoviral Penton Protein Improves Transfection Efficiency of mRNA and Plasmid DNA in Primary Neuroglial Cells

Langer DJ, Fisher KJ, Malone RW, Hecker JA, Barnathan ES, Welsh FA* Dichter MA, Kariko K. Div. Neurosurgery & Cardiology, Univ. of PA, Phila., PA 19104, Dept. of Med. Pathol. UC Davis, Davis CA 95616

Cationic lipid delivery of RNA and DNA has shown utility both in vitro and in vivo. The mechanism of transfection with these nonviral systems has been shown to involve endosomal uptake, progressive acidification, and lysosomal fusion resulting in nucleic acid degradation and decreased transfection efficiency. In contrast, adenovirus transfection efficiency is thought to be higher, due at least in part to promotion of endosomal lysis enabling escape from lysosomal degradation. We hypothesized that the adenoviral penton protein itself might similarly enhance cationic lipid-mediated RNA or DNA transfection. Primary neonatal rat hippocampal neuroglial cells and NIH 3T3 cells were transfected with either firefly luciferase mRNA or DNA in the presence or absence of purified penton protein and Lipofectin. Control transfection resulted in a ~100 fold increase in luciferase activity over background. Penton protein addition resulted in a dose dependent, further 3-4 fold increase of luciferase level in both cell types whereas a control protein had no such effect. Similar stimulation was observed with DNA plasmid transfection as well. Addition of chloroquine, which blocks lysosomal degradation, did not block the stimulation, suggesting a potentially novel mechanism of action for penton. Penton-mediated delivery is a promising approach for gene transfer in neural cells.

Supported in part by NINDS Grant NS-29331

378.6

RAPID GENERATION OF STABLE CELL LINES FOR DRUG DISCOVERY

R. A. Horlick, K. Sperle, L. A. Breth, E. S. Shen, C. M. Reid, G. M. Cooke, S. G. Culp* and B. L. Largent. Applied Biotechnology and CNS Departments, The DuPont Merck Pharmaceutical Co., Wilmington, DE 19880.

A human 293 cell line that stably expresses the Epstein Barr nuclear antigen 1 (EBNA1) supports the episomal replication of plasmids containing the Epstein Barr virus origin of replication (EBV ori). A 293EBNA (293E) cell line expressing the human corticotrophin releasing hormone receptor subtype I (CRHR1) from an episomal plasmid was generated (293CR1s), analyzed, adapted to spinner culture and scaled-up for production in less than six weeks. Fifty stable CHO cell lines transfected with CRHR1 were also isolated. Expression of the receptor in the best of these lines, CHO-R22, was compared to 293CR1s. Results indicate that use of episomal vectors in 293E cells (1) rapidly generates stable cell lines suitable for scale-up; (2) these lines retain a stable copy number of episomes, (3) express highly abundant levels of CRHR1 mRNA and (4) produce a steady level of CRHR1 even after 4 months in culture. The 293CR1s line expresses significantly more CRHR1 than the CHO-R22 line that was prepared using traditional methods. Our data support the idea that using the EBV ori-driven episomal system for gene expression results in greater production of protein in a comparatively very short period of time.

378.7

TARGETED mRNA DISPLAY AND RT-PCR APPROACH TO IDENTIFY GENES INVOLVED IN ISCHEMIC BRAIN TISSUE INJURY AND REGENERATION.

U. Utans, H.P. Lorez, J. Sepinwall* and W. Lesslauer. F. Hoffmann-LaRoche Ltd, Basel;*Hoffmann-LaRoche, Inc., Nutley, NJ

To identify novel genes potentially active in brain ischemic tissue injury and regeneration, a mRNA display screen was established using biased primer designs targeting non-coding regulatory AU-rich elements common to a number of genes known to be active in tissue stress, injury and repair, and host defense mechanisms. The screen strategy was validated in a permanent MCAO rat stroke model; of 26 display bands analysed, 7 known genes were identified, 5 of which belonged to the targeted class of genes (eg. IL-1, VEGF, c-fos). In addition, several sequences were identified which were not found in data base searches and therefore might represent candidate genes of interest in the ischemic disease context. The identification of proinflammatory mediator expression in and around the ischemic tissue points to a neuroinflammatory reaction which may propagate and aggravate pathology. Therefore the time-sequential expression of other mediator and related genes was analysed in a semiquantitative RT-PCR study comparing infarct core, border zone and contralateral cortical tissue. Early and persistent induction of TNFa, IL-1b and IL-6 mRNA was found in core (1h to 2 weeks) and border zones (1 - 24 h). In contrast, TGFb1 transcripts were increased only at late times (24 h - 2 weeks) in the core, but not in border or contralateral zones. Elevated transcript levels of iNOS were identified in infarct core, but only a moderate increase at early time points was seen in the border zone (1 - 24 h). Differentially spliced isoforms of amyloid precursor protein (APP) were detected late and exclusively in the core.

378.8

ANTISENSE TARGETING OF OXYTOCINERGIC HYPOTHALAMUS NEURONS INDUCES CYTOPLASMIC TRIPLE HELIX- LIKE IMMUNOREACTIVIY.

Jirikowski G.F.*[1], Prüfer K.[1], and J.S. Lee[2]. [1]Dept. of Anatomy II, Friedrich Schiller Universität Jena, Germany and [2]Dept. of Biochemistry, University of Saskatchewan, Saskatoon, Canada.

Intracerebroventricular injections into intact rats with oligonucleotide probes complementary to oxytocin encoding transcripts resulted in a decrease of systemic and central oxytocin levels. Immunoreactive peptide and oxytocinergic hybridization signal in the magnocellular hypothalamic perikarya was reduced while signal for oxytocin within the neurohypophysial projections remained unaffected. Immunocytochemistry with a monoclonal antibody to triple helix resulted in an accumulation of cytoplasmic reaction product in many of the magnocellular oxytocin immunoreactive neurons. A fraction of the hypothalamo neurohypophysial processes and Herring bodies in the posterior lobe of the pituitary contained also specific triple helix immunostaining. Animals treated with the respective sense probe were devoid of cytoplasmic or axonal triple helix immunoreactivity. Immunostaining in antisense treated samples could be abolished by pretreatment of sections with RNase H. Our findings indicate that oxytocinergic transcripts form triple helix like aggregates upon specific antisense targeting rather than being degraded by endogenous RNases. Complexation of mRNA may in part be responsible for the onset of de novo transcription, thus accounting for the observed increased levels of oxytocinergic hybridization product. Cytoplasmic and axonal RNA complexes may be untranslatable and in addition may interact with peptide secretion from neurohypophysial nerve terminals. It is likely that further effects observed upon antisense treatment like protooncogene- or cytokine expression are also linked to these mechanisms.

378.9

THE YEAST SPLIT-HYBRID SYSTEM: IDENTIFYING FACTORS THAT DISRUPT PROTEIN:PROTEIN INTERACTIONS.

P.S. Goldman[1], H.M. Shih[1], A.J. DeMaggio[2], M.F. Hoekstra[2], and R.H. Goodman[1]*. Vollum Institute[1], Oregon Health Sciences University, Portland OR 97201 and ICOS Corp., Bothell WA 98021.

We have developed a variation of the yeast two-hybrid system that allows the identification of factors which disrupt the association of two interacting proteins. The "split-hybrid" system can also be used to screen for mutations which block specific associations. This yeast system turns the disruption of a protein:protein interaction into a positive selection by using the *E. coli* tet-repressor (TetR). We have engineered LexA binding sites upstream of the TetR gene, and have placed TetR binding sites (tet operators) upstream of a reporter gene, HIS3. Interaction of one protein fused to the LexA DNA binding domain with a second protein fused to the VP16 activation domain results in the expression of TetR, which binds to the tet operators, shutting off expression of HIS3 and consequently preventing yeast growth in media lacking histidine. As with the conventional yeast two-hybrid system, this method allows large numbers of clones to be screened rapidly and easily. In addition, the interaction of TetR with tet operators is a very high affinity, specific reaction, which can be abolished by tetracycline (Tc). Addition of Tc is useful when the protein fused to the LexA DNA binding domain has intrinsic activating capability. Likewise, the drug 3-aminotriazole, a competitive inhibitor of the histidine pathway, can be used when the interaction of two proteins is relatively weak and unable to produce enough TetR to block histidine production fully. We have demonstrated that the interaction of CREB and its binding protein CBP are able to prevent the growth of yeast in the split-hybrid system. We have also shown that expression of CBP and a CREB mutant (CREB-M1; Ser133Ala), which is unable to bind CBP, does not produce TetR, and allows the yeast to grow in the absence of histidine. This system should be useful for identifying proteins, peptides, and or drugs which disrupt the CREB:CBP interaction and perhaps interactions of other pairs of proteins as well.

Support has been provided by NIH RO1DK50014 and F32DK09396.

378.10

ENKEPHALIN-ENCODING SEQUENCES REPRESENT TARGETS FOR SPECIFIC DNA-BINDING FACTORS.

G. Bakalkin*, T. Yakovleva, M. Telkov and L. Terenius. *Department of Clinical Neuroscience, Karolinska Institute, S-17176 Stockholm, Sweden.*

The DNA targets for a variety of sequence-specific DNA-binding factors are generally located in the enhancer/promoter regions of different genes, at matrix associated regions, origins of replication *etc.* We here report that short, conserved and repetitive sequences encoding the Leu- or Met-enkephalin fragments in the opioid prohormone genes represent the targets for specific DNA binding factors, LEF and MEFs, respectively. Whereas LEF demonstrated high affinity and specificity for the Leu-enkephalin-encoding sequences (*PNAS 92, 9024-9028, 1995*), MEF1 and MEF2 are targeted either to the 3′-terminal Met-enkephalin-encoding sequence in the proenkephalin gene or to the Met-enkephalin/β-endorphin encoding fragment in the POMC gene, respectively. All factors are located in the cell nucleus. The expression of the factors is tissue-specific and developmentally regulated. LEF inhibited the transcription of reporter genes through the binding to the Leu-encoding fragment inserted between the transcription initiation site and coding region of the reporters. LEF, a 67 kDa protein was purified to apparent homogeneity and biochemically characterized. These observations suggest that the enkephalin-encoding sequences in the opioid prohormone genes also have regulatory functions realized through interaction with the specific DNA-binding factors.

Supported by grants from the Swedish Medical Research Council (Project 3766) and NIDA (DA-05186-04).

378.11

DIFFERENTIAL GENE REGULATION BY DISTINCT PATTERNS OF ELECTRICAL ACTIVITY. A. Buonanno*, J. Stauffer, S. Calvo, J. Cheng and M. Nakayama, UMB, LDN, NICHD, NIH, Bethesda, MD 20892-4480.

Eccles and colleagues elegantly demonstrated in the 1960's that the contractile properties of skeletal muscle are plastic, and are regulated by the specific type of innervation and activity the muscle receives. We have analyzed the transcriptional regulation of the troponin I slow (TnIs) and fast (TnIf) genes by activity, as a model to identify the factors that control activity-dependent plasticity. We found that innervation, and depolarization of denervated skeletal muscle with specific frequencies that mimic endogenous motoneuron activity (10 Hz or 100 Hz), differentially regulated either the TnIs or the TnIf gene, respectively. Successive deletions of TnI promoter sequences were analyzed in transgenic mice and cultured myocytes to identify the cis- and trans-acting factors regulating TnI transcription in either slow- or fast-twitch muscles. A 128 bp slow enhancer was identified that, when linked to the -0.5 kb TnIs promoter, specifically confers transcription to slow-twitch muscles. Sequences directing fast-specific transcription were identified in transgenic mice harboring a construct containing the TnI slow -0.5 kb promoter fused to a 144 bp fast enhancer derived from the quail TnIf gene. Mice harboring this TnIf/TnIs chimera CAT construct expressed the reporter gene in fast but not in slow muscles. These results indicate that the 2 enhancers (128 and 144 bp) are sufficient to confer fiber-type-specific transcription. Sequence alignment of the rat TnIs and the quail TnIf enhancers indicates that there is a conserved spatial organization of cis-acting elements, namely an E box, a CCAC box, a homeotic-like A/T-rich sequence (homologous to a MEF-2 site), and a previously uncharacterized motif we denoted the CAGG box. These core elements were shown to bind their cognate regulatory factors using electrophoretic mobility shift assays. Mutations of the cis-elements demonstrated that these sites are necessary for muscle-type specific expression in transgenic mice. Our results suggest that the interaction of closely related transcriptional protein-DNA complexes are utilized to restrict TnI expression to specific fiber-types in response to specific depolarization frequencies.

378.12

ANALYSIS OF THE FUNCTION OF NEURONAL INTERMEDIATE FILAMENTS IN MAMMALIAN EMBRYOS BY TARGETING MOUSE PERIPHERIN GENE. M.Simonneau[1*], M.Santha[2], J. Zakany[3], A. Zvara[2], T. Kalmar[2], C. Mas[1] and I. Rasko[2]. [1] Neurologie du Développement & INSERM U120, Hôpital Robert Debré, 75019 Paris, France. [2] Institute of Genetics, Biological Research Center, H-6701 Szeged, Hungary. [3] Department of Zoology and Animal Biology, University of Geneva, Switzerland.

Peripherin is a neuron-specific type III intermediate filament protein expressed in well-defined populations of neurons projecting towards peripheral targets. Its biological function can be approached by generating null mutant mouse carrying a disrupted peripherin gene.

We first constructed a targeting vector containing the bacterial neomycin resistance and *lacZ* genes between two homologous regions of the peripherin gene (5.8 and 1.1 kb). Analysis of transgenic mouse lines expressing this construct showed that these upstream and intragenic sequences contains all regulatory elements essential for both spatial and temporal expression of the mouse peripherin gene during embryogenesis (Leconte *et al.*, Dev. Brain Res., 1996). The replacement targeting vector contains the diphteria toxin (DT-A) gene added at the 3'end of the vector. This replacement vector was introduced into either D3 or R1 embryonic stem (ES) cells, by electroporation. With D3 ES cells, 4 homologous recombinant clones out of 96 neo resistant colonies were obtained, indicating the high efficiency of DT-A gene as a negative selection marker. We did not get chimeric animals using aggregation of these clones with eight-cell stage embryos. With R1 embryonic cells, we obtained a high degree of chimerism and germ line transmission. Null mutant mice for the peripherin gene are under investigation. Supported by ARC, DRET n° 93-137, AFM, Fondation de France and Phare Accord 0157.

378.13

MICE LACKING MAOB SHOW MARKED INCREASES IN β-PHENYLETHYLAMINE AND ANTIDEPRESSANT LIKE RESPONSES IN THE PORSOLT'S FORCED SWIM TEST. J. Grimsby[1], M. Toth[2], F.F. Karoum[3], L. Klaidman[1], J.D. Adams[1], K. Chen[1] and J.C. Shih*[1]. [1]Dept Molec Pharmacol. & Toxicol, Sch Pharmacy, USC, Los Angeles, CA 90033, [2]Dept Pharmacol, Cornell Univ Med Col, New York, NY 10021, [3]Dept Psychol & Pharmacol, NIMH, St Elizabeth's Hosp, Washington, DC 20032.

Monoamine oxidase (MAO) A and B are isoenzymes that play important roles in regulating biogenic amine levels. To examine the *in vivo* function of MAOB, we have specifically inactivated MAOB expression by targeted disruption of the mouse MAOB gene. In contrast to MAOA deficient mice, no changes in brain concentrations of serotonin, norepinephrine, dopamine and their metabolites were observed in MAOB knockout (KO) mice. Interestingly, MAOB deficient mice showed a 10-fold increase in urinary excretion of β-phenylethylamine (PEA) compared to wild-type mice. Mutant animals were less immobile in the Porsolt's forced swim test, suggesting that MAOB activity can mediate moods by regulating PEA levels. These results demonstrate that MAOA and MAOB degrade different biogenic amines and their deficieny results in stikingly different behaviors in mice. The MAOB KO mice will provide a valuable animal model for studying the molecular mechanism of antidepressants. (Supported by NIMH grants R37 MH39085 (MERIT Award), K05 MH00796 (Research Scientist Award), R01 MH37020 and Welin Professorship).

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION II

379.1

POSSIBLE ROLE OF THE GLT-1 GLUTAMATE TRANSPORTER IN REGULATING POA AND MBH GLUTAMATE LEVELS DURING THE PREOVULATORY LH SURGE. R. Unda, V. B. Mahesh*, and D. W. Brann, Dept. of Physiology and Endocrinology, Medical College of Georgia, Augusta, GA

Glutamate is the dominant excitatory neurotransmitter in the mammalian brain implicated in facilitating the LH surge. Previous work in our laboratory has demonstrated that progesterone suppression of glutamic acid decarboxylase (GAD), the enzyme that converts glutamate to GABA, appears to be a mechanism for increasing glutamate levels in the hypothalamus during the LH surge. In addition to GAD, GLT-1, EAAC1, GLAST, and EAAT are several glutamate transporters which are expressed in the hypothalamus and function to remove glutamate from the synaptic cleft. After standardizing the hybridization conditions with northern blots, dot blot studies revealed that the mRNA for the glial type glutamate transporter (GLT-1) was significantly reduced in the POA of the adult cycling animal during proestrus at 1200, 1400, 1600, and 1800 hrs. when compared to 1200 h metestrus. This reduction was attenuated by RU486 at 1200, 1400, and 1800 hrs. and abolished at 1600 h. Conversely, in the MBH GLT-1 mRNA levels showed dramatic increases at 1400 h and 1600 h in proestrus animals. The increase in GLT-1 mRNA levels during proestrus was prevented by the administration of RU486. Corresponding Western Blot analysis demonstrated that GLT-1 protein levels were also significantly reduced in the MBH by the administration of RU486 during proestrus at 1400 h and 1800 h. These studies indicate that in addition to changing the activity of GAD, progesterone regulation of GLT-1 transporters levels may also play an important role in regulating glutamate levels in the POA and MBH during the preovulatory gonadotropin surge. Supported by Grant R29HD28964.

379.2

FSH-RELEASING FACTOR(S) ARE PRESENT IN THE HYPOPHYSEAL PORTAL CIRCULATION OF THE EWE. J.S. Lee, F.J. Karsch, R.C. Thompson, P.C. Andrews, V. Padmanabhan*. Depts of Biological Chemistry, Pediatrics, Physiology, and the Reproductive Sciences Program, University of Michigan, Ann Arbor, MI 48109-0404.

By exploiting the hypophyseal portal collection technique to characterize the secretory patterns of FSH close to the release site in sheep, we have shown that: 1) FSH secretion is episodic; 2) each GnRH pulse is associated with an FSH pulse; and 3) non-GnRH-associated pulses of FSH exist in both ovariectomized and luteal phase ewes. Further, injection of the GnRH antagonist, Nal-Glu, completely abolishes LH pulsatility and GnRH-associated FSH pulses but fails to block FSH pulses not associated with GnRH pulses (Soc. Neurosci.: 20, Part 2:1058, 1994; Soc. Study Fertil., Abstr. Ser. #15:41, 1995). This suggests the non-GnRH-associated pulses of FSH are truly GnRH-independent. To test the hypothesis that hypophyseal portal blood contains factor(s) capable of releasing FSH, we validated an ovine pituitary cell bioassay sufficiently sensitive to detect FSH-releasing activity in hypophyseal portal blood. The assay involves culturing ovine pituitary cells in the presence of Nal-Glu to block GnRH action. Hypophyseal portal plasma stimulated FSH but not LH during the 4h bioassay period. Adding activin, a potent but slow stimulator of FSH, neither elicited FSH release nor increased the threshold of FSH sensitivity to GnRH, within the time frame of the bioassay. Thus, the FSH-releasing activity in the hypophyseal portal blood is also independent of activin. Preliminary characterization studies suggest the FSH releasing activity in portal plasma is heat labile, not extractable into organic solvents, and unable to pass through membranes with molecular weight cut-offs of 10,000 or 30,000. Overall these studies suggest that hypophyseal portal blood contains FSH-RF(s) other than GnRH. Supported by NIH grants U54 29184 and P30 HD 18258.

379.3

IS AN INCREASE OF NITRIC OXIDE EFFLUX IN THE MEDIAL PREOPTIC AREA COUPLED TO THE PREOVULATORY GnRH SURGE? S. Pu*, P.S. Kalra and S.P. Kalra, Depts. of Neuroscience and Physiology, Univ. Fla. Col. Med., Gainesville, FL 32610

The medial preoptic area (MPOA) contains abundant nitric oxide (NO) synthase-positive neurons which lie in close proximity to GnRH neurons. Pharmacological studies from our laboratory demonstrated that NO is involved in both the preovulatory and the ovarian steroid-induced LH surge. We recently reported that measurement of extracellular cGMP levels by microdialysis in the MPOA is a reliable index of NO efflux. Therefore, microdialysis was performed in freely-moving rats to test the hypothesis that the MPOA NO efflux may increase in association with the LH surge. A guide cannula was implanted in the MPOA of cycling and long-term ovx rats between 1700-1800 h. On the next day, a CMA/12 probe was inserted and perfused at a flow rate of 5 μl/min with aCSF. The cGMP content was determined by RIA in dialysates collected at 20 min intervals between 1100-1800 h. Intriguingly, a significant afternoon increase of cGMP levels ($p < 0.05$) with a similar profile was evident not only on proestrus, but also on diestrus II and in ovx rats. While the onset of cGMP increase occurred at slightly different times, peak cGMP values (about 400% of basal) did not different statistically among groups. This afternoon increase was absent in pentobarbital anesthetized proestrous rats. An increase of cGMP efflux was also observed in estrogen-primed ovx rats in association with the LH surge, and this increase was augmented by progesterone. Cumulatively, these results show that there is an ovarian steroid-independent diurnal increase of the MPOA NO efflux which may trigger the GnRH surge only when the neural circuitry responsible for the genesis of this surge is appropriately primed with estrogen and progesterone. (Supported by NIH grant HD08634).

379.4

EFFECT OF L-ARGININE ON NEUROPEPTIDE Y RELEASE *IN VIVO* IN THE FEMALE RHESUS MONKEY. C.L. Nyberg, W. Saltzman* and E. Terasawa, Wisconsin Regional Primate Res. Ctr., Univ. of Wisconsin, Madison, WI 53715

The mechanism regulating pulsatile LHRH release is not fully understood. In previous studies we have shown that in the rhesus monkey pulsatile LHRH release in the stalk-median eminence (S-ME) is modulated by neuropeptide Y (NPY): NPY pulses occur simultaneously with or preceding LHRH pulses, and immunoneutralization of endogenous NPY suppresses LHRH pulses, while infusion of NPY stimulates LHRH release. Since nitric oxide (NO), in the form of L-arginine (L-arg), stimulates LHRH release, NO-induced LHRH release may be mediated by NPY neurons. In this study we examined the effects of L-arg on NPY release from prepubertal (15.50±1.66 months of age, n=4) and pubertal (38.38±1.35 months of age, n=8) female rhesus monkeys using push-pull perfusion. Perfusates were continuously collected in 10-min fractions for 14 h, and L-arg at 0.1 nM, 10 nM and 1μM was directly infused into the S-ME for 10 min at 90-min intervals. NPY and LHRH in aliquots of the same perfusates were measured by RIAs. L-arg induced significant increases in both NPY release ($p<0.05$ for all doses) and LHRH release ($p<0.05$ for all doses). Both NPY and LHRH peaks occurred at 10 min after L-arg infusions in prepubertal and pubertal monkeys. Infusion of D-arg at all concentrations or of vehicle failed to induce any significant responses in NPY or LHRH in either group of monkeys. These results indicate that NO does stimulate both NPY release and LHRH release in the non-human primate, and that NO-induced LHRH release may be mediated by NPY. However, it is also possible that NO may be a common stimulator of NPY and LHRH pulses. The results further suggest that NO is not critical for the mechanism triggering the onset of puberty, since L-arg increased the release of NPY and LHRH similarly in prepubertal and pubertal monkeys. (Supported by NIH grants HD11355, HD15433 & RR00167).

379.5

INTRACELLULAR Ca^{2+} OSCILLATIONS IN CULTURED LHRH NEURONS DERIVED FROM EMBRYONIC OLFACTORY PLACODE OF THE RHESUS MONKEY. W. K. Schanhofer, and E. Terasawa*, Wisconsin Regional Primate Res. Ctr. & Neurosci. Training Prog., Univ. of Wisconsin, Madison, WI 53715

Previously, we have reported that cultured LHRH neurons derived from the olfactory placode of monkey embryos release LHRH in a pulsatile manner, and that the presence of extracellular Ca^{2+} is necessary for LHRH pulsatility. To study the mechanism of LHRH pulse generation, we have examined whether cultured LHRH neurons exhibit intracellular Ca^{2+} $[Ca^{2+}]_i$ oscillations. The olfactory placode and the migratory pathway of LHRH neurons from monkey embryos at E35-37 were dissected out and cultured on glass coverslips in a growth medium. Two to five weeks later cultured cells were labeled with the calcium dye fura-2 and examined for $[Ca^{2+}]_i$ oscillations. $[Ca^{2+}]_i$ changes were recorded every 10 sec for 30 to 100 min using a video imaging system. After recording, cells were fixed and immunostained for LHRH. Three types of cells were observed based on $[Ca^{2+}]_i$ activity: Type1 cells exhibited periodic $[Ca^{2+}]_i$ oscillations; Type 2 cells showed irregular $[Ca^{2+}]_i$ elevations; Type 3 cells showed no $[Ca^{2+}]_i$ flux. In 11 cultures 168 Type 1 cells (5-39 cells/culture) were recorded. $[Ca^{2+}]_i$ oscillations of Type 1 cells occurred with an interpulse interval (IPI) of 3.6-17.7 min (9.9±0.5, Mean±SE, n=168) and a pulse duration of 25-145 sec (87±3). IPIs and durations within a single cell were very consistent. Age of cultures was not related to the presence or periodicity of oscillations. Type 1 cells appeared to be LHRH neurons, while Type 2 and 3 cells appeared to be non-LHRH cells: Type 1 cells had long neurites and were found along long fiber bundles associated with the placode, which is characteristic of migratory LHRH neurons, while Type 2 and 3 cells lacked these characteristics. All cultures with Type 1 cells contained immunopositive LHRH neurons. These results indicate that LHRH neurons derived from embryonic olfactory placode exhibit spontaneous $[Ca^{2+}]_i$ oscillations. Whether $[Ca^{2+}]_i$ oscillations are related to pulsatile LHRH release remains to be determined. (Supported by NIH grants: HD15433, HD11355 & RR00167).

379.6

THE MEDIAN EMINENCE: A POTENTIAL SITE FOR DEVELOPMENTAL MODULATION OF PULSATILE GnRH RELEASE IN THE RHESUS MONKEY **A. R. Durrant and T. M. Plant*. Dept. of Cell Biology and Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.**

Puberty in primates is triggered by reamplification of a pulsatile discharge of gonadotropin-releasing hormone (GnRH), a hypothalamic peptide that is secreted into the hypophysial portal circulation and drives the pituitary-gonadal axis. Although the mechanisms underlying this developmental event are poorly understood, previous studies in the monkey indicate that PSA-NCAM is expressed in hypothalamic areas containing GnRH neurons and that the pubertal amplification of pulsatile GnRH release is correlated with synaptic plasticity at the level of the GnRH perikaryon. The present study extended these observations by examining ultrastructure within the GnRH neuronal network at the level of the median eminence (ME): the convergence point where neuroendocrine GnRH neurons terminate and synchronously secrete intermittent pulses of their peptide into the portal vasculature. Specifically, we used immunohistochemistry and electron microscopy to examine whether afferent neuronal processes impinge on GnRH end boutons. Two adult ME were serially sectioned, and the GnRH end boutons were identified by post-immunogold-labeling with a GnRH-specific antibody (LR1). Electron micrographs revealed that GnRH end boutons contain small, clear synaptic and large dense core vesicles; GnRH was exclusively localized in the large dense core vesicles. As previously recognized in other species, GnRH end boutons were in direct apposition with neurophenotypic and astrocytic processes. These data suggest the possibility that cotransmission and multiple signals at the level of the GnRH end bouton may developmentally modulate the pulsatile secretion of GnRH into the hypophysial portal vasculature. (Supported by HD13254 & HD08160.)

379.7

PROGESTERONE RECEPTOR ANTISENSE OLIGONUCLEOTIDES BLOCK LH SURGES IN OVARIECTOMIZED, ESTROGEN-PRIMED RATS. P.E. Chappell and J.E. Levine*. Dept. of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

Release of preovulatory LHRH and LH surges occurs subsequent to estrogen (E_2) actions in brain and pituitary, and stimulation via a daily neural signal. We have proposed that integration of E_2 feedback and neural signals mediating surge initiation occurs through ligand-independent activation of E_2-induced progesterone receptors (PRs). To further test this hypothesis, we assessed whether blockade of hypothalamic PR expression attenuates or blocks the surges in E_2-primed animals. Female rats were ovariectomized (OVX) and implanted stereotaxically with 22 ga. icv cannulae (anterior IIIrd ventricle). After 7d, rats received 30μg estradiol benzoate at 0900hrs to induce an LH surge on the following day. At 1800 hrs, rats were infused, via icv cannulae, with 1μl of 4nm PR antisense, missense, or sense oligos. A repeat infusion was administered at 0600 hrs the following day. Hourly atrial blood samples were taken from 1200hrs-2200hrs. In additional experiments, infusions were conducted similarly in intact proestrus females. RIA revealed that LH surges in rats receiving PR antisense oligos were completely blocked. By contrast, most rats receiving PR missense and sense oligos exhibited LH surges that were 6- to 8-fold higher. These data demonstrate that the LH surge in rats is dependent upon hypothalamic PR expression, even in the absence of ovarian P secretion. Our observations are consistent with previous findings using PR antagonists, and directly support the hypothesis that neuroendocrine integrative mechanisms leading to LHRH surges involve P-independent PR activation by neural signals. (Supported by NIH RO1-HD20677, P30-HD28048, PO1-HD21921).

379.8

CO-LOCALIZATION OF PROGESTERONE RECEPTORS WITH ENKEPHALIN mRNA IN THE MEDIOBASAL HYPOTHALAMUS: IMPLICATIONS FOR PHENOTYPIC CHANGE DURING HYPERPROLACTINEMIA. R. A. Baker* and G. E. Hoffman, Dept. of Neurobiology and Center for Research in Reproductive Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15261

Physiologically elevated levels of prolactin (PRL), as occur during lactation or experimentally induced hyperprolactinemia, result in induction of enkephalin (ENK) mRNA in tuberoinfundibular dopamine (TIDA) neurons in the dorsomedial division of the hypothalamic arcuate nucleus (Arc). Recent evidence suggests that for a maximal effect of elevated PRL, progesterone must also be present. If so, one must also postulate that TIDA neurons that express ENK during hyperprolactinemia have progesterone receptors. Thus, we investigated the extent of co-localization of progesterone receptors (PRs) with ENK mRNA in the Arc using a combination of immunocytochemistry and non-radioactive *in situ* hybridization histochemistry. In intact female rats rendered hyperprolactinemic by pituitary implant under the kidney capsule, nearly all PR positive neurons in the dorsomedial Arc contained ENK mRNA. These same neurons also contained TH immunoreactivity. In contrast, ovariectomized estrogen-treated (OVX + E) rats had little or no ENK mRNA in the dorsomedial Arc, although PR-ir neurons there were prominent. In another hypothalamic area, the ventromedial nucleus, nearly all PR-containing neurons co-localized ENK mRNA, in both OVX + E rats and hyperprolactinemic rats. The data demonstrate that the TIDA neurons of the dorsomedial Arc that express ENK also contain PR, thus providing a morphological substrate for contribution of PR in the induction of ENK mRNA under conditions of high prolactin. Supported by NS 28730 and HD 07332.

379.9

PLASTIC CHANGES IN LHRH TERMINALS, GLIA AND PORTAL CAPILLARIES OF THE MEDIAN EMINENCE AS A FUNCTION OF OVARIECTOMY. J.C. King*, P.M. Ronsheim and B.S.Rubin. Dept. of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA 02111.

We have been investigating the median eminence (ME) of adult rats as a site of local regulation of LHRH secretion, leading to the LH preovulatory surge and ovulation. Previously, we delineated plastic changes in the relationship of LHRH terminals to portal capillaries on the day of proestrus, using confocal microscopy and immunocytochemistry. Primarily, the LHRH terminals of proestrous females were free of glial obstruction, i.e.the end-feet of tanycytes, which intruded between LHRH terminals and portal capillaries of females sacrificed on estrus or diestrus. In this study, we examined these relationships following ovariectomy. Tanycytic end-feet were interposed between LHRH terminals and portal capillaries in ovariectomized animals, potentially reducing the quantity of LHRH reaching the portal capillaries. In contrast, loops of portal capillaries originating from the primary plexus were as extensive in their invagination into the ME of ovariectomized animals, as in intact proestrous rats. These extensive capillaries provide expanded sites for secretion of LHRH and may counterbalance the glial barrier to the portal capillaries. The factors that generate or maintain these capillary loops in ovariectomized animals are currently undefined, but clearly are not dependent on ovarian steroids. Finally, a reduction in LHRH is indicated by the loss of LHRH processes and terminals from specific regions of the ME and the smaller size of variocosities and terminals. This loss may result from the uninterrupted secretion of LHRH in ovariectomized rats, despite continual synthesis, as suggested by our earlier electron microscopic studies.

NSF IBN-9310267 (J.C.K), HD 19174 (B.S.R).

379.10

SECRETION OF LUTEINIZING HORMONE-RELEASING HORMONE BY ENZYMATICALLY DISPERSED RAT HYPOTHALAMIC CELLS IN A PERIFUSION CELL CULTURE IS PULSATILE. M.J. Woller*, E. Nichols, B. Hackett, K.H. Hoeft, T. Herdendorf, M. Jarzynski. Biological Sciences, University of Wisconsin-Whitewater, Whitewater, WI 53190.

Hypothalamic release of luteinizing hormone-releasing hormone (LHRH) has been demonstrated to be pulsatile in a variety of species and model systems. After a considerable effort by many research groups, the driving force behind the generation of pulsatile LHRH release remains highly disputed. To address the question of how LHRH neurons coordinate their secretory events to elicit pulsatile release, we used cell perifusion of primary cultured enzymatically dispersed rat hypothalami. Each chamber contained cells derived from 1/2 of a hypothalamus. Perifusion media flowed past the cells at 1ml/10min and was collected at 10min intervals for 8-10hrs. Samples were frozen at -70C until measurement of LHRH by radioimmunoassay. We measured LHRH release from cells derived from 8 rats; 5 secreted LHRH at levels measurable in 50µl aliquots of media. PULSAR analysis of the assay results from these 5 cell preps revealed 41 pulses in a total of 45 hours of sampling, a pulse frequency of 0.91 pulses/hr. Mean pulse amplitude for the cell preps ranged from 1.28 to 3.5pg/ml, with an overall mean of 2.25pg/ml. These results demonstrate clear pulsatile release of LHRH from cells in which all of the physical cell-cell connections have been disrupted by enzymatic dispersion. This suggests not only an endogenous hypothalamic 'pulse generator', but implicates an endocrine mechanism for the coordination of this pulse generator. Our use of this model has yielded a very interesting piece of evidence in our quest for an answer to how LHRH neurons communicate, and provides a line of inquiry which may lead to our 'grail' of the mechanism underlying the generation of coordinated pulsatile LHRH release by the LHRH neurons which are not synaptically connected. Supported by UW-W Grant to MW and a McNair award to EN.

379.11

CHANGES IN GONADOTROPIN RELEASING HORMONE RECEPTOR (GnRH-R) mRNA CONTENT OF THE RAT MEDIOBASAL HYPOTHALAMUS DURING THE ESTROGEN-PROGESTERONE-INDUCED LH SURGE. L. Jennes* and A. Centers. Dept. Anatomy & Neurobiol., Univ. Kentucky, Coll. Medicine, Lexington, KY 40536.

GnRH-R mRNA content in the mediobasal hypothalamus has been shown to change significantly during the course of a 4 day estrous cycle with highest levels during early morning of proestrus and lowest levels during the afternoon of proestrus. The present study was designed to characterize in detail with *in situ* hybridization the changes in GnRH-R content in the arcuate and ventromedial nuclei at 2 hour intervals beginning at 0800 and ending at 2200 hours and to correlate GnRH-R expression with circulating LH levels. The results show that in the arcuate nucleus GnRH-R mRNA levels peak between 0800 and 1000 hours before they decline rapidly by about 50% at 1200 hours. They remain low until 1600 hours before they begin to rise by 1800 hours and peak again at 2000 hours which is followed by a steep decline at 2200. GnRH-R mRNA content in the ventromedial nucleus parallels the changes in the arcuate nucleus for all time points except for the evening rise which was significantly smaller. Plasma LH levels begin to rise at 1600 hours and peak at 1800 hours.The results indicate that GnRH-R synthesis in the mediobasal hypothalamus is stimulated in the early morning, probably as a result of positive estradiol feedback in preparation of the GnRH-mediated LH surge as well as after the surge, probably as a result of homologous upregulation. Supported by NIH HD 24697.

379.12

TRANSCRIPTIONAL ACTIVATION OF THE HUMAN GNRH GENE BY THE IGF-1 RECEPTOR INVOLVES RAS AND IMMEDIATE-EARLY GENES IN THE NLT GNRH NEURONAL CELL LINE S. Zhen, and S. Radovick*, Division of Endocrinology, Children's Hospital/Harvard Medical School, Boston, MA 02115.

An immortalized GnRH-expressing neuronal cell line (NLT) established in this laboratory was used to investigate the signaling pathways involved in the regulation of human GnRH gene expression by IGF-1. NLT cells were developed by targeting the expression of the SV 40 T-antigen to the GnRH neurons in transgenic mice with the hGnRH gene 5'-promoter sequence. These cells display neuronal morphology and express neuron-specific markers MAP-2 and Tau but not the glial cell marker GFAP. An RNase protection assay and immunocytochemistry demonstrated the expression of GnRH mRNA and the decapeptide, respectively, in NLT cells. Receptor binding assays indicated the presence of IGF-1 receptors on the cell membrane. Scatchard analysis revealed a single class of high affinity binding sites for IGF-1, with a K_d of $5.0x10^{-10}$M and a receptor number of $3.2x10^5$/cell. The NLT cells were transfected with DNA constructs containing hGnRH gene regulatory sequences fused to the luciferase reporter gene. A significant increase in reporter activity was induced by IGF-1 treatment of cells transfected with -412 bp of the hGnRH gene promoter. Sequence analysis revealed the presence of a unique AP-1 binding site in the human GnRH gene promoter region located between -402 and -396 bp. Mutation of this AP-1 binding site rendered the promoter unresponsive to IGF-1 stimulation. A significant increase in the expression of c-jun and c-fos was induced by IGF-1 treatment of the NLT cells. Transfection of these cells with a constitutively active Ras plasmid produced a similar increase in -412 hGnRH reporter activity and in the expression of c-jun and c-fos after treatment with IGF-1. Transfection of a dominant-interfering Ras plasmid decreased the IGF-1 induction of the hGnRH reporter construct. These results demonstrate that 1) IGF-1 receptors are expressed in the NLT GnRH-expressing neurons; 2) activation of IGF-1 receptors stimulates hGnRH gene expression; 3) Ras, c-jun and c-fos are components of the signaling pathways by which the IGF-1 receptor activates hGnRH gene expression.

NIH HD30040, ACS DB-78

OPIOID RECEPTORS III

380.1

µ-OPIOID RECEPTOR IMMUNOLABELING IS PRESENT IN GABAERGIC NEURONS AND THEIR TARGETS IN THE RAT NUCLEUS ACCUMBENS. A.L. Svingos*[1], A. Moriwaki, J.B. Wang, G.R. Uhl and V.M. Pickel[1]. Cornell Univ. Med. Col., Dept. of Neurol. & Neurosci., NY, NY, 10021[1], Intramural Res. Prog., NIDA and Depts. of Neurol. & Neurosci., Johns Hopkins Univ., Baltimore, MD, 21224. Stimulation of µ-opioid receptors (MOR) in the nucleus accumbens (NAC) can produce both hypo- and hyperactivity, that have been attributed to modulation of activities of local neurons, many of which contain γ-aminobutyric acid (GABA). To anatomically test this hypothesis, we combined immunogold-silver detection of MOR with immunoperoxidase labeling of GABA. MOR-like immunoreactivity (MOR-LI) was mainly seen at extra-synaptic plasmalemmal sites in GABA-immunoreactive spiny and aspiny neurons. From 524 examples of MOR/GABA associations, 46% were in dendrites that colocalized MOR and GABA immunolabeling. The remainder of MOR/GABA associations included colocalization in soma and axon terminals, or synaptic and appositional contacts between differentially labeled neurons. The latter group included convergence of separately labeled MOR and GABA terminals onto common dendrites, and contact between terminals containing only GABA and MOR-labeled dendrites. MOR has a subcellular distribution consistent with opiate roles in modulating 1) postsynaptic responses in dendrites of GABAergic neurons, and 2) presynaptic release of GABA from axons in the NAC. MOR ligands may also alter presynaptic and/or postsynaptic responses in non-GABAergic neurons subject to GABA inhibition. The multiple sites of MOR are likely to contribute to the diverse locomotor and rewarding effects of opiates in the NAC. (Supported by the Aaron Diamond Foundation to ALS, NIDA DA04600 to VMP and the NIDA Intramural Res. Prog.).

380.2

REGIONALLY SPECIFIC UP-REGULATION OF MU AND DELTA OPIOID RECEPTOR BINDING IN ENKEPHALIN KNOCKOUT MICE. L. S. Brady*, M. Herkenham, R. B. Rothman, J. S. Partilla, M. König, A. M. Zimmer, and A. Zimmer. Section on Functional Neuroanatomy (LB, MH) and Unit on Developmental Biology (MK, AMZ, AZ), Laboratory of Cell Biology, NIMH, Bethesda, MD 20892 and Clinical Psychopharmacology Section (RR, JP), Addiction Research Center, NIDA, Baltimore, MD 21224.

Enkephalin (Enk) -/- mice, generated by gene targeting, were used to examine the physiological role of enkephalins in regulating the levels and functions of opioid receptors in brain. In fresh frozen brain sections, mu and delta receptors were labeled with [^{125}I]D-ala^2, N-me-phe^4, gly^5-ol-enkephalin or [^{125}I]deltorphin-II, respectively. In wild type Enk +/+ mice, moderate levels of mu binding were seen in the hypothalamus, amygdala, midline thalamus, rostral striatum, and nucleus of the solitary tract; lower levels of binding were seen in caudal striatum, cortex, hippocampus, brainstem, and spinal cord. In the Enk -/- mice, mu binding was greatly elevated in the ventral pallidum, preoptic area, hypothalamus, and amygdala. Increases in mu binding were also seen in the ventromedial striatum, midline and intralaminar thalamic nuclei, tectum, locus coeruleus, parabrachial nucleus, and nucleus of the solitary tract. In the Enk +/+ mice, moderate to dense levels of delta binding were seen in olfactory bulb, striatum, cortex, amygdala, and hippocampus. Significant elevations in delta binding in the Enk -/- mice were restricted to the neostriatum. The striking up-regulation of mu receptors in limbic areas, notably the amygdala, is consistent with the increased emotional and aggressive behaviors observed in the Enk -/- mice.

(Support: NIMH and NIDA Intramural Research Programs)

380.3

DISRUPTION OF THE MU-OPIOID RECEPTOR GENE IN MICE BY HOMOLOGOUS RECOMBINATION. H. Matthes, F. Simonin, S. Slowe, I. Kitchen, P. Dolle, R. Maldonado and B. L. Kieffer*. CNRS UPR 9050; ESBS Parc d'innovation Bld S. Brand 67400 ILLKIRCH, France.

Opioid receptors mediate the strong analgesic and addictive properties of opiates. Recently their genes have been cloned (for rev see Kieffer B. L. 1995 Cell Mol Neurobiol vol 15, 615) allowing study of their role *in vivo* by genetic tools. The mu receptor is considered as the primary target of morphine. To assess the specific contribution of this opioid receptor type in pain control and reward, we have disrupted its gene by homologous recombination. A 12 kb genomic fragment was obtained from a mouse library and the neomycin phosphotransferase gene (neo) under control of the PGK promoter was inserted into the coding sequence. The targeting vector was electroporated into D3 embryonic stem cells. We analysed 92 neomycin resistant colonies which we screened using a 5' BamHI probe. We found 7 positive clones, representing a targeting frequency of 1/13. Southern analysis using a 3' BamHI probe and the neo probe confirmed accurate integration of the neo gene. One positive clone was microinjected into mouse blastocysts and gave rise to chimaeric mice which in turn were bred with C57BL/6 females. Germline transmission occurred, leading to heterozygous offsprings which were further bred for the obtention of homozygous mice. Opioid receptor expression and response to morphine of these mice is currently under investigation.

380.4

DESENSITIZATION AND DOWN-REGULATION OF THE HUMAN KAPPA OPIOID RECEPTOR. J. Zhu*, L.-Y. Luo, C. Chen, and L.-Y. Liu-Chen Dept. of Pharmacology, Temple Univ. Sch. of Med., Philadelphia, PA 19140

Activation of the κ opioid receptor inhibits adenylate cyclase, enhances K^+ conductance or reduces Ca^{++} conductance via pertussis toxin-sensitive G proteins. We recently cloned a human κ opioid receptor. In addition, we demonstrated that activation of the human κ receptor expressed in CHO cells enhanced [^{35}S]GTPγS binding. In this study, we examined desensitization and down-regulation of the human κ opioid receptor after exposure to the κ agonist U50,488H. Preincubation with U50,488H (1 μM) from 15 min to 4 h reduced in a time-dependent manner the magnitude of increase in [^{35}S]GTPγS binding by the subsequent application of U50,488H. The B_{max} values of [^{3}H]diprenorphine binding to the κ receptor were significantly reduced after a 4-h exposure to U50,488H (1 μM), but not after 15-min or 1-h exposure. Pretreatment of cells for 4 h with 0.1 nM to 1 μM of U50,488H decreased in a dose-dependent fashion [^{35}S]GTPγS binding by the subsequent U50,488H incubation. Pretreatment with 10 nM or 100 nM U50,488H decreased B_{max} values by about 30%, similar to 1 μM of U50,488H, whereas 0.1 nM U50, 488H had no effect. Thus, desensitization and down-regulation of the κ receptor occur following agonist exposure and represent two different adaptation processes. (Supported by NIH grants DA04745 and T32 DA7237)

380.5

AGONIST-SELECTIVE INTERNALIZATION OF OPIOID RECEPTORS IN VITRO AND IN VIVO. Chris Evans*@, Duane Keith Jr.@, Paulette Zaki@, Benito Anton@, F. Ivy Carroll^, George Brine^, Mark von Zastrow#. @Dept. of Psychiatry, UCLA, Los Angeles, CA 90024, # Dept. of Psychiatry, UCSF, San Francisco, CA 94143, ^Research Triangle Institute, Research Triangle Park, NC 27695.

Using 293 cells transfected with epitope-tagged mu and delta opioid receptors, we have studied agonist-induced internalization using flow cytometry and immunocytochemistry. We have previously shown that opioid peptides and the alkaloid agonist etorphine induce rapid internalization of mu and delta receptors, whereas morphine does not induce internalization of either receptor. We have tested a number of additional opiate ligands for their ability to internalize receptors in order to ascertain which structural components are required for internalization. We have also analyzed internalization of mu opioid receptors in rat brain. As seen in the cell line, etorphine, but not morphine, triggers a dramatic redistribution of mu receptors from plasmalemma to intracellular vesicles. This internalization occurs in virtually all areas containing detectable receptor in somata and neuropil. In addition, after etorphine treatment there is enhanced staining in deeper layers of neocortex and in matrix-like compartments in the caudate putamen. In untreated animals, these areas contain little or no receptor staining. The mu receptor containing vesicles are shown to be intracellular when viewed by confocal microscopy. Double labeling with tranferrin receptor in cells that contain both receptors suggests that these vesicles are early endosomes.

Work supported by NIDA grant # DA-05010

380.6

IMMUNOLOGICAL CHARACTERIZATION OF OPIOID BINDING SITES IN NG108-15 SUBCELLULAR FRACTIONS. C. J. Coscia*, E. G. Ignatova, and M. M. Belcheva, Dept. Biochem. and Mol. Biol., St. Louis Univ. Sch. of Med. St. Louis, MO 63104

C- and N-terminal-directed antipeptide antibodies to the δ opioid receptor were used to characterize opioid binding sites in NG108-15 subcellular fractions. In immunoblotting experiments, the C-terminal antibody detected two major bands in both plasma membrane-enriched (P_{20}) and nuclear matrix preparations. The higher mol. wt. protein migrates at 64 kDa and another band was found at 33 kDa. In P_{20} preparations the N-terminal antibody detected two lower mol. wt. proteins at 38 and 33 kDa in addition to higher mol. wt. bands. The intensity of all bands except that of the 38 kDa protein were reduced in nuclear matrix preparations. To determine which of the proteins are glycosylated, P_{20} membranes were solubilized and passed over wheat germ agglutinin columns. The N-terminal antibody detected a single band (72 kDa) in N-acetyl-D-glucosamine eluates. The results are consistent with cross-linking data suggesting that nuclear matrix associated opioid binding sites represent truncated forms of the glycosylated δ opioid receptor found in P_{20} membranes. Supported in part by NSF grant IBN-9121750.

380.7

CORTICOSTERONE REGULATES GENE EXPRESSION OF OPIOID RECEPTORS IN THE HIPPOCAMPUS. **A. Ableitner, D. Fischer, J. Deicke, V.K. Patchev*, O.F.X. Almeida. Max Planck Institute of Psychiatry, Clinical Institute, 80804 Munich, Germany**

The action of glucocorticoids upon the hippocampal formation plays an important role in the regulation of the hypothalamo-pituitary-adrenal axis and the adaptation to stress. The hippocampal opioidergic system which is activated by several stressors might be a candidate for glucocorticoid-dependent adaptive changes resulting from alterations in the gene expression of opioid peptides or receptors. Using ribonuclease protection assays, the present studies were designed to examine the possibility of alterations in the gene expression of hippocampal μ-, δ- and k-opioid receptors in response to varying corticosterone levels. Removal of endogenous corticosterone by adrenalectomy (1 week) had no effect upon k-opioid receptor mRNA levels, while a trend for an increase in δ-opioid receptor transcripts was observed. Concentrations of mRNA encoding μ-opioid receptors were, however, significantly reduced in adrenalectomized animals, as compared to sham-operated controls, and this decrease was prevented by supplementation with physiological doses of corticosterone. These data indicate an interaction between glucocorticoids and the hippocampal opioidergic system, which may have implications in the behavioural and endocrine response to stress.

Supported by the Max Planck Society and a grant from the Federal Ministry of Education and Research (BMBF).

380.8

OPIOIDS REGULATE THE DEVELOPMENT OF NEURONS, ASTROCYTES AND OLIGODENDROCYTES: MULTIPLE ACTIONS AND RECEPTORS SEGREGATE OPIOID FUNCTIONS AMONG DISSIMILAR CELL TYPES. K.F. Hauser*, A. Stiene-Martin, W. Maxson, and P.E. Knapp, Dept. of Anatomy & Neurobiology, Univ. of Kentucky Med. Ctr., Lexington, KY 40536.

Opioids modify nervous system development through both direct and indirect mechanisms. To identify which classes of cells are directly affected by opioids during maturation, we systematically examined the role of μ, δ, and κ opioid receptors on the maturation of postnatal murine neuroblasts, astrocytes, and oligodendrocytes *in vitro*. Opioid receptor expression was assessed immunologically (antibodies courtesy Dr. R.P. Elde), and functionally by assessing growth. Cerebellar neuroblasts uniformly express μ and δ, but not κ, opioid receptors. Treatment with δ agonists significantly inhibited cellular differentiation in newly postmitotic neurons, and preliminary results suggest that δ agonists also inhibited neuroblast division. In contrast, immature oligodendrocytes uniformly expressed μ, but not κ or δ, opioid receptors, and treatment with μ agonists caused about a 300% increase in DNA synthesis. Lastly, subpopulations of astrocytes possessed μ, κ, and/or δ opioid receptor immunoreactivity. Exposure to agonists selective for μ or κ opioid receptors significantly inhibited DNA synthesis in distinct subpopulations of astrocytes. Proliferation was unaffected by δ receptor activation; however, few astrocytes expressed δ receptors compared to μ or κ receptors. Importantly, our findings suggest that opioids intrinsically regulate neural development by differentially affecting the maturation of neurons, astrocytes, and oligodendrocytes. In addition, findings that opioid receptor types are segregated among classes of cells suggest a mechanism by which the endogenous opioid system discriminates among dissimilar cell types. Opioid diversity may permit the independent regulation and/or the coordination of separate developmental events in different neural cell types. Support NIH DA06204.

380.9

OPIOIDS REGULATE CYTOSKELETON PROTEINS IN EMBRYONIC NEURONS. D. Mangoura* and S. Leung. Peds, The Univ. of Chicago Sch. of Med, Chicago, IL 60637.

We have been studying the mechanism of action of opioids in regulating the cytoskeleton in developing neurons. Using early postmitotic, presynaptic cultured neurons derived from chick embryo cerebral hemispheres (E6CH), we previously showed that the G-protein opioid receptor causes protein tyrosine phosphorylation of both kinases and cytoskeleton-associated proteins. Specifically, 1μM met-enkephalin activated $pp125^{FAK}$, a focal adhesion tyrosine kinase, and $pp60^{src+}$, the neuron-specific isoform of the cytosolic tyrosine kinase src. Met-enkephalin also caused tyrosine phosphorylation of vinculin, an actin binding protein. We have extended our studies to other cytoskeleton-associated proteins and known src substrates. We now report that p80/85, which forms complexes with src upon agonist stimulation, is tyrosine phosphorylated in response to opioids. The phosphorylation was seen as early as 2 min of incubation with met-enkephalin , peaked by 5 min, and declined by 15 min. All met-enkephalin-stimulated signaling was inhibited by the antagonist naloxone. We also found that several growth factors (namely EGF and FGF) acting through receptors with intrinsic tyrosine kinase activity, increased the phosphotyrosine content of p80/85 in E6CH neurons. These data further elucidate the actions of opioids early in development. Furthermore they suggest that cytoskeleton proteins may be the converging points of two different signaling pathways and therefore that the regulation of such proteins may serve as a crosstalk point between two pathways in orchestrating the complex homeostasis of the neuronal cytoskeleton. (HD 09402 and BRF).

380.10

STATISTICAL PARAMETRIC MAPPING (SPM) ANALYSIS OF PET IMAGES OF BRAIN MU OPIOID RECEPTORS REVEALS HIGHER REGIONAL BINDING IN HEALTHY WOMEN COMPARED TO MEN. N. Ilgin, B. Bencherif, M.Kruger, M.J. Stumpf, J.J. Frost*.The Johns Hopkins Medical Institutions, Baltimore, Maryland.

Sex differences in brain structure and function have been described but little is known about the relations of sex and opioid receptors in the human brain. Mu-opioid receptor binding was measured with PET and C-11 carfentanil (CFN) in 10 male and 10 age-matched female controls. PET data was acquired with a GE 4096 PET scanner (FWHM resolution 7mm) following i.v. administration of 20±2 mCi C-11 CFN. Positioning in the PET scanner was standardized by using a thermoplastic mask and the amygdala-occipital cortex plane was localized using MRI. Specific binding images were obtained after summing images from 36-82 min. and correcting for nonspecific binding by use of the occipital cortex counts ([region-occipital]/ occipital). Data were then spatially normalized to the Talairach atlas and smoothed (10 mm Gaussian filter). AnCova analysis was generated using adjusted means for the 2 pooled groups (males vs females) and the resulting SPM(t) maps were transformed to SPM(z) maps (threshold=2.33, $p \leq 0.05$). Significantly higher mu opioid receptor specific binding was found in females compared to male controls in the hypothalamo-thalamic region ($Z=3.54$, $p<0.05$) and the same trend of higher binding was observed in anterior cingulate gyrus, amygdalae and temporal cortex with lesser Z scores. These results demonstrate that SPM analysis can detect higher brain mu opioid receptor binding in healthy females compared to age matched males. Differences in regional mu opioid receptors may play a role in mediating emotional and cognitive differences in men and women.

380.11

IDENTIFICATION OF NEW, HIGHLY ACTIVE LIGANDS FOR THE ORPHANIN RECEPTOR USING COMBINATORIAL LIBRARIES R.A. Houghten*, C. Spaeth, L. Toll[1], C. T. Dooley, Torrey Pines Institute for Molecular Studies, San Diego, CA, 92121; [1]SRI International, Menlo Park, CA, 94025.

Orphanin FQ (nociceptin) is the natural ligand for ORL1, and it is thought that antagonists to this peptide may have a useful role in pain therapy. A tritiated form of the natural heptadecapeptide ligand for ORL1 has been prepared, and was used to develop a radioreceptor assay using rat brain homogenates. Binding data indicate the presence of a single binding site with a dissociation constant of 5 ± 1.1 nM and Bmax of 535 ± 85 fmoles/mg protein. Thirty-four analogues of orphanin FQ, including a complete alanine "scan" of orphanin FQ, and truncation analogues from both the N- and C- terminals were synthesized and tested. The data obtained indicate that the N-terminus plays a more critical role in binding than the C-terminus and that residues 1, 2, 4, and 8 are essential for binding. Using combinatorial libraries, new ligands from libraries containing millions of different compounds were identified. The compounds were found to be agonists, inhibiting electrically induced contractions of the mouse vas deferens.

Funded by Houghten Pharmaceuticals and NIDA Grant DA09410.

DEVELOPMENTAL GENETICS III

381.1

THE REELIN PARADOX: LACK OF CORRELATION BETWEEN EXPRESSION OF REELIN AND THE REELER PHENOTYPE. C. Lambert de Rouvroit, B. Bernier, S. Schiffmann, V. de Bergeyck, A.M. Goffinet*. Dept. Physiology, FUNDP Sch. Med., Namur, Belgium

Expression of the reelin gene is affected in five alleles of the reeler mutation so that alterations of reelin are most probably the cause of the reeler phenotype.

In the present work, the expression of reelin mRNA and protein was studied during mouse brain development from embryonic day 13 (E13) to maturity. mRNA expression was followed using *in situ* hybridization of radiolabeled oligonucleotides or digoxigenin riboprobes, while the reelin protein was revealed with monoclonal antibodies generated against an *E.coli* fusion protein corresponding to an N-terminal segment of the reelin sequence. Both methods revealed consistent results, with strong reelin expression in developing Cajal-Retzius cells, olfactory bulb, cerebellar granule cells, spinal cord and retina, all of which are minimally affected in homozygous reeler mice. Moderate to low expression was found in tectum, striatum, basal forebrain, while no expression was seen in the cortical plate, radial glial cells, Purkinje cells and most of the hindbrain, all of which have been shown to be abnormal in reeler mice.

Those results demonstrate a lack of correlation between reelin mRNA and protein expression and the reeler phenotype. Those discrepancies probably indicate an indirect action of reelin on radial glial cells and/or on postmigratory neurons. Such an action could be explained by postulating that reelin acts in the extracellular matrix as a cell-repulsive molecule.

381.2

SCRAMBLER AND REELER: MORPHOMOLECULAR ANALYSIS OF MUTANT CEREBELLA INDICATE ONE PHENOTYPE FROM TWO DIFFERENT GENES. D. Goldowitz*, E. Laywell, D. Steindler, G. D'Arcangelo[1], M. Sheldon[1], T. Curran[1], M. Davisson[2], K. Johnson[2], H. Sweet[2]. Dept. of Anat. & Neurobiol., Univ. of Tenn. Coll. of Med. and [1]Dept. Devel. Neurobiol., St. Jude Children's Res. Hosp., Memphis, TN, 38163; [2]The Jackson Laboratory, Bar Harbor, ME.

The reeler mutation has profound effects on neuronal migration and cortical structure in the mouse. The gene mutated in reeler mice has been shown to code for a novel extracellular protein called Reelin (D'Arcangelo et al., 1995). More recently, a new neurological mutant mouse, scrambler, has been found that exhibits a phenotype very similar to that of reeler — but maps to a different region of the mouse genome (Sweet et al., in press).

Developing and adult scrambler cerebella were studied to assess the number and placement of Purkinje cells, molecular markers of cerebellar compartments, and *reelin* gene expression. The scrambler cerebellum is marked by ectopic nests of calbindin-positive Purkinje cells that lie deep to a greatly reduced population of granule cells. The size, number, and placement of these Purkinje cell nests are similar to those seen in the reeler cerebellum. The number of ectopically and normally placed Purkinje cells is similar in both scrambler and reeler cerebella (Heckroth et al., 1988). Finally, scrambler Purkinje cells are partitioned into Zebrin II-positive and Zebrin II-negative parasagittal bands like those seen in the reeler cerebellum (Edwards et al., 1994). At postnatal day 6, GFAP and tenascin immunoreactivites are co-extensive and encircle the nests of scrambler Purkinje cells, as well as marking a midline raphe. These features are virtually identical to what is seen in the reeler mouse.

In spite of numerous phenotypic similarities, genetic analyses indicate that the scrambler and reeler genes are different. First, the scrambler gene maps to mouse Chromosome 4 while reelin maps to 5. Second, mRNA analysis shows normal *reelin* expression in the brain of scrambler mice. Thus while the scrambler mutant appears to be a neurological phenocopy of the reeler mouse, the genetics point to separate genes. The *scrambler* gene product may interact with Reelin itself, or influence cellular events subsequent to Reelin action. One tantalizing possibility is that the *scrambler* gene may encode a Reelin receptor expressed on target cells.Supported by NS23475, NS29225, P40 RR01183, BIR 9502221, P30 CA21765, and ALSAC.

381.3

DEVELOPMENTAL CHANGES IN mRNA LEVELS IN SINGLE CAJAL-RETZIUS NEURONS P.B. Crino*1, T. Pribyl 2, K. Nakajima 3, T. Miyata 3,4, M. Ogawa 4, K Mikoshiba 5, A. Campagnoni 2, J Eberwine 1. 1 Univ. of Pennsylvania Sch. of Med., Philadelphia, PA ; 2 UCLA Hospital, Los Angeles CA; 3 RIKEN, Japan; 4 Kochi Medical Sch., Japan; 5 Univ Tokyo, Japan;

Cajal-Retzius (C-R) neurons arrive first in layer I of the cortical plate and may provide trophic cues to neurons destined for layers II-VI. The patterns of gene expression in C-R neurons during development may provide insight into how C-R cells effect cortical lamination. We used immunohistochemistry with single cell mRNA amplification to assess mRNA levels in individual C-R cells. Rat brain sections at (E)16 and (E)18 were labeled with Golli-MBP (Golli) antibodies which label C-R and other neurons. Sections were also labeled with the CR-50 antibody to confirm the Golli C-R neurons. CR-50 specifically recognizes C-R neurons. cDNA synthesis from poly(A) mRNA was performed using an oligo-dT-T7RNA polymerase primer-promoter and Avian myeloblastosis reverse transcriptase on Golli stained sections. Single Golli stained C-R neurons were aspirated into microelectrodes and double stranded cDNA was synthesized. ^{32}PCTP radiolabeled aRNA was generated with T7 RNA polymerase and was used as a probe for reverse Northern blotting. Numerous candidate mRNAs were detected including mRNA encoding cytoskeletal elements (neurofilaments, internexin, and activity regulated cytoskeleton associated protein), neurotrophic factor receptor and transcription factor mRNAs at differing abundances between E16 and E18. GAD65 and several GABA A receptor subunit mRNAs were also detected at differing abundances which supports the hypothesis that C-R neurons are GABAergic. Changes in the relative abundance of multiple mRNAs during development in single immunohistochemically defined C-R neurons provides important insight into the role that C-R neurons play in cortical lamination. cDNA libraries from these single cells will help to determine the molecular fingerprint of C-R neurons and potentially provide novel C-R cell markers for each stage of cortical development.

P.Crino is a Howard Hughes Medical Institute Fellow; JE grant AG9900

381.4

INDUCTION OF NEURAL GENE EXPRESSION IN MUSCLE BY ECTOPIC EXPRESSION OF DOMINANT NEGATIVE REST. **M. Frohman*, J. Wells, and Y. Zhang. Institute for Cell and Develop. Biology, SUNY Stony Brook, Stony Brook, NY 11794-8651**

Numerous genes are expressed exclusively in neurons, such as the type II Na Channel, Synapsin, and SCG10. Exploration of such restricted neural specific gene expression in neural and non-neural cell lines has led to the general finding that most such genes are controlled in the important sense via negative regulation.The genes contain positive enhancer elements that encourage wide-spread expression, and negative enhancers that restrict expression to neural cells only. REST is a zinc-finger transcription factor that was identified as a silencing (repressing) factor that bound to the negative element of the Type II Na Channel gene and SCG10; it is now appreciated that at least 20 neural-specific genes contain this element and are potentially regulated by REST. Consistent with this hypothesis, REST is expressed in embryos in all non-neural cells. In addition, REST is expressed in neuroblasts, which also don't express the neural-specific genes described above. To determine the role of REST in vivo, we have ectopically expressed REST in vivo using the engrailed promoter to direct expression to the cerebellum, and have ectopically expressed dominant-negative REST in vivo to muscle, using a myosin-light-chain promoter. Inhibition of and ectopic expression of the neural genes described above was monitored using in situ hybridization. Preliminary results indicate that REST regulates neural differentiation, and that REST in muscle cells represents the only mechanism through which widespread neural gene expression in non-neural cells is normally prevented.

This work was funded by grants from the NIH to MAF.

381.5

TISSUE- AND DEVELOPMENTAL-SPECIFIC HUMAN MAP1A GENE EXPRESSION IS DETERMINED BY 1.5 KB OF 5' FLANKING SEQUENCE. A. Kume, J. Wilkowski, S.M. Jones, S. Rainier, T. Reinglass, T.L. Saunders, J.K. Fink*. Univ. Michigan, Dept. of Neurology and GRECC, VAMC, Ann Arbor, MI 48109.

Microtubule associated proteins (MAPs) regulate microtubule stability in axons and dendrites. Developmental changes in MAP gene expression suggest that MAPs present before birth (eg. MAP1b) are involved in neuronal cytoskeleton formation; MAPs present after birth (eg. MAP1a) are involved in maintaining the neuronal cytoskeleton. We are interested in determining what regulates the developmental switch in MAPs gene expression. Previously, we cloned human the MAP1a gene, its 5' flanking sequence contained a consensus promoter (TATA) element. For the current study, we placed the β-gal gene under the control of 1.5 kb of MAP1a's 5' flanking sequence and prepared transgenic mice. 16 founders and their progeny were examined histologically for transgene expression. Transgene expression was confined to the CNS and appeared only in neurons and ependymal cells. The magnitude of transgene expression was variable between animals. We compared transgene expression at embryonic day 14, at birth, and older than 2 mo of age. Day 14 embryo showed no expression; mice examined at birth had approximately 10% of adult levels of β-gal. Our results correlate with developmental changes in MAP1a protein concentrations: in rats, MAP1a is detectable several days before birth and increases to a maximum by day 20. Our results indicate that 1.5 kb flanking MAP1a contains cis-acting elements that regulate developmental and tissue-specific MAP1a gene expression. This information will expedite discovery of trans-acting factors that regulate MAP1a gene expression and advance our understanding of the molecular regulation of the neuronal cytoskeleton.

381.6

DEVELOPMENTALLY REGULATED EXPRESSION OF SENSE AND ANTISENSE TRANSCRIPTS OF *HSP70.2* GENE IN MICE. A. K. Murashov* and D. J. Wolgemuth, Dept. of Obstetrics & Gynecology and Genetics & Development, Columbia Univ. Coll. of Physicians & Surgeons, NY 10032.

We have recently shown that the murine gene *hsp70.2*, discovered by virtue of its abundant expression in germ cells during spermatogenesis, is also expressed in adult mouse brain. Moreover, we have made the surprising observation of *hsp70.2* antisense transcription in several brain areas such as the cortex, hippocampus, superior and inferior colliculi, thalamic nuclei, cerebellum, and brainstem detected on both Northern and in situ levels. We now report that *hsp70.2* sense and antisense transcripts are expressed in specific spatio-temporal patterns in the developing mouse central nervous system. In situ hybridization analysis revealed overlapping expression of the *hsp70.2* sense and antisense transcripts in developing brain from embryonic d 9.5 to postnatal day 17 mice. In midgestation embryos the expression of sense and antisense transcripts was found in developing spinal cord, hindbrain, neocortex, and spinal ganglions. During postnatal development, particularly strong expression of both transcripts was observed in developing cerebellar and cerebral cortexes, hippocampus and olfactory bulbs. Immunocytochemical analysis of the hsp70.2 protein encoded by the *hsp70.2* sense strand was performed using A2 antibody (generously provided by M. Eddy) revealed that the pattern of hsp70.2 protein expression in the developing brain corresponded to that of its mRNA, suggesting that regulation at the level of translation is not a critical feature of the *hsp70.2* antisense transcript. RT-PCR analysis using pairs of gene-specific primers showed that the *hsp70.2* sense and antisense transcripts share at least 2.3 kb of genomic sequence.

(Supported by NASA NAGW 4462 and ONR N00014-96-1-0563)

381.7

A RANDOM MUTAGENESIS SCREEN FOR NEUROCRANIAL MUTATIONS IN THE MOUSE. P.M. Nolan*, S.A. Alivizadeh, C. Lo# and M. Bucan. Depts. of Psychiatry and Biology#, University of Pennsylvania, Philadelphia, PA 19104.

A random mutagenesis screen for dominant behavioral mutations in the mouse provides us with an additional class of mutations - those that affect neurocranial development. While in some cases abnormal behavior in heterozygous mice is indicative of disrupted neuronal development, the simple observation of heterozygotes with abnormal craniofacial features can also hint at a striking neurodevelopment defect in homozygotes (Matsuo et al., *Genes and Development* 9, 2646-2658, 1995). Based on the high incidence of mice with craniofacial anomalies in our F1 screen, we hypothesize that the C57BL/6J strain of mice, used here as a genetic background, is highly sensitized to neurocranial defects.

Here, we present phenotypic analysis of two mutations that affect craniofacial development: a novel mutation 4Z and the previosly described *Whl* mutation (Nolan et al., *Genetics* 140, 245-254, 1995; Pickard et al., *Brain Research* 705, 255-266, 1995). *Whl* has a complex neurological and behavioral phenotype in heterozygotes, while homozygotes die *in utero* between embryonic day (E) 9.5 and E11.5. Histological analysis indicates that homozygote embryos have severely underdeveloped forebrain and midbrain structures. The mutant locus maps to the subcentromeric region of mouse chromosome 4. Two candidate genes, *Brain2 (Brn2)* and *Embryo brain kinase (Ebk)* , and other embryonic markers are being used to further characterize the neurodevelopmental defect caused by the *Whl* locus.

Supported by AFOSR F49620-94-1-0234 and NIH HD28410 (MB)

382.1

TRACKING VIRTUAL TRAJECTORIES. Leland S. Stone*, Brent R. Beutter, & Jean D. Lorenceau, NASA Ames Res. Ctr., Moffett Field, CA 94035-1000.

Current models of smooth pursuit eye movements assume that it is largely driven by retinal image motion (e.g. Krauzlis & Lisberger, 1991). We tested this hypothesis by measuring pursuit of elliptical motion (3.2s, 0.9 Hz, 1.4° x 1.6°, 4 randomly interleaved phases) of either a small spot ("real" motion) or of a line-figure diamond viewed through apertures such that only the motion of four isolated oblique line segments was visible ("virtual" motion). Each segment moved sinusoidally along a linear trajectory yet subjects perceived a diamond moving along an elliptical path behind the aperture (Lorenceau & Shiffrar, 1992). We found, as expected, that real motion produced accurate tracking (N = 2) with mean gain (over horizontal and vertical) of 0.9, mean phase of -6° (lag), mean relative phase (H vs V) of 90±8° (RMS error). Virtual motion behind an X-shaped aperture (N= 4 with one naive) yielded a mean gain of 0.7, mean phase of -11°, mean relative phase of 87±15°. We also measured pursuit with the X-shaped aperture using a higher segment luminance which prevents the segments from being grouped into a coherently moving diamond while keeping the motion otherwise identical. In this incoherent case, the same four subjects no longer showed consistent elliptical tracking (RMS error in relative phase rose to 60°) suggesting that perceptual coherence is critical. Furthermore, to rule out tracking of the centroid, we also used vertical apertures so that all segment motion was vertical (N = 3). This stimulus still produced elliptical tracking (mean relative phase of 84±19°), albeit with a lower gain (0.6). These data show that humans can track moving objects reasonably accurately even when the trajectory can only be derived by spatial integration of motion signals. Models that merely seek to minimize retinal or local stimulus motion cannot explain these results. *NASA RTOP 199-16-12-37*

382.2

MICROSTIMULATION IN DORSOMEDIAL FRONTAL CORTEX AFFECTS SMOOTH PURSUIT EYE MOVEMENTS S.J. Heinen*, The Smith-Kettlewell Eye Research Institute, San Francisco, CA

Smooth eye movements have been previously evoked with electrical microstimulation from the dorsomedial frontal cortex (DMFC) in anesthetized monkeys (Tian, 1995). Single-unit recording experiments in our lab have implicated the DMFC in visually-guided (Heinen, 1995) and predictive smooth-pursuit control (Heinen, 1994). We show here that stimulation can affect smooth pursuit in alert primates. Results were obtained from a *Macaca mulatta* monkey that was trained to track a spot which moved at a constant velocity in discrete trials. We used relatively high target velocities (40-70 deg/sec), and a 300 msec gap to facilitate anticipatory eye movements. Stimulation was applied through a tungsten microelectrode that was lowered stereotaxically through a recording chamber mounted over the DMFC, at a location where single-unit activity related to pursuit had been recorded. Bipolar current was used with intensities and durations typically ranging from 100-300 μA and 100-500 msec respectively. We found that microstimulation applied during ongoing pursuit reduced eye velocity. However, microstimulation applied at the beginning of the trial facilitated initial eye acceleration (measured 90 msec after pursuit initiation) and/or anticipatory eye velocity (measured @ target onset). Interestingly, enhanced eye movements appeared to depend on the time of stimulation *offset*. This was evidenced by the finding that at the same site, stimulation that ended before pursuit initiation could facilitate pursuit, but stimulation that extended beyond pursuit initiation suppressed it. Sometimes stimulation at a given site affected both anticipation and initiation, but not always in the same direction. The results strengthen the notion that the DMFC plays a role in smooth pursuit, and that neuronal signals in this area may be involved in timing predictable target motion.

Supported by The Smith-Kettlewell Eye Research Institute.

382.3

SPATIAL SUMMATION OF MOTION SIGNALS IMPROVES SMOOTH PURSUIT. S.N.J. Watamaniuk*[1] & S.J. Heinen[2]. Wright State University, Dayton, OH 45435 and The Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115

Previous studies have shown that the smooth pursuit response is enhanced when stimuli larger than the traditional single spot are tracked (Pola & Wyatt, 1985; van den Berg & Collewijn, 1986). However, it is not known if this enhancement is due to the presence of more motion or more position information in the stimulus. Using a stimulus devoid of consistent position cues, we investigated the effects of increasing the motion signal on the smooth pursuit response. Eye movements were measured to random-dot cinematograms (RDCs) in which all dots moved in a single direction (left or right) at a constant speed. The width of the RDC was always 10 deg. We varied both the height of the display (dot density constant at 2 dots/deg^2), and the density of the display (display height constant at 2 deg). Gain, initial acceleration, and latency were determined for each eye movement record. For the largest aperture size (10x10 deg), gain at the end of the open-loop period was consistently higher than for a single spot target. Eye acceleration, determined over a 20 msec bin centered at 90 msec after pursuit onset, *increased* when either aperture size or density were increased. In addition, pursuit latency *decreased* when either aperture size or density were increased. When equated for the number of moving dots, increasing aperture size had a larger effect on eye acceleration and latency than did increasing density. The differential effect of density and aperture size supports the notion that the input to the pursuit system arises from a nonlinear spatial summation of inputs from a population of motion detectors.

Supported by NIH EY10838 and The Smith-Kettlewell Eye Research Institute.

392.4

SMOOTH PURSUIT EYE MOVEMENTS FOLLOW THE 2/3 POWER LAW [§§]P. Viviani* and [§]C. de'Sperati [§]Faculty of Psychology and Educational Sciences, University of Geneva, Carouge, Switzerland and [§§]Department of Cognitive Science, Scientific Institute S. Raffaele, Milan, Italy.

Curvature and velocity of voluntary hand movements covary according to the so-called Two-thirds Power Law (Viviani & Schneider, 1991). Neurophysiological findings (Schwartz, 1995) suggest that this covariation originates from the dynamic properties of the motor cortex. We provide further confirmation of this hypothesis by showing that the Two-thirds Power Law applies also to the smooth pursuit movements of the eye. In the main experiment scleral search coils were used to record eye position while tracking 2D elliptic targets with 3 different eccentricities. In all cases the average velocity of the target was 8 deg/s. For each eccentricity, 7 targets were tested, only one of which complied itself with the power law. In the others the relation between velocity and curvature violated by a controlled amount this biological regularity. All aspects of oculomotor performance were affected by the amount of mismatch between the kinematics of the target and that predicted by the power law. Number and amplitude of catch-up saccades increased with the mismatch, and so did the retinal position error during the smooth phases of the pursuit. Moreover, the trajectory of the target was restituted faithfully only with biologically compatible targets. All the failures of the oculomotor system can be accounted by the hypothesis that, irrespective of target kinematics, the system is forced to comply with the Two-thirds Power Law. In a control experiment we ruled out the possibility that pursuit failures were due to the high accelerations present in non-biological targets. This was done by using non-elliptic targets having the same distribution of velocity as in the main experiment, but whose distribution of curvature along the trajectory was designed so as to satisfy the power law. In this case pursuit performance was considerably improved. The fact that a motor system whose plant dynamics is entirely different from that of the hand complies with the same motor rule strongly suggests a central origin of the rule.

382.5

SHORT-LATENCY OCULAR FOLLOWING: EFFECTS OF CONFLICTING IMAGES MOVING IN THE SAME OR DIFFERENT DEPTH PLANES. C. Busettini*, G. S. Masson, and F. A. Miles. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892 and CRNC/CNRS, Marseille, France.

Movements of large patterns elicit ocular following (OFR) at ultra-short latency (monkeys, <60 ms; humans, <80 ms) and the very earliest responses can be elicited only by images moving in the plane of fixation: images with binocular disparities exceeding a few degrees are ineffective (Busettini et al, 1996). In past studies, only one pattern was present, and we now report the effect on the initial OFR (of two monkeys) of introducing a second (interfering) pattern that moves with conflicting motion in the same or a different depth plane.

Visual stimuli, produced by back-projection onto a tangent screen, consisted of regularly spaced horizontal bands (3.36° thick) of random dots that could be moved horizontally to induce ocular following. However, the bands were organized into two groups such that alternate bands moved always together and opposite in direction to the intervening bands. Using multiple projectors and crossed polarizers to confine images to one or other eye, one set of "drive" bands was imaged always in the plane of fixation, while the other set of "interfering" bands was imaged within or outside the plane of fixation by adjusting their horizontal disparity (range, 0-12.8° crossed and uncrossed). Trials started with all images stationary in the plane of the screen. In the wake of 10° centering saccades, shutters blanked the scene for 15 ms, after which the two sets of bands were revealed to be moving at 40°/s in opposite directions.

For both monkeys, the initial OFR induced by the motion of the "drive" images showed strong dependence on the disparity of the "interfering" images. Based on measures of the change in version over the time period 60-77 ms (from the end of the blanking), initial OFR was weakest when the disparity was <0.5° and increased to a maximum as disparity increased to 2-5°; in 3/4 cases, measures declined with further increases in disparity (inverted Mexican hat *vs.* inverted bell profile). These data indicate that initial OFR can use binocular disparity to distinguish images moving in the plane of fixation from those moving in other depth planes.

Supported by The National Eye Institute and Le Ministere de la Recherche.

382.6

SHORT-LATENCY VERSION AND VERGENCE EYE MOVEMENTS TO LARGE-FIELD MOTIONS: EVIDENCE FOR SHARED MOTION DETECTORS. G. S. Masson*, C. Busettini and F. A. Miles. CRNC/CNRS, Marseille, France and Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Large-field motions applied dichoptically to the two eyes elicit tracking at ultra-short latency (<60 ms): conjugate motion at the two eyes (i.e., in the plane of fixation) evokes version responses, *Vs*, and opposite motion (i.e., motion in depth) evokes vergence responses, *Vg* (Miles et al 1986; Busettini et al 1996a,b). We here report on the velocity tuning curves for these two kinds of response in 3 monkeys.

Animals faced a large tangent screen onto which two identical patterns were back-projected. A system of crossed polarizers ensured that each eye saw only one of the patterns, and mirror galvanometers controlled the horizontal positions of the images. Each trial started with the patterns superimposed and so imaged in the plane of the screen (distance, 33 cm). Then, the two patterns moved horizontally at the same constant speed (range, 5-240°/s) in either the same or the opposite direction.

Velocity tuning curves for *Vg* for motion-towards-the-observer and for *Vs* (based on ΔVg and ΔVs over time 60-77 ms measured from stimulus onset), had very similar forms--an initial steep rise to a peak followed by a gradual decay--with the peaks for both occurring at about the same retinal image speed (≈60°/s). The exact form of these *Vg* velocity tuning curves could be predicted by summing the *Vs* responses to matching monocular stimuli, i.e., summing ΔVs for rightward motion when the right eye was patched with ΔVs for leftward motion when the left eye was patched. For motion-away-from-the-observer, only 1 animal produced appreciable ΔVg measures, again predictable from the ΔVs for matching monocular motion stimuli; ΔVg for the other 2 animals to motion-away was extremely weak. It is known that the visual processing underlying these *Vg* and *Vs* responses to large-field binocular motions is *not* purely monocular (in which each eye independently tracks the motion it sees) but involves binocular signals encoding motion-in-depth and motion-in-the-plane-of-fixation, respectively (Busettini et al, 1996a,b). However, the present data suggest that these *Vg* and *Vs* responses may rely on the same (monocular) motion detectors.

Supported by Le Ministere de la Recherche and The National Eye Institute.

382.7

MOTION DISPARITY AND LOOMING CUES FORM HIERARCHICAL INPUTS FOR SMOOTH PURSUIT OF TARGETS MOVING IN 3 DIMENSIONS. Gal A. Cohen* and Stephen G. Lisberger, Dept. of Physiology and W.M. Keck Foundation Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143.

Several cues are available to guide visual pursuit of 3D target motion. To dissociate the influence of looming from that of relative target motion on the two eyes, we presented virtual reality pursuit targets to 2 monkeys.

1) In the initial 100 ms of pursuit, the pursuit trajectory of one eye was influenced by visual input to the other eye. If looming was absent, and the right eye saw the same target motion over all trials, then the right eye's movement varied as a function of the left eye's input.

2) Visual input to one eye was sufficient to induce vergence pursuit motion in both eyes. Targets started straight ahead, on the plane of the screen, and were extinguished for the left eye at the time when the target began moving towards the monkey, so that the right eye saw nasal target motion. Under these conditions, a normal-looming monocular target induced both the right and left eyes to move nasally, and produced 60% of the convergence pursuit seen with a stereo target, as measured 300 ms after pursuit initiation. Inverse-looming monocular targets induced only 9% of the vergence observed with normal-looming monocular targets.

3) Looming is a much stronger cue for vergence pursuit in the absence of motion disparity information. Stationary monocular targets which grew or shrank in size successfully elicited strong convergence or divergence pursuit, respectively, in the absence of translational motion. Stereo targets that moved towards the monkey, but got smaller rather than larger (inverse-looming), induced ~85% of the vergence velocity seen with normal-looming stereo targets that had the same trajectory.

These data suggest that the pursuit system integrates target input from the 2 eyes into a 3D trajectory that is then transformed into commands for motion for the two eyes. When stereo information is ambiguous or underconstrained, monocular cues strongly influence 3D pursuit trajectory. *Supported by ONR grant N00014-94-1-0269 and by NIH grant EY06544.*

382.8

FAST DISCONJUGATE SACCADE ADAPTATION. L. Averbuch-Heller*, R.F. Lewis, D.S. Zee. Dept. of Neurology, Univ. Hosp., Cleveland, OH 44106 and The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21287

Horizontal saccades become immediately disconjugate when associated with gaze shifts in depth. The mechanism of this disconjugacy and its relation to disconjugate adaptation and phoria adaptation is unclear. Using prism-induced disparity, we tested disconjugate adaptation in one set of orbital positions, and examined generalization to other positions. Four subjects faced an LED array at 125 cm in front of the head. Movements of both eyes were recorded using search coils. Saccades and static ocular alignment were determined by subtracting left eye (LE) from right eye (RE) position, and measured during refixation between left 20, 0, and right 20° (L-C-R). A 10 diopter base-out prism, calling for convergence, was placed in front of the central field of RE, so that at L and R the eye viewed outside the prism. During 15 min of training, subjects made repetitive saccades viewing binocularly in the right field of vision (C-R-C). This required divergence for rightward (centrifugal) and convergence for leftward (centripetal) saccades. We compared static and dynamic alignment in both fields of vision (C-R-C-L-C) before and after training, during LE viewing. Following training, all subjects increased divergence for C-R saccades (range, 0.48 to 1.24°), convergence for R-C saccades (-0.36 to -1.12°), and divergence for C-L saccades (0.33 to 0.65°); the changes were significant at the 95% confidence level. No consistent changes occurred for L-C saccades (-0.35 to 0.56°). Static alignment after training showed a tendency toward convergence in all positions of fixation (L: -0.36 to -2.08°; C: -0.31 to -2.36°; R: -0.28 to -1.13°). In 2 subjects relative convergence was greater on L fixation, in contrast with increased divergence during C-L saccades. Our results indicate some degree of transfer of disconjugate saccade adaptation to non-trained positions. Changes in dynamic alignment did not always correlate with changes in static alignment, suggesting differential control of these two processes. Supported by NIH grants EYO1849, NSO1656

382.9

UNEQUAL AMPLITUDE SACCADES: THE USE OF DISPARITY INFORMATION AS A FUNCTION OF VIEWING DISTANCE.

J. Van der Steen [1] and P.Bruno[2]. Dept. of Physiology, Erasmus University Rotterdam[1], The Netherlands and Dip. di Elettrotecnica, Univ. of Trieste, Italy[2]. (SPON: European Neuroscience Organisation)

Under aniseikonic conditions, the ability to generate saccades with unequal amplitudes depends on viewing distance (Vision Res (35): 3459-3471, 1995). The finding that intrasaccadic vergence errors are small compared to far distance when only disparity information is available, is either an indication of a more effective use of disparity information at close distance (sensorial effect), or is directly related to the state of convergence of the two eyes as a function of viewing distance (motor effect). To test these two alternatives, we instructed three subjects to make horizontal saccades (amplitudes 10, 20, 30, 40 and 50 degrees) while viewing two dichoptically presented aniseikonic images. Size differences between the two images were 0, 8 and 16 %. By shifting the relative horizontal positions of the two images in front of each eye, we simulated viewing distances varying between 100 and 0.25 meters. In all three subjects intrasaccadic vergence errors during leftward saccades, requiring divergence, were inversely related to viewing distance. For rightward saccades, requiring convergence, the opposite trend existed, although the effects were much smaller. On the other hand the maximum disparity that was tolerated before saturation occurred, was dependent on viewing distance. In conclusion, the effects of viewing distance on the capability to generate unequal amplitude saccades, can partially be explained by a distance dependent use of disparity information, partially by a more mechanistic mechanism based the initial state of convergence of the two eyes.

382.10

MEMORY EVOKED SACCADES TO ANISEIKONIC TARGETS. Z. Kapoula*, M.P. Bucci. LPPA, CNRS-Collège de France, Paris, France.

Saccade disconjugacy appears immediately when the image is made larger for one eye and persists under monocular viewing. This suggests a fast learning mechanism. We propose an associative learning mechanism based on the pairing between disparity and saccades rather than a low-level adaptive readjustment of the saccades. To test this idea we examined memory evoked saccades to aniseikonic targets.

Four subj wore an afocal magnifier (8%) in front of one eye. They were seated 123 cm in front of an arc of LEDs. Subj fixated a central LED spot. A target (a normal or a backward E subtending 0.4° x 0.2°), was then flashed to the left or to the right at 5°, 10°, 15° or 20°. The target size and its eccentricity was 8% larger in the eye with the magnifier. One second and a half after the flash, the central spot was turned off and the subj was asked to make a saccade in complete darkness to the remembered target location. Three subj were trained for 20 min and one subj for 10 min. Before and after training memory evoked saccades were also recorded under monocular viewing. Saccades were recorded binocularly with an IR device.

Before training, memory evoked saccades showed only a small divergent disconjugacy (0.28°). Within 2 min of training, for all subj memory evoked saccades became larger in the eye with the magnifier. For the subj trained for 20 min the disconjugacy increased over time (mean of all training saccades 0.51°) and persisted under subsequent monocular viewing (0.89°). These results indicate that disparity information from a small target briefly flashed in the periphery can be memorized for a few seconds and used to program a disconjugate saccade. With longer training, a disconjugacy command becomes linked automatically to the saccade: memory evoked saccades remain disconjugate even in the absence of recent storage of peripheral disparity information. These findings support the idea of associative learning based on short or longer term memorization of visual disparity and capable of altering saccade conjugacy rapidly.

382.11

Eye-Head Interactions During Coordinated Gaze Shifts in the Head Unrestrained Rhesus Monkey. Edward G. Freedman* and D. L. Sparks. Dept. of Psychology, Univ. of Pennsylvania, Phila., PA 19104.

We analyzed both the metrics and kinematics of over 8,000 gaze shifts made during the delayed gaze shift paradigm in order to assess the nature of the interactions between the eyes and head. We found that during gaze shifts directed along the horizontal meridian, average eye velocity increased as gaze shift amplitude increased from 5°-25°. During larger gaze shifts with significant head contributions, there was a pronounced reduction in eye velocity associated with the onset of the head component. As the head accelerated a clear reduction in the velocity of the eye component was observed, and this reduction was often larger than the increase in head velocity. During many movements there was a subsequent re-acceleration of the eyes before the end of the gaze shift. No re-acceleration of the head was observed, and as a result, during some phases of gaze shifts the eyes were decelerating while the head was accelerating, and subsequently the eyes were accelerating while the head was decelerating. Average eye velocity declined by ~50% as gaze amplitudes increased over a range from ~25°-70°, and this decrease in velocity was greater than the concurrent increase in head velocity over this same range of gaze shifts.

During oblique gaze shifts the head contributed preferentially to the horizontal component, and the effect of head velocity on the eye velocity was along the vector of the head movement. As a result the duration of the horizontal components of oblique eye *and gaze* movements were often considerably longer than the duration of the vertical component. In contrast, the durations of the horizontal and vertical head movement components were approximately equal. Based upon this data and reports in the literature regarding the gain of the VOR during large amplitude gaze shifts, it is unlikely that this eye-head interaction is the result of the VOR. Some other source of head velocity information must be used to reduce the eye velocity of coordinated gaze shifts. Support: NEI grant# R37EY01189 and NIH grant# MH17168.

382.12

DYNAMIC PROPERTIES OF HUMAN GOAL-DIRECTED HEAD MOVEMENTS H. Scherberger, D. Straumann, V. Henn, M. R. Duersteler*. Neurology Dept., Zurich University Hospital, CH-8091 Zurich, Switzerland.

In head-unrestrained gaze shifts the contribution of the head is considerably smaller for vertical than horizontal movements, an anisotropy which might be due to the dynamic properties of head movements.

We investigated head displacements during paradigms in which subjects were forced to move the head in the full amplitude of a gaze shift. Subjects were seated in front of a tangent screen (distance 1.2 m) with a central fixation spot. Additional fixation spots were placed in horizontal, vertical, and oblique directions at 30 deg distance from center. The left eye was occluded and head position was continuously monitored by the subject using a head-fixed laser beam projecting onto the screen. Without further instructions, subjects were asked to alternatively shift the laser beam from the center to the peripheral spots upon acoustic triggers, while rotational head velocity was recorded by an angular rate meter (sample frequency 1000 Hz).

In the four subjects tested we found peak angular head velocities higher in the horizontal (mean 67 deg/s) than vertical (56 deg/s) direction, while deviations from the correct target direction varied much stronger in oblique (SD 13.1 deg/s) than in purely horizontal or vertical directions (SD 8.1 deg/s).

The slower dynamics of the vertical component might explain, at least in part, the smaller head movement contribution during vertical gaze shifts. In addition, increased deviations of trajectories during oblique head displacements suggest separate horizontal and vertical channels for head movement control.

Supported by Swiss National Foundation #31-40484.94.

383.1

SYSTEMIC KAINIC ACID INDUCES A PERSISTENT THERMAL HYPERALGESIA IN THE MOUSE, S.L. Giovengo* and A.A. Larson. Department of Veterinary PathoBiology, University of Minnesota, St. Paul, MN 55108.

Noxious stimuli that lead to induction of a persistent hyperalgesic state are thought to be mediated, in part, by excitatory amino acid (EAA) activity. We tested the possibility that kainic acid (KA) activity at receptors on primary afferent C-fibers is sufficient to induce hyperalgesia. Injected intraperitoneally (i.p.), KA causes neurotoxicity in areas of the CNS containing high densities of KA receptors. We found that a subconvulsant dose of KA injected i.p., induces a long-term, irreversible (2 week) thermal hyperalgesia, as demonstrated by decreased latencies of response in the hot plate assay at 52.5°C and tail flick assay at temperatures of -10°C, 49°C, and 53°C. In contrast, there was no effect of KA on either chemical nociception or tactile allodynia. Neonatal capsaicin treatment alone, which causes degeneration of primary afferent C-fibers, increased the latency of nociceptive responses only when tested using the tail-flick assay at 49°C. However, neonatal capsaicin prevented KA-induced hyperalgesia when tested at higher temperatures, i.e. the tail-flick at 53°C and hot plate assay at 52.5°C. Hyperalgesia thus requires activity along primary afferent C-fibers for thermal nociception that is triggered by higher temperatures (52.5-53°C) and normally transmitted along larger, perhaps Aδ fibers. However, large-diameter, cold-sensitive (-10°C) pathways and small diameter, C-fiber pathways sensitive to warm (49°C) temperatures, do not require C-fibers for the induction of KA-induced hyperalgesia and may, therefore, be brought about by an action of KA at other sites in the brain or spinal cord. [Supported by NIH grant DA04090]

383.2

PCR DIFFERENTIAL DISPLAY IDENTIFIES RAT DORSAL ROOT GANGLIA mRNAs WHICH ARE REGULATED IN A NEUROPATHIC PAIN MODEL K.E. Rogers*, M.G. Erlander, K.D. Wild, S.K. Yagel, J.E. Galindo, and R.B. Raffa. Drug Discovery, The R. W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477 and La Jolla, CA 92121.

Animal models have recently been developed for the study of neuropathic pain (NP). The "Chung" model mechanism (Kim & Chung, Pain 50:355-363, 1992) is particularly attractive since the animals develop characteristics which closely mimic the clinical disorder, including allodynia, hyperalgesia, and lack of sensitivity to opioid analgesics. We have chosen to analyze this NP model in a differential display paradigm to identify specific transcriptional changes which occur with the development of this type of pain. Rat spinal nerves, L_5 and L_6 were tightly ligated and animals were tested for sensitivity of hind paw to stimulation with von Frey filaments. In order to meet the NP criteria and be included in the differential display analysis, animals needed to respond to < 4 g of pressure on post-operative (PO) day 7 by lifting the hind paw. Animals meeting the criteria were sacrificed on PO day 8 and L_5 and L_6 dorsal root ganglia (DRG) from both the ligated and non-ligated sides were removed. Total RNA was extracted from dissected DRGs and cDNA synthesis was subsequently performed to generate a template for differential display analysis. Differentially expressed mRNAs in ligated vs. contralateral control have been identified and will be presented.

383.3

SIZE DISTRIBUTION OF FOS IMMUNOHISTOCHEMICAL LABELED NEURONS IN THE DORSAL HORN OF RAT SPINAL CORD. S-F. WANG, M-Y. TASI,* W-Z. SUN, B-C. SHYU . Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, 11529, R.O.C.

Laser stimulation induced c-fos expression in both superficial and deep layers in the dorsal horn of spinal cord. Repeated experiment showed high correlation (r=0.75). The labeled nuclei in the deep layer are significantly ($p<0.05$) larger than the ones in the superficial layer. The same phenomena happened while stimulating with 4, 8 or 12 W of laser stimulation. The labeled nuclei induced by 12 W laser stimulation were also significantly larger than the one induced by 8 W or 4W laser stimulation ($p<0.05$). This result demonstrated that laser induced FOS protein labeled by immunocytochemistry can be quantified and are repeatable. Therefore, it could be served as objective index of pain measurement. Furthermore, due to the size and location of the labeled FOS nuclei, the discovery of different layers of pain related dorsal horn neurons with different sizes may reflect different functions in sensory processing. The phenomena of higher intensity induced FOS protein in larger nuclei may reveal the possibility of recruitment order in the sensory system. This result could be applied to the studies of the therapeutic intervention of pain management by physical or chemical and pharmacological agents. With this established methods, the phenomena of chronic pain, such as reflex sympathetic dysfunction, could be studied in greater details in the spinal cord. Granted NSC 84-2331-B-0020089.

383.4

CHANGES IN DISTRIBUTION OF CGRP PEPTIDE FOLLOWING SPINAL CORD HEMISECTION PROVIDE A MECHANISM FOR CHRONIC CENTRAL PAIN STATES, C.E. Hulsebosch* and M.D. Christensen. Anat.& Neurosci., Univ. of Texas Medical Branch, Galveston, TX, 77555-1069.

Spinal cord injury results in chronic pain states in which the underlying mechanism is poorly understood. To begin to explore possible mechanisms, calcitonin gene-related peptide (CGRP), a neuropeptide confined to fine primary afferent terminals in laminae I and II in the dorsal horn of the spinal cord and implicated in pain transmission, was selected. To test the temporal and spatial distribution of CGRP, immunocytochemical techniques were used to examine the spinal cord following T-13 spinal cord hemisection in adult male Sprague Dawley rats compared to controls (N=5) using standard immunocytochemical techniques. Spinal cords from spinally hemisected rats (N=5, per time point) were examined on post operative day (POD) 3, 5, 7, 14 and 108 following surgery. Image analysis techniques demonstrated an increased density of reaction product that was statistically significant, in laminae III and IV, both ipsilateral and contralateral to the lesion which extended at least two segments rostral and caudal to the hemisection site by POD 14, and remained significantly elevated as long as POD 108. Increases in density of CGRP were found in the dorsal roots, in Lissauer's tract, and in motor neurons in the ventral horn ipsilaterally. Sham operated rats displayed CGRP immunoreaction product in laminae I and II, Lissauer's tract and the dorsal roots. Since upregulation of CGRP would occur in an acute temporal window, these results are interpreted to be invasion of laminae III and IV by sprouting of fine primary afferents. These data indicate changes in density and distribution of CGRP following spinal hemisection which could contribute to the development of various pain states in human SCI. (Supported by Kent Waldrep National Paralysis Foundation and NS 11255.)

383.5

SUBTLE NEGLECT OF A NON-PAINFUL PART OF THE BODY IN A CHRONIC PAIN PATIENT REVERSED BY INTRALAMINAR THALAMOTOMY. F.A. Lenz*, A.Hillis, R.H. Gracely, L.H. Rowland, M.C.Delano, T.S.Eckel, R.N.Bryan, P.M. Dougherty. Depts Neurosurgery Radiology, Hopkins Hospital, Baltimore, MD 21205 & NAB:NIDR:NIH.

The role of the intralaminar nuclei in nociception is suggested by findings in a 67 year old woman undergoing medial thalamotomy for treatment of intractable left dental pain without current somatic cause or testable sensory loss. Pre- and post-operative testing for inattention included dual simultaneous presentation of cutaneous, vibratory, proprioceptive, graphesthetic, visual and painful (mechanical and temperture) stimuli plus appropriate sensory controls. Cutaneous stimuli were also applied simultaneously to the face and contralateral hand (simultaneous bilateral heterogeneous stimulation - SBHS). Facial sensory stimuli were applied to the skin overlying the painful area. During preoperative testing the patient neglected stimulation of the right hand on 44% of presentations to the left cheek and right hand but never neglected the stimulation of the hand on presentations (three blocks, total of 156 presentations) to the right cheek and left hand ($P<0.0001$, Fisher exact). Postoperatively, the response to SBHS had changed significantly ($P<0.0001$, Fisher exact) so that the patient did not neglect either hand (three blocks, n=145). The MRI was negative pre-operatively but demonstrated a lesion of central median and parafascicularis nuclei postoperatively, when registered with the Schaltenbrand atlas. The reversal of subtle neglect following thalamotomy suggests that the intralaminar nuclei may have a role in directed attention to the painful area of the body. Support: NIH P01 NS32386-Proj. 1.

383.6

TRANSCRANIAL ELECTROANALGESIA (TEA): MRI STUDY OF INTRACRANIAL CURRENT DENSITIES (CD) AND PATHWAYS (CP) IN RABBIT. V. Lebedev*, M. Joy, J. Gati. Lab. of Electroanagesia, Pavlov Inst of Physiology, S.-Petersburg 199034, Russia, Inst. of Biomed. Engineering, Univ. of Toronto, Toronto, M5S 1A4, Canada.

It was shown (Lebedev et al., '83, '88) that impulse current (C) of special parameters applied sagittally (AS) could elicit a naloxone-reversible analgesia in animals and humans, whereas the same C applied bilaterally (AB) was ineffective. Present study was done to estimate noninvasively, by means of MRI processing (Scott, 1991), intracranial CD and CP of CAS and CAB through the coronal and sagittal sections of rabbit brain. In contrast to CAB, which passed through brain tissue and intracranial spaces rather diffusely, CAS passed through areas containing cerebro-spinal fluid. The highest CD levels were observed below the brain in basal cisternas connected in series, and inside the brain in ventricles (V). The intaventricular CP included anterior horns of lateral V - foramina Monro - 3rd V - aqueduct - 4th V - foramina Luschka. The intreventricular and cisternal CP were interconnected in areas of infundibulum (through the ventral recess and thin bottom of 3rd V) and in lateral parts of medulla (through lateral recesses of 4th V). These MRI data demonstrated that CAS has an opportunity to pass near the parts of brainstem-antinociceptive structures in hypothalamus, midbrain and medulla, and provided the explanation of efficacy of TEA elicited by CAS.

Supported by Can. Natur. Sci. Res. Counc., OGPIN 335.

383.7

CEREBRAL ACTIVITY IN ACUTE AND CHRONIC VISCERAL PAIN IN HUMANS. D.H.S. Silverman*, J. Munakata, H Ennes, C.K. Hoh, M. Mandelkern, E.A. Mayer. Depts. of Medicine and Nuclear Medicine, UCLA Medical Center and West LA VA Medical Center, Los Angeles, CA 90095.

Perception of pain is altered in patients with certain chronic pain syndromes. The purpose of this investigation was to determine regional cerebral responses to an acute visceral pain stimulus, in subjects with and without a chronic visceral pain disorder. Effects of distal intestinal pressure stimuli on regional cerebral blood flow were assessed with O-15-water positron emission tomography (PET) in 16 subjects, half with a chronic intestinal pain disorder, the irritable bowel syndrome (IBS). A balloon catheter was placed in the distal intestine 30 min prior to imaging sessions. Following IV administration of 40 mCi O-15 water, PET data were obtained in a 10-sec frame followed by 18 5-sec frames, and summed for all frames proceeding entry of tracer into the brain. PET scans were obtained at baseline, during delivery of non-painful pressure stimuli, and during both actual and simulated delivery of painful stimuli. Scans were interpreted using statistical parametric mapping and region of interest methods of analysis, and results were compared according to condition, and to subjects' ratings of perceived intensity of painful stimuli. In healthy subjects, perception of pain during actual or simulated delivery of anticipated painful stimuli was significantly associated ($p < 0.01$) with activity of the anterior cingulate cortex (Brodmann's areas 24 and 32), while no anterior cingulate response to delivery or perception of non-painful stimuli was seen. In IBS patients, in contrast, the anterior cingulate cortex failed to respond to either actual or simulated delivery of painful stimuli. During the latter, however, significant activation ($p < 0.01$) of the left prefrontal cortex (maximal in Brodmann's area 10) of IBS patients was seen. In conclusion, perception of intestinal pain was associated with a specific pattern of regional cerebral activity involving most significantly the anterior cingulate cortex in healthy subjects. This pattern was aberrant in patients with a chronic intestinal pain disorder. *Supported by funds from the National Institutes of Health, the Veterans Administration, and the Mathers Charitable Foundation.*

383.8

Non-dominant hemispheric preference for pain affect: PET studies. Jen-Chuen Hsieh[1,2,*], Bjorn A. Meyerson[2], Jan Hannerz[2], Per Hansson[3], Martin Ingvar[2]. [1]Dept. Anesthesiology, Veterans General Hospital-Taipei & Institute of Neuroscience, National Yang-Ming University,Taiwan, R.O.C. [2]Dept. Clinical Neuroscience, [3]Neurogenic Pain Unit, Karolinska Hospital, Sweden.

We reported three positron emission tomographic (PET) studies of pain syndromes to elucidate the preference of the right hemisphere in attributing emotional valence and attention to pain suffering. Right-handed patients with ongoing painful peripheral mononeuropathy (PMN), episodic cluster headache (CH) or painful trigeminal neuropathy (PTN) were investigated using PET. Pain alleviation was obtained using regional nerve block for PMN and electric extradural precentral gyrus stimulation for PTN. Nitroglycerin was employed to provoke CH attack. Comparisons were made between ongoing/provoked painful state vs. pain-alleviated/resting state. Regardless of painful side, the right Brodmann area (BA)24 of the ACC and the BA9/32, adjoining the MPFC (BA9) and the ACC (BA32) were preferentially engaged.The right temporopolar region (area 38), known to be involved in the emotion of fear, was additionally activated during the experience of excruciating CH attack. Our studies not only verifies the preferential role of the right hemisphere in the appreciation of pain suffering but further supports that sustained chronic pain, being devoid of the motivational component of an escape response, targets the right hemisphere. The differences between the activation patterns of acute intense pain and chronic sustained pain conform to the current neuropsychological theories of hemispheric asymmetries in the limbic regulation of affect and cognition.

383.9

RIGHT-HEMISPHERE PREPONDERANCE OF RESPONSES TO PAINFUL CO_2 STIMULATION OF NASAL MUCOSA. R. Hari*, K. Portin, B. Kettenmann, V. Jousmäki and G. Kobal. Brain Research Unit, Low Temperat. Laboratory, Helsinki University of Technology, 02150 Espoo, Finland, and Dept. Experimental and Clinical Pharmacology, Univ. Erlangen-Nürnberg, 91054 Erlangen, Germany.

We recorded whole-scalp (Neuromag-122™) cerebral magnetic fields from 9 healthy adults to painful CO_2 pulses (duration 200 ms, concentration 65–90%), embedded in a continuous airflow (140 ml/s, 36.5°C, humidity 80%). The stimuli were led to the left or right nostril in successive experiments once every 20 or 30 s. The subjects were trained to breathe through the mouth to avoid respiratory air flow in the nasal cavity. The recording passband was 0.03–90 Hz and 16 single responses were averaged per run. Five out of the 9 subjects showed replicable reponses 280–400 ms after stimulus onset, with shorter latencies with higher CO_2 concentrations. The signals originated in the insular region, close to the SII cortex, and were considerably stronger over the right than the left frontotemporal region. The right-to-left ratio of mean signal amplitudes, calculated across 16 channels over each hemisphere, was 2.3 for both left and right nostril stimuli. The results imply a clear right-hemisphere dominance in responses to painful CO_2 stimulation of the nasal mucosa.

Supported by the Academy of Finland, the EC's HCM Programme (Large-Scale Facility BIRCH at the LTL), and DFG Ko 812/5-1.

383.10

DEFINING PROPERTIES OF TEMPORAL SUMMATION FOR SECOND PAIN SENSATIONS IN HUMANS. C. J. Vierck*, R. L. Cannon and A. J. Acosta-Rua. Dept. of Neuroscience, Univ. of Florida Col. of Med., Gainesville, FL 32610-0244.

A method of thermal stimulation is described which produces dramatic temporal summation of pain sensations, akin to the phenomenon of "windup" of central neuronal responses to input from unmyelinated nociceptors. Windup has been attributed to activation of NMDA receptors, and abnormal NMDA activation and temporal summation are frequently mentioned as mechanisms underlying certain chronic pain conditions. Therefore, it would be advantageous for clinical testing to assess whether temporal summation of pain sensations is abnormal, which requires definition of the time course of temporal summation and decay in normal subjects. The method of stimulation involved naturalistic brief contact (700 msec) of a preheated thermode (6 cm^2) with the glabrous skin of either hand. Trained subjects rated the peak magnitude of second pain sensations (latencies greater than 2 sec) with a numeric rating scale that is linked to verbal descriptors (e.g., 20 = pain threshold; 90 = very, very strong pain). At repetition rates of 0.33 Hz, temperatures of 45 to 53 deg. C produced ratings below pain threshold on the first presentation, and temporal summation increased with temperature. Ratings of 80 (very strong pain) were obtained after 12 contacts at 53 deg. C. Temporal summation was inversely proportional to interstimulus interval and was absent at 7 sec. The decay of temporal summation was tested by presenting two series of 10 contacts (at 0.33 Hz) that were separated by intervals of 4, 5 or 6 sec. A rapid rate of decay was observed in these normal subjects (complete by 6 sec). Comparisons of temporal summation of sensations were made with skin temperature recordings from a thermistor inserted beneath the epidermis of anesthetized monkeys. Phasic increases in skin temperature immediately following each stimulus were constant over the repetition rates, and baseline temperatures (immediately before each stimulus) were not related to interstimulus interval in the same manner as the sensory ratings. Supported by NINDS grant NS 07261.

COGNITION: MEMORY I

384.1

COGNITIVE PERFORMANCE AND NUTRITIONAL STATUS IN INDIVIDUALS EVALUATED FOR SENILE DEMENTIA. G. Shor-Posner*, J. Button, J. Bean, J. Demirovic, MK Baum and RJ Prineas. Dept of Epidemiology and Public Health, U Miami School of Medicine, Miami, Florida.

An important role for B vitamins in neuropsychological function has been demonstrated. The present study evaluated cognitive performance and nutritional status in a subsample (n=120: 37 men, 83 women) of 2,575 elderly subjects being examined for dementia. Cognitive impairment, as measured by SPMSQ error scores (adjusted for ethnicity and education), was observed in 28 men and 55 women (69% of the subsample, mean age 78.8±7 yrs). Logistic regression indicated that age and vitamin B_{12} status were significantly related to cognitive performance ($p<0.05$). Although serum albumin levels were within the normal range in the impaired and non-impaired groups, vitamin B_{12} levels and dietary B_{12} intake were significantly lower in the impaired group: 476±261 vs 634±351 non-impaired, ($p<0.01$), and 13±13 vs 28±45 mg/d ($p<0.01$), respectively. Moreover, serum vitamin B_{12} level was significantly correlated with SPMSQ adjusted error scores ($p=0.05$). Vitamin B_6 intake, which was significantly correlated with B_{12} intake ($r=0.81$, $p<0.0001$) was lower in the impaired group (ns), and positively related to Direct Assessment of Function Status scores in the women ($r=0.35$, $p<0.004$), but not the men ($p<0.13$). These results suggest that adequate intake of vitamins B_{12} and B_6 may be particularly important in maintaining cognitive function in aging individuals, and underscore the importance of gender-specific nutritional asssessments in relationship to cognitive performance.

Support:NIH-NIA-1-RO1-AG09461

384.2

INFORMATION PROCESSING IN MILD HYPOGLYCEMIA: A NEW METHOD FOR EXAMINATION R. Lobmann[1], B.G. Trümper[1], K. Wagner[1], G. Pottag[1], H.G. Smid[2], H.J. Heinze[2] and H. Lehnert[1]. [1]Dept. of Endocrinology, [2]Dept. of Neurophysiology, Otto-von-Guericke-University, Magdeburg, Germany

Little is known about distinctive and topographically related effects of hypoglycemia on cognitive functions. We employed novel event- related potential recording techniques (ERP's) in a hybrid selective-attention/ response-choice task. We presented prespecified features for example red color. Other stimuli also occur in the task but were presented in different color. So we could differenciate relevant and irrelevant stimuli, including different cognitive reaktion. Then we messured the onset of selection negativity as well as the response selection after stimulus selection. To study single components (stimulus selection, response choice, reaction time) of cognitive function impairement during a stepped hypoglycemic clamp (< 3mmol/l), two groups of 12 healthy volunteers were studied. One group performed different cognitive tasks during the following phases: euglycemia-hypoglycemia-euglycemia. The second group had only three euglycemic phases (randomized, single blind). The tasks consisted of recognition memory performance, selective attention and choice motor responses to colored letters. We also investigated 12 diabetic patients with IDDM and a group of 12 NIIDM patients. The ERP's related to selective identification of colored letters, to central activation of motor responses in primary motor cortex and reaction times to these letters were delayed during hypoglycemia as compared with the first phase of normoglycemia (32 msec, 72 msec, 27 msec, ps all <.05). In particular we found that hypoglycemia impairs recognition memory and primarily affects early components of cognitive responses such as motor cortex activation and selection of input information. Our results suggest that hypoglycemia delays stimulus selection, with consecutively delayed central and motor processing.

384.3

EFFECTS OF SCOPOLAMINE ON THE PERFORMANCE OF RATS IN A DELAYED MATCHING TO POSITION TASK IN AN 8-ARM RADIAL MAZE. Poorheidari G, Stanhope KJ & Pratt JA SPON: Brain Research Association. Dept. of Physiology & Pharmacology, Univ. of Strathclyde, Glasgow UK. Cerebrus Ltd. Dept. of Neuropharmacology, Silwood Park, Ascot, Berkshire UK.

Scopolamine-induced "memory impairment" has been extensively used in various animal models of dementia including the radial arm maze. However, it has still to be resolved whether the putative cognitive effects can be separated from, or indeed are a consequence of, other psychopharmacological effects of the drug. A delayed matching to position task in the radial maze was employed to examine the effects of scopolamine. Male Long-Evans hooded rats were trained to find the only baited arm (as the sample) from the 8-arm radial maze (first trial). Then after a delay of 5 hours, rats were returned to the maze to find the baited arm (the same as the sample) on the second trial. In three experiments, scopolamine (0.25 mg/kg, i.p.) or saline were administered 20 min before the first trial, immediately after the first trial, and 20 min before the second trial, respectively. Three measurements were recorded: 1) the number of the arm entries before entering the baited arm in the second trial (errors); 2) time taken for the rats to find the baited arm in the first trial; and 3) time taken for the rats to find the baited arm in the second trial. In a separate experiment, the effect of scopolamine (0.25 mg/kg, i.p.) on locomotor activity was also measured. Analysis of the radial maze data revealed that when the drug was administered before either the first trial or the second trial, it took significantly longer for the scopolamine-treated group than the saline-treated group to find the baited arm on either of the trials. There was no significant differences between the two groups in the other measurements. However, scopolamine significantly increased the locomotor activity behaviour. These results question the specificity of the "cognitive" impairment induced by scopolamine in many radial arm maze tasks.

This work was supported by SHERT and Iranian Ministry of Health

384.4

PRIMING AND INTERFERENCE PHENOMENA IN A MODEL OF COGNITIVE RESPONSE GENERATION, Vijaykumar Gullapalli[2] and Jack Gelfand*[1]. Dept. of Psychology[1] and Dept. of Mech. Eng.[2], Princeton University, Princeton, NJ 08544.

We present a general model of the brain mechanisms operating in cognitive tasks that involve the generation of responses to external stimuli. The model is consistent with the anatomy and physiology of cortical and subcortical structures involved and includes the dynamics of cortico-thalamo-basal ganglionic loops (Gullapalli and Gelfand, Proc. NYAS, 769:375-380, 1995). This model uses groups of neurons corresponding to cortical columns to form distributed representations of the internal state of the organism or external state of the environment. These representations, including the representation of the goal of the task, are used by dynamical processes in prefrontal cortico-thalamo-basal ganglionic loops to generate appropriate responses to stimuli in various tasks. In this study, we report on priming and interference phenomena that result from these dynamical processes in the model. We found that activation of a feature that is relevant to the task produces priming if the feature is related to the appropriate response given the task and the stimulus. If the feature is relevant but inappropriate for the task/stimulus combination, it results in interference, slowing down the selection of the response. We discuss this model in terms of the representation of input stimuli and goals and the role of prefrontal cortex circuits in working memory

Supported by a grant from the James S. McDonnell Foundation.

384.5

PRESERVED AND IMPAIRED MEMORY PERFORMANCE IN RETROGRADE AMNESIA : A CASE STUDY. E.A. Phelps*, J.D. Sweder, and K.S. LaBar. Dept. of Psychology, Yale University, New Haven, CT 06520.

We report a patient, T.A., who became amnesic following encephalitis. T.A. is a 50 year old man who performs in the low average range on most neuropsychological tests with the exception of those assessing memory where his performance is impaired, indicating a moderate to severe anterograde amnesia. In addition, T.A. reports no memory for events prior to his illness, suggesting a retrograde amnesia. On examination, T.A. is at chance on recognizing events that occurred over the past 40 years, although he does show above chance recognition of events that occurred in the first 10 years of his life. This temporally graded retrograde amnesia affects personal events, public events, pictures of famous individuals and family members, and meanings for words that were commonly used in only limited periods of his life. He also shows a marked change in personality and new preferences for food and music.

In contrast, T.A. shows relatively intact semantic memory as demonstrated by his ability to use language, solve simple arithmetic problems, and describe the system of government in the U.S.. This spared semantic memory is consistent with other reported cases of retrograde amnesia (e.g., Tulving et al., 1988; O'Connor et al., 1992). In addition, he demonstrates intact memory for complex skills, such as driving, billiards, bowling, and archery. T.A.'s intact memory for skills suggests a distinction between lifelong memory for events and complex skills.

384.6

HIPPOCAMPAL ACTIVATION DURING MEMORY ACQUISITION AND RETRIEVAL USING FUNCTIONAL MRI S.Y. Bookheimer*, M. Dapretto, G. Small, J. Wang, M. Cohen. Brain Mapping Division, UCLA School of Medicine, Los Angeles, CA 90024.

Lesions of the human hippocampus (HC) produce deficits in acquiring new memories, though retrieval of previously acquired information remains essentially intact. Nonetheless, single unit recording studies in animals and humans reveal neural activity in HC during memory retrieval as well as acquisition. We used functional MRI to measure changes associated with cerebral blood flow during the learning and recall of unrelated paired associates. This task is markedly impaired in patients with left mesial temporal lesions. Six normal volunteers underwent fMRI during 4 auditory presentations of 7 unrelated word pairs, interspersed with an attention control task in which they monitored for a tone. These were followed by retrieval blocks in which the first word in each pair was presented, and subjects silently generated the correct associate. Rest blocks were obtained prior to and after the activation sequence. Images were acquired on a GE 1.5 Tesla Signa MR scanner with an SPGR sequence (TR=70ms; TE=50ms; FA=45°; 128x256; 0.75 NEX) using oblique slices along the longitudinal axis of the left HC. Regions of interest (ROI's) were made on the basis of co-planar anatomical scans in the anterior hippocampal formation, and signal intensity (SI) was determined for each activation block. In comparison to the control tasks, the MR signal intensity in the HC was greater for both learning and recall. The magnitude of the SI changes increased over trials in correlation with the rate of learning, both in the learning and the retrieval blocks. During the interspersed attention control blocks, the SI returned to baseline levels. Our data are consistent with patient studies implicating the HC in forming new associations, and with single unit recording studies which demonstrate increased neural activity in the HC during memory retrieval. The data support a model HC function positing that, in addition to forming new associations, the HC is important in reconstructing memories from partial cues.

Supported in part by NIH Grant 1RO1 AG13308-01 and the UCLA Center on Aging

384.7

NEURAL SUBSTRATES OF REASONING: AN fMRI STUDY OF RAVEN'S PROGRESSIVE MATRICES TEST. V. Prabhakaran[1*], J.A.L. Smith,[2] J.E. Desmond[2], G.H. Glover[3], J.D.E. Gabrieli[2]. Program in Neurosciences[1], Depts. of Psychology[2] and Radiology[3], Stanford University, Stanford, CA 94305.

The Raven's Progressive Matrices Test is a widely used measure of reasoning ability that reliably predicts performance in reasoning tasks from a number of different domains. fMRI was utilized in order to identify neural substrates involved in performing this reasoning task. Three types of problems were adapted from the Raven's test: 1. Easy problems consisted of a 3X3 block of patterns related by the pairwise constant rule (Carpenter & Just, 1990). The problems can be solved by a visuospatial strategy . 2. Hard problems consisted of patterns related by different rule sets (Distribution of 3, Distribution of 2, etc.) These problems cannot be solved by simple visuospatial strategy but require abstract/analytical reasoning. 3. Match problems consisted of nonsense blocks of 3x3 patterns. Subjects matched a pattern to one of eight choices given. The match problems served as a control for sensory and motor areas of activation irrelevant to the cognitive factors of interest. Three combinations of problem sets (match-hard, match-easy, and easy-hard) were presented to subjects in the scanner. Each set contained 12 problems (6 cycles of alternating problem types). Subjects were allowed 30 seconds per problem. Presentation of problem sets was counterbalanced across subjects(n=6). Imaging was performed at 1.5T, using a T2* sensitive gradient echo spiral sequence (TR=720 msec, TE= 40msec, flip angle=65°). Extensive activation was seen bilaterally in posterior parietal, inferior temporal, extra-striate, prefrontal, and premotor areas in the match-hard condition. Less activation was found for these areas in the match-easy condition. In the hard-easy condition high activation occurred in the left posterior parietal area and bilateral prefrontal areas.

This study was supported by NIH grants MH53673 and AG12995.

384.8

NEUROPHYSIOLOGY OF DUAL TASK PERFORMANCE: A PET O15 STUDY. T. E. Goldberg,* K. Fleming, K. F. Berman, J. Van Horn, G. Esposito, J. Ostrem, J. M. Gold, D. R. Weinberger. Clinical Brain Disorders Branch, IRP, NIMH Neurosciences Center at St. Elizabeths, Washington, DC 20032 USA

Introduction. The neurophysiological response to manipulations of information processing demands in dual task paradigms has received little attention in functional neuroimaging. Thus, it has been unclear whether the effects of excessive cognitive load would increase or decrease local metabolism. Method. Fourteen normal subjects participated. As a primary working memory task they received a computerized, paced Wisconsin Card Sorting Test (WCST). As a secondary task they received rapidly paced shadowing (in which the subjects hear, then repeat words). PET cerebral blood flow measurements were made using the O15 water technique. Data were analyzed by Statistical Parametric Mapping. Results. A number of different conditions were contrasted. In the key comparison we examined whether the pattern of brain activation was different for simultaneously performing the WCST and shadowing words as opposed to the pattern when the two tasks were performed individually and then "summed." Significant deactivations were noted in prefrontal regions. This stands in contrast to numerous activation foci in prefrontal cortex produced when the WCST was performed alone. Thus, shadowing during the WCST produced an activation pattern "more" than the sum of its constituent tasks. In addition, large increases in cerebellar blood flow were observed. Discussion. A fundamental characteristic of prefrontal cortex appeared to be an attenuation of activation in the face of supramaximal demands for stimulus processing. A second physiological response to dual tasks that demand rapid attentional shifts and multiple stimulus response mappings involved large increases in neocerebellar activation, perhaps reflecting a time sharing function for this structure

384.9

EXTRASTRIATE AND FRONTAL CONTRIBUTIONS TO FACE AND LOCATION WORKING MEMORY: AN FMRI STUDY S.M. Courtney*, J.Ma. Maisog, L.G. Ungerleider, J.V. Haxby. SFBI, LPP, NIMH, Bethesda MD 20892.

Differences in the organization of working memory (WM) function in the dorsal/spatial and ventral/object vision pathways were investigated using functional magnetic resonance imaging. Face and location WM tasks were performed during 2 sessions, each with 8 sets of 88 scans (TR 3s) by 3 healthy volunteers. Subjects were presented with an alternating series of WM and sensorimotor control trials. Each WM trial consisted of 3 face pictures, shown one at a time, each in a different location on the screen, followed by a 9 sec delay, which was then followed by the presentation of a single face in a single location. For the face task, subjects indicated whether the fourth face was the same as one of the three faces in the memory set. For the location task, subjects indicated whether it appeared in one of the three locations. Time series data were analyzed using multiple regression in order to determine the relative contributions of visual stimulation, perceptual processing, and sustained activity during the memory delay. Both tasks produced activation in occipital, posterior ventral temporal, parietal, and prefrontal cortices. In prefrontal cortex, sustained memory delay activity in the posterior superior frontal sulcus, bilaterally, was greater during the location task than during the face task. Memory delay activity in bilateral inferior and mid-frontal cortices was greater during the face task than during the location task. In extrastriate cortex, bilateral regions in superior parietal and intraparietal sulcal cortex showed memory delay activity during the location but not the face task. By contrast, during the face task, a bilateral ventral temporal region showed memory delay activity that was smaller and less consistently observed than the parietal activity during the location task. These results demonstrate differences in the functional organization of WM processing in the dorsal and ventral pathways. Different prefrontal regions are associated with spatial and object WM. Moreover, extrastriate areas in the dorsal pathway play a greater role in location WM maintenance than extrastriate areas in the ventral pathway do in object WM.

(Supported by NIMH-IRP)

384.10

ACTIVATION OF IDENTICAL PREFRONTAL REGIONS BY DELAY AND NON-DELAY COMPONENTS OF A WORKING MEMORY TASK: A fMRI STUDY. M D'Esposito,* GK Aguirre, E Zarahn, C Thompson. Dept. Neurology, Univ of Pennsylvania, Phila, PA 19104.

Neurons in macaque dorsolateral prefrontal cortex increase their activity during delayed response tasks. In these tasks, neuronal activity is observed in response to memory and non-memory components. Using echoplanar fMRI, we tested the hypothesis that prefrontal activity in humans could be observed during processes in addition to working memory. Subjects performed a three condition experiment (memory, attention, rest). In the memory condition, subjects observed serially presented stimuli and determined if each stimulus was the same as that presented two stimuli back. Stimuli were either letters or white squares presented in various locations. In the attention condition, subjects were asked to identify a single predetermined letter or location. Three runs of each condition were performed and combined for analysis, allowing observation of significant activity within individuals. Data within prefrontal cortex were analyzed using a modified general linear model for correlated observations. In three subjects, both the verbal and spatial working memory conditions activated dorsolateral prefrontal cortex within middle frontal gyrus (areas 9/46). In addition, activation to a lesser degree was observed during the attention task (as compared to rest), within these same prefrontal regions. We conclude that this region of prefrontal cortex supports processes in addition to working memory, (i.e. the retention of information for subsequent action) such as sustained attention. The region of prefrontal cortex activated in this study may comprise distinct neuronal populations, beyond the spatial resolution of fMRI, that support separable components necessary for bridging perception to action across time. These results illustrate the need to consider the neural correlates of the control task in cognitive subtraction paradigms.

Supported by McDonnell-Pew and NIH grant NS01762.

384.11

DELAY-SPECIFIC ACTIVITY WITHIN PREFRONTAL CORTEX DEMONSTRATED DURING A WORKING MEMORY TASK: A FUNCTIONAL MRI STUDY. E Zarahn,* GK Aguirre, M D'Esposito
Department of Neurology, University of Pennsylvania, Phila, PA 19104

Single-unit recordings in non-human primates have revealed neurons in the prefrontal cortex that increase their firing during a delay between the presentation of information and its later use in behavior. Using the "cognitive subtraction" technique, several imaging studies have claimed that activity observed within human prefrontal cortex is a consequence of this delay period. It is possible, however, that this activity is also related to unsubtracted stimulus presentation and response components of the task. Using echoplanar fMRI at 1.5 T, we tested the hypothesis that the prefrontal cortex increases its functional activity specifically during the delay period of a spatial delayed response task. Subjects were presented pseudo-randomized no-delay and delay trials. Six runs were performed and combined for analysis, allowing observation of significant activity within individuals. A general linear model for correlated observations was used to assess the effects of three independent variables: 1) stimulus presentation, 2) delay, 3) response. This approach allowed signal changes to be correlated directly in time with various temporal components of the delayed response task. In two subjects, a region within prefrontal cortex displayed activity attributable to the delay period of the task. Premotor regions displayed activity in response to all three components of the task. In contrast, primary sensorimotor cortex displayed activity correlated only with response. The different profiles of activity observed in our task agree with findings of single unit electrophysiology experiments. This study identified and differentiated regions involved in stimulus processing, temporary storage of information, and response execution. This method of analysis represents an alternative to "cognitive subtraction" and may be suitable for analysis of other cognitive activation paradigms.

Supported by McDonnell-Pew and NIH grant NS01762.

384.12

RECALL OF TOPOGRAPHIC APPEARANCE AND POSITION: A DISSOCIATION DEMONSTRATED WITH fMRI. GK Aguirre,* E Zarahn, M D'Esposito Dept. of Neurology, Univ. of Penn., Phila., PA 19104

Patients with selective topographical disorientation appear to fractionate into those unable to recognize the visual features of places and those unable to recall the spatial relationships between places. To identify the neural basis of this dissociation we used fMRI to observe ss during their recall of topographic appearance and position information. Two ss learned to criterion a virtual-reality town composed of sixteen distinctive, named, "places". During whole-brain scanning ss were presented with snapshots of the environment and a place name. During the Appearance {**A**} task, ss indicated if the name matched the pictured place, while during the Position {**P**} task, ss indicated the direction of the named place relative to the scene. A Control {**C**} task required ss to alternate left-right responses to scrambled visual scenes and names. All tasks were 60 seconds in duration and self-paced. Multiple runs were collected and analyzed using a modified general linear model for correlated observations, allowing the observation of statistically significant results in a single subject. Maps were thresholded at $p < 0.0167$ (corrected). Both the **A-C** and **P-C** comparisons demonstrated bilateral posterior cingulate (area 30), posterior cuneus (19), posterior parietal (7) and left premotor (6) activity for both ss. Inferior, medial, temporal-occipital cortex (19/37) was active bilaterally in both subjects for only the **A-C** condition. The direct **P-A** comparison revealed a dorsal/ventral dissociation. Activity in the temporal-occipital area was significantly greater during the **A** condition (t=-5.4) while activity in the left premotor (both subjects, t=4.5, 5.4) and left posterior parietal cortex (one subject, t=4.9) was significantly greater during the **P** condition. The dissociated areas of activity observed during the recall of topographic appearance and position information are consistent with lesion sites that have been identified for the classes of topographical disorientation. This study provides evidence that representation of large scale space is in part subserved by separable neuro-anatomical components.

Supported by McDonnell-Pew and NS01762

SEROTONIN RECEPTORS

385.1

IN VITRO AUTORADIOGRAPHIC STUDY OF GUANYLYL-5'-[γ-[^{35}S]THIO]-TRIPHOSPHATE BINDING STIMULATED BY 5-HT$_1$ AGONISTS IN GUINEA PIG BRAIN SECTIONS. C. Waeber and M.A. Moskowitz*. Stroke and Neurovascular Regulation Laboratory, MGH, Harvard Medical School, Charlestown MA 02129.

We have measured the stimulation of [^{35}S]GTPγS binding in guinea pig brain slices using the technique described by Sim et al (PNAS 92, pp. 7242-7246). Briefly, 18 μm-thick sections were incubated in the presence of 2 mM GDP and 0.04 nM [^{35}S]GTPγS for 2 hours at 25°C. After 2 x 2 min. washing, the slides were dried and exposed to Kodak Biomax MR films along with ^{14}C standards. In the absence of agonist, [^{35}S]GTPγS labeling was heterogeneous throughout the brain and was drastically decreased by increasing concentrations of GDP (from 1 to 8 mM). Under the study conditions (2 mM GDP), basal [^{35}S]GTPγS binding was very high in the superficial laminae of the spinal cord and trigeminal nucleus and was overall higher in gray matter (in particular, substantia nigra, interpeduncular nucleus, hippocampus, neocortex, striatum) than in white matter. The 5-HT$_{1A}$ agonist 8-OH-DPAT (10 nM to 1 μM) dose-dependently stimulated [^{35}S]GTPγS binding in hippocampus and deep cortical layers. At 1 μM, hippocampal binding was stimulated 3-fold over basal binding, this effect was inhibited by 1 μM NAN-190. The stimulation obtained with the 5-HT$_{1D/1F}$ agonists sumatriptan and CP-122,288 (0.1 to 10 μM) was much weaker: 10 μM sumatriptan and CP-122,288 induced a 30%, respectively 50%, increase in binding in the substantia nigra. Comparable increases were also found in the striatum, neocortex, central gray and superior colliculus. Interestingly, 2- (sumatriptan) and 5-fold (CP-122,288) increases in binding were observed in hippocampus, with a distribution identical to that seem with 8-OH-DPAT. The non selective agonist metergoline (10 μM) did not enhance binding in the substantia nigra but stimulated more than 5-fold in hippocampus. These results suggest that, in this system, 5-HT$_{1A}$ receptors display a more efficient coupling than 5-HT$_{1D/1F}$ receptors, and that sumatriptan and CP-122,288, despite their 10-fold lower affinity at 5-HT$_{1A}$ receptors, activate the latter to a larger extent than 5-HT$_{1D/1F}$ receptors.

Research supported by NIH 1P01NS35611-01 (MAM) and The Migraine Trust (CW)

385.2

P19 MOUSE EMBRYONAL TERATOCARCINOMA CELLS EXPRESS THE 5-HT$_{1A}$ RECEPTOR IN THEIR NEURONALLY DIFFERENTIATED STATE. D. Benjamin* and L.A. Pohorecky. Division of Neuropharmacology, Center of Alcohol Studies, Rutgers University, Piscataway, New Jersey 08855.

The P19 mouse embryonal carcinoma cell line is used to study neuronal differentiation processes and provides a useful model system for NMDA receptor-mediated neurotoxicity. Four days of treatment with retinoic acid (500 nM) induces the P19 cell to terminally differentiate to a neuronal phenotype. In our ongoing studies of the 5-HT receptors, we tested P19 cells for the expression of 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ receptors. Receptor binding studies with [^{125}I]-LSD did not detect 5-HT$_2$ receptors on the P19 cells in their undifferentiated or neuronally differentiated state. Reverse-transcription polymerase chain reaction did not detect mRNA for the 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors in the P19 cell in either state. The 5-HT$_{1A}$ receptor, however, was expressed at high density in the differentiated, but not undifferentiated P19 cells. Receptor binding studies with [^{3}H]-8-OH-DPAT showed high affinity (Kd $\cong$ 2 nM), specific, and saturable binding in neuronally differentiated P19 cells. Reverse transcriptase-polymerase chain reaction studies confirmed the presence of mouse 5-HT$_{1A}$ message; studies are in progress to verify full sequence identity. Because the P19 cell is, to our knowledge, only the second cell line to constitutively express the mouse 5-HT$_{1A}$ receptor it should provide a useful model system to study the regulation of the 5-HT$_{1A}$ receptor, and because it also expresses a functional NMDA receptor it should be useful to study the effect of 5-HT on NMDA toxicity. (Partial support from MH52293 and AA10124).

385.3

CELLULAR LOCALIZATION OF 5-HT_{2A} AND 5-HT_4 RECEPTORS IN ENTERIC NERVE AND SMOOTH MUSCLE E. Fiorica-Howells[1], S. Garlow[2] and M.D. Gershon[1]*. [1]Dept. Anat. & Cell Biol., Columbia Univ. P.&S., New York, NY 10032 & [2]Dept. Psych., Emory Univ. Atlanta, GA 30322.

Over 95% of the body's 5-HT is located in the gut. Because many subtypes of 5-HT receptor are present on enteric nerves, muscle, and epithelium, cellular expression of receptors must be known to understand 5-HT's function. Studies were carried out to locate cells that express 5-HT_{2A} or 5-HT_4 receptors. RNA was extracted from isolated guinea pig myenteric ganglia and mucosa-free rat small intestine, reverse transcribed, and amplified by PCR. mRNAs encoding 5-HT_{2A} and 5-HT_4 receptors were detected respectively in the guinea pig ganglia and rat intestine. Immunocytochemistry and *in situ* hybridization (^{35}S-riboprobe) indicated that the 5-HT_{2A} receptor is expressed by myenteric neurons throughout the gut, by submucosal neurons in small and large intestines, and by circular muscle where 5-HT_{2A}-immunoreactive nerve fibers were also especially abundant. mRNA encoding the 5-HT_4 receptor was detected by *in situ* hybridization in enteric neurons (most abundantly in ileum and proximal colon), but not in muscle or mucosa. These data suggest that 5-HT_{2A} receptors play a role in enteric neuronal as well as muscle function; 5-HT_4 receptors are exclusively neuronal, but there are regional differences in the abundance of 5-HT_4-expressing neurons. Supported by NIH grant NS12969.

385.4

ANTISENSE INHIBITION OF 5HT_{2A} RECEPTOR IMPAIRS CONTAINMENT OF STRESS RESPONSE. E. Sibille[1], Z. Sarnyai[2], D. Benjamin[3], and M. Toth[1]* [1]Department of Pharmacology, Cornell Univ. Medical College, [2]Laboratory of Neuroendocrinology, Rockefeller University, and [3]Center of Alcohol Studies, Rutgers University.

Stress causes an abrupt and large change in neurotransmitter release. While this activation of neurochemical systems is beneficial in the body's and brain's response to stress, it is imperative that these responses are shut down when they become excessive. If containment does not occur, a vicious cycle may ensue in which elevated sympathetic activity and glucocorticoid level lead to diseases such as depression. We are interested in the involvement of the 5HT2A receptor (5HT2AR) in adaptation since it is well documented that its level is increased in suicide victims. Since drugs are not specific to 5HT receptors, a genetic approach was chosen to assess the relationship between 5HT2AR and behavior. Antisense oligo-nucleotides (AS) can specifically downregulate gene expression. A 19-mer AS, corresponding to the 5' translated region of the 5HT2AR or a control mismatched oligonucleotide (MS) were injected icv in mice (10μg, every 12 hours for 4 days). Animals were subjected to a swim stress that normally results in a gradual decrease of escape directed behavior as an adaptive containment of stress response. AS but not MS treatment decreased the development of this response to stress. In addition to hyperreactivity to stress, AS treated animals showed an increased ethanol tolerance, indicating a higher arousal state. Our results suggest that 5HT2AR is involved in the termination and containment of stress induced responses, such as hyperreactivity and hyperarousal. When 5HT2AR level is sub-optimal, delayed and incomplete containment can lead to excessive maladaptive responses to stress, resembling certain symptoms of depression. Supported by NARSAD.

385.5

A NF-AT LIKE TRANSCRIPTION FACTOR, FACTOR *B*, IS ESSENTIAL FOR THE EXPRESSION OF HUMAN SEROTONIN 5-HT_{2A} RECEPTOR GENE. Q.S. Zhu* and J.C Shih. Dept. of Mol. Pharm. and Tox., Sch. of Pharmacy, Univ. of Southern California, 1985 Zonal Ave., Los Angeles, CA 90033.

A novel transcription factor, factor *b*, binding site was identified 62 bp upstream of the 5' most transcription initiation site of the human serotonin 5-HT_{2A} receptor (5-HT_{2A}R) gene. The DNA binding sequence of factor *b* was similar (15 out of 21 bp identical) to that of human NF-AT, a transcription factor for interleukin-2 (IL-2) production in activated T cells and the factor *b* DNA binding was displaceable by human NF-AT oligonucleotides in gel retardation assay. These results suggest that similar transcription factors may be involved in regulation of the 5-HT_{2A}R and the IL-2 genes. Furthermore, the presence of factor *b* complex was correlated with 5-HT_{2A}R mRNA level and promoter activity in all cell lines tested. Site-directed mutagenesis of the binding sequence abolished the factor *b*-DNA complex in a gel retardation assay and reduced the promoter activity to 35% of the wild type in a transient transfection assay (human growth hormone as a reporter gene). These results suggest that factor *b* may be essential for the expression of the 5-HT_{2A}R gene. UV-crosslinking with synthetic factor *b* binding sequence showed a specific protein band with a molecular weight of ~49 kDa. Further characterization of this protein will help us better understand the nature of the factor *b* complex. (Supported by NIMH grants R37 MH39085 (MERIT Award), K05 MH00796 (Research Scientist Award), R01 MH37020 and Welin Professorship).

385.6

A SINGLE POINT MUTATION DIFFERENTIALLY AFFECTS TRYPTAMINE AND PHENYLISOPROPYLAMINE EFFICACY AT 5-HT2A RECEPTORS. BL Roth*, N Khan, and MS Choudhary, Departments of Biochemistry and Psychiatry, Case Western Reserve University Medical School, Cleveland, OH 44106.

Understanding the mechanisms by which drugs bind to and activate G-protein coupled receptors (GPCR) remains a major unsolved problem for biochemists and pharmacologists. We have used the 5-HT2A receptor as a model system for understanding how agonists bind to and activate receptors. We have recently discovered a single point mutation (F340L) which appears to differentially alter the ability of structurally diverse agonists to activate second messenger production. For these studies, stably transfected cell lines expressing the native and mutant 5-HT2A receptors were constructed (Roth et al, JPET, 1995). We discovered that the abilities of tryptamine agonists (5-HT, N,N'-DMT, 5-O-Me-DMT,α-Methyl-5HT) to activate phosphoinositide hydrolysis was virtually abolished by the F340L mutation, while several phenylisopropylamines (DOI, DOB, DOM) had efficacies ranging from 50-100% of that seen with the native receptor. For all groups of compounds, potencies were diminished to a much greater extent than binding affinities. In several cases, potencies were diminished by greater than 1000-fold while binding affinities for the high-affinity state was affected by less than 20-fold. Our results are consistent with a model in which agonist-induced movements of Helix 6 are an essential feature of receptor activation. Supported by RO1GM52213 to BLR.

385.7

REDUCED DENTATE GYRUS LTP AND IMPAIRED PROCESSING OF SPATIAL CONTEXT IN 5-HT_{2C} RECEPTOR MUTANT MICE. L. Tecott, S. Das, S.F. Logue*[1], J.M. Wehner[1] and J.A. Kauer[2]. UCSF Dept. Psychiatry, S.F., CA 94143; [1]Inst. Behav. Genetics, Univ. Colorado, Boulder, CO 80309; [2]Dept. Neurobiology Duke Univ., Durham, NC 27710.

Serotonin systems have been implicated in the regulation of cognition and hippocampal activity. To determine the contributions of the 5-HT_{2C} receptor to the serotonergic modulation of these processes, a 5-HT_{2C} receptor mutant mouse strain was examined (Tecott et al *Nature* 372: 542 '95). In slice preparations, long term potentiation (LTP) was examined at each of four principle regions of the hippocampal complex: the dentate gyrus, CA3, CA1 and the subiculum. Using standard double blind methods of LTP induction, potentiation at medial perforant path-dentate gyrus synapses was selectively reduced (by approx. 50%) in mutant mice. To assess behavioral correlates of altered hippocampal function, contextual fear conditioning and defensive withdrawal responses were examined. Twenty-four hours after training for contextual fear, wild type mice froze more to the trained context than the altered context, while the mutants did not discriminate between the two contexts. In the second paradigm, the latencies of mice to emerge from a small enclosure into a brightly lit open field were assessed. Emergence neophobia has been found to correlate with levels of dentate gyrus LTP in rats (Maren et al *Psychobiology* 21: 2 '93). A 70% reduction in emergence latency was observed in the mutants, consistent with the reduction in perforant path LTP in these animals. Together, these results indicate that 5-HT_{2C} receptor mutant mice have impaired processing of contextual stimuli associated with a selective impairment of dentate gyrus function. *Supported by the EJLB Foundation and DA00282 (LT); MH-48663 (SFL, JMW); NS30500-05 (JAK).*

385.8

LOCALIZATION OF 5-HT_{2C} RECEPTORS BY IMMUNOHISTOCHEMISTRY AND CONFOCAL MICROSCOPY: PRESYNAPTIC 5-HT_{2C} RECEPTORS? J.R. Backstrom, S. Schroeter*, R.D. Blakely, and E. Sanders-Bush. Department of Pharmacology and Center for Molecular Neuroscience, Vanderbilt University School of Medicine, Nashville, TN 37232.

Precise localization of serotonin 5-HT_{2C} receptors has not been possible due to the absence of 5-HT_{2C}-selective reagents. We have developed specific antibodies (anti-2C-CT) against a peptide within the carboxy-terminus that has enabled us to localize 5-HT_{2C} receptors with cellular resolution. Specificity of affinity-purified anti-2C-CT was examined in two cell lines expressing the 5-HT_{2C} receptor or the related 5-HT_{2A} receptor. Anti-2C-CT recognized proteins on immunoblots from the 5-HT_{2C} but not the 5-HT_{2A} receptor cell line. In addition, anti-2C-CT only labeled cells expressing 5-HT_{2C} receptors in immunocytochemical experiments. 5-HT_{2C} receptor immunoreactivity was localized in 40 μm sections of paraformaldehyde-perfused rat brain and detected by fluorescence or peroxidase. Confocal images of sections double-labeled with anti-2C-CT and anti-GFAP, to identify glial elements, revealed a distribution of 5-HT_{2C} receptors within the choroid plexus and neuronal layers including the hippocampus, cortex, and cerebellum. Anti-2C-CT also intensely labeled olfactory regions such as the olfactory cortex and medial habenula. Receptor immunoreactivity was localized to axon-like structures which terminate in a network of fibers surrounding the surface of neuronal soma. Future experiments will include colocalization with presynaptic markers and lesioning of serotonergic nuclei to test the hypothesis that 5-HT_{2C} receptors are located at axon terminals. (Supported by NIH research grants DA07390, DA05181, MH34007, training grant MH19732, and a fellowship from PhaRMA Foundation)

385.9

IDENTIFICATION AND CHARACTERIZATION OF RNA EDITING WITHIN THE SEROTONIN 2C RECEPTOR. C.M. Burns*, H. Chu, S.M. Rueter, E. Sanders-Bush and R.B. Emeson. Dept. of Pharmacology, Vanderbilt University, Nashville, TN 37232-6600.

Transcripts encoding the 2C-subtype of serotonin receptor (5-HT_{2C}R) undergo RNA editing events in which genomically-encoded adenosine residues are represented as guanosines in the 5-HT_{2C}R cDNA. Editing events at four major positions, termed A, B, C, and D, are predicted to alter amino acids within the second intracellular loop of the protein, encoding unique receptor isoforms with one to three amino acid substitutions (I157V, N159S and I161V). Sequence analyses of individual cDNA isolates from microdissected rat brain regions have predicted the tissue-specific production of eight major protein isoforms encoded by eleven distinct RNA species. 5-HT_{2C}R transcripts isolated from the choroid plexus demonstrated lower levels of editing at the A and B sites (25%) than other brain regions (85%). The C site appeared to be edited with the most consistency in all areas examined (30-35%) while editing at the D site was most variable (45-80%).

Examinations of 5-HT_{2C}R pre-mRNA sequences identified the presence of a putative RNA duplex formed by the 3'-end of exon 3 and the proximal region of the third intron. Mutational analyses within this structure indicated a dependence of editing upon this duplex region similar to that previously observed for GluR-B RNA processing. The development of an *in vitro* 5-HT_{2C}R RNA editing system, using rat brain nuclear extracts fractionated by cation exchange chromatography and a 375 nt 5-HT_{2C}R transcript, revealed that this modification results from an adenosine to inosine conversion rather than the adenosine to guanosine alteration inferred from isolated cDNA sequences. Further characterization of cation-exchange fractions identified two distinct adenosine deaminase activities responsible for the processing of 5-HT_{2C}R transcripts. The first activity, mediating RNA modifications at the A, B and C sites, co-eluted with the activity responsible for the processing of GluR-B at intronic position +60. The second peak of activity mediated the editing of the 5-HT_{2C}R D-site as well as the Q/R site of GluR-B. These results suggest that similar, or identical activities, are responsible for the editing of both 5-HT_{2C}R and GluR-B RNA transcripts. Supported by NIH grant NS33323.

385.10

FUNCTIONAL CHARACTERIZATION OF RAT SEROTONIN 5-HT_{2C} RECEPTOR EDITING ISOFORMS. H. Chu, C. Burns, H. Canton, R.B. Emeson and E. Sanders-Bush*. Dept. of Pharmacology, Vanderbilt University, Nashville, TN 37232-6600.

We have accumulated evidence that RNA transcripts encoding the rat 5-HT_{2C} receptor undergo RNA editing events similar to those identified for the B-subunit of the AMPA subtype of glutamate receptor. Several adenosine to guanosine transitions were discovered within the exon that encodes the putative second intracellular loop of the receptor. The major products of 5-HT_{2C} RNA editing encode unique proteins with one to three amino acid substitutions (I157V, N159S and I161V) in the second intracellular loop of the predicted protein. Analyses of mRNA from microdissected brain regions have revealed the existence of region-specific editing patterns predicting the production of eight major protein isoforms encoded by eleven edited RNA species. To assess the significance of RNA editing in 5-HT_{2C} receptor function, the major edited species were subcloned into the mammalian expression vector pCMV2 and transiently transfected into NIH 3T3 fibroblasts. Competition binding analyses revealed similar affinities of different ligands for all edited isoforms. Pharmacological characterization of the edited receptors in this model system showed that the potency of serotonin to activate the inositol lipid signaling cascade was markedly reduced in the fully edited form ($5HT_{2C}$-VSV) compared to the non-edited form($5HT_{2C}$-INI); the EC_{50} for serotonin activation was 106.3 ± 21.1 vs 11.7 ± 4.7 nM, respectively. A similar functional difference was observed for another edited species ($5HT_{2C}$-ISV) that differed by only two amino acids. In both cases, the $5HT_{2C}$ receptor edited species with a serine at position 159 and a valine at position 161 were less potent than those with asparagine and isoleucine at the same positions. We propose that the simultaneous amino acid alterations at these two sites, as a result of RNA editing events, generates receptor isoforms which couple less efficiently to the intracellular signaling machinery and represents a novel mechanism for the regulation of G-protein coupled receptor activity. (Supported by NIH grant MH34007; H.Chu is supported by The National Defense Medical Center, Taipei, Taiwan R.O.C.)

385.11

WITHDRAWN

AXON GUIDANCE MECHANISMS AND PATHWAYS I

386.1

THE EPH RECEPTOR CEK5 AND ITS LIGAND, CEK5L, HAVE COMPLEMENTARY DISTRIBUTIONS IN THE EARLY RETINA. J.A. Holash*, C. Soans L.D. Chong, and E.B. Pasquale, The Burnham Institute, La Jolla, CA 92122

Members of the EPH subclass of receptor tyrosine kinases appear to influence pattern formation. We have shown that the Eph receptor Cek5 has a polarized distribution in the chick retina suggesting that it plays a role in establishing retinotectal topography along the dorsal-ventral axis. Others have found that a similar, yet distinct, role is likely played by Cek4, which is expressed in a nasal-temporal gradient in the developing retina (Cheng et al., Cell 82:371-381, 1995). Cek4 and its ligand, Elf-1, have complementary distributions in the tectum. We have compared the distribution of Cek5 and an activating ligand, Cek5L, in the visual pathway using immunohistochemistry and *in situ* hybridization. In the embryonic day 8 chick tectum, neither receptor nor ligand can be detected to be expressed in a gradient, suggesting that their role in visual pathway development occurs prior to axons reaching the tectum. Interestingly, in the retina Cek5 and Cek5L have complementary distributions during axonogenesis, but as development progresses, their patterns of expression become overlapping. Both Cek5 and Cek5L are expressed in retinal ganglion cells. To examine the interaction of Cek5 and Cek5L, we prepared chimeric molecules consisting of either Cek5 or Cek5L fused to a human IgG domain. These molecules were used as substrates for cells dissociated from dorsal or ventral retina. Cells derived from ventral retina (expressing high levels of Cek5) showed enhanced attachment to ligand-coated plates, whereas cells from dorsal retina (expressing high levels of ligand) attached and spread more readily on receptor-coated plates. In both instances the cells were capable of extending neurites. This suggests that Cek5-Cek5L interaction mediates cell adhesion and does not prevent neurite outgrowth. Funded by NIH and MRC-Canada.

386.2

EPH FAMILY RECEPTORS AND LIGANDS ARE DISTRIBUTED IN COMPLEMENTARY DOMAINS IN THE MOUSE RETINOFUGAL PATHWAY. R.C. Marcus*, R. Dhavan, N.W. Gale, C.S. Harlan, M.E. Morrison, C.A. Mason and G.D. Yancopoulos. Dept. of Pathology, Columbia Univ. Coll. of P &S, New York, NY 10032 and Regeneron Pharmaceuticals, Tarrytown, NY 10804.

Eph family receptor tyrosine kinases and their ligands can be grouped into either GPI-linked or transmembrane (TM) subclasses. We used receptor- and ligand-antibody fusion proteins from each subclass to localize their corresponding ligands and receptors in the developing mouse retinofugal pathway. In agreement with previous reports, we find that in the embryonic day 14 (E14) retina, receptors of the GPI-linked and TM subclasses are distributed in a high temporal-low nasal and a high ventral-low dorsal gradient, respectively. We now report that ligands from each subclass are also distributed in gradients within the retina, and that these gradients are opposite to those of their corresponding receptors. The presence of ligand in the retina suggests an intra-retinal role for Eph family members in addition to their proposed role in retinotectal mapping. The distinct distributions of the GPI-linked and TM members suggest that the different subclasses control retinal development in the naso-temporal and dorso-ventral axes.

Eph family receptors and ligands also appear in complementary domains in the E14 optic chiasm. Retinal axons diverge to opposite sides of the brain within a radial glial palisade that straddles the chiasmatic midline. We find receptors of both the GPI-linked and TM subclasses localize in the zone occupied by these radial glia. Ligands of the GPI-linked subclass are located posterior to the chiasm, in a region occupied by a population of neurons thought to be inhibitory to retinal axon growth.

Thus Eph-family receptors and their ligands may direct retinal ganglion cell growth at multiple sites along the developing visual pathway. Supported by NRSA EY06510 (RCM) and NS27615, PO1 NS30532 (CAM).

386.3

Regulation of Topographic Projection in the Brain: Elf-1 in the Hippocamposeptal System. P.-P. Gao[1], J.-H. Zhang[1], M. Yokoyama[2], B. Racey[3], C. F. Dreyfus[3], I. B. Black[3], and R. Zhou[1]. [1]Laboratory for Cancer Research, College of Pharmacy, Rutgers University; Piscataway, NJ 08855. [2]M. Yokoyama, Trophix Pharmaceuticals, Inc., 40 Cragwood Road, South Plainfield, NJ 07080. [3]B. Racey, C. F. Dreyfus, I. Black, Department of Cell Biology and Neuroscience, Robert Wood Johnson Medical School, Piscataway, NJ 08855

Although topographic organization and projection are general features of brain functional architecture, organizing mechanisms remain undefined. We now report that Elf-1, a membrane-bound eph family ligand, is a candidate molecular tag for the genesis of the hippocampo-septal topographic projection. Neurons project as a gradient from the medio-lateral hippocampus to dorso-ventral septal targets. Elf-1 was expressed in an increasing gradient from dorsal to ventral septum. Furthermore, Elf-1 selectively allows growth of neurites from topographically appropriate lateral hippocampal neurons, while inhibiting neurite outgrowth by medial hippocampal neurons, thereby playing a critical role in the development of the topographic projection. Complementary to the expression of Elf-1, an eph family receptor, Bsk, is expressed in the hippocampus in a lateral to medial gradient, consistent with a function as a receptor for Elf-1. Further, Elf-1 specifically bound Bsk, eliciting tyrosine kinase activity. We conclude that the Elf-1/Bsk ligand-receptor pair exhibits traits of a chemoaffinity system for the organization of hippocamposeptal topographic projections. (Supported by NSF IBN-9409930 and Trophix Pharmaceuticals Inc.)

386.4

PARTIAL RETINAL ABLATION IN EMBRYO ALLOWS THE UNCROSSED PROJECTION TO DEVELOP.

J.S.H. Taylor*
Dept. Human Anatomy, University of Oxford, Oxford. OX1 3QX. U.K.

The optic chiasm is where the retinal axons from the two eyes meet and a sub-population turns into the ipsilateral optic tract forming the uncrossed projection. It has been shown in both mice (Godement et al., 1987. J. comp. Neurol. **255**:97) and ferrets (Taylor, J.S.H. & Guillery, R.W. 1995. J. comp. Neurol. **357**:331) that very early eye removal stops the uncrossed projection from the other eye from growing into the ipsilateral optic tract. However a contradictory finding has been published suggesting early enucleation has no effect on the uncrossed projection in mice (Sretavan & Reichardt 1993. Neuron **10**:761).

Using exo-utero surgery, the dorso-nasal or temporo-ventral retina was removed on embryonic day 12 or 13. Embryos were fixed on E17.5 and DiI used to label the ipsilateral projection of the intact eye retrogradely or anterogradely. Ipsilaterally projecting labelled cells were counted in retinal flat mounts and showed no diminution of the temporal crescent when compared with control retinae. DiI labelling of the temporo-ventral retina in the intact eye showed a normal uncrossed projection had formed at the optic chiasm.

We have found no clear evidence for a differential effect of partial retinal lesion and conclude that there is no specific sub-population of contralaterally projecting axons that is required to guide the ipsilaterally projecting axons. These results may have implications for the interpretation of experiments in which monocular enucleation has been suggested to have no effect on the development of the ipsilateral retinal projection.

This work is supported by the Wellcome Trust.

386.5

RETINAL AXON PATHFINDING: DIFFUSIBLE CUES FROM THE CHIASM AND THE FLOOR PLATE SUPPRESS GROWTH OF ALL RETINAL AXONS L.-C. Wang*, A. Augsburger, R.A. Rachel, R.C. Marcus, and C.A. Mason, Depts. Pathology., Anat. and Cell Biol., Physiol. and Cell. Biophys., Cntr. Neurobiol. Behav., Columbia Univ., NY, NY 10032.

Retinal axons diverge to both sides of the brain near the midline of the optic chiasm. Recent in vitro studies demonstrated that cells from the chiasm elicit differential growth of crossed and uncrossed fibers, in patterns that mimic avoidance or growth across the midline (Wang et al., Neuron, 15:1349, 1995). These effects are believed to be contact-mediated. We also showed that diffusible cues from the chiasm suppress growth of axons from all poles of the retina to an equal extent (Wang et al., Soc. Neurosci. Abstr. 21: 1296, 1995). Such cues, if released and bound to the ECM, could produce divergence, or could slow axon growth in decision regions, thereby permitting responses to other more specific guidance cues. Here we demonstrate that this general growth-retarding activity is not present in the cerebellum and cortex, but is even stronger in another midline site, the floor plate, suggesting that the floor plate contains similar diffusible factors that suppress retinal axon growth. Since the floor plate is known to be rich in netrin-1, which can act as an attractant or repellant on different axon populations, retinal explants were cocultured with netrin-1-transfected COS cells (construct gift of M. Tessier-Lavigne) in collagen gels. Netrin-1 did not mimic the general growth-retarding effect, but, instead, promoted retinal axon growth. Further, neurites did not appear to orient toward netrin-1-positive cells, implying a trophic but not tropic influence on retinal neurite growth. These data suggest that a non-netrin-1 inhibitory factor is present at different axial levels, and together with our previous studies, emphasize that multiple guidance mechanisms may underlie retinal axon divergence.

Supported by NIH grants NS27615 and PO NS30532 to C.A.M. and NS27113 to J. Dodd (A.A.).

386.6

MIDLINE CROSSING MUTATION AFFECTS MOTONEURON PATHFINDING AND TARGET RECOGNITION B. Wolf, L. Faiman,* & A. Chiba Dept. of Cell & Struct. Biol., Univ. of Illinois, Urbana, IL 61801

We are interested in how disruptions in midline crossing of CNS axons influence the axons' subsequent responsiveness to local molecular cues. One model system is the *commissureless* (*comm*) mutants in *Drosophila* embryos. The *comm* gene encodes a putative transmembrane protein expressed on midline glia and when absent causes the failure of axons to cross the midline. The expression pattern has also been reported during early peripheral tissue development. We found that immunocytochemistry labeling of motoneurons using anti-Fasciclin II antibody in *comm* null mutants embryos (late stage 16) revealed abnormalities in the SNb nerve branch which contains midline-crossing motoneurons including RP3 and RP1. Using an enhancer-trap line which labels RP3 and RP1, two midline-crossing neurons that innervate muscles 6 and 7, and 13 respectively, we were able to observe the effects of *comm* mutations at the single cell level. In *comm* null mutants, RP3 axons extend ipsilaterally along the normal pathway and reach the ventral muscle region containing muscles 6 and 7, but fail to innervate the 6/7 cleft. Much of the axon pathway remains normal despite failing to cross the midline; only the final target recognition and innervation seemed to be affected. Other nerve branches which may or may not contain midline-crossing axons (SNa, SNc, and Snd) were also examined using anti-Fasciclin II. We observed similar abnormalities in *comm* null mutants, suggesting the existence of previously uncharacterized midline-crossing motoneurons or a direct interaction between those axons which normally cross the midline and those that do not. The results raise the possibility that midline crossing is responsible for altering molecular expression pattern in growth cones and is required to specify aspects of subsequent pathfinding and target recognition.

Supported by NIH and NSF.

386.7

A CAM SIGNALING COMPLEX WITH POTENTIAL FOR INTEGRATING ADHESION SIGNALS FOR AXON GROWTH. H.E. Beggs, S. C. Baragona, J.J. Hemperly, and P.F. Maness*. Department of Biochemistry, Univ. of North Carolina, School of Medicine, Chapel Hill, NC 27599.

Growth cones respond to different substrates by rapid morphological changes and growth rate modification. We have shown that the *src* family tyrosine kinase p59*fyn* is necessary for NCAM-mediated neurite outgrowth (Beggs et al., *J. Cell Biol.* 127, 825-833). A molecular complex of the cell adhesion molecules NCAM and p59*fyn*, together with L1, was revealed by immunoprecipitation from developing mouse cerebellum. When different NCAM isoforms were transfected into COS cells or rat B35 neuroblastoma cell lines, NCAM 140 was found to associate strongly with p59*fyn*, whereas NCAM 180 associated to a much lesser degree. The presence of the VASE isoform in NCAM140 did not alter the association. In contrast, pp60*c-src* was not associated with either isoform of NCAM. Similarly, L1 bound strongly to p59*fyn* but not to pp60*c-src*+ in transfected cells. p59*fyn* kinase was transiently activated upon crosslinking of NCAM140 with antibodies to the extracellular region, indicating a functional association. *In vitro* kinase assays revealed an additional protein besides p59*fyn* as a component of the NCAM signaling complex, which was identified as the focal adhesion kinase p125FAK. This CAM/*fyn*/FAK complex may represent an important biochemical signal transduction mechanism for integrating multiple adhesion pathways during growth cone guidance. This work was supported by NIH grant NS52269.

386.8

In the absence of axonin-1 and NrCAM interactions the floor plate is inhibitory for the ingrowth of cultured commissural axons

E.T. Stoeckli, P. Sonderegger[1], G.E. Pollerberg [2], and L.T. Landmesser*, Case Western Reserve University, Cleveland, OH 44106;[1]Institute of Biochemistry, University of Zurich, CH-8057 Zurich, Switzerland, [2]MPI for Developmental Biology, 72076 Tuebingen, Germany

In vivo perturbation studies have shown that axonin-1 and NrCAM, two cell adhesion molecules of the Ig superfamily, are required for the normal growth of chick commissural axons across the floor plate in the chicken embryo spinal cord (Stoeckli, E.T., and L.T. Landmesser, Neuron *14*(1995), 1165-1179). To further characterize their role in commissural pathfinding, we studied the behavior of commissural growth cones when they were interacting with floor plate explants in culture. In control cultures, commissural axons rapidly entered such explants. When either axonin-1 or NrCAM function was interfered with, commissural axons grew up to, but failed to enter the floor plate explants. Time lapse imaging revealed that in the presence of anti-axonin-1 antibodies filopodial contact with the floor plate triggered full collapse of the growth cones. In the presence of anti-NrCAM antibodies growth cones did not exhibit full collapse, but appeared unable to grow into the floor plate. These studies show that axonin-1 and NrCAM are crucial for the intimate interactions of commissural axons with the floor plate, which in turn is required for the guidance of these axons across the ventral midline.

Supported by NIH grant NS19640.

386.9

FASCICLIN III MEDIATES BOTH AXON FASCICULATION AND TARGET RECOGNITION IN *DROSOPHILA*

H. Kose, X. Zhu, D. Rose, A. Chiba , & E. Meisami* Neurosci. Program, Univ. of Illinois, Urbana, IL 61801

Fasciclin III, an Ig-CAM, appears in specific subsets of motoneurons and muscle cells during *Drosophila* neuromuscular development and has been proposed to act as a "positive target recognition molecule" for at least one Fasciclin III-positive motoneuron (RP3) which normally innervates the Fasciclin III-positive target muscle cells. Here we further tested how this Ig-CAM may regulate growth cone pathfinding and/or target recognition. First, to determine Fasciclin III's possible roles during axon navigation, we generated both heat-shock inducible Fasciclin III overexpresser fly lines and an enhancer trap line ectopically expressing Fasciclin III in a small subset of motoneurons (e.g. aCC) which do not normally express this molecule. When the overexpresser embryos are heat-shocked before motoneuron axons reach the peripheral target regions and thereby all motoneuron axons are made to express Fasciclin III, the axons continue to extend but fail to defasciculate from each other at appropriate nerve branch points. In contrast, in the enhancer trap embryos, where only one or a few motoneuron axons ectopically express Fasciclin III in a given nerve, defasciculation occurs normally. This suggests that Fasciclin III can mediate axon-axon fasciculation through homophilic adhesion. Second, to determine how Fasciclin III may mediate the motor growth cone-muscle interaction, we conducted a series of molecular (Fasciclin III) mismatch experiments using various genetic manipulations. The newly developed laser-mediated single cell gene activation method has also facilitated our ability to control the site and timing of Fasciclin III ectopic expression. The results suggest that the decision for a motoneuron growth cone to stop and initiate synaptogenesis on Fasciclin III-positive muscle cell(s) may require either Fasciclin III itself or other yet uncharacterized Fasciclin III receptors on the responding growth cone. Supported by NIH and NSF.

386.10

MECHANISMS OF GROWTH INHIBITION INDUCED BY THE NG2 CHONDROITIN SULFATE PROTEOGLYCAN

Joel M. Levine* and Chang-lin Dou[1], Dept. Of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY and [1]Dept. Of Cell Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY

Proteoglycans (PG) comprise a heterogeneous class of glycoproteins that can inhibit or enhance neurite extension in vitro. One growth inhibitory chondroitin sulfate PG is NG2, a high molecular weight PG that is found on the surfaces of oligodendrocyte precursor cells in developing and adult animals. Neurite growth from neonatal rat cerebellar neurons on either laminin or L1-coated surfaces is inhibited by the NG2 core protein but the growth of embryonic DRG neurons is inhibited by NG2 only on laminin substrates (J.Neurosci., 14:7616,1994).

This growth inhibition may be mediated by specific cell surface receptors for NG2 that are linked to intracellular signaling pathways. In support of this hypothesis, we show that NG2 binds specifically and with high affinity to cerebellar granule neurons but not to 3T3 cells. Rat DRG neurons had approximately half the NG2 binding sites as did cerebellar neurons. Protein crosslinking and immunoprecipitation identified a 280kD protein from cerebellar neurons as an NG2-binding site. The inhibition of neurite outgrowth by NG2 on L1 surfaces was reversed by treating the neurons with pertussis toxin suggesting that the NG2 binding site is linked, either directly or indirectly, to G-protein-dependent signaling mechanisms. Pharmacological agents that raise intracellular calcium also reversed the NG2-induced growth inhibition. Interestingly, growth inhibition on laminin was not reversed by pertussis toxin.

These observations suggest that the ability of the NG2 PG to inhibit neurite growth on L1 substrates is due to an interaction of the PG with a neuronal receptor. NG2 binding to this receptor may modify the ongoing activity of L1-stimulated second messenger systems. Supported by NIH grant # NS 21198 to JML.

386.11

HB-GAM (HEPARIN-BINDING GROWTH ASSOCIATED MOLECULE) AND N-SYNDECAN PLAY A ROLE IN THE FORMATION OF THALAMOCORTICAL CONNECTIONS ?

A. Kinnunen*, M. Niemi, R. Nolo, T. Kinnunen, M. Kaksonen and H. Rauvala, Laboratory of Molecular Neurobiology, Institute of Biotechnology, P.O.Box 56, 00014 University of Helsinki, Finland.

ECM-molecules such as laminin, tenascin, chondroitin sulphate proteoglycans (CSPG) and heparan sulphate proteoglycans (HSPG) have been suggested to have "signpost" and directing roles in the formation of axonal projections and in the cortical development. We have discovered that the distribution of HB-GAM, an 18 kD neurite-outgrowth promoting protein, in the developing rat brain is spatio-temporally associated with the developing thalamicortical pathways. Using *in situ* hybridization, thalamic neurons were shown to express N-syndecan mRNA, an HSPG shown to act as a receptor for HB-GAM. Thalamic E16 neurons showed repeatedly a typical growth pattern resembling the HB-GAM distribution when cultured on living brain slice preparation. *In vitro* thalamic neurons expressed more neurite outgrowth on HB-GAM than on laminin-coated matrixes. We also tested the effect of soluble heparin on the neurite outgrowth of E16 thalamic neurons *in vitro* on living slice-culture preparation and the effect of soluble heparin, N-syndecan, HB-GAM and chondroitin sulphate on culture wells coated with HB-GAM, laminin and poly-L-lysine. Neurite outgrowth of thalamic neurons was partially perturbed by soluble heparin on living brain slices and clearly dimished by soluble HB-GAM, N-syndecan and heparin on HB-GAM coated wells. Our results suggest that HB-GAM may function as an ECM bound cue for thalamic neurons that possess N-syndecan on their cell membrane. *Supported by the Academy of Finland and Sigrid Jusélius Foundation.*

386.12

ERROR CORRECTION IN REELER BRAIN DEVELOPMENT.

Nuwan C. Kurukulasuriya and Walter L. Salinger*, Biology and Psychology Departments. University of North Carolina at Greensboro, NC 27412

In the reeler neocortex, many neurons achieve normal patterns of connections even though they are ectopically placed. This achievement requires changes in the timing and routing of axonal growth. Thus, DiI (Molecular Probes) labeled thalamic axons in reeler traverse the full extent of cortex by E15 while in the normal, the thalamocortical axons do not invade until much later, E18-P0 and never traverse the entire, developing cortex. How is molecular regulation of axonal growth altered in the reeler to permit these changes? Glycosylated protein conjugates, GPC's, labeled with biotinylated peanut agglutinin, PNA (Vector), are expressed much earlier in the reeler cortex, at least by E15, as opposed to E18-P0, in the normal. Furthermore, in the E15 reeler, PNA labeling is present throughout the thickness of the developing cortex, mottled and without evidence of the radially oriented concentration gradient that is typical for the normal. Contributing to the mottled PNA labeling pattern at E15 in the reeler are cell-sparse channels that are richer in GPC label than the surrounding cortex which also displays PNA label. However, these GPC rich channels do not seem essential for guidance of the invading thalamocortical axons since many such axons grow through GPC labeled cortical tissue outside of the GPC rich channels. The reeler timetable for and spatial distribution of PNA labeled GPC expression in developing neocortex thus reveals that GPC's may be permissive rather than instructive for thalamocortical axons in developing cortex. The deviations in reeler neocortex from normal patterns of GPC expression account for the altered timetable for the invasion of neocortex by thalamocortical axons in the reeler but not for their distinctive growth trajectories. The mechanism by which the temporal and spatial patterns of expression of GPC's in reeler brain are adjusted in compensation for ectopia remains to be described. (Supported by UNCG-RCG #3-19563)

POSTSYNAPTIC MECHANISMS: ELECTRICAL EXCITABILITY AND Ca^{2+} SIGNALLING

387.1

Phase Resetting of High Potassium Spiking Activity in Hippocampal Slices. A. Jahangiri and D.M. Durand* and J.C. Lin, Depts of Biomedical Engineering, Case Western Reserve University, Cleveland, OH. 44106.

The theory of phase resetting can be used to predict the response of spontaneous oscillators to perturbations. Some oscillators have a property known as strong resetting in which a perturbation applied during the period of oscillation can shift the occurrence of the next period and thereby reset the oscillation. It has been shown that systems with this property also present a singularity. At the singularity, the response of the system is unpredictable and the perturbation can lead to annihilation of the response. We have applied the theory of phase resetting to quasi-periodic signals generated by epileptogenic agents in the hippocampus in-vitro. The purpose of the study was to determine whether the resulting oscillatory systems had the property of strong resetting and whether a singularity could be found. Hippocampal slices were perfused with high potassium solutions generating quasi-static periodic activity with a period of .8 to 1.3 Hz in the CA3 region. Current pulses in the range of 1 to 250uA were applied at various times between spikes (coupling intervals) with an electrode located in the CA3 somatic layer. The results indicate that for current amplitude below 80uA weak resetting (no effect) is observed. For amplitude greater than 150uA, strong resetting is generated. For amplitudes between 90 and 110 uA at coupling latencies around .7, periodic activity was abolished and the latency of spikes was unpredictable. This singularity was observed in 5 out of 5 slices tested. This analysis of the interactions between interictal spikes and current stimulation has shown that the periodic epileptiform activity generated in high potassium is vulnerable and could potentially be annihilated with electrical stimulation. Supported by NSF grant # IBN 93 19591.

387.2

IMAGING THE STEADY-STATE MEMBRANE POTENTIAL DISTRIBUTION OF PURKINJE CELLS *IN SITU* USING VOLTAGE-SENSITIVE DYES

M.O.Thomann*, D.Heck and A.Borst. Friedrich-Miescher-Laboratory of the Max-Planck-Society, Spemannstraße 37-39, D-72076 Tuebingen, Germany

A new method, previously described for leech ganglion cells (Borst,A., Z. Naturforsch. 50c, 435-438, 1995), is applied which allows to image the steady-state distribution of membrane potential of single Purkinje cells *in situ*. The method consists of staining the tissue extracellularly with a voltage-sensitive dye (Di-4- or Di-8-ANEPPS) and injecting current into a single neuron using the whole-cell patch clamp configuration. A large series of images of the cell is taken at the appropriate excitation wavelength of the dye with a CCD camera while the neuron's membrane potential is shifted by a periodic current injection (PCI). Afterwards two groups of images are averaged separately: those images with the cell at rest and those while the membrane potential of the cell was manipulated. After subtraction of these averaged images, the resulting difference image only shows the cell whose membrane potential was manipulated periodically. The signal-to-noise ratio improves with the square-root of the number of images taken within an experimental session. Drift noise contaminating control and stimulus images in approximately the same way is further reduced in the resulting difference image due to the PCI averaging method. The method is applied to Purkinje cells in cerebellar slices of young rats (p11-17). The two-dimensional arborization pattern of these cells' dendrites makes them a favorable object to optically examine their passive membrane properties. Provided that the whole dendrite is aligned planparallel to the optics, one can image the steady-state voltage distribution in the whole cell. We see very little decrease of the optical voltage signal in the difference image from the soma to even distant dendritic branches. From this, we conclude that Purkinje cells of young rats are electrically very compact. These data are in accordance with what is known about their passive membrane properties so far (Shelton,D.P., Neuroscience 14, 111-131, 1985).
This work was supported by the Max-Planck-Society.

387.3

MOTION IN THE PREFERRED AND NULL DIRECTION CO-ACTIVATES EXCITATORY AND INHIBITORY INPUT TO VISUAL INTERNEURONS.

S. Single*, J. Haag and A. Borst. Friedrich-Miescher-Laboratory of the Max-Planck-Society, Spemannstr. 37-39, D-72076 Tübingen, Germany.

In the original model of visual motion detection (Reichardt W., Z. Naturforsch. 12b, 448-457, 1957) direction selectivity (DS) is aquired in two steps (Borst A. and Egelhaaf M., PNAS USA 87, 9363-9367, 1990): First local luminance values derived from neighboring retinal locations are correlated with each other after one of them has passed a temporal filter. This results in a signal with only a weak DS. Full DS is acquired by subtracting the output signals of two such operations performed in a mirror-symmetrical way. The subtraction step has been proposed to be implemented on the large dendrites of the fly lobula plate tangential cells (LPTCs) by a combination of excitatory and inhibitory input synapses (Brotz T. and Borst A., J. Neurophysiol., submitted, 1996). If this were true, visual motion, e.g. in the preferred direction (PD), is predicted to co-activate excitatory and inhibitory inputs onto these cells. We can clearly demonstrate such a co-activation by measuring the motion-induced change in input resistance of LPTCs before and after application of 10^{-4} M Picrotoxinin (a chloride channel blocker). In normal fly saline, PD-motion leads to a depolarisation (ΔV= +4.5mV) and ND-motion to an hyperpolarisation (ΔV= -3.5mV). During PD-motion the input resistance drops by 14% (ND-motion: 13%). After PTX-application the PD-response increases and the ND-response becomes positive. This fact can be explained in two ways: Either, PTX acts presynaptically and disinhibits excitatory inputs to the cells, or PTX blocks inhibitory inputs to the LPTCs which normally become co-activated. We find that after PTX, the input resistance during PD-motion drops only by 9%. This finding can only be reconciled by the second of the above alternatives and demonstrates that PD-motion and ND-motion both co-activate excitatory as well as inhibitory input to fly tangential cells.

This work was supported by the Max-Planck-Society.

387.4

INFLUENCE OF DENDRITIC SODIUM CHANNELS ON TRANSIENT RESPONSE PROPERTIES OF VISUAL INTERNEURONS.

J. Haag* and A. Borst, Friedrich-Miescher-Laboratory of the Max-Planck-Society, Spemannstrasse 37-39, D-72076 Tuebingen, Germany

Voltage dependent sodium channels have been found in the dendrites of many nerve cells studied to date. To investigate the functional implications of active membrane properties in signal processing, we compared the transient responses of two types of non-spiking, motion-sensitive interneurons (CH- and HS-cells) of the fly visual system, only one of which (HS-cell) is equipped with a fast sodium inward current. Dual electrode recordings revealed that the fast sodium channels are located in the axon as well as in the dendrite of HS-cells. We stimulated CH- and HS-cells in two different ways: a) with oscillatory visual motion stimuli of various discrete frequencies, and b) with white noise motion stimuli. We found that cells with fast activating sodium currents can respond to temporal changes in their synaptic input signals up to higher frequencies than can those which lack such currents. A direct way of demonstrating the contribution of voltage-gated currents to the normal motion response of HS-cells is to inject a large depolarizing current into the cells, which inactivates voltage-gated sodium currents, while stimulating them synaptically with visual motion. At stimulus frequencies above 20 Hz, the response amplitudes are much reduced compared to the non-depolarized state. Thus, fast sodium currents lead to a frequency-dependent amplification of synaptic signals, enhancing cellular responses specifically to transient inputs which otherwise would be attenuated due to the passive properties of the dendritic tree.

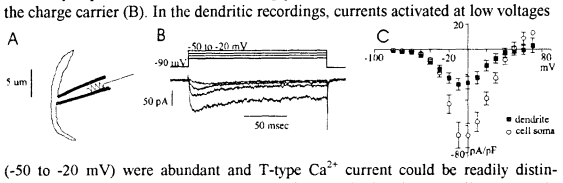
This work was supported by the Max-Planck-Society

387.5

CALCIUM CURRENTS IN ISOLATED HIPPOCAMPAL DENDRITES.

E.T. Kavalali*, M. Zhuo, H. Bito and R.W. Tsien. Dept. of Mol. and Cell. Phys., Beckman Center, Stanford University School of Medicine, Stanford, CA 94305.

We used the whole-cell recording configuration to study Ca^{2+} channel currents in isolated dendrites acutely dissociated from rat hippocampi (A). Dendrites were isolated by gentle trituration of hippocampal slices following enzymatic treatment, and their identity was verified by immunocytochemistry. Ca^{2+} channel currents were evoked by depolarizations from a holding potential of -90 mV using 5 mM Ba^{2+} as the charge carrier (B). In the dendritic recordings, currents activated at low voltages



(-50 to -20 mV) were abundant and T-type Ca^{2+} current could be readily distinguished by its characteristic kinetics, including slowly deactivating tail currents. At -30 mV, for example, dendrites and cell bodies displayed similar current densities (-15.2 ± 2.8 pA/pF vs. -18.3 ± 3.3 pA/pF, n = 20 for both)(C). In contrast, with stronger depolarizations, the dendrites showed significantly smaller current density than somata (at -10 mV: -27.5 ± 4.6 pA/pF vs. -67.5 ± 10.2 pA/pF). Currents activated at high voltages were reduced by nimodipine, ω-conotoxin GVIA or ω-conotoxin MVIIC, indicating the contributions of L-, N- and P/Q-type Ca^{2+} channels. The total current blocked by these compounds in combination was ~65% at 0 mV. These results provide a direct estimate of the contribution of low voltage-activated Ca^{2+} channels in dendrites, in support of previous studies indicating their critical role in dendritic signal transduction. (*Supported by a grant from NIH NS 24067*)

387.6

FAST IMAGING OF CALCIUM TRANSIENTS IN DENDRITIC SPINES OF CULTURED HIPPOCAMPAL NEURONS.

E. Korkotian and M Segal*, The Weizmann Institute, Rehovot 76100 Israel

Dendritic spines are unique calcium compartments, able to raise [Ca]i to high levels, in relative independence of their parent dendrites. Current methods for imaging [Ca]i have not been able to resolve differences in fast kinetics of [Ca]i changes in spines ([Ca]s) and dendrites ([Ca]d), and it is not entirely clear where are [Ca]i changes initiated and what are the sources of elevated [Ca]i in the spines. To address these questions we used a fast (0.75 ms time resolution) single line scan mode in a confocal laser scanning microscope, and monitored [Ca]i variations in dendritic spines of cultured hippocampal neurons. Cells were loaded with Calcium Green-1 through a micropipette and their dendritic arbors were scanned with a 63x water immersion objective. Low intensity laser light was focused through the long axis of the spine and its parent dendrite. Micropipettes containing glutamate or caffeine were placed near the recorded dendrite/spine pair and the responses of the pair to a puff application of the drug were monitored. Glutamate application caused a larger calcium change in the spine than in the dendrite. The latency to the spine response was shorter than that of the dendrite response by a mean of 15msec (n=18 spines). This may be caused by a difference in glutamate receptor density or properties or by travel time of a calcium wave from the spine to the dendrite. Topical application of caffeine caused a similar rise of both [Ca]s and [Ca]d (n=11 spines). The caffeine induced rise of [Ca]i, unlike that of glutamate, was not caused by an influx of calcium from the extracellular space, indicating release of calcium from spine-associated stores. In a separate series of experiments done with ratio imaging of Fura-2 loaded neurons, the response to glutamate was reduced significantly by the calcium pump antagonist, thapsigargin, indicating that part of the calcium rise seen with glutamate is also caused by mobilization of calcium from internal stores.

Supported by a grant from the US Israel binational Science foundation.

387.7

A SUBTYPE OF ENDOPLASMIC RETICULUM IS A MAJOR CALCIUM BUFFER IN CA3 PYRAMIDAL DENDRITES.

S. B. Andrews, L. D. Pozzo–Miller, N. Pivovarova, R. A. Buchanan, T. S. Reese*, and R. D. Leapman[1]. Laboratory of Neurobiology, NINDS, and [1]Biomedical Engineering and Instrumentation Program, NCRR, NIH, Bethesda, MD 20892-4062.

The endoplasmic reticulum (ER) in neuronal dendrites is thought to buffer intracellular Ca^{+2} fluctuations via ATP-mediated uptake. However, there has been no direct correlation of the Ca content of ER with different states of neuronal activity. Here we report the effect of high-frequency afferent synaptic activity on the elemental composition, especially the total Ca content, of the dendritic ER of CA3 pyramidal neurons. After establishing stable evoked field potentials in the CA3 region of 7-day-old hippocampal slice cultures, slices were given either: 1) a single train of 50 shocks at 50 Hz; 2) four such trains at 0.06 Hz; or 3) four trains in the presence of 5 μM TTX. Within 3 min of the last volley, slices were frozen against an LN_2-cooled metal block. Elemental concentrations in ER, mitochondria, and cytoplasm were measured in a VG HB501 scanning transmission electron microscope by EDX microanalysis of freeze-dried, ultrathin (90 nm) cryosections. This method gives the *total* concentrations of elements in mmol/kg dry weight, which for Ca is typically 10^3-10^4 higher than the corresponding free Ca^{+2} concentration. Control slices frozen with or without 15-30 min incubation with 5μM TTX showed typically low values for ER Ca—4.9±1.2 mmol/kg dry weight (SEM). Following a single stimulus train, the total Ca within a subset of ER (~35% of the cisterns in a dendrite), increased to >15 mmol/kg; the Ca content of a few cisterns exceeded 50 mmol/kg. Following four tetani, the Ca concentration increased even more dramatically in a slightly larger subset of ER (~45%) to 80 mmol/kg (mean of non-Gaussian distribution), with some cisterns exceeding 100 mmol/kg. No Ca increase was found after stimulation in the presence of TTX. In all cases, there was no significant increase in mitochondrial Ca, and only a graded, modest rise in cytoplasmic Ca. Tetanic stimulation increased Na in all compartments without changes in K, Mg, Cl, P and S. These findings identify a subtype of ER as a major, high-capacity cytoplasmic Ca^{+2} buffer in CA3 pyramidal dendrites, during both mild and strong synaptic activity. Supported entirely by NIH Intramural Research Program.

387.8

TARGETING OF AUTOPHOSPHORYLATED CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II TO POSTSYNAPTIC GLUTAMATE RECEPTORS AND PROTEIN PHOSPHATASES.

S. Strack* and R. J. Colbran. Dept. Mol. Physiol. & Biophys., Vanderbilt Univ., Nashville, TN 37232-0615

Ca^{2+}/calmodulin-dependent kinase II (CaMKII) is a major brain enzyme particularly enriched in the postsynaptic density (PSD), where it is thought to regulate synaptic efficacy by phosphorylating key substrates such as receptors and ion channels. We studied the mechanism by which CaMKII is targeted to the PSD using an *in vitro* binding assay with purified PSDs and recombinant [^{35}S-Met] CaMKII. Ca^{2+}-dependent autophosphorylation at Thr286 (T286-P), which makes CaMKII independent of further stimulation by Ca^{2+}, strongly enhances the binding of CaMKII to PSDs (app. EC_{50} = 1.1 μM). We also localized [T286-P]CaMKII binding in brain sections using biotinylated CaMKII. Heaviest binding was observed to hippocampus, labeling dendrites and neuronal somata, but sparing neuronal nuclei, axons, and glia. We then analyzed the dephosphorylation of [T286-P]CaMKII by PSD-bound protein phosphatases (PPs). Previously bound [T286-P]CaMKII is not released from the PSD, even after extensive dephosphorylation. Soluble [T286-P]CaMKII is predominantly dephosphorylated by PP2A, whereas PSD-bound CaMKII is a selective substrate for PSD-bound PP1, which has itself been implicated in synaptic plasticity. This results suggests that soluble and PSD-bound CaMKII may be differentially regulated by dephosphorylation. Upon binding to the PSD, [T286-P]CaMKII increases phosphorylation of the GluR1 subunit of the AMPA-type glutamate receptor and many other PSD proteins. Our results suggest a mechanism by which a rise in intracellular calcium mediated by synaptic activity leads to a translocation of activated CaMKII to the postsynaptic density, where it phosphorylates glutamate receptors leading to enhanced synaptic efficacy. This mechanism may also be involved in the translocation of CaMKII to the PSD seen under ischemic and hypoglycemic conditions. [Supported by NIH grant GM47973 to RJC, an AHA Established Investigator]

387.9

MECHANISMS OF CALCIUM-CALMODULIN KINASE II DIRECTED POTENTIATION OF POSTSYNAPTIC SENSITIVITY. **A.M. Shirke* & R. Malinow, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, and Dept. of Physiology & Biophysics, U. of Iowa, Iowa City, IA**

Calcium calmodulin kinase II (CaMKII) has been implicated in the induction and maintenance of long-term potentiation (Malinow, et al., 1989) by actions on postsynaptic receptors (McGlade-McCulloch, et al., 1993). Here we use theoretical and experimental techniques to examine possible mechanisms by which CaMKII might affect postsynaptic sensitivity.

Using the program Neuron (M. Hines), we employ a five-state model for receptor function which includes desensitization (Ambros-Ingerson & Lynch, 1993). Various hypothetical mechanisms whereby CaMKII might act predict different effects on the magnitude and timecourse of responses to transmitter.

The model predicts that either a decrease in desensitization rate or an increase in opening rate produce a larger, slower response. Changes in single channel current produce a change in response size, but not timecourse or the coefficient of variation (CV) across responses. Changes in the number of active receptors would change response size and CV but not timecourse.

We have also experimentally examined responses to puffed kainate. Kainate responses act as predicted for changes in desensitization (by applying cyclothiazide) and of single channel current (by changing driving force). We find that internal perfusion of pre-activated CaMKII (courtesy of T. Soderling, OHSU) increases kainate responses without a change in timecourse. In addition, kainate responses show a decrease in CV. These observations are consistent with the hypothesis that CaMKII potentiates responses by increasing the number of active postsynaptic receptors.

Supported by Mathers Charitable Foundation.

387.10

DECREASED PAIRED-PULSE FACILITATION ASSOCIATED WITH SYNAPTIC POTENTIATION INDUCED BY ACTIVATION OF POSTSYNAPTIC Ca^{2+}/CaM SIGNAL PATHWAYS. PAUL T. KELLY* & JIN-HUI WANG. *Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, Texas 77030.*

We have shown that synaptic potentiation induced by postsynaptic injection of Ca^{2+}/CaM is associated with significant decreases in paired-pulse facilitation (PPF) of synaptic transmission at CA1 synapses of hippocampal slices (*J. Neurophysiol. in press*). Since occlusion experiments showed that Ca^{2+}/CaM-induced synaptic potentiation and tetanus-induced LTP share certain common mechanisms, we re-examined if PPF changes during LTP. We observed significant decreases in PPF (45±7% in intracellular recordings and 48±10% in field potential recordings) during LTP induction and maintenance relative to baseline (100%). Interestingly, postsynaptic injections of pseudosubstrate inhibitors of CaM-KII and PKC [CaMKII(281-302)/PKC(19-31)] reversed these decreases in PPF to baseline values while blocking LTP maintenance in pre-potentiated compared to control pathways. In addition to synaptic potentiation and PPF attenuation induced by activating postsynaptic Ca^{2+}/CaM pathways, we observed that inhibiting postsynaptic calcineurin (CaN) activity with FK-506 or CaN autoinhibitory peptide induced synaptic potentiation (212±18% or 203±12% vs. baseline 100%, respectively) and PPF attenuation (39±7% or 53±6% vs. baseline). The effects of postsynaptic FK-506 injections were blocked by co-injecting BAPTA, a calmodulin-binding peptide, a mixture of CaMKII(281-302) and PKC(19-31), or heparin plus dantrolene. Together, our results indicate that postsynaptic Ca^{2+}/CaM pathways play a dual role in synaptic plasticity, synaptic potentiation and simultaneous attenuation of synaptic facilitation. We speculate that the same postsynaptic mechanisms enhance synaptic transmission and limit the degree of synaptic facilitation at central synapses.

ALZHEIMER'S DISEASE: TAU AND ApoE

388.1

TAU PROTEIN PHOSPHORYLATION IN HEAT-SHOCKED RATS FOLLOWING GONADECTOMY WITH AND WITHOUT SEX HORMONE REPLACEMENT THERAPY. S. Ch. Papasozomenos*. Dept. of Path., Univ. of Texas Med. Sch., Houston, TX 77030.

We have shown that heat shock induces rapid dephosphorylation of τ in both female and male rats followed by hyperphosphorylation only in female rats (J. Neurochem. 66:1140, 1996). To further investigate whether gonadal hormones play a role in the phosphorylation state of τ, 180 male and female rats were gonadectomized or sham-operated and heat-shocked at 42°C for 15 min following daily subcutaneous injections of testosterone propionate (1 mg/100 g) or estradiol benzoate (50 μg/250 g) or sesame oil for 2-3 wk. SDS cerebral extracts were analyzed by qualitative and quantitative immunoblotting using 4 monoclonal anti-τ antibodies that recognize phosphorylated and nonphosphorylated epitopes and the peroxidase-antiperoxidase technique or ^{125}I-labeled protein A. None of the above treatments prevented the rapid dephosphorylation of τ at 0 h after heat shock. In both orchiectomized and ovariectomized rats, τ became hyperphosphorylated between 3 and 6 h after heat shock. The degree of hyperphosphorylation decreased in orchiectomized and heat-shocked rats that were treated with testosterone propionate. Also, in orchiectomized but not heat-shocked rats the degree of phosphorylation of τ was lower following daily injections of 2 mg than 0.5 mg of testosterone propionate. No differences were found between ovariectomized and heat-shocked rats with and without replacement therapy with estradiol benzoate. These findings suggest that (1) the heat shock-induced rapid dephosphorylation of τ is gonadal hormone independent; and (2) the subsequent hyperphosphorylation of τ is prevented and can be modified by circulating androgens in male rats but is estrogen-independent in female rats. Since τ is hyperphosphorylated in Alzheimer's disease, androgens may exert a protective effect.

388.2

BUTYROLACTONE I, A CYCLIN-DEPENDENT KINASE INHIBITOR, INDUCES DEPHOSPHORYLATION OF TAU AND DIFFERENTIATES SY5Y CELLS. [1,2]T. Tanaka*, [1]K. Iqbal, [1]E. Trenkner, [2]M. Takeda and [1]I. Grundke-Iqbal. [1]Institute for Basic Research in Developmental Disabilities, Staten Is., NY 10314, USA. [2]Dept. Neuropsychiatry, Osaka Univ. Med. Sch., Osaka, 565 Japan.

The microtubule associated protein tau of the human neuroblastoma cell line SY5Y is hyperphosphorylated at several of the same sites as tau in Alzheimer disease (AD P-tau). Furthermore, like AD P-tau, the tau in SY5Y cells does not bind to microtubules (FEBS Lett. 360:5-9, 1995). Proline-directed kinases, MAP kinase, cdc2 kinase, cdk5, and glycogen synthase kinase-3 phosphorylate tau *in vitro* at sites found in AD P-tau. However, the identity of the kinase(s) responsible for the phosphorylation of tau in living cells is not established. Butyrolactone-I (BL-I, a generous gift from Dr. Okuyama, Banyu Pharmaceutical Company, Tsukuba, Japan), a non-cytotoxic and specific inhibitor of the cdk family including the neuron specific cdk5, was employed to investigate the phosphorylation of tau in SY5Y cells. By 2 days BL-I induced the extension of long neurites whereas under similar conditions neurites extension by treatment with retinoic acid was unremarkable. In the BL-I-treated cells, phosphorylation of tau at the PHF-1 site (Ser 396/404) was markedly decreased and the microtubule binding activity was restored . In contrast, olomoucine which inhibits both MAP kinase and cdk was strongly cytotoxic and was far less effective in differentiating cells. This study suggests that in SY5Y cells differentiation might be regulated by cdk and that the site(s) on tau critical for microtubule binding are phosphorylated by this kinase(s). The restoration of the biological activity of tau and neuritogenesis by inhibition of cdk might have therapeutic implications in AD. (Supported by the NY State Office of Mental Retard. and Development. Disabil., the NIH grants NS18105, AG05892, AG08076 and Zenith Award from Alzheimer's Assoc. USA.)

388.3

CHEMICAL SIMILARITIES OF Al-INDUCED NEUROFIBRILLARY DEGENERATION IN RABBITS WITH THOSE OF ALZHEIMER'S DISEASE USING PROBES FOR TAU, APP, β/A4, α1-ANTICHYMOTRYPSIN, AND UBIQUITIN. J. Savory, Y. Huang, M.R. Wills and M.M. Herman*, Departments of Pathology, Biochemistry and Internal Medicine, University of Virginia, Charlottesville, VA 22908; and Clinical Brain Disorders Branch, NIMH Neuroscience Center at St. Elizabeths, N.I.H.,Washington, DC 20032.

Administration of aluminum (Al) compounds directly into the CNS of rabbits produces marked neurofibrillary degeneration (NFD) consisting of argyrophilic accumulations. Early studies indicated that these aggregates consisted of hyperphosphorylated neurofilament proteins (NF) and consequently were of limited value as an experimental animal model for Alzheimer's disease. We now show that other proteins characteristically seen in neurofibrillary tangles of Alzheimer's disease are also present in NFD in rabbits. We administer Al maltolate (2.5 µmole) via the intracisternal route to 6- month -old male and female New Zealand white rabbits. Clinical symptoms are seen as early as 2 days and animals are sacrificed on days 2 through 7. Marked argyrophilic accumulations are observed by day 3, increasing to day 7. Immunohistochemical studies and Western blot analysis with tau mAbs (Tau-2, AT8, PHF-1, and Alz50) demonstrate that the accumulations contain abnormal tau. Amyloid precursor protein (APP), ubiquitin and α1-antichymotrypsin (ACT) show increased immunohistochemical staining intensities in neurons containing NFDs, and the elevations are confirmed by Western blot analysis. β/A4 is detected in neurons of Al-treated rabbits using immunohistochemistry with mAb β amyloid. Time studies demonstrate that intraneuronal immunostaining for APP, ACT, β/A4 and ubiquitin is distinctly positive by day 3 which correlates with the appearance of NFD by day 3; abnormal tau accumulation is a later (day 6-7) event. Studies in aged rabbits (4-5 years) indicate that Al susceptibility is greater than in young adults as demonstrated by more severe neurological symptoms following intracisternal administration of 0.8 µmole of Al maltolate/kg of body weight to animals in both age groups. This animal system provides a means of studying temporally-related changes in NFD and how aging may affect these events. Supported by the Virginia Center on Aging.

388.4

LONGITUDINAL STABILITY OF CSF TAU LEVELS IN MID-STAGE ALZHEIMER PATIENTS. T. Sunderland*, B. Wolozin, D. Galasko, J. Levy, R. Motter, R. Dukoff, M. Bahro, S. Molchan, D. Rubinow, T. Lehtimaki, P. Seubert. National Institute of Mental Health, Bethesda, MD 20892-1264, Department of Neurosciences, University of California, San Diego, CA 92093, Department of Biomedical Sciences, University of Tampere, Finland and Athena Neurosciences, San Francisco, CA.

Antemortem levels of tau in the cerebrospinal fluid (CSF) of Alzheimer's disease (AD) patients have repeatedly been demonstrated to be elevated when compared to controls. While CSF tau has been found to be elevated even in very mild AD, it is unknown how tau levels change during the course of the disease. We have followed 29 mild-moderately affected AD subjects over two years with repeat CSF taps and found no significant overall change in CSF tau levels (548 ±355 vs. 557±275 pg/ml, NS). During this same period, CSF somatostatin levels have been shown to decrease significantly (42±13 vs. 34±13 pg/ml, p=0.009) suggesting that not all CSF markers are stable over time. Similarly, clinical measures of dementia severity (Clinical Dementia Rating Scale, Global Determination Scale and Mini-Mental Status Examination) all showed significant changes consistent with a progression in the disease. None of these variables was significantly correlated with either baseline measures of CSF tau or delta CSF tau (last-first). The only significant relationship we found was between baseline CSF tau itself and delta tau (r= -0.72, p<0.01). Baseline CSF tau levels spanned a wide range but did not correlate with measures of clinical severity of APO E phenotype. These data suggest that CSF tau levels are stable over extended periods of time in a group of mild-moderately demented AD subjects and that tau levels do not predict the severity or rate of progression of AD.

388.5

CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II PHOSPHORYLATES TAU AT SER 262 BUT ONLY PARTIALLY INHIBITS ITS BINDING TO MICROTUBULES. T. J. Singh[1]*, J. Wang[1], M. Novak[2], E. Kontzekova[2], I. Grundke-Iqbal[1] and K. Iqbal[1]. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314; [2]Institute of Neuroimmunology, Slovak Academy of Sciences, 842-46 Bratislava, Slovak Republic.

PHF-tau, which is phosphorylated at ten ser/thr-pro and eleven non-ser/thr-pro sites, is unable to promote microtubule assembly. Phosphorylation of the non-ser/thr-pro site, ser 262, is reported to be primarily responsible for this. The identities of kinase(s) responsible for ser 262 phosphorylation are still to be clarified. In this study we have used the monoclonal antibody 12E8, which recognizes P-ser 262 and P-ser 356 on tau, to survey different kinases for their abilities to phosphorylate ser 262 on human tau 3L (tau_{410}). In decreasing order of effectiveness we found that ser 262 and ser 356 phosphorylation can be catalyzed by CaM kinase II >>C-kinase>>GSK-3=A-kinase>>CK-1. CaM kinase II and C-kinase were shown to phosphorylate both ser 262 and ser 356. The binding of tau to taxol-stabilized microtubules was decreased by 35% and 42% after phosphorylation by CaM kinase II and C-kinase, respectively. Of the fraction of tau that bound to microtubules, about 50% was phosphorylated at ser 262 and ser 356. These results suggest that ser 262 and ser 356 are very good substrates for CaM kinase II but their phosphorylations are not sufficient to achieve maximal inhibition of tau binding to microtubules. [Supported in part by NIH grants AG08076, AG05892 (K.I.), NS18105 (I.G.-I.), AG11932 (T.J.S.), and Howard Hughes Medical Institute grant 75195-547401 (MN, EK)]

388.6

A NEW MOLECULAR INTERACTOR FOR TAU PROTEIN. G. Lee, S.L. Kwei, S.T. Newman, M. Lu* and Y. Liu. Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

Tau protein is a microtubule-associated protein found in neuronal cells and is a prominent component of the neurofibrillary tangles of Alzheimer's disease. Using the yeast two hybrid system, we have identified a new protein that interacts with tau. This protein contains several copies of the WD repeats that are found in G protein β subunits as well as other proteins involved in signal transduction, regulation of cytoskeletal assembly, cell cycle, and RNA processing. Database searching revealed that this protein is likely to be the human homolog of the Drosophila extra sex combs (esc) gene product. The esc protein is involved in the stable long term repression of the homeotic genes and therefore is involved in maintaining spatial specific expression of genes. In addition, at various stages of Drosophila development, the esc protein is reported to be neuronal specific. We have isolated full length cDNA clones for the human protein from brain cDNA and have found that the amino terminus is alternatively spliced. Transfection of the isoform most commonly isolated indicated that the protein is cytoplasmic, consistent with the finding that the amino terminal sequence did not contain the nuclear targeting sequence identified in the Drosophila homolog. Conservation between the sequences was greatest in the domain containing the WD repeats. Examples of other known cytoskeletal proteins containing WD repeats are coronin and an intermediate chain of Chlamydomonas outer arm dynein.
Supported by NSF and ADRDA.

388.7

IDENTIFICATION AND CHARACTERIZATION OF TAU PHOSPHOPEPTIDE EPITOPES RECOGNIZED BY ANTI-PAIRED HELICAL FILAMENT MONOCLONAL ANTIBODIES. R. Hoffmann, V.M-Y. Lee and L. Otvos, Jr.*. The Wistar Institute and Department of Pathology, University of Pennsylvania Medical School, Philadelphia, PA 19104

Increasing number of evidence indicates that theoretically abnormally hyperphosphorylated tau protein could be detected in normal body fluids (e.g. CSF) by diagnostic antibodies. The currently available anti-PHF mAbs, however, are not specific enough for further development. In the current study we used single, di- and triphosphorylated tau peptides to identify the location and required number of phosphate groups for full recognition by a panel of known and newly developed mAbs that recognized PHF-tau but not normal tau. We concentrated on peptides encompassing clustered phosphoamino acids between residues 210-220, 230-240 and 395-405. For all mAbs the governing feature of the antigen recognition could be identified as single phosphoserines or phosphothreonines. Some of the antibodies, however, needed multiple phosphates on the peptide antigens for full binding. For example, mAbs PHF-6 and AT180 recognized phosphorylated Thr231, and their recognition was not increased after phosphorylation of Ser235. In contrast, the antigen recognition of mAb PHF-27 (that recognizes the same Thr231) was increased after phosphorylation of Ser235. As the reverse-turn conformation of the corresponding non-phosphorylated, and single phosphorylated peptides was stabilized after dual phosphorylation, it appears that while mAbs PHF-6 and AT180 see only the presence of the phosphate group, mAb PHF-27 recognizes both the presence of the phosphate and the secondary structure of the antigen.

We model the extracellular stability of peptides with stability studies in human serum. As the addition of phosphate groups to the epitopes may or may not increase the serum half-life of the synthetic peptides, these serum stability studies identify amino acid residues the phosphorylation of which can result in increased protease resistance and consequently help the accumulation of the hyperphosphorylated tau protein in the affected brains.

Supported by NIH grant AG10670.

388.8

NEURONS EXPRESSING PHOSPHORYLATED TAU PROTEIN ARE MORE RESISTANT IN VITRO TO APOPTOSIS INDUCED BY NMDA. M. Lesort, C. Yardin, F. Terro, C. Blanchard, J. Hugon*. Neurobiol. & Cell. Pathol. Unit, Lab. Histology and Cell Biology, Fac. Medicine, Limoges, 87025 FRANCE

Apoptosis is a programmed cell death occuring during brain development and in certain neurological diseases such as Alzheimer's disease (AD). Tau protein is a microtubule associated protein promoting microtubule polymerisation and stabilization. Phosphorylated tau protein is the major constituent of paired helical filaments which accumulate in neurons of patients suffering from AD. The goal of the present study is to compare the rate of apoptosis induced by the glutamate agonist NMDA in neurons expressing or not phosphorylated tau and in neurons expressing the Amyloid Precursor Protein (APP). Rat cortical neurons were exposed for 16 hours to 20 µM NMDA or vehicule alone. After exposure, the percentages of apoptotic neurons evaluated by the TUNEL method are 32.4 % ± 6.6 % in treated cultures and 11.5 % ± 4.3 % in control cultures. Treated and control cultures were processed for immunofluorescence study using AT8 antibody (Innogenetics) directed against tau phosphorylated epitopes, tau 1 antibody (Boehringer) directed against a non phosphorylated epitope or ß amyloid 1/40 (Sigma) and stained with propidium iodide to evaluate apoptotic nuclei. The percentages of apoptotic neurons immunopositive for AT8 antibody are not stastistically different in NMDA-treated and control cultures (101 % of controls). At the opposite, the percentages of apoptotic neurons immunopositive for tau 1 and 1/40 antibodies stastistically increased in NMDA-treated cultures (respectively 230 and 190 % of controls). In addition a cellular APP release was observed around apoptotic neurons. In conclusion, AT8 positive neurons are clearly resistant in vitro to apoptosis compared to neurons expressing tau 1 and apoptosis is associated with the accumulation of extracellular and potentially amyloidogenic APP derivates.

388.9

MONOCLONAL ANTIBODY PHF-9 RECOGNIZES SER404 OF TAU AND LABELS PAIRED HELICAL FILAMENTS. F.P. Zemlan* and G.E. Dean. Alzheimer's Research Center, University of Cincinnati Col, Med., Cincinnati, OH 45267-0559.

Alzheimer's paired helical filaments (PHF) consist of phosphorylated MAP-tau. In PHF, phosphorylation occurs at ser/thr tau residues. Several of these ser/thr phosphorylation sites lie immediately C-terminal to the tau tubulin binding domain. The C-terminal ser^{396} to thr^{413} tau region contains two or more phosphorylated residues and eight possible ser/thr phosphorylation sites. Immunologic studies and mass spectroscopy have identified ser^{396} as one of the phosphorylation sites but identification of more C-terminal phosphorylated residues has been hampered by the lack of Mabs that recognize defined epitopes in this region. We have raised Mabs against PHF purified from Alzheimer's brain. One of these Mabs, PHF-9, showed phosphorylation-dependent binding to purified PHF and recognized a phosphorylated epitope in the C-terminal portion of cyanogen bromide-digested PHF. Epitope mapping studies employing synthetic tau phosphopeptides indicated that PHF-9 labeled a 13-mer tau peptide phosphorylated at ser^{404} but not the corresponding non-phosphorylated peptide. PHF-9 demonstrated no immunoreactivity with a phospho-ser^{396} peptide indicating that the PHF-9 epitope is C-terminal to ser^{396}. In conclusion, the present study describes a Mab, PHF-9, which recognizes phosphphorylated ser^{404} of tau independently of phosphorylated ser^{396} and indicates that ser^{404} is phosphorylated in PHF.

388.10

INCREASING CEREBROSPINAL FLUID TAU LEVELS IN ALZHEIMER PATIENTS WITH APOLIPOPROTEIN E ALLELE ε4 DURING 14 MONTHS FOLLOW-UP. L. Lannfelt*, M. Blomberg, M. Jensen, H. Basun, L.O. Wahlund. Karolinska Institute, Department of Geriatric Medicine, Huddinge University Hospital, S-141 86 Huddinge, Sweden

We and others have shown that tau concentrations are elevated in cerebrospinal fluid (CSF) from Alzheimer disease (AD) patients, suggesting that tau in CSF may be a useful biochemical diagnostic marker for this disorder. We have investigated CSF tau concentrations in AD (n=18), mild cognitively impaired individuals (MCI, n=9), and other dementing diseases (OD, n=9) by ELISA (Innogenetics hTau Antigen, Belgium). CSF was obtained on two occasions with a mean of 14 months in between. Mean CSF tau levels on the first occasion for AD, MCI and OD were 948±498 (pg/ml, SD), 372±90 and 568±258, respectively. Tau levels were significantly higher in the AD group than in MCI and OD groups. Twelve of the AD patients demonstrated increasing values of tau at follow-up and six showed diminished values. All AD patients with increasing tau were carriers of one or two ε4 alleles of the apolipoprotein E (APOE) gene. Of those AD cases with decreasing tau levels only three individuals had the ε4 allele. This difference was significant ($p < 0.05$). The MCI and OD groups did not show an increased CSF tau at follow-up. These preliminary findings suggest that there may be APOE isoform specific differences of tau regulation in AD.

This work was supported by the Swedish Medical Research Council (project no 10819).

388.11

WITHDRAWN

388.12

THE ABSENCE OF AN APOLIPOPROTEIN ε4 ALLELE IS ASSOCIATED WITH A MORE AGGRESSIVE FORM OF ALZHEIMER'S DISEASE Y. Stern*, J. Brandt, M. Albert, D.M. Jacobs, K. Bell, K. Marder, M. Sano, S Albert, C. D. Castenada, F. Bylsma, B. Tycko and R. Mayeux. Neurology Dept., Columbia Univ., New York, NY 10032.

We investigated the relationship between *APOE* genotype and rate of disease progression and survival in 99 patients with probable Alzheimer's disease who were followed biannually for up to 6 years. The rate of decline in modified Mini-Mental State Examination scores was slower ($p<.05$), the presence of extrapyramidal signs was decreased ($p<.01$), the development of myoclonus occurred later (RR= 0.08; CI=0.01-0.51), and the risk of mortality was decreased (RR= 0.38; CI=0.17-0.84) among patients with at least one *APOE* ε4 allele compared to patients with other genotypes. Because the decline in mental ability as well as the development of myoclonus and extrapyramidal signs are consistent manifestations of disease progression, our results imply that *APOE* ε4 is associated with a less aggressive form of Alzheimer's disease.

Supported by NIH Grants AG07370, AG10963, AG080702 & RR00645

388.13

MOLECULAR AND ANATOMICAL CHARACTERIZATION OF THE LOW DENSITY LIPOPROTEIN RECEPTOR RELATED PROTEIN (LRP), AN APOE AND APP RECEPTOR, IN ALZHEIMER'S DISEASE. B. T. Hyman*, A. Clatworthy, O. Berezovskaja, R. Christie, K. Page, G.W. Rebeck, and D. Strickland. Mass General Hosp, Boston, MA 02114 & Am Red Cross, Rockville, MD 20855

LRP is an APP-KPI receptor and the major neuronal apoE receptor. LRP is a >600KDa protein, whose gene contains 89 exons. We screened genomic DNA for polymorphisms, including a published polymorphism in the 5' untranslated region, and so far have found no allelic differences between AD and controls. We also examined the multiple ligand binding domain regions of cDNA but did not detect evidence for any splice form variants. By both immunostaining and in situ hybridization, LRP was prominent on neurons. The hippocampal formation and cerebellum contained highest levels of signal. By confocal microscopy of human brain and cultured H4 neuroglioma cells, the majority of LRP was observed in the Golgi and ER; LRP was also present in clustered patches on the cell surface and on proximal axons and dendrites. LRP colocalizes with the 39 kDa Receptor Associated Protein intracellularly but not on the cell surface. These data suggest that the expression of LRP on the neuronal cell surface is tightly regulated: it is expressed in brain as the full length protein, in association with RAP, and ultimately reaches the cell surface where it is highly localized, perhaps in clathrin coated pits. Supported by NIH AG12406

388.14

HIPPOCAMPAL VOLUMES IN APOLIPOPROTEIN E TYPE 4 HOMOZYGOTES BEFORE THE ONSET OF ALZHEIMER'S DEMENTIA. A. Uecker*, E.M. Reiman, S. Lewis , R.J. Caselli, D. Bandy, M. deLeon, S. DeSanti, and A. Convit. U. Ariz, Mayo Clinic, New York Univ., and the Samaritan PET Center, Good Samaritan Reg. Med. Center, Phoenix, AZ 85006

Patients with Alzheimer's dementia have abnormal reductions in regional glucose metabolism using positron emission tomography (PET) and abnormal reductions in hippocampal volume using magnetic resonance imaging (MRI). Subjects homozygous for the apolipoprotein E type 4 (APOE-ε4) allele have similar PET abnormalities before the onset of cognitive abnormalities(Reiman et al, *New Engl J Med*, 1996). In this study, we investigated the ability of MRI measurements of hippocampal volume to provide preclinical evidence of AD in the same subject group.

APOE genotypes were characterized in 235 volunteers 50-65 yearsof age who reported a family history of probable AD. Neurologic, psychiatric and neuropsychological evaluations, PET measurements of regional glucose metabolism, and a T1-weighted volumetric MRI were performed in 11 ε4 homozygotes and 22 controls matched for gender, age, and educational level. A blind rater characterized hippocampal volume (97% intra-rater reliability) and intracranial volume (99% intra-rater reliability) in each MRI.

We failed to detect significantly lower hippocampal volumes (one-tailed t-test, p=.11), significantly lower ratios of hippocampal-to-intracranial volume (p=.34), or significantly greater differences between right and left hippocampal volumes (p=.17) in the cognitively normal ε4 homozygote group. Using available image-acquisition and image-analysis techniques, PET measurements of posterior cingulate glucose metabolism appear to provide a more sensitive index of AD pathology than MRI measurements of hippocampal volume before the onset of cognitive impairment.

LONG-TERM POTENTIATION: PHYSIOLOGY II

389.1

Cerebellar LTD is Induced by NO+Ca^{2+} or cGMP+Ca^{2+}. Varda Lev-Ram[1*], Jason Wood[2], David S. Lawrence[2], and Roger Y. Tsien[1,3], [1]Dept. of Pharmacology and [3]Howard Hughes Med. Inst., UCSD, La Jolla, CA 92093-0647; [2]Dept. Of Chemistry, SUNY Buffalo, NY 14260-3000

Long-term depression (LTD) in the cerebellum is a prolonged reduction in the efficacy of the parallel fiber (PF) synapse onto Purkinje cells (PCs) and is physiologically induced by activating PFs simultaneously with postsynaptic depolarization. We have previously shown in acute slices from young adult rats that nitric oxide (NO) uncaged inside PCs can completely replace PF activation (Lev-Ram *et al.* (1995) *Neuron* 15:407-415). We now find that Ca^{2+} uncaged from nitr-7 or azid-1 can completely replace postsynaptic depolarization and synergize with either PF activity or uncaged NO to trigger LTD. The ability of simultaneously uncaged NO and Ca^{2+} to induce LTD without any electrical activity proves that these two messengers are sufficient and that no other ionic signals are necessary. Ca^{2+} elevation must begin $\leq$10 ms after the end of NO production, suggesting a very short lifetime and limited diffusion distance of NO, and a precise coincidence detection mechanism. As previously suspected, NO acts via cGMP production, because ODQ and LY83583, which inhibit soluble guanylate cyclase (GC), block LTD induction by PFs or uncaged NO. This blockade could be circumvented by uncaged cGMP, but only when combined with a Ca^{2+} transient. Inhibition of cGMP-dependent protein kinase (PKG) with three different types of blockers, Rp-8Br-PET-cGMPS as a cGMP antagonist, KT5823 as an ATP-site inhibitor, and a novel pseudosubstrate peptide GRTGRRN(D-Ala)I-NH_2 all prevented LTD induction by PFs or uncaged NO. Therefore, simultaneous NO and Ca^{2+} are necessary and sufficient for LTD induction; their coincidence is detected with $\approx$10 ms time resolution; NO acts via GC and PKG rather than other proposed transducing molecules; and cGMP elevation still has to coincide with a Ca^{2+} increase to induce LTD. Supported by HHMI and NIH NS27177.

389.2

INDUCTION OF LTD IN VISUAL CORTICAL NEURONS WITH STOCHASTIC PATTERNS OF AFFERENT STIMULATION. S. M. Dudek*, D. Egelman†, P. R. Montague†, and M. J. Friedlander. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294, and †Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Many neural network theories today make use of Hebb-type learning rules, resembling the phenomena of long-term potentiation (LTP) and long-term depression (LTD). One theory, put forth by Bienenstock, Cooper, and Munro (BCM), inspired a line of experiments leading to the development of a now commonly used method of inducing long-term depression (LTD) in the hippocampus (Dudek & Bear, 1992) and neocortex (Kirkwood et al., 1994). While these experiments established that the induction of LTD is dependent upon the frequency of afferent stimulation, the use of sustained trains at regular stimulation rates in those studies differs considerably from the less regular input that neurons may experience *in vivo*. To determine whether a more realistic version of this "afferent noise" can also induce LTD, we delivered afferent stimulation in pseudo-random, stochastic patterns. The average frequency of the 900 pulses of stimulation was set by the experimenter in the range of 1 to 10 Hz and delivered with interstimulus intervals in a Poisson interval distribution to produce the specified value. Intracellularly recorded compound PSPs from layer 2/3 neurons in slices of guinea pig visual cortex were evoked by stimulation in layer 4 and assessed for the expression of a long-lasting depression (>30 min.). LTD was induced in 10 of 10 cells that were given stimulation averaging 1 or 2 Hz ($\bar{x}$=-32.4±5.1% and -23.4± 3.5%, for 1 and 2 Hz respectively). Significant depression was not induced in any of the 4 cells tested at the 10 Hz-average stochastic stimulation ($\bar{x}$=+1.4±3.0%). Induction of significant depression was highly variable at intermediate frequencies: LTD in 4 of 5 cells at 3 Hz-average ($\bar{x}$=-28.7±9.8%) and in 3 of 6 cells at 5 Hz-average ($\bar{x}$=-15.3±7.3%). The similar character of LTD induction for regular and stochastic stimulation suggests strongly that LTD induction is not an artifact of fixed rate stimulation. Supported by NIH grant EY05116 and HFSP grant RG69193 to M.J.F., and NIMH grant R0152797 to P.R.M.

389.3

A LATE PHASE OF CEREBELLAR LONG-TERM DEPRESSION (LTD) IS DEPENDENT UPON PROTEIN SYNTHESIS. D.J. Linden* Department of Neuroscience, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Cerebellar LTD is a cellular model of information storage in which a persistent attenuation of the parallel fiber-Purkinje neuron (PN) synapse is induced by conjunctive stimulation of parallel fiber and climbing fiber inputs. LTD may be induced in the cultured mouse PN when glutamate pulses and PN depolarization, which mimic the effects of parallel and climbing fibers respectively, are co-applied. Amphotericin-patch recording from PNs with Cs-based internal saline has allowed for the recording of input-specific LTD which persists for >120 min. Bath application of the translation inhibitor anisomycin (10 uM), immediately, but not 30 min after glutamate/depolarization conjunction, produced a blockade of the late phase of LTD which became evident ~60 min later. No effect of anisomycin was seen on a separate control input which did not receive conjunctive stimulation. A similar blockade of late-phase LTD was seen using the transcription inhibitors actinomycin D (30 uM) or DRB (30 uM). Fura-2 microfluorimetry revealed that these three drugs did not significantly alter depolarization-induced Ca influx or mGluR-induced Ca mobilization at the concentrations used, suggesting that their effects were indeed mediated by protein synthesis inhibition, rather than inhibition of processes previously shown to be necessary for LTD induction. Finally, LTD was produced using glutamate/depolarization conjunction in a perforated outside-out macropatch of dendritic membrane. LTD in this preparation, which lacks nuclear material, returned to baseline with a time-course similar to that produced by protein synthesis inhibitors in intact cultured PNs, and was not further altered by application of actinomycin D. However, application of anisomycin to the dendritic macropatch resulted in a further shortening of the depressed time-course suggesting that local mRNA translation in the dendrite may contribute to the late phase. Supported by PHS MH51106, NARSAD, and the Klingenstein, McKnight and Develbiss Funds.

389.4

INDUCTION OF THE LATE PHASE OF SCHAFFER COLLATERAL LTP LEADS TO AN INCREASE IN THE NUMBER OF SITES FOR SYNAPTIC TRANSMISSION. V.Y. Bolshakov*, H. Golan, S.A. Siegelbaum and E.R. Kandel. HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032.

LTP in the Schaffer collateral pathway between CA3 and CA1 pyramidal neurons exhibits an early phase (E-LTP) that is independent of protein synthesis and a late phase (L-LTP) that requires protein synthesis and cAMP. We previously studied the unitary synaptic connections between single CA3 and CA1 neurons and found that the EPSC amplitude distributions showed only a single quantal peak. This indicates that a single CA3 neuron makes only a single synaptic contact with a single CA1 neuron and that this contact contains only a single release site which releases a single quantum. With the production of E-LTP there was an increase in the probability of transmitter release (fewer failures) but no increase in the number of release sites or quantal amplitude. Does the same mechanism underlie L-LTP? To induce L-LTP we pretreated slices for 15 min with Sp-cAMPs (50 μM). Recordings of unitary synaptic transmission, 2-4 hrs after washout of cAMP showed two major differences from untreated slices: 1. Similar to E-LTP, the fraction of failures of transmission was decreased; 2. Unlike E-LTP, there was an increase in the number of peaks in the EPSC amplitude distribution. Quantal amplitude was not altered. Both effects were blocked by the protein synthesis inhibitor anisomycin. These results suggest that L-LTP involves the addition of new sites of synaptic transmission (see Golan et al. below). Supported by HHMI and NIMH IP50 MH50733.

389.5

MECHANISMS FOR INCREASED NUMBER OF SITES OF SYNAPTIC TRANSMISSION DURING THE LATE PHASE OF CA3-CA1 LTP. H. Golan, V.Y. Bolshakov, E.R. Kandel, and S.A. Siegelbaum*. HHMI, Ctr. Neurobiology & Behavior, Columbia Univ., NY, NY 10032.

Several mechanisms could explain the increased number of peaks in the EPSC amplitude histogram that appear during L-LTP (see Bolshakov et al., above). First, there could be an insertion of new clusters of postsynaptic receptors under a single release site (with multiple peaks corresponding to multiple clusters of active receptors). We ruled this out by showing that elevation of external Ca shifted the EPSC amplitude distribution (more events now appear in peaks composed of multiple quanta) consistent with an increased number of release sites. We next ruled out the unmasking of silent synapses (sites with no active AMPA receptors but active NMDA receptors) during L-LTP. The NMDA (in Mg^{2+}-free solution) and AMPA components of the unitary EPSCs exhibit a similar fraction of failures in control slices suggesting the initial absence of silent synapses. We thus conclude that L-LTP involves an increase in the number of release sites. Surprisingly L-LTP was accompanied by a 2-fold increase in the mean size and CV of spontaneous MEPSCs. However, this effect appears presynaptic since substituting Sr^{2+} for Ca^{2+} in the extracellular solution, so as to desynchronize release, decreased MEPSC size back to control values. Following the proposal of Korn and Faber that synchronized release of MEPSC can occur when a single bouton contains multiple active zones, our results are most consistent with the formation of perforated synapses by splitting of active zones into multiple sites. Supported by HHMI and NIMH IP50 MH50733.

389.6

AMPA RECEPTOR EXPRESSION INCREASES DURING THE LATE PHASE OF ELECTRICALLY INDUCED LTP. A.S. Nayak* and M.D. Browning University of Colorado Health Sciences Center, Denver, CO 80262.

LTP is a dramatic form of synaptic plasticity that is believed to underlie certain types of learning and memory. Considerable interest has focused on the potentiation of postsynaptic AMPA receptors in hippocampal LTP. Here we report that tetanus-induced LTP is accompanied by an increase in synthesis of GluR1 and GluR2/3 subunits of the AMPA-receptor. High frequency stimulation (HFS) was delivered to CA1 mini-slices using a special multi-electrode array (the "rake", a linear array of four monopolar electrodes), in order to optimize the fraction of synapses in each slice that received stimulation. Three hours after stimulation, tetanized slices showed a 51.6±10.5% increase in GluR1 synthesis and a 28.6±5.1% increase in GluR2/3 synthesis over unstimulated control slices, as determined by ^{35}S-methionine incorporation. Ninety minutes after stimulation, there was a similar but smaller increase in the expression of these subunits (not statistically significant). Western blot analysis confirmed that the increases observed at 3 hours were in total protein level and not merely in turnover rate. The effects at three hours were completely blocked by 100μM APV or 10μM MK-801, NMDA-receptor antagonists which block the induction of LTP. Frey et al. (1993, *Science*, **260**,1661-4) have defined a late-phase LTP which begins three hours after HFS and requires protein synthesis and protein kinase A (PKA). The increases in GluR expression we observed 3 hours after HFS also require PKA, as these effects were completely blocked when slices were stimulated in the presence of 100μM Rp-cAMPS, a membrane permeable inhibitor of PKA. Interestingly, Sp-cAMPS, an activator of PKA, was able to induce a significant but small increase in GluR expression even in the absence of high frequency stimulation. These data suggest that increases in AMPA-receptor expression contribute to LTP-associated increases in synaptic responsiveness, and that cAMP/PKA may play a pivotal role in the expression of LTP by orchestrating these postsynaptic changes. Supported by MH-10757.

389.7

STUDIES OF LTP IN MICE WITH KNOCK-OUT OF THE NEURONAL AND ENDOTHELIAL FORMS OF NOS. H. Son*, R.D. Hawkins, E.R. Kandel, P.L. Huang‡, M.C. Fishman‡. HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., New York, NY 10032; ‡Cardiovasc. Res. Ctr., Mass. Gen. Hosp., Harvard Med. School, Charlestown, MA 02129.

Nitric oxide (NO) has been implicated as a retrograde messenger in hippocampal LTP. However, LTP is normal in mice with a knockout of the neuronal form of NO synthase ($nNOS^{-/-}$), suggesting that the endothelial, but not neuronal, form of NOS may be required for LTP (O'Dell et al., 1994). Therefore, we tested LTP in mice with a knock-out of neuronal NOS, endothelial NOS ($eNOS^{-/-}$), or a double (neuronal and endothelial NOS) knock-out. LTP in the stratum radiatum of CA1 was normal in both $nNOS^{-/-}$ mice (212 ± 17%, n=4) and $eNOS^{-/-}$ mice (199 ± 6%, n=6) compared to wild-type (211 ± 8%, n=16), but LTP was significantly reduced in double knock-out mice (142 ± 4%, n=8). The amplitude of field EPSPs, paired-pulse facilitation, and LTD were normal in the double knock-out mice. In preliminary experiments, LTP in the stratum radiatum was significantly reduced by the NOS inhibitor, nitroarginine, in wild-type (133 ± 8%, n=10) but not in double knock-out (141 ± 4%, n=2) mice. However, LTP in stratum oriens was not affected by nitroarginine (192 ± 5%, n=10) compared to control (200 ± 7%, n=10) in wild-type mice, indicating that it is NOS-independent (Haley and Madison, 1995). LTP in stratum oriens was normal in the double knock-out mice (202 ± 24%, n=2), suggesting that the deficit in stratum radiatum LTP is not due to nonspecific effects in the double knock-out mice. These genetic results therefore support the idea that NO is involved in LTP, and suggest that the neuronal and endothelial forms can compensate for each other in the single knock-outs. Supported by NIMH and HHMI.

389.8

NO ACTS AS A RETROGRADE MESSENGER DURING LONG-TERM POTENTIATION IN CULTURED HIPPOCAMPAL NEURONS. O. Arancio*, V. Lev-Ram, R.Y. Tsien, E.R. Kandel, and R.D. Hawkins. HHMI, Columbia Univ., NY, NY 10032, and UC San Diego, La Jolla, CA 92093

Previous results suggest that long-term potentiation (LTP) at synapses between hippocampal neurons in dissociated cell culture involves presynaptic guanylyl-cyclase (Arancio et al., *Nature* **376**:74-80, 1995). Since guanylyl-cyclase can be activated by several substances including NO, we have examined the role of NO in LTP using dissociated cell culture. LTP was blocked by postsynaptic but not presynaptic injection of an NO-synthase inhibitor, L-NMMA, and bath application of NO produced activity-dependent long-lasting potentiation (Arancio et al., *Soc. Neurosci., Abstr.* **21**:1811, 1995). These results suggest that NO is involved in LTP, and that NO-synthase is located in the postsynaptic neuron. We now report that LTP was also blocked by delivery of a NO scavenger, oxymyoglobin to the bath (10 μM, n=16) or through the patch pipette (50 μM) to either the postsynaptic (n=10) or presynaptic (n=11) neuron. By contrast, potentiation by exogenous NO was blocked by oxymyoglobin in the presynaptic (n=8) but not the postsynaptic (n=8) neuron. These results suggest that NO must travel through the extracellular space to the presynaptic neuron to produce potentiation. As an additional test of the site of action of NO, we used a membrane-impermeant NO donor, CNO-4, that releases NO only upon photolysis with UV light. Intracellular injection of CNO-4 (20 μM) into the postsynaptic neuron followed by photolysis (estimated to release 10 nM NO) paired with activity caused long-lasting potentiation (n=8) that was blocked by extracellular oxymyoglobin (n=5). By contrast, photolytic release of NO in the presynaptic neuron paired with activity caused potentiation that was not blocked by extracellular oxymyoglobin (n=14), suggesting that the site of action of NO is in the presynaptic neuron. Taken together, these results strongly support the hypothesis that NO can act as an activity dependent retrograde messenger during LTP. Supported by NIMH and HHMI.

389.9

OVER-EXPRESSION OF Cu/Zn SOD and S100β GENES IS LINKED TO IMPAIRED LEARNING AND HIPPOCAMPAL LTP E. Gahtan, J.M. Auerbach*, Y. Groner and M. Segal The Weizmann Institute, Rehovot 76100, Israel.

A link between Alzheimer's Disease (AD) and Down Syndrome (DS) has been based on the finding that most DS patients, who are characterized by trisomy of chromosome 21, develop AD pathology at young age. To investigate the role of chromosome 21 genes in brain and behavior, transgenic (Tg) mice were produced that overexpressed the human Cu/Zn SOD and S100β genes, located on human chromosome 21. Over-expression of SOD and S100β in brains of these mice was confirmed immunohistochemically.

Tg mice were found to be significantly more active then controls in an open field and in activity-monitoring cage. Tg mice also had fewer defecations and greater frequency of rearing in the open field, suggesting decreased emotionality relative to controls. Tg mice demonstrated impaired performance in the Morris Water Maze. The Tg group had longer mean escape latencies across each of the 12 training trials, suggesting a deficit in spatial learning, and were impaired in a test of spatial memory conducted 24 hours after the last training trial. No difference was observed between Tg and controls in escape latency to a visible platform, indicating that sensory, motor and motivational factors are equivalent between groups.

Population EPSP slopes in region CA1 of hippocampal slices from Tg and controls showed similar degrees of post-tetanic potentiation in response to tetanic stimulation (100Hz, 1s) of stratum radiatum fibers, but potentiation in Tg slices dropped off rapidly, falling to approximately half its initial level 10 min post tetanus. In contrast, potentiation in control slices remained stable for at least 30 minutes post tetanus.

The present experiments were conducted with Tg mice over-expressing 2 genes. Future studies will examine the contribution of each gene to the observed deficits. Supported by NIH HD21229 and Weizmann Institute grants.

389.10

HIGH-FREQUENCY TETANUS RELEASES A FACTOR THAT BLOCKS HIPPOCAMPAL LONG-TERM POTENTIATION LOCALLY. Julie A. Kauer* and Lori M. McMahon. Dept. of Neurobiology, Duke University Med. School, Durham, NC 27710.

Extracellular recordings were made in the CA1 region of hippocampal slices from 3-4 week old rats. One stimulating electrode was placed in s. radiatum within 200 μm of the recording electrode ("close"), with another >500 μm away ("far"). Field EPSPs elicited by alternating stimulation to each site were stable over time. A brief high-frequency tetanus (100 Hz, 1 sec., 2 trains) to the close stimulating electrode produced little potentiation (15 min. post-tetanus: 126+/-9%, n=8). Subsequent tetanus to the far pathway triggered normal LTP at the same recording site (15 min. post-tetanus: 170 +/- 14%, n=6), suggesting that the postsynaptic recording region is apparently unharmed by nearby tetanic stimulation. Fiber volleys were closely monitored to ensure that presynaptic fibers were not damaged by a close tetanus.

We hypothesized that during a tetanus a diffusible factor that blocks LTP may be released locally. We therefore tetanized close and far pathways simultaneously. In support of the hypothesis, LTP was substantially attenuated in both, while LTP was triggered by a subsequent tetanus to the far pathway alone. We cannot overcome the block of LTP in a close pathway using antagonists of $GABA_A$ or adenosine receptors (50 μM picrotoxin; n=13 or 2-20 μM CPT; n=4). Inhibition of NO synthase (100 μM N-methyl-L-Arg; n=3) or of protein phosphatases involved in LTD (1 μM calyculin A; n=3) is also ineffective. PTP is significantly smaller after close vs. far tetanus, suggesting a presynaptic action. We speculate that during high-frequency activity, axons in CA1 release a locally-acting factor that blocks LTP. Supported by NS30500-05 to J.A.K.

389.11

ROLE OF THE TRK FAMILY OF RECEPTOR TYROSINE KINASES IN NEUROTROPHIN- AND SYNAPTICALLY-INDUCED POTENTIATION IN ADULT RAT HIPPOCAMPUS. H.Kang[1]*, A.Welcher[2], E.M.Schuman[1], [1]Division of Biology 216-76, Caltech, Pasadena, CA 91125 and [2]Amgen Inc., Thousand Oaks, CA 91320

During development, the neurotrophins exert their effects through the Trk family of receptor tyrosine kinases. The widespread expression of Trk receptors in the adult hippocampus suggests the possibility that the neurotrophin-induced potentiation we have observed is mediated by these same receptors. Our previous studies with the K252 compounds support this possibility since K252a, an inhibitor of receptor tyrosine kinases, prevents this enhancement (Kang & Schuman, *Science*, 1995). However, to circumvent the potential non-specific effects of pharmacological inhibitors, we utilized specific function-blocking Trk antibodies to ascertain directly which receptors the neurotrophins interact with to bring about the synaptic enhancement. Pre-incubation of slices with TrkB antibody (Ab) dramatically attenuated the induction of synaptic enhancement upon exposure to brain-derived neurotrophic factor (BDNF, 50 ng/ml), although adjacent slices pre-incubated with pre-immune serum showed normal enhancement [mean % of baseline 1 hour after exposure: pre-immune, 186.6 ± 12.2 (p<0.05, n=5); TrkB Ab, 110.3 ± 5.2 (ns, n=5)]. Immunoprecipitation/western blot analysis of hippocampal slice homogenates also indicates that the full-length TrkB receptors are phosphorylated in response to BDNF treatment. In examining the relationship between neurotrophin-induced potentiation and synaptically-induced LTP, we previously showed that prior treatment with the neurotrophins does not significantly occlude the subsequent induction of early-phase LTP. In addition, the neurotrophin-induced potentiation was not affected by the presence of NMDA receptor antagonist. Here, we investigated whether treating slices with TrkB Ab would affect the induction of LTP by high-frequency stimulation. The same antibody which completely prevents BDNF-induced potentiation did not have any effect on tetanus-induced LTP [mean % of baseline 1 hour post tetanus: 180 ± 18.9 (p<0.05, n=5)]. These results support the idea that these two forms of plasticity involve at least partially independent cellular mechanisms for their early phases. *This work was supported by the Alfred P. Sloan Foundation, John Merck Fund, and the PEW Charitable Trusts.*

389.12

LONG-TERM POTENTIATION INDUCES PHOSPHORYLATION OF CREB IN THE HIPPOCAMPUS *IN VIVO* AND *IN VITRO*. P.J. Voulalas* and J.M. Sarvey. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

The transcription factor CREB has been implicated as a key regulatory protein in the mediation of long-term changes in synaptic efficacy thought to underlie memory formation. Increases in CREB phosphorylation on Ser^{133} reflect functional activation of this protein. We have carried out a time-course analysis of changes in the phosphorylation state of CREB following induction of LTP in rat hippocampal dentate gyrus *in vivo* and *in vitro*. Electrophysiological analyses were accompanied by Western blot analyses utilizing an antibody which specifically recognizes the phosphorylated form of CREB. LTP, which lasted several hours, was induced by theta burst stimulation in anesthetized rats, or by a 100Hz, 2s train in hippocampal slices. Increases in CREB phosphorylation in the anesthetized rat occurred within 10' after delivery of a train, and remained elevated for up to 60'. In the *in vitro* slice, CREB phosphorylation increased 5' after delivery of the train, and returned to baseline levels within 30'. NMDA receptor antagonists blocked these increases. These results imply that the time-course of phosphorylation of CREB occurs at a time between activation of membrane receptors (which increase intracellular calcium and cAMP) and increases in protein synthesis events. Supported by NIH NS23865.

GENESIS OF NEURONS AND GLIA: ADULT

390.1

SPATIAL AND TEMPORAL PATTERNS OF NEUROGENESIS AND NEURONAL RECRUITMENT IN THE POST-LARVA AND ADULT FISH BRAIN. P. M. Ma* and A. Gilligan, Marine Science Center, Northeastern University, Nahant, MA 01908.

Adult fish brains retain the capacity to produce new neurons and to re-generate after ablation. To investigate the proliferative activity and the extent to which new neurons contribute to the functional organization of the normal and regenerated brain, we use bromodeoxyuridine immuno-cytochemistry to identify and follow newly-formed neurons in the post-larva and adult guppy and zebrafish brains. Major neurogenetic zones are seen along the ventricles in the telencephalon, preoptic and supra-chiasmatic areas (SA) , hypothalamus, tectum, and cerebellum. Activity within these zones is age-dependent. Neuronal recruitment occurs by two modes: active migration and displacement. Nuclei removed from the ventricles, such as nucleus glomerulosus (NG), recruit cells by active migration. In other areas, such as the dorsal telencephalon, younger neurons displace older ones from the ependyma. Recruitment is also age-dependent. For example, NG recruits neurons born between post-larval days 1-4, but rarely those born later. Neurons generated at the SA between days 1-5 remain in this area, whereas some of those born later migrate into the pituitary. Newly-formed neurons can apparently migrate to the correct destination and integrate into existing functional circuits in many regions of the adult fish brain and may contribute to functional recovery. [supported by NIMH]

390.2

POSTNATAL NEUROGENESIS IN THE DORSAL LATERAL GENICULATE NUCLEUS (LGN) OF THE CAT. L.P. Fedynyshyn* and R.E. Kalil. Center for Neuroscience and Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison, WI 53706.

The neurons of the mammmalian central nervous system are derived from the mitotic division of undifferentiated neuronal precursor cells, and with few exceptions this neurogenesis occurs during embryonic development and prior to birth. However, many mitotic figures are seen in the LGN of early postnatal kittens. To explore the possibility that neurogenesis in the LGN of the cat may occur after birth, we have used immunofluorescent techniques to determine if cells undergoing mitosis in the postnatal LGN are neurons.

Neonatal kittens were perfused with 4.0% paraformaldehyde and 0.5% glutaraldehyde and sections through the LGN were labeled immunofluorescently with monoclonal antibodies specific for low (68kD), middle (160kD) and high (200kD) molecular weight neurofilaments (NFP) or for class III β tubulin. The primary antibodies were omitted in control sections. The labeled sections were counterstained for mitotic figures with propidium iodide and examined with epifluorescence/differential interference contrast microscopy or with a Bio-Rad MRC 1024 confocal microscope.

Present results suggest that both NFP and β tubulin class III immunoreactivity appear to co-localize with propidium iodide-labeled mitotic figures. These observations provide evidence for postnatal neurogenesis in the cat central visual pathway. Additional experiments with other probes are underway to confirm these results, and to rule out possible artifacts such as cross-reactivity with nonneuronal proteins.

This work was supported by NEI grant EYO 1331.

390.3

NEUROGENESIS IN THE DENTATE GYRUS OF THE ADULT TREE SHREW B.S. McEwen*, E.Gould, P.Tanapat, L.A.M.Galea and E.Fuchs. Lab. of Neuroendocrinology, Rockefeller Univ., New York, NY 10021 and German Primate Center, 37077, Gottingen, Germany.

Although adult neurogenesis has been well-documented in the dentate gyrus of rodents, the existence of this process in the brains of primates has not yet been described. The tree shrew (*Tupaia belangeri*) is considered to be phylogenetically between insectivores and primates. In order to determine whether the dentate gyrus of the adult tree shrew continues to produce new granule cells, we performed immunohistochemistry for the thymidine analog bromodeoxyuridine (BrdU) as well as for cell-specific markers on the brains of adult tree shrews. Adult tree shrews were injected with BrdU and perfused either 2 or 24h after injection. BrdU-IR cells were detected in the dentate gyrus of all adult tree shrew brains examined. Most of these BrdU-IR cells were located in the subgranular zone (sgz), the region between the granule cell layer (gcl) and the hilus. The vast majority of these cells had the morphology of immature granule neurons, i.e., medium-sized, round or oval cell bodies. A significant increase (>2x) in the number of BrdU-IR cells was detected between the 2 and 24h survival time points indicating that cells which had incorporated BrdU divided at least once in the 24h period. Examination of tissue stained for vimentin, a marker of immature glia, revealed numerous cells with radial glial morphology. The triangular cell bodies of these glia typically resided in the sgz with radial processes extending through the gcl, suggesting that these cells might participate in the migration of immature granule cells.

Supported by MH52423 to EG.

390.4

ALTERED NEUROGENESIS IN THE ADULT DENTATE GYRUS FROM SPREADING DEPRESSION. D. Dziewulska* P.E. Kunkler, J.D. Hunter and R.P. Kraig. Department of Neurology, The University of Chicago, Chicago, IL 60637

The cellular and molecular mechanisms by which neural tissue might be salvaged or even regenerated after injury are only beginning to be explored. Glial cells are thought to affect both injury severity and the ability of brain to regenerate. Accordingly, we are examining how gliosis influences neurogenesis and plasticity within the rodent dentate gyrus (DG) where neurogenesis continues in adulthood.

Spreading depression (SD) was initiated within hippocampus by micro-injection of KCl in caudal hippocampus for 3 hrs and confirmed via more rostral DC recordings. Animals were allowed to recover and processed for GFAP and PCNA-based immunohistochemical measurement of cellular proliferation. In controls we found significantly more ($p<0.05$; $n=5$) PCNA-positive cells in the subgranular zone of the lower (compared to upper) limb of the DG. This area also showed a significant increase ($p<0.05$) in PCNA-positive cells after SD compared to controls. Increased PCNA immunostaining was seen throughout the rostral caudal length of the DG. Total PCNA-positive cell counts within the entire DG after SD increased from controls ($p=0.06$; $n=5$). Finally, SD induced astrogliosis within the hippocampus, as evidenced by increased GFAP immunostaining compared to controls.

Cameron and colleagues (J. Neurosci, 1995) have shown that lesions of the entorrhinal cortex and blockade of excitatory synaptic transmission increase DG neurogenesis while increased excitatory activity by NMDA receptor activation retards neurogenesis. SD not only involves a massive release of glutamate to the interstitial space but also includes a propagating electrical silence. Thus, if events associated with SD and those ablative and pharmacologic experiments noted above are similar, our results suggest that a decrease in dentate neurogenesis is influenced more by electrical silence than glutamate release. Perhaps, diminished neuronal activity can trigger neurogenesis by co-activating astrocytes. (Supported by NS-19108.)

390.5

APOPTOSIS AND SPREADING DEPRESSION. P.E Kunkler* and R.P. Kraig, Department of Neurology, The University of Chicago, Chicago, IL 60637

Spreading depression (SD) induces gliosis in the CNS but not necrosis. Reactive gliosis is mostly thought of as a response to injury, but some evidence suggests that gliosis may also play a role in synaptogenesis and plasticity. Apoptosis occurs in the CNS under various physiological and pathophysiological conditions. It is possible that SD induced gliosis may be an event secondary to apoptotic cell death, however it is not known if apoptosis occurs following SD. Here we show that apoptotic cell death is not induced following cortical SD but is elevated following SD in the hippocampus. SD was elicited in Wistar male rats by micro-injection of 0.5M KCl into the left parietal cortex every 9 minutes for 3 hours. After survivals times of 0, 6, 24 or 72 hrs, sections collected throughout the rostral caudal extent of the brain were processed with ApopTag In Situ apoptosis detection kit. Counts of ApopTag positive cells were conducted and compared to the contralateral non-exposed hemisphere and to untreated controls. Hippocampal SD was elicited in a similar manner with the micro-injection placed instead in the caudal aspect of the hippocampus. Isolated ApopTag positive cells were found randomly throughout all cortical layers, though occasionally clustered. On average, 1-2 positive cells were observed per section, with an ocassional section containing up to 10 cells. Statistical analysis revealed no left to right difference between the exposed and non-exposed contralateral hemisphere or with untreated controls, though fewer cells overall were observed in the experimental animals. A nonsignificant left to right increase in the presence of small apoptotic bodies was observed in the dentate gyrus (DG) following hippocampal SD, but not in the proliferative subgranular zone of the DG. When compared to untreated brains, the total number of positive cells within the DG of hippocampal SD exposed animals approached significant values. The absence of either necrotic or apoptotic cell death following SD suggest that gliosis with SD may be approaching a physiologic phenomena which may more accurately reflect glial changes associated with brain plasticity rather than injury. (Supported by NS-19108.)

390.6

ADRENAL STEROIDS AND NMDA RECEPTOR ACTIVATION REGULATE NEUROGENESIS IN THE ADULT RAT DENTATE GYRUS THROUGH A COMMON PATHWAY. H. A. Cameron*, P. Tanapat, and E. Gould. Lab of Neuroendocrinology, Rockefeller Univ, NY, NY 10021.

The rate of granule cell birth in the adult rat dentate gyrus is regulated by both adrenal steroids and NMDA receptor activation. Parallels between the actions of these two factors suggest that they may affect cell birth through a common pathway. To test this hypothesis, we altered both of these factors simultaneously and determined whether their effects were additive, implying independent mechanisms, or whether one factor could block the effect of the other, indicating a common pathway. Adult male rats injected with the NMDA receptor antagonist MK-801 had significantly more ^{3}H-thymidine-labeled cells in the dentate gyrus than saline-injected controls (≈3x increase), following a short (2 hour) survival period, while rats injected with corticosterone (cort) had significantly fewer labeled cells than uninjected controls (≈1/3x). In rats treated with both MK-801 and cort, the labeled cell density (≈3x control value) was not significantly different from that of rats treated with MK-801 alone, indicating that the NMDA receptor blockade completely blocks the effect of cort. In the converse experiment, removal of adrenal steroids through adrenalectomy (ADX) significantly increased ^{3}H-thymidine-labeled cell density compared to sham-operated controls (≈3x), and NMDA injection significantly decreased labeled cell density compared to saline injection (≈1/4x). Following ADX and NMDA injection, the density of labeled cells (≈1/3x control value) was not significantly different from that of NMDA treatment alone, indicating that NMDA receptor activation can block the effect of ADX on cell proliferation. These findings suggest that adrenal steroids act upstream of NMDA receptors in a common pathway.

Supported by MH52423 and NARSAD (E.G.).

390.7

NEUROGENESIS IN THE DENTATE GYRUS OF ADULT MICE.
G. Kempermann*, H. G. Kuhn and F. H. Gage. Salk Institute for Biological Studies, Laboratory of Genetics, 10010 N. Torrey Pines Rd., La Jolla, CA 92037, USA., E-mail: gerd_kempermann@qm.salk.edu

Neurogenesis can be described by the three consecutive steps of proliferation, migration and differentiation. We compared two mouse strains (Balp/C and C57) at two ages (3 months and 1 year) to study neurogenesis in the adult hippocampus. Proliferating cells were labeled with bromodeoxyuridine (BrdU) and detected with antibodies against BrdU. In all four groups cell proliferation could be detected in the subgranular zone of the dentate gyrus. The number of proliferating cells decreased with the age of the mice and showed strain differences. Four weeks after injection of BrdU the number of BrdU-positive cells was very low, but some of these remaining newborn cells expressed the neuronal marker NeuN, while others stained positive for the astrocytic protein GFAP. In contrast to the analogous cells in rats, however, these cells had not migrated into the granule cell layer and did not express calbindin D28K which is a marker protein for differentiated granule cells. The polysialylated form of the neural cell adhesion molecule (PSA-NCAM) is considered to be involved in the regulation of migration and differentiation in certain distinct nerve cell populations. As PSA-NCAM is expressed in the subgranular zone of adult mice, we conclude that the expression of PSA-NCAM alone is not sufficient to promote neuronal migration and granule cell differentiation in the adult dentate gyrus. Thus mice might differ from rats with regard to other still unknown regulatory factors that are intrinsic to the population of precursor cells or to the environment of the dentate gyrus. These results emphasize that proliferation, migration and differentiation are independently regulated. Mice transgenic for genes or trophic factors that are involved in these distinct events might therefore provide a useful approach to studying the regulation and possible inducibility of neurogenesis in the adult brain.

Funded by NIH AG 08514 and DFG (Ke 615/1-1).

390.8

EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF EGF AND FGF-2 ON PROLIFERATION AND DIFFERENTATION OF NEURAL PROGENITORS IN ADULT RATS: II. HIPPOCAMPUS AND SPINAL CORD. J. Winkler*, H.G. Kuhn, G. Kempermann, L.J. Thal and F.H. Gage. Dept. of Neurosciences, UCSD, La Jolla, CA 92161 and Laboratory of Genetics, Salk Institute, La Jolla, CA 92037.

To define EGF and FGF-2 actions on neural precursor cells in the adult brain we examined the hippocampus and the thoracic spinal cord of rats treated with 2 weeks of intraventricular EGF or FGF-2 (360 ng/day) or artificial cerebrospinal fluid (aCSF). Bromodeoxyuridine (BrdU; 50mg/kg) was injected daily during treatment. At the end of treatment, the hilus and the granule cell layer of EGF-treated animals showed an increase in BrdU-labeled cells in comparison with FGF-2 or aCSF-treated animals. Four weeks following withdrawal, the density of BrdU-labeled cells did not differ between the groups. However, in the molecular layer EGF-treated animals showed a significant increase in BrdU-labeled cells in comparison with all other groups at the end of the treatment and after withdrawal. In the thoracic spinal cord EGF and FGF-2 induced an increase of BrdU-labeled cells in comparison with aCSF-treated animals at both time points. These data indicate that EGF and FGF-2 caused a topographically selective proliferation of precursor cells in the adult CNS. Further characterization with glial and neuronal markers will reveal the phenotype of the newborn cells.

Supported by NIH, Brookdale National Fellowship and Hereditary Disease Foundation.

390.9

EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF EGF AND FGF-2 ON PROLIFERATION AND DIFFERENTIATION OF NEURAL PROGENITORS IN ADULT RATS: I. SUBVENTRICULAR ZONE, STRIATUM AND OLFACTORY BULB. H.G. Kuhn*, J. Winkler, G. Kempermann, L.J. Thal and F.H. Gage. Laboratory of Genetics, The Salk Institute, La Jolla, CA 92037 and Dept. of Neurosciences, UCSD, San Diego, CA 92161.

In recent years the existence of proliferating neural progenitor cells in the adult brain has been demonstrated by both *in vitro* and *in vivo* studies. The current experiment analyzed the effects of chronic intracerebroventricular infusion of growth factors on progenitor populations in adult rat brain. Animals received EGF, FGF-2 (360ng/day) or artificial cerebrospinal fluid for 2 weeks via osmotic minipumps into the lateral ventricle, accompanied by daily systemic injections of BrdU (50mg/kg). At the end of the infusion and 4 weeks after withdrawal BrdU-positive subependymal cells of the lateral ventricle wall were significantly increased in EGF and FGF-2 treated rats. EGF infusion resulted in a massive thickening of the subependymal zone. BrdU positive cells were also found in the adjacent striatum of EGF-treated animals. In accordance with a recent study performed in mice (Morshead et al., 1996, J.Neurosci. 16:2649-58), confocal analysis partially identified these striatal cells as newborn neurons and glia. In contrast, EGF infusion decreased BrdU-positive cells in the olfactory bulb, in particular, in the rostral migratory stream, when analyzed at the end of infusion interval, and in the granule cell layer at 4 weeks post-infusion. These data indicate that EGF is capable to expand the population of subventricular progenitor cells. However, retarded rostral migration of neuronal progenitor cells into the olfactory bulb is observed following EGF infusion. *Supported by NIH, Hereditary Disease Foundation and Brookdale National Fellowship.*

390.10

POLYSIALYLATED NEURAL CELL ADHESION MOLECULE IS ASSOCIATED WITH GABAERGIC NEURONS LOCATED ON THE VENTRICULAR SURFACE OF THE ADULT RAT FOREBRAIN. G. Alonso*, M. Prieto, N. Chauvet, A. Legrand and A. Privat. INSERM U 336, University Montpellier II, 34000 Montpellier, France.

In the developing central nervous system, virtually all the neurons express an isoform of the neural cell adhesion molecule that is highly enriched in polysialic acid (PSA-NCAM). In the adult, the expression of this molecule is restricted to areas that contain either newly formed neurons or neurons that exhibit a particular capacity for morphological plasticity. In the present study, 50 to 75 µm thick sections cut frontally or sagitally through the forebrain of adult rats were treated for single, double or triple fluorescence immunostaining in order to detect and characterize PSA-NCAM-immunoreactive (IR) cells located on the ventricular surface. In intact brains, intense PSA-NCAM immunostaining was found to be associated with small spherical or fusiform cells located on the surface of (i) the third ventricle bordering the median eminence, and (ii) the lateral ventricles bordering the lateral septum or the dorsal portion of the hippocampus. These PSA-NCAM-IR cells were co-labelled by antibodies against GABA, MAP2, MAP5, and neurofilament. In brains that have received various types of surgical lesions through the forebrain, an increased number of such PSA-NCAM-IR neurons was detected on the surface of the lateral and third ventricles where they appeared either (i) as small spherical cells aggregated into clusters of various size, or (ii) as large isolated cells exhibiting very extended processes. In addition to the different neuronal markers detected in intact brains, these PSA-NCAM- and GABA-IR neurons were also found to be intensely immunoreactive to B-50 (GAP-43). It is suggested that this population of PSA-NCAM-IR GABAergic neurons represent neuronal precursors that remain on the ventricular surface of the rat forebrain throughout adulthood.
(Supported by IRME)

390.11

EXPRESSION OF NOS, NCAM-H AND S100 PROTEIN IN THE GRANULE CELL GENERATION PATHWAY OF THE ADULT GUINEA PIG OLFACTORY BULB. A. T. M. S. Islam, K. Nakamura, T. Seki*, A. A. Banu, K. Hirata and M. Kawabuchi*. Dept. of Anatomy, Faculty of Med., Kyushu Univ., Fukuoka 812-82, Japan; *Dept. of Anatomy, Sch. of Med., Juntendo Univ., Hongo, Tokyo 113, Japan.

In order to reveal the role of nitric oxide (NO) in the persistent adult neurogenesis and neuro-glial coherence in the neuronal migration process, we demonstrated a double-labeled immunofluorescence cytochemistry under confocal laser scanning microscope, using antibodies against nitric oxide synthesizing enzyme, nitric oxide synthase (NOS), highly polysialylated isoform of neural cell adhesion molecule (NCAM-H), and astroglial marker in the brain, S100 protein (S100) in the whole length of subependymal layer as well as in the internal granular layer (IGr) of the adult guinea pig olfactory bulb. Different populations of blast like, beaded, clustered immature cellular elements positive for both NCAM-H and S100 were densely interlaced throughout the entire length of germinal subependymal layer; some S100 positive ependymoglial cells or tanycytes gave off side extensions into NCAM-H positive clusters. This layer was devoid of anti-NOS immunoreactivity. Dense network of irregular, punctate, radially oriented immature cellular elements both positive for NOS and NCAM-H were intermingled, overlapped and sparsely colocalized in the inner part of IGr, but in the outer part, NCAM-H expression was gradually being diminished, and mature bipolar, spherical- or sphindle- shaped granule cells with uniform cellular contour were only immunostained with anti-NOS antisera. Radially oriented astroglial cellular elements expressed close contact with NOS and NCAM-H immunoreactive cellular elements in IGr. Our results showed the morphological basis of astroglial role for neuronal migration and maturation; and specific spatio-temporal relation between NOS and NCAM-H in the course of continuous generation and growth of granule cells in the adult olfactory bulb.

390.12

INTRAVENTRICULAR INFUSION OF BDNF IN ADULT RATS INCREASES THE PROLIFERATION OF CELLS DESTINED FOR THE OLFACTORY BULB. T. Zigova*[1], S.J. Wiegand[2], R.M. Lindsay[2] and M.B. Luskin[1]. [1]Dept. of Anat. and Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA 30322 and [2]Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

The olfactory bulb (OB) is one of the few regions of the brain that acquires new neurons in the adult; the neurons originate from progenitor cells of the subependymal zone (SEZ). In this study we examined the effect of BDNF on the proliferation and differentiation of cells destined for the olfactory bulb. The right ventricles of adult rats were infused with either BDNF (12 µg/day) or PBS. To label dividing SEZ cells, one group of animals received 5 i.p. injections of BrdU over an 8 hour interval on the 4th day of BDNF infusion; in the other group BrdU was added to the osmotic minipump used to deliver BDNF. All animals were perfused on day 12, and their olfactory bulbs were immunostained to reveal BrdU(+) cells. The number, position and phenotype of the BrdU(+) was determined. Neurons were identified by the neuron-specific antibody TuJ1, and astrocytes by anti-GFAP. Rats that received i.p. BrdU injections with BDNF infusion had > 40% more BrdU(+) cells in their OB compared to PBS controls. The majority of the BrdU(+) cells were in the SEZ. Moreover, many of these cells were TuJ1(+); only a few were GFAP(+). Following continuous infusion of BDNF combined with BrdU, numerous BrdU(+)/TuJ1(+) cells were present in both the SEZ and overlying granule cell layer. These results suggest that BDNF increases the proliferation and/or survival of cells with a neuronal phenotype that originate from the adult SEZ. Supported by NIH, March of Dimes, Emory Center for Neurological Sciences and Regeneron Pharmaceuticals, Inc.

390.13

POTENTIAL MYELIN REPAIR OF THE ADULT SUBVENTRICULAR ZONE, FOLLOWING CHEMICALLY INDUCED DEMYELINATION. Nait-Oumesmar B., Lachapelle F., Vignais L., Bachelin C., Rougon G*. and Baron-Van Evercooren A. U 134 INSERM, Hôpital de la Salpêtrière, 75651 Paris cedex 13, France.

Several studies have provided evidence for the existence of oligodendrocyte progenitors and stem cells around the subventricular zone (SVZ) of the adult central nervous system (CNS). Such cells might be expected to be more responsive to demyelination and more competent to generate new oligodendrocytes. In order to demonstrate the involvement of the adult SVZ cells in remyelination, we have induced a chemical demyelinating lesion by stereotaxic injection of 2µl of lyso-phosphatidylcholine (LPC, 1%) into the corpus callosum of adult mouse. Using immunohistochemistry for embryonic N-CAM (E-NCAM) and Platelet-Derived Growth Factor α-receptor (PDGF-αR), we have studied the SVZ at various time points after demyelination. After one week, cells expressing E-NCAM within the SVZ strongly increased over controls. E-NCAM-positve cells emerged from the SVZ and were observed along the fibre tracts of the lesioned corpus callosum, suggesting their migration towards the demyelinated area. These cells expressed also the PDGF-αR, thus suggesting that these cells were immature cells of the oligodendocyte lineage. Size increment of the SVZ was more obvious, two weeks after LPC injection. The number of cells, expressing the PDGF-αR and the embryonic form of N-CAM, which were localized along the corpus callosum white matter tracts, strongly increased. These cells had the typical bipolar morphology of migrating cells. At this time, numerous PDGF-αR positive oligodendocyte progenitors were also visualized within the demyelinated area. These results demonstrate that cells from the adult SVZ, may be responsible for the generation of new oligodendrocytes following myelin damage. The involvement of adult progenitors in CNS remyelination will be further analyzed.
Supported by INSERM and ARSEP. BNO is a fellow of the Myelin Project and Societe des Amis des Sciences.

390.14

FGF-2, EGF AND PDGF-A DIFFERENTIALLY ACTIVATE IN VIVO THE POTENTIAL OF GRAFTED CELLS DERIVED FROM THE ADULT SUBVENTRICULAR ZONE TO GENERATE MYELIN-FORMING OLIGODENDROCYTES Lachapelle F., Nait-Oumesmar B. , Avellana-Adalid V., Bargeu N., Seilheim*, D.and Baron-Van Evercooren A. U 134 INSERM, Hôpital de la Salpetrière F75651 Paris France

In order to identify new sources of oligodendrocytes in the adult CNS, fragments of various regions of the SVZ from 6 months-old normal mice were dissected, Hoechst-labelled and grafted into the corpus callosum of newborn shiverer mice. The absence of myelin basic protein (MBP) in the shiverer myelin allows to detect the myelin synthesized by grafted normal cells and Hoechst allows to vizualize their nuclei. Brains were examined 60 to 90 days after grafting. Hoechst-positive cells were found in 54% of the recipient brains . Five % contained MBP-positive myelin located around and at a short distance from the graft (less than 1 mm). In order to enhance the potential of the adult SVZ to produce myelin-forming cells, donors were treated with a single intraperitoneal injection of FGF-2, EGF, or PDGF-A (5ng/g of living body) 17 to 72h before dissection and engraftment. FGF-2 not only increased the proportion of surviving grafts (84%) but also the proportion of brains containing MBP-positive myelin (84%). This effect was time-dependent since fragments grafted 17-24h after treatment produced small patches of myelin located around and at short distance from the graft (less than 2mm), while SVZ fragments grafted 48-72h after treatment, synthesized large patches of myelin covering the entire section of the corpus callosum or the fimbria, and migrations were observed over more than 4 mm. Treatment with EGF only slightly increased the number of grafted brains containing Hoechst-positive cells (64%) and MBP-positive myelin (33%)which were found around the graft only. PDGF-A dramaticaly increased the number of grafted brains containing Hoechst-positive cells (94%) with only 55% containing small MBP-positive myelin patches which were dispersed and frequently located around blood vessels. Our data thus demonstrate that the adult SVZ is an effective source of myelin forming Ols in vivo and that injections of growth factors modulate the survival, migration and differentiation of these cells. Suported by INSERM and ARSEP

391.1

GAP JUNCTIONS PLAY A ROLE IN NEURONAL DIFFERENTIATION OF NTERA2/D1 HUMAN EMBRYONAL CELLS. M. Bani-Yaghoub, J.F. Bechberger, and C.C.G. Naus*, Department of Anatomy and Cell Biology, The University of Western Ontario, London, Ontario, N6A 5C1, Canada

Gap junction intercellular communication (GJIC) provides a mechanism for coordination and regulation of neighboring cells through the exchange of metabolic materials (MW<1200 D) and electrical signals. We have used Ntera2/D1 (NT2/D1) cells as human CNS neuronal precursors to study the role of gap junctions in neuronal differentiation. These cells are capable of differentiating to post-mitotic neurons (NT2-N cells) following retinoic acid (RA) treatment for 4-5 weeks and mitotic inhibitors for 3 weeks, respectively. In order to block gap junctions, NT2/D1 cells were treated continuously with 18 α-glycyrrhetinic acid (AGA) and dye-coupling in these cells was examined by pre-loading techniques. Cell proliferation was determined by estimating incorporation of [^{3}H]thymidine into DNA of NT2/D1 cells with or without AGA treatment during their differentiation upon RA induction. There was a reduction of about 40% and 80% in the number of cells incorporating [^{3}H]thymidine when the GJIC in these cells was blocked with AGA for 1 day and 2 weeks, respectively. The proliferation was shown to be decreased to similar levels during neuronal differentiation of AGA treated NT2/D1 cells upon RA treatment for 2 weeks. The cell viability test with trypan blue showed a healthy state (more than 85% viable) in all the samples used in this experiment. Thus, the prominent reduction of proliferation of AGA and RA treated NT2/D1 cells was not due to cytotoxicity. NT2/D1 cells treated with RA for 4 weeks expressed microtubule associated protein (MAP2) while cells treated with both RA and AGA for 4 weeks did not express MAP2. Interestingly, these cells started to express MAP5, MAP2 and neurofilament (NF200) after removing AGA from medium. On the other hand, vimentin expression, which is lost through neuronal differentiation of NT2/D1 cells, was present all time when the GJIC was blocked with AGA. There was no significant changes in cx43 immunoreactivity levels and localization between the untreated cells and those of treated with AGA. These results show that gap junction blockage inhibits neuronal differentiation in NT2/D1 cells. (Supported by MRC of Canada).

391.2

TEMPORAL EXPRESSION OF DIFFERENT GAP JUNCTIONS DURING NEURONAL DIFFERENTIATION. M. Morales, R. Rozental*, M. Urban, M.F. Mehler, R. Dermietzel, J.A. Kessler and D.C. Spray. Depts. of Neurosci. & Neurol., Albert Einstein College of Medicine, Bronx, NY 10461 and Inst. Biophys. "Carlos Chagas Filho", Fed. Univ. Rio de Janeiro, Brazil.

We previously showed that cytokine treatment of immortalized hippocampal progenitor cells (MK31) regulates neuronal differentiation *in vitro* (*Nature* **362**: 62, 1993), and that the onset of neuronal membrane excitability coincides with a decrease in strength of coupling *via* gap junction channels between cells (*Dev. Biol.* **167**:350, 1995). Here, we have identified and characterized the types of gap junction proteins (connexins) expressed by these cells using immunocytochemical, molecular biological and electrophysiological techniques. Single channel conductances (γ_j) in untreated progenitor cells were 55 pS, typical of Cx43; presence of this Cx-type was confirmed by Northern blot, immunocytochemistry and RT-PCR assays. In differentiated neuroblasts (treated with IL-7, bFGF and TGFα), γ_j was 60 pS. By contrast, in neuroblasts induced to intermediate differentiation (treatment with IL-7 alone), γ_j were 40 pS and 160 pS, conductances consistent with Cx37 and Cx40. RT-PCR analysis of theses cells, however, detected Cx33. Because Cx33 is reported to form nonfunctional channels, our findings raise the possibility that the progressive decline in coupling observed during neuronal differentiation may be due, in part, to the expression of a gap junction protein with dominant negative function.

391.3

INHIBITION OF DNA SYNTHESIS IN CEREBELLAR GRANULE NEURON PROGENITORS BY DEPOLARIZING CONCENTRATIONS OF KCL. R.F. Bulleit* and X lin. Dept. of Pharmacology, Univ. of Maryland Sch. of Med., Baltimore MD 21201.

Cerebellar granule neurons arise during postnatal development from a proliferative population of cells in the external granule layer (EGL). Local cues in the EGL may direct granule cell progenitors to exit the cell cycle and begin terminal differentiation. These local cues may include depolarization by neurotransmitter receptor activation. To begin testing this hypothesis we examined whether depolarizing concentrations of KCl can inhibit DNA synthesis in granule neuron progenitors maintained *in vitro*. We isolated progenitors from the cerebellum of postnatal day 7 mice. Granule neuron progenitors were purified by percoll gradient sedimentation followed by panning steps on poly-L-lysine coated plates to remove residual glia. The cells were plated in 96 well tissue culture plates in serum free medium (MEM containing 6 mg/ml glucose, 10 mg/ml BSA, 100 ug/ml transferrin, 10 μM putrescine, 20 nM progesterone, 30 nM selenium). These cells form aggregates during over night culture. The next day we added KCl and ^{3}H thymidine to the cultures and continued incubation for 24 hours. We collected cells using a cell harvester and thymidine incorporation was determine by liquid scintillation counting. KCl (25 mM) significantly reduced thymidine incorporation. This reduction could be prevented by the addition of 1.6 mM EGTA to the medium. Thus, KCl appears to inhibit DNA synthesis by increasing calcium influx into granule neuron progenitors. We are presently examining whether glutamate receptor activation has a similar effect on DNA synthesis. We will also use BrdU incorporation as an alternative measure of DNA synthesis.

391.4

THE SALVAGE PATHWAY OF PYRIMIDINE SYNTHESIS: DIVERGENCE OF SUBSTRATE SPECIFICITY IN BRAIN CELLS OF TWO RELATED SPECIES OF TELEOST FISH. G.K.H. Zupanc* and I. Horschke. Dpt. Physical Biology, Max Planck Institute for Developmental Biology, D-72011 Tübingen, FRG.

For nucleotide synthesis, cells use purine and pyrimidine nucleosides generated either through *de novo* synthesis or through utilization of salvage pathways. In the pyrimidine salvage pathway, thymidine (dThd) is taken up by transport proteins and phosphorylated by the enzyme thymidine kinase to thymidine monophosphate. All vertebrates analyzed thus far are able to use radioactively labelled dThd for the biosynthesis of nucleotides in brain tissue. However, when standard techniques for the detection of the incorporation of tritiated dThd and the dThd analogue 5-bromo-2'-deoxyuridine (BrdU) into DNA of postnatally generated brain cells were applied to two species of gymnotiform fish, a divergence in substrate specificity has been revealed.

Intraperitoneal injection of BrdU and processing of brain sections for BrdU immunohistochemistry resulted in a large number of labelled cells in each of the 60 individuals of *Apteronotus leptorhynchus* and in each of the 15 fish of *Eigenmannia sp.* examined. In contrast, experiments employing [^{3}H]dThd autoradiography showed a picture completely divergent for the two species. In 14 individuals of *Apteronotus leptorhynchus*, a total of 649 sections through various regions of the cerebellum (which, based on the results obtained by BrdU immunohistochemistry, were expected to display high levels of mitotic activity) revealed not a single labelled cell. The identical protocol employed in *Eigenmannia sp.* resulted in labelled cells in 20 out of 21 fish. Similar results were obtained by quantifying the degree of incorporation of [^{3}H]dThd into DNA of whole brain by liquid scintillation spectrometry.

We hypothesize that the inability to use dThd for nucleotide synthesis is caused either by a defect in the transport system mediating the uptake of dThd or by a deficiency in the thymidine kinase of *Apteronotus leptorhynchus*.

Supported by grants from NINCDS, FES, MPG, and BMFT.

391.5

ASCORBIC ACID INDUCES BIOCHEMICAL AND MORPHOLOGICAL CHANGES ON NEURAL PROGENITOR CELLS E.Bazán, C. Redondo, M.A.López-Toledano, M.J. Asensio, C.L. Paino* and A.S.Herranz. Depto. Investigación, Hospital Ramón y Cajal, INSALUD, 28034-Madrid, SPAIN.

As a water-soluble vitamin, ascorbate participates in a number of life sustaining functions, including absortion of iron from the gut and the detoxification of organic radicals. During development, high levels of ascorbate have been found in the brain, indicating that ascorbate may be critical for the growth and initiation of proper biochemical functioning of certain neurons and glial cells.

The aim of this work is to study the effects of ascorbate upon the differentiation process of neuroepithelial progenitors cells.

Progenitor cells, obtained from E 15 rat embryo striatal eminence, were grown in DMEM:F-12 (1:1) supplemented with glucose, N2 components and 20 ng/ml EGF for a minimum of 5 passages, and then plated on poli-L-ornithine coated dishes or coverslips. Deprivation of EGF was performed at 3 days postplating, and ascorbate was added to the culture medium (0.5 mM) and maintained for 14 days. The cellular morphology was studied by immunocitochemistry, and the endogenous content of free amino acids neurotransmitters by HPLC (OPA-precolumn derivatization and fluorimetric detection).

Fourteen days of treatment with 0.5 mM ascorbate increases the number of neurons, stained with antibodies to β-tubulin III, as well as the length of the neurites, when we compare with control cultures. No changes were observed in the shape and number of glial cells, stained with antibodies to GFAP. The concentration of aspartate, glutamate, glutamine, and taurine was reduced about a 50% of control values, while GABA levels remained unchanged.

Our results suggest that ascorbate might play a role inducing the neuronal phenotype during the process of stem cell *"in vitro"* differentiation.

Partially supported by FIS 93/555 and 94/0490

391.6

LOW DOSES OF METHYLMERCURY AFFECT CELL CYCLE PROGRESSION IN DEVELOPING CORTEX. N.L.Hayes*[1], D.Fu[1,2], K.R.Reuhl[2] and R.S.Nowakowski[1]. [1]Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, and [2]Dept. of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ 08854.

Methylmercury (MeHg), a widespread environmental neurotoxicant, has a profound effect on cell proliferation in the developing CNS. A single injection of MeHg (2.5 or 10 μg/g, i.p.) or saline was given to pregnant CD-1 mice on embryonic day 14 (E14) between two S-phase markers: 1 hr after ^{3}H-thymidine (TdR) and 3 hrs before bromodeoxyuridine (BUdR). Thirty minutes after the BUdR injection embryos were fixed and processed for visualization of the two markers. Labeled and unlabeled nuclei in the ventricular zone of the neocortical anlage were counted and labeling indices (LI) calculated. Each tracer labels the nuclei in S-phase at the time of the injection; progression through the cell cycle is assessed by comparing the LI for the first, second and both tracers. LI:TdR-only is a measure of the proportion of nuclei which left S-phase during the interinjection interval, LI:BUdR-only is a measure of the proportion of nuclei which entered S-phase during the interinjection interval, and LI:TdR+BUdR is a measure of the proportion of nuclei that remained in S during the entire experiment. We found: 1) a decrease in both LI:TdR and LI:BUdR in both dosage groups with greater decreases following the higher dose of MeHg, 2) an increase in LI:TdR+BUdR that was slightly less than the sum of these decreases and 3) abnormally large nuclei labeled only with TdR between the S-phase zone and the ventricular surface. These data indicate that MeHg reduces entry of nuclei into S-phase by lengthening G1 and slows or blocks passage of nuclei through M. That these changes occur even at low doses of MeHg (i.e., 2.5 μg/g) suggests that human developmental pathology produced by MeHg is consequent to such cell cycle progression changes. Supported by EOHSI, NIH (NS33443 and ES04976) and NASA (NAG 2-950).

391.7

DNA SYNTHESIS FIDELITY AT SITE DIRECTED LESIONS AND IN VITRO ORIGIN-DEPENDENT REPLICATION USING EXTRACTS PREPARED FROM VARIOUS BRAIN CELLS., Efrati E.[1], Eritja R.[2], Goodman M.F.[1] & Tocco G.[1*], [1]Univ. Southern California, Los Angeles, CA 90089-2520., [2]European Molecular Biology Organization, Heidelberg, Germany 60912.

The ability of neuronal cells to maintain the integrity of their DNA is especially important in view of their relative long differentiated life span. It is generally assumed that DNA polymerase β is the enzyme primarily involved in the repair process. We therefore wanted to characterize β polymerase activity in cells of various cell lineage and in different cell cycles.

Lysates from cultured neuroblastoma and astrocytoma cells as well as from rat brains were used to measure the fidelity of DNA synthesis using primed DNA templates. Fidelity measurements were made at normal template sites and at abasic and 8-oxo-G site directed lesions. Using a variety of inhibitors for the different polymerases we identified the presence of both α and β activities in all the examined lysates. However β activity was more important. These lysates were also capable of performing abasic lesion bypass synthesis preferentially in reaction conditions favoring β polymerase activity. Furthermore lysates from established cell lines were capable of supporting SV40 origin-dependent DNA replication.

Our results suggest that it is possible to use crude cell lysates to measure various DNA polymerase activities. Furthermore, lesions bypass synthesis can occur in these extracts predominantly in conditions favoring β polymerase activity.

391.8

IDENTIFICATION OF REGIONS OF MEF2C THAT MODULATE TRANSCRIPTIONAL ACTIVATION. D. Leifer*, Y. Chen and Y. Li. Dept. of Neurology, Yale University School of Medicine, New Haven, CT 06520.

The MEF2 transcription factors are expressed abundantly in brain and muscle and appear to be involved in cellular differentiation. One of these factors, MEF2C, is found preferentially in post-mitotic inhibitory interneurons in the cerebral cortex. The amino terminal region of the MEF2 proteins is necessary and sufficient for binding to the MEF2 DNA element ((C/T)T(A/T)$_2$AAATA(A/G)). To identify regions of MEF2C that modulate transcription, we constructed plasmids with deletions of the carboxyl terminus of MEF2C. We transfected COS cells with these plasmids and with plasmids containing a reporter gene under control of the MEF2 element. Deletion of a small portion of the carboxyl terminus of MEF2C enhanced transcriptional activation, but mutants with larger deletions activated transcription less well. A new short isoform of MEF2C that we cloned from human brain has a variant carboxyl terminus and activates transcription less well than the previously identified isoforms. The MEF2C mutants that we constructed and the naturally occurring isoforms were all localized almost entirely to the cell nucleus by immunostaining. They all also have intact amino terminal DNA-binding domains, so the differences in their ability to activate transcription are likely to reflect altered interactions with other proteins. Our results suggest that the carboxyl terminus of MEF2C can repress transcriptional activation mediated by regions of the protein located towards the middle of its amino acid sequence.

Support: Amer. Heart Assoc., March of Dimes, and NARSAD.

391.9

COORDINATION OF PROLIFERATION AND NEURONAL DIFFERENTIATION: ROLE OF p130 AND E2F1. M. Miyaijma and T. Neuman*. Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

Molecular mechanisms that regulate cessation of proliferation and initiation of differentiation of neuroblasts are largely unknown. Expression of Rb family member Rb2/p130 is induced in several neuronally developing cells such as cortical neurons and teratocarcinoma PCC7 cells. Blocking expression of Rb2/p130 in PCC7 cells results in inhibition of neuronal differentiation. Also, cells with no induction of Rb2/p130 expression do not stop proliferation. Induction of Rb2/p130 during neuronal differentiation correlates well with the reduced activity of E2F transcription factors. Over-expression of E2F1 in neuronally differentiating PCC7 cells prevents neuronal differentiation and suppresses expression of neurofilament L (NF-L) gene at the transcriptional level. E2F1 protein binds to the sequences close to the transcriptional start site of NF-L gene and reduces transciptional activity.

Induction of Rb2/p130 expression in neuronally differentiating cells is likely regulated by the NeuroD family transcription factors. 5′ regulatory region of the Rb2/p130 gene contains several E-box sequences and over-expression of NeuroD family members stimulates significantly Rb2/p130 promoter activity in transient CAT assays. (Supported by the Spinal Cord Society).

391.10

ISOLATION AND CHARACTERIZATION OF NEURONAL CELLS IMMORTALIZED WITH THE TEMPERATURE-SENSITIVE SV40 TUMOR (T) ANTIGEN. P. Yamdagni[1], C. Mellilo[2], B. Berechid[3], H.G. Polites[3], P. Sander*[1], N. Riedel[3], E.K. Dutton[1], Molecular Neurobiology[1], Transgenics[2], Director of Research[3], Neuroscience Therapeutic Domain, Hoechst Marion Roussel, Inc., Bridgewater, NJ 08807.

Neuronal cell lines have greatly facilitated investigations into cellular mechanisms and neuronal functions over the past years. Most neuronal cell lines, however, have been generated from the fusion of some proliferative glial cell and a neuron. Thus, neuronal cell lines often have glial characteristics and may not possess all the qualities of a neuron. Because of this, we have attempted to produce new immortalized neuronal cell lines that maintain neuronal properties by preparing neuronal cultures derived from transgenic mice harboring the temperature-sensitive SV40 T antigen gene driven by a human vimentin promotor.

Embryonic (E9-E15) neuronal cultures prepared from two transgenic lines were characterized by morphology, cell growth, and expression of neuronal specific proteins. Cells proliferated at 33° C in both fetal bovine and horse serum (H_S) containing medium. Morphologically, these cells were non-neuronal. When these cells were shifted to a neuronal differentiation and maintenance medium containing B27, without serum or with 1% HS, cells differentiated, even at 33° C. In HS/B27 medium, a monolayer of non-neuronal cells was maintained which supported differentiated neuronal-like cells. In B27 medium alone, there were few non-neuronal cells and more neuronal-like cells. To date, cells have been maintained at 33° C in serum containing medium and passaged at least 10 times. We are currently testing various conditions to select for a pure population of neuronal-like cells. Preliminary immunofluorescence results demonstrated T-antigen immunoreactivity at 33° C with less T-antigen immunoreactivity at 37° C. In addition, neurofilament immunoreactivity was observed in differentiated neuronal-like cells.

391.11

PATHOLOGICAL CHARACTERIZATION OF TWO TRANSGENIC MOUSE LINES HARBORING THE TEMPERATURE-SENSITIVE SV40 TUMOR (T) ANTIGEN. E.K. Dutton*[1], P. Yamdagni[1], C. Mellilo[3], B. Berechid[3], E. Soly[2], D. Cramer[2], T. Adams[2], H.G. Polites[3]. Molecular Neurobiology[1], Drug Safety[2], Transgenics[3], Neuroscience Therapeutic Domain, Hoechst Marion Roussel, Inc., Bridgewater, NJ 08807.

Transgenic mice harboring the temperature-sensitive SV40 large tumor (T) antigen gene driven by the human vimentin promoter were produced for the generation of new immortalized neuronal cell lines. The T antigen expressed is a transcription factor that will bind DNA at 33° C but not at 39° C. Six founders in two different backgrounds (ICR and B6SJL) were produced with four of the six demonstrating mRNA transcription. Three mRNA positive lines tested produced T antigen protein as demonstrated by Western Blot.

Gross pathological analysis of G1 offspring from two lines revealed thymus enlargements that often encompassed the heart. G2 offspring appeared to develop such pathology at an earlier age with some animals developing enlargements in the neck, groin and axillary regions, and on the fore and hindlimbs. In addition, some G2's demonstrated poor hindlimb motor function with no apparent gross pathology presenting itself peripherally or in the CNS. One animal, however, with severe motor dysfunction, did display major necrosis of the cerebellum. Two additional animals, with no apparent dysfunction, had minor necrosis of the frontal cortex. Animals from two founder lines with different backgound strains demonstrating the most robust gross pathology were analyzed microscopically. Pathological analysis will be presented.

391.12

MYELIN BASIC PROTEIN GENE EXPRESSION DURING EARLY HUMAN DEVELOPMENT, M.Tosic, J.-M.Matthieu* and N.Zecevic[1].CHUV, Lausanne 1011, Switzerland;[1] Univ Connecticut, Farmington, Ct 06030

Myelin basic protein (MBP) is considered to be a marker for oligodendrocyte differentiation and myelination. Although the first myelinated axons in humans were observed at 11 gestation weeks (gw), MBP was detected in isolated neuroepithelial cells by immunocytochemical reaction already in 6 gw human embryos (Zecevic et al, 1994). Here we confirm early expression of the MBP gene using in situ hybridization on paraffine embedded sections of human embryos and fetuses obtained from medically indicated abortions with postmortem delay of 0-3 hours. 7-8mm sections were hybridized with DIG labelled antisense RNA probe transcribed from mouse cDNA clone corresponding to 14kD MBP isoform. Sense RNA from the same clone was used as a negative control. Expression of the MBP gene was followed during human development from 6 to 20 gw. In early embryos (6 gw) MBP specific mRNA was detected in rare isolated neuroepithelial cells from spinal cord to prosencephalon. Same type of cells had shown positive reaction using MBP specific antibodies. For the moment it is not possible to predict if these cells will develop in neuronal or glial types, neither to define the function of the MBP in nonmyelinating cells. At 10 gw, MBP specific mRNA positive cells were observed in the cortical anlage as well. Number of cells expressing MBP increased from age 13 gw to 20 gw, and their distribution reflected regions of myelination. Spinal cord and cerebellum were the regions with highest expression of the MBP gene. Since in this study we have confirmed MBP gene expression in human embryos much earlier that the beginning of myelination, our next goal is to distinguish between several possible transcripts on the MBP or Golli-MBP genes in early embryonic stages. (Support: SNSF and NIMH)

391.13

EPITOPE RECOGNIZED BY AN ANTINEURONAL AUTOANTIBODY ASSOCIATED WITH PARANEOPLASTIC NEUROLOGICAL SYNDROME ARE CONSERVATIVE BETWEEN VERTEBRATE.

K. Yoshimura*[1], K. Kagai[2], A. Kaneko[2], Y. Sakamoto[1], K. Kitamura[1], T. Hosokawa[2], K. Hamaguchi[2] and M. Nomura[1]. Dept. of Physiology[1]and Neurology[2], Saitama Medical School, Moroyama, Iruma-gun, Saitama 350-04, Japan

Paraneoplastic encephalomyelitis and paraneoplastic sensory neuropathy are usually associated with small cell lung cancer. We previously found that a patient with mediastinal neoplasm and paraneoplastic neurological syndrome harbored antineuronal autoantibody, named anti-Ta antibody in serum. At 25th Annual meeting for Neuroscience, we presented that anti-Ta antibody was similar to anti-Hu antibody, which reacts proteins with molecular weight of 39-44 kDa and recognizes cytoplasm and nucleus of neurons, considering neurological syndrome of the patient, immunohisto-and cytochemical study, and Western blot analysis using anti-Ta antibody.

At the present study, we examined whether anti-Ta antibody reacted or not with neurons of nervous system from human, rabbit, rat, mouse, frog, and carp in order to immunologically characterize the epitope of the antigens recognized. Neurons from all species investigated were recognized well by anti-Ta antibody. Since Hu antigens are believed to be RNA-binding proteins, which may paly a role in development and maintenance of neurons as well as Elav, the immunohistochemical study for anti-Ta antibody suggests that the epitopes of antigens would be conservative and the antigens may play a basic role associated with development and maintenance in neurons in vertebrate investigated. We examined the localization of the antigens in neurons using electron microscopy.

391.14

THE POSTEMBRYONIC DEVELOPMENT OF SOMATOSTATIN IMMUNOREACTIVITY IN THE CENTRAL POSTERIOR/PREPACEMAKER NUCLEUS OF *APTERONOTUS LEPTORHYNCHUS*: A DOUBLE-LABELLING STUDY. T. Stroh* and G.K.H. Zupanc. Dpt. Physical Biology, Max Planck Institute for Developmental Biology, D-72011 Tübingen, FRG.

The neuropeptide somatostatin (SS) is widely distributed in the central nervous system (CNS) of vertebrates. While the embryonic development of SS in the CNS has been well-examined, nothing is known about the postembryonic development of this peptide. One well-suited brain system for studying this phenomenon is the central posterior/prepacemaker nucleus (CP/PPn) of *Apteronotus leptorhynchus*, a weakly electric gymnotiform fish. This nucleus consists of roughly 10,000 cells. In the ventricular zone of the CP/PPn, new cells are generated continuously and at a high rate throughout adulthood. Within the first days of their life, these cells migrate laterally and develop features characteristic of neurons (Zupanc GKH and Zupanc MM 1992: *Proc Natl Acad Sci USA* **89**, 9539-9543; Zupanc GKH and Horschke I 1995: *J Comp Neurol* **353**, 213-233).

Since 5-10% of the total number of cells in the CP/PPn express SS, we set out to examine the development of SS-like immunoreactivity in the cells born during adulthood. This was achieved by labelling mitotically active cells with 5-bromo-2'-deoxyuridine (BrdU) and by subsequent immunocytochemical detection of SS. By employing survival times of 2, 3.5, 5, 10, 28, and 100 days after the administration of BrdU, we investigated whether newborn cells express SS within this time frame.

Expression of SS could not be detected at 2 days. However, in three out of four fish at 3.5 days, in all animals with survival periods of up to 28 days, and in three of four fish at 100 days double-labelled cells were found. In these fish, 1.0-7.6% of all BrdU-labelled cells were SS-positive. This percentage corresponds well to the fraction of somatostatinergic cells among the total population of cells in the CP/PPn.

Supported by the Friedrich Ebert Foundation and the Max Planck Society.

CELL LINEAGE AND DETERMINATION IV

392.1

THE USE OF RETROVIRAL VECTORS TO INVESTIGATE THE STEM CELL-LIKE BEHAVIOR OF EGF-GENERATED MULTIPOTENT SUBVENTRICULAR ZONE PROGENITOR CELLS. R.E. Gross*[1] and J.A. Kessler[2] Departments of [1]Neurosurgery, [2]Neurology, and [3]Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

In contrast to hematopoietic cells, there are no definitive antigenic markers to characterize and isolate neural stem cells. The definition of neural stem cells relies on the demonstration of multipotentiality and self-renewal. Cortical progenitors have been demonstrated to fulfill these criteria in single cell culture (Davis and Temple, 1994). Progenitor cells from embryonic and adult subventricular zone (SVZ) can be expanded in the presence of epidermal growth factor (EGF) and likely contain stem cells by the above criteria, but the difficulty in culturing these cells under clonal conditions limits further investigations of their stem-like properties.

To address this issue we have utilized infections of EGF-generated SVZ progenitor cells with retroviral vectors at clonal titers. We used a vector expressing *lac-Z* appended to a nuclear localization signal to facilitate analysis of dissociated, plated, serially-passaged SVZ cells cultured in the presence of EGF. The frequency of clones and the number of labelled cells per clone was analyzed to estimate the frequency of stem-like cells within EGF-generated neurospheres. Infected cells were then selected with G418 and subsequently passaged and differentiated to fulfill the criteria necessary to characterize EGF-generated SVZ progenitor cells as stem-like cells. Using this technique, we are evaluating the effects of various factors on the lineage-commitment of SVZ stem-like cells.

392.2

A CELL SURVIVAL FACTOR ALTERS THE FATE OF PROLIFERATING SUBEPENDYMAL CELLS IN THE ADULT MAMMALIAN FOREBRAIN. C.M. Morshead* and D. van der Kooy. University of Toronto, Neurobiology Research Group, University of Toronto, Toronto, Ontario, Canada.

The adult mammalian forebrain contains a population of constitutively proliferating subependymal cells which line the lateral ventricles and extend rostrally to the olfactory bulb. These mitotically active cells have a cell cycle time of 12.7 hours and comprise up to 33% of the subependyma in the rostral dorsolateral corner of the lateral ventricle. Under baseline conditions the constitutively proliferating cells proliferate in a steady-state mode of division whereby a single cell gives rise to two progeny, one of which continues to proliferate and the other undergoes cell death. Some of the progeny of the constitutively proliferating cells will not die but continue to proliferate and migrate to the olfactory bulb (ob). The relative percentage of cells which undergo these 2 possible fates (i.e. cell death versus migration to the ob) is not known.

We have used a cell survival factor N-acetyl-L-cysteine (NAC) which is known to have survival effects without concomitant proliferative effects on cells in vitro. We hypothesized that infusion of this factor in vivo would result in the survival of the cells which normally undergo cell death and hence we could examine the resulting fate of cells spared from death. Accordingly, we can ask if preventing cell death is sufficient to keep cells in a proliferative mode. By examining the magnitude of the increase following infusion we can further address the percentages of the cells dying versus those migrating to the ob.

Animals received injections of a replication deficient retrovirus (RV) containing the beta-galactosidase (B-gal) gene. RV injections were made into the lateral ventricles on day 0 followed by implantation of osmotic pumps on day 1. Saline or 0.1M NAC was infused for 5 days at which time the animals were sacrificed (day 6). Preliminary findings reveal a 5X increase in the total number of B-gal positive cells in NAC animals compared to controls. The cells were confined to the subependyma and not observed in the surrounding parenchyma. The same relative increase in total number of cells was observed in the ob and the subependyma surrounding the lateral ventricles. These data suggest that infusion of a cell survival factor is able to keep the normally dying cells alive, some of which are able to migrate to the ob. (MRC Canada and Neurospheres Inc)

392.3

IN VITRO DIFFERENTIATION OF NEURONS AND ASTROCYTES FROM JUVENILE SVZ CELLS: RESCUE OF PROGENITORS FATED TO DIE? Young, G.M., Albrecht, P.J., and Levison, S.W*., Dept. of Neuroscience and Anatomy, M.S. Hershey Med. Ctr. Penn State College of Medicine, Hershey, PA 17033

The juvenile subventricular zone (SVZ) normally produces oligodendrocytes. To determine whether cells residing within this germinal zone were determined glioblasts, we retrovirally labeled juvenile SVZ cells in vivo by stereotactic injection and determined the cellular composition (using cell morphology and antigen expression) within clones that had expanded for 1 week in vitro. Many homogeneous clones of neurons, astrocytes or oligodendrocytes were observed, with large type 1 astrocyte clones most common; however, mixed oligodendrocyte/type 1 astrocyte clusters represented 15% of the total clones. Immature neurons were present in 25% of the total clusters, and 8% of the clones contained neurons, astrocytes and oligodendrocytes (of 128 clones examined). Analyzing apoptosis in the juvenile brain by in situ end labeling revealed that over 60% of all apoptotic cells were within the SVZ and some apoptotic cells in the SVZ expressed markers of differentiated cells. We conclude that the juvenile SVZ is a mixture of lineage restricted, bipotential and multipotential neural progenitors, and suggest that the limited output of the SVZ to a single lineage in the juvenile animal is due to a non-permissive environment rather than the absence of potential neuroblasts or glioblasts. Supported by NS 33251 (awarded to SWL).

392.4

ORIGIN OF ADULT NEURAL STEM CELLS, EPENDYMA OR SUBEPENDYMA? Bernard J. Chiasson*, Vincent Tropepe, Cindi M. Morshead and Derek van der Kooy. Neurobiol. Res. Group, Dept. of Anat. and Cell Biol., Univ. of Toronto, Toronto Ont. Canada, M5S 1A8.

The tissue surrounding the lateral ventricle of the adult mammal consists of a 1 cell thick ciliated ependymal layer closest to the ventricle and an immediately adjacent 2-4 cell thick layer called the subependyma. In the rostral forebrain subependyma up to 1/3 of the cells are cycling with a cell cycle time of approximately 12 hours (constitutively proliferating) whereas another population of cells surrounding the ventricle, much smaller in number, cycle approximately every 2-4 weeks (relatively quiescent). The relatively quiescent cells are believed to be neural stem cells which give rise, in vitro, to clonal aggregates called neurospheres in the presence of epidermal or basic fibroblast growth factor (EGF or bFGF). Earlier work established that the tissue surrounding the adult lateral ventricle is necessary and sufficient for the generation of neurospheres, however, it is not clear whether the population of neural stem cells reside within the subependyma and/or ependyma. Preliminary observations using EGF and bFGF receptor (EGFR and bFGFR) antibodies suggested that a population of cells in the adult forebrain ependyma may possess EGFR and bFGFR, while a population of subependymal cells slightly farther from the ventricle possesses only EGFR. Since bFGF is capable of giving rise to neurospheres in culture this suggests that at least some neural stem cells could be located within the ependyma. Here we attempt to isolate the neural stem cells further by subdividing the subependyma from the ependyma. Dissections of the adult mammalian forebrain were performed and the ependyma was physically isolated from the subependyma. Staining for ependymal markers, such as S-100β, and labelling the constitutively proliferating subependymal population with bromodeoxyuridine allowed us to establish a protocol for separating these cell layers. We cultured ependymal and subependymal cells separately to examine the feasibility of generating neurospheres from either tissue in the presence of EGF or bFGF. Our preliminary evidence suggests that the ependymal cells may also give rise to clonal aggregates. Some aggregates contain ciliated cells and show rotational behaviour in suspension, further suggesting their origin from ciliated ependymal cells. However, we have yet to determine if these aggregates are self-renewing and multipotential as are neurospheres. Supported by MRC and NCE Canada.

392.5

A NEW SPONTANEOUS MUTATION AFFECTING LATER STAGES OF NEURAL DEVELOPMENT IN THE RAT. K.S. Bittman, V. Leung, K. Borodezt, J.J. LoTurco* and S.R. D'Mello. Dept. of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269.

Mutations that disrupt specific aspects of neural development can be used to define unique developmental stages and to ultimately identify the molecules underlying these stages. We report here an initial description of Flathead (*fh*), an autosomal recessive mutation that arose spontaneously in an inbred colony of Wistar rats. Affected rats display resting tremor, impaired coordination of hindlimbs, are unable to rear, and die on postnatal day 23-27. On the day of birth *fh/fh* rats and unaffected littermates have comparable overall body size, however, the brains of *fh/fh* rats are reduced in size by 40%. The reduction in brain size is not associated with hydrocephaly, and is most pronounced in the forebrain and cerebellum. The reduction in brain size is clearly detectable at birth, but not at E15, indicating that the primary deficit is during late or post neurogenesis. In addition to a reduction in overall brain size there is a dramatic dysgenis within the cerebellar cortex and dentate gyrus. At PO there are far fewer cells in the external granule cell layer of cerebellar cortex, and by P21 the inner granule cell layer has failed to form with cells persisting in the *egl*. In the hippocampus, there is a drastic reduction in the number of dentate granule cells, and the remaining cells fail to form a well defined layer. BrDu incorporation and TUNEL assays were performed at PO and P3 in order to determine whether the developmental abnormalities in *fh/fh* are due to a decrease in proliferation, or to an increase in cell death. Within the *egl* of cerebellum the mitotic index is comparable in affected and non-affected brains. The number of apoptotic cells, as judged by positive TUNEL staining is currently being determined.

Supported by grants from the National Science Foundation to S.R.D. and from the Ester and Joseph Klingenstein fund to J.J.L.

392.6

NESTIN MAPPING IN THE ADULT RAT BRAIN

I. Johnson, U. Hasselrot, D. S. K. Magnuson* and D. J. Morassutti, Dept of Neurological Surgery, U. of Louisville, Louisville, KY. 40292

Nestin is a novel intermediate filament, expressed in embryonic radial glia, neuronal precursors, myoblasts, schwann cells (Lendahl et al., Cell 1990), and reactive astrocytes (Clarke et al., Neuroreport 1994). It has recently been found in adult EGF-responsive CNS stem cells which are derived from the adult rat lateral ventricle subependymal zone (Morshead et al., Neuron 1994). In this study we further characterized the distribution of nestin in the adult rat/mouse CNS.

We utilized a double-label immunocytochemical technique to examine the expression of Nestin, Vimentin, and Glial Fibrillary Acidic Protein (GFAP) and NF160 (neurofilament) in the CNS of normal, striatal ibotenic acid lesioned adult Sprague-Dawley rats and normal CD-1 mice. Nestin is distributed in the subependymal zone where it appears in several subpopulations, some of which colocalize GFAP or Vimentin. It is also present in cells of the median eminence, spinal central canal, dentate gyrus and in a sparse population of cells in the septal nucleus and striatum. Most interestingly, it is expressed around capillary vessels throughout the brain, where it is colocalized with GFAP. This colocalization is best seen in the reactive astrocytes found within the injured striatum.

Our data suggest that nestin is expressed in several subpopulations of neural precursor and non-precursor cells, and may play a role in the blood-brain barrier. Supported by the Alliant Community Trust Fund.

392.7

CLUSTERS OF RAT CNS PROGENITOR CELLS ECTOPICALLY PLACED IN THE EMBRYONIC RAT BRAIN DIFFERENTIATE ACCORDING TO THEIR ORIGIN. L.Magrassi*[1],M.Ehrlich[2],G.Butti[1],S.Pezzotta[1],S.Govoni[3],E.Cattaneo[4] 1.Neurosurgery Dept. of Surgery, IRCCS S. Matteo 27100 Pavia; 2.NYU Medical Center, NY,USA; 3.Institute of Pharmacology, Pavia; 4.Institute of Pharmacological Sciences, University of Milano, 20133 Milano, IT

CNS progenitor cells dissociated from the embryonic striatum and implanted into the brain of embryos of the same species generate both cells that disperse and integrate as single cells into the host brain and cells that reaggregate together forming easily recognizable clusters (Cattaneo,1994). We were interested to study if the cells in the clusters differentiate according to their final location as shown for some of the single integrated cells (Campbell, 1995; Brustle1995; Magrassi 1996) or if they were able to acquire a striatal phenotype. We transplanted into the embryonic rat brain cells dissociated from the E14 rat striatum primordia as well as from the two ganglionic eminences. We found clusters of BrdU and DiI labelled donor cells located in the central grey, colliculi, midbrain, hypothalamus, cortex, striatum and hippocampus. There was no apparent preferential bias for the striatum, the region where their progenitors were harvested. This was true both at short survival and long survival times. Immunocytochemical analysis was performed on cryostat sections of brains of animals that survived 21 days and 3 months after the in utero transplantation. We found that many cells within the clusters stained positively with antibodies against DARPP-32 and ARPP-21 two antigens typically present in striatal cells. Only occasional cells within the clusters were GFAP positive. Nestin expression was not detected. Clusters located close to substantia nigra were always TH negative, while a strong staining for DARPP-32 and ARPP-21 was present. These data suggest that cells in the clusters differentiate according to their origin despite their ectopic integration and their single cells exposure for the first hours after the transplant to the host environment (partially supported by the Hereditary Disease Foundation, USA and by the Center Grossi Paoletti, Italy).

392.8

Fate Restriction and Differentiation of CNS Stem Cells. K.K. Johe, T.G. Hazel, T. Muller, K. Wolter, C. Vicario-Abejon*, R.D.G. McKay. Laboratory of Molecular Biology, NINDS, National Institutes of Health, Bethesda, MD 20892.

Identifying the mechanisms that generate cellular diversity is fundamental to understanding the development of the central nervous system. We have isolated from embryonic rat CNS multipotential cells that can be clonally expanded through multiple passages and retain their capacity to differentiate into neurons, oligodendrocytes, and astrocytes. Lineage analysis of accutely dissociated E16 hippocampal cells by retroviral marking suggests that these multipotential cells are abundant in vivo. We have identified several extracellular factors that influence the pathways of determination and differentiation of CNS stem cell into mature cell types in culture. CNTF can stably restrict mitotic multipotential stem cells into unipotential astroblasts. T3 generates unipotential oligodendroblasts. PDGF acts to restrict the differentiating stem cells toward the neuronal fate. In contrast to these factors which influence lineage determination, other extracellular factors such as EGF and neurotrophic factors influence the differentiation and maturation of CNS stem cells. These results suggest that progressive action of growth factors plays a critical role in the differentiation of multipotential precursors in the vertebrate CNS.

392.9

INDUCTION OF THE HOMEOPROTEIN PBX1 FOLLOWING NEURONAL DIFFERENTIATION OF CORTICAL PROGENITOR CELLS BY NT-3. M.C. Mione[1], L. Edelmann[2], J.G. Parnavelas[1], and M.A. Morabito[3]*. [1] Dept. of Anatomy, UCL, Gower St., London WC1E 6BT, U.K., [2] Biocenter, Univ. of Basel, Klingelberg Str. 70, Basel, Switzerland, [3] Dept. Pharmacology, Yale Univ. School of Medicine, New Haven, CT.

The regulation of proliferation, cell fate determination, and differentiation of cortical progenitor cells is thought to be achieved by the modulation of their transcription factors activity and gene expression in response to various extracellular signals. The homeodomain protein PBX1, a cofactor of other homeotic proteins, is expressed in the developing telencephalon indicating that it may play a role in cortical neurogenesis [L. Redmond, S. Hockfield, and M.A. Morabito (1996) J. Neuroscience 16: 2972-2982].

An antibody generated against recombinant rat PBX1 protein was used to investigate the expression of PBX1 during the differentiation of cortical progenitor cells in cultures prepared from E16 rat dorsal telencephalon and maintained in serum-free medium. In these cultures, bFGF was found to regulate the proliferation of undifferentiated progenitor cells, whereas the addition of NT-3 promoted cell cycle withdrawal and neuronal differentiation. After treatment with bFGF for 3 days, most cells showed features of undifferentiated progenitors and labeled by BrdU uptake; they showed low levels of PBX1 immunoreactivity. Subsequent addition of NT-3 induced expression of MAP2 (a marker of postmitotic neurons) and reduced the uptake of BrdU in these cultures. The MAP2 cells were strongly immunoreactive for PBX1.

The induction of PBX1 immunoreactivity following neuronal differentiation of cortical progenitor cells by the addition of NT-3, indicates that PBX1 expression is positively regulated by this neurotrophin and suggests that PBX1 may regulate the transition from proliferating progenitor cells to terminally differentiated neurons.

392.10

SPECIFICTY OF AN INDUCTIVE SIGNAL THAT REGULATES LIMBIC FATE IN THE DEVELOPING CEREBRAL CORTEX. K.L. Eagleson* and P. Levitt. Dept. of Neurosci. and Cell Biol., Robert Wood Johnson Medical School-UMDNJ, Piscataway, N.J. 08854.

The differentiation of the cerebral cortex includes the parcellation of functional areas in the tangential domain. We previously utilized the expression of the limbic system-associated membrane protein (LAMP), a cell adhesion molecule unique to limbic cortical areas, to identify an interaction between transforming growth factor (TGF)α and collagen type IV that induces this area-specific phenotype *in vitro*. When grown in the presence of this inductive signaling system, precursors derived from the sensorimotor cortex, a non-limbic region of the cerebral wall, express LAMP. In contrast, when grown in non-inductive environments, which include laminin or fibronectin as the matrix component, only precursors derived from presumptive limbic regions express LAMP. In the present study, we further examine the specificity of the TGFα/collagen type IV inductive signal. When collagen type I is used in combination with TGFα, LAMP induction in the sensorimotor population is variable and, at best, only half that seen on collagen type IV. In addition, the profile of LAMP induction in precursors derived from presumptive visual cortex, a different non-limbic region of the cerebral wall, is identical to that in the sensorimotor population when grown on laminin, collagen I and collagen IV, both in the presence and absence of TGFα. Thus, collagen type IV is most effective in inducing LAMP expression, most likely by more efficiently modulating the activity of TGFα or enhancing the responsiveness of the precursors to the growth factor signal; other members of the collagen family, however, are able to partially substitute as the matrix component. The observation that the same signal can induce a limbic fate in different non-limbic progenitors indicates that, during corticogenesis, spatially segregated signals within the ventricular zone may operate to produce neurons with a molecular feature unique to a discrete cortical domain. Supported by NIMH grant MN45507 and March of Dimes Basic Research Grant 95-1023.

392.11

SPECIFICATION OF LAMINAR-SPECIFIC CHARACTERISTICS OF DEVELOPING CORTICAL NEURONS. J.M. Weimann*, K. Mackarehtschian and S.K. McConnell. Dept. Biological Sciences, Stanford University, Stanford, CA 94305.

Previous transplantation studies have demonstrated that cortical progenitors become committed to a specific laminar fate during their final cell cycle. Here we ask whether all layer specific characteristics of cortical neurons are specified in a single step or rather in sequential specification events. To address this question we asked when newly born neurons acquire their laminar-specific axonal and dendritic morphologies and their physiological membrane properties.

Cortical progenitors from E29 ferrets, which are generating deep layer neurons, were dissociated and labeled with ^{3}H thymidine and rhodamine (PKH26), and then transplanted into the VZ of P1 ferrets, which are generating superficial neurons. As previously reported (McConnell & Kaznowski, 1991), transplanted neurons migrated into either layer 6 or layer 2/3 depending on their position in the cell cycle at the time of transplantation. Recording from transplanted neurons with Neurobiotin electrodes revealed that these cells had axonal and dendritic morphologies and physiological properties appropriate for the layers into which they had migrated. This result suggests that transplanted neurons can integrate normally into the cortex and that their projection patterns and physiological properties are specified at or after the time that laminar position is determined.

To determine whether the laminar-specific characteristics are specified in the VZ or during later stages of cortical development, labeled E29 progenitor cells were placed ectopically into layer 2/3 of P23 ferrets. PKH26-labeled neurons displayed two phenotypes when viewed 4 days after transplantation: One type had a pyramidal morphology with axonal branches in layers 2/3 and the deep layers, typical of normal layer 2/3 neurons. The second type extended axons 1-3mm along layer 1. These data suggest that local cues within layer 2/3 can induce the formation of upper layer neurons from some early progenitors.

Supported by DRWW Fellowship DRG1227 and NIH EY08411.

392.12

COORDINATED REGULATION OF CNS LINEAGE DEVELOPMENT BY CYTOKINES, EXTRACELLULAR MATRIX, AND CELL CONTACT. P. Mabie, M.F. Mehler, R. Marmur, and J.A. Kessler*, Dept. of Neurology and Neuroscience, Albert Einstein Col. of Medicine, Bronx, NY 10461.

Multipotent progenitor cells (MPs) from forebrain proliferative zones generate lineage-restricted progenitor intermediates that give rise to all three CNS lineages. In addition to the influence of soluble factors (growth factors), cell surface and extracellular matrix (ECM)-associated signals are likely to modulate the fate of MPs and their progeny. To examine how non-diffusible signals modulate the responsiveness of MP progeny to growth factors, dissociated EGF-derived progenitors from mouse embryonic day 15 cortex were plated on alternate substrates and treated with a series of growth factor paradigms designed to enhance specific lineage development. The relative proportion of undifferentiated progenitors, neurons, and glia could be manipulated by specific combinations of soluble growth factors and substrates. The optimal conditions for selecting immature post-mitotic neurons were with low concentration EGF on poly-D-lysine (PDL) or with no added growth factor on laminin. Optimal astrocyte differentiation occurred with BMP treatment; this maturational effect was associated with either cell cycle exit (PDL) or with proliferation (laminin). Undifferentiated progenitors were optimally selected by EGF treatment on a non-adhesive substrate. The rate of differentiation was delayed when cell-cell contact was preserved by plating cells as undissociated neurospheres. These studies suggest that the lineage fate of MP progeny can be selectively modulated by interactions between soluble and non-diffusible cell surface and ECM-associated signalling pathways. Further, they provide a basis for investigating the relationship between extracellular signalling, MP cell cycle regulation and lineage commitment necessary for designing rational regenerative therapies for CNS disorders. (Sponsored by MDA and Hirschl Trust (MFM) and NIH (JAK) grants).

392.13

NEURAL STEM CELLS CAN BE ISOLATED IN bFGF, INDEPENDENT OF EGF SIGNALLING. V. Tropepe*, M. Sibilia#, C.G. Craig, E.F. Wagner# and D. van der Kooy. Neurobiology Research Group, Department of Anatomy & Cell Biology, University of Toronto, Toronto, Canada M5S 1A8; #Institute of Molecular Pathology, Vienna, Austria A-1930.

The presence of self-renewing, multipotential neural stem cells in the mammalian embryonic ventricular zone and adult forebrain subependyma suggests that a population of neural stem cells is established very early in neural development and that this population self-renews throughout life, retaining the capacity to generate progeny that give rise to both neuronal and glial lineages. We asked whether the known mitogens for mouse neural stem cells in serum-free media, epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), have differential roles in stem cell behaviour during development. Embryonic day (E)14 forebrain neural stem cells respond to either EGF or bFGF to generate clonal aggregates of cells (neurospheres) *in vitro* that are self-renewing and multipotential. At low concentrations EGF is an order of magnitude more effective than bFGF in generating neurospheres, but at higher concentrations both growth factors produce the same plateau number of neurospheres. However, the generation of E14 neurospheres in bFGF is not dependent on EGF signalling. The number of E14 neurospheres generated in EGF from the brains of EGF-receptor null (EGFR$^{(-/-)}$) mice is decreased by >90%, but the generation of large numbers of E14 neurospheres in bFGF alone was not affected in these mutants. A similar pattern of results was seen using a synthetic inhibitor which selectively blocks intracellular signalling through the EGF receptor. Consistent with the idea that bFGF allows for the isolation of E14 neural stem cells independently of EGF signalling, we found that neurospheres can be generated in serum-free media from E8.5 anterior neural plate in the presence of bFGF alone, but not in EGF alone. There are two possible explanations for these results. The first is that the neural stem cell possesses only bFGF receptor signalling at E8.5, but by E14 has developed both bFGF and EGF receptor signalling capacity, either of which alone is sufficient to activate the neural stem cell in defined media. The second possibility is that the bFGF-responsive stem cell at E8.5 is the precursor in a lineage to the EGF-responsive stem cell, and that both of the separate EGF and bFGF responsive stem cells are present in the E14 forebrain ventricular zone and adult subependyma.

Supported by MRC and NCE Canada.

392.14

Glial cells exhibit differential effects on the survival of two immortalized neuronal cell lines. E.Bongarzone, L.Foster[1], V.Schonmann, S.Howard* and A.T.Campagnoni. Mental Retardation Research Center, UCLA, Los Angeles, CA. [1] La Jolla Cancer Research Foundation, La Jolla, CA.

Primary cell cultures established from embryonic (E13) and newborn (P2) mouse brains were conditionally immortalized by infection with a retrovirus containing the temperature-sensitive mutant SV-40 large T antigen oncogene. Two clonal cell lines (CN1.4 from E13 cultures and SJ3.6 from P2 cultures) were isolated and stably maintained in vitro. They express a number of neuron specific markers like neurofilaments, GAD 67, the BG21 isoform of Golli-MBP and NSE but do not express any glial marker. Both cell lines proliferate well at the permissive temperature of 34°C. Under these conditions, CN1.4 cells have small pyramidal cell bodies with short processes and SJ3.6 cells show small round cell bodies and their processes are longer. When CN1.4 cells are grown at 39°C, there is an abrupt decrease in the proliferation rate accompanied with an elongation in some of the neurites but the cell survival is not compromised. In contrast, SJ3.6 cells do not survive beyond 3 days at 39°C. The death of SJ3.6 cells is accompanied by fragmentation and condensation of the DNA within the nucleus. Interestingly, when these cell lines are co-cultured with either oligodendrocytes or astrocytes, there is a marked increase in the SJ3.6 survival and outgrowth of their neurites but there is no effect on the CN1.4 cells. This differential capacity to survive in vitro suggests that these two cell lines might represent neuronal cells at different stages of maturation. SJ3.6 cells seem be more mature than CN1.4 since their ability to survive in vitro depends on the presence of glial cells. In fact, the absence of survival signals from glial cells might activate a programmed cell death mechanism. These results also indicate that the survival effect of glial cells might depend upon the differentiation state of the neurons. Glial cells might promote survival and differentiation in more mature neurons but not in less mature neurons. (Supported by NIH grants NS23022 and NS33091).

CELL DIFFERENTIATION AND MIGRATION VI

393.1

ISOLATION AND ANALYSIS OF GENES WHICH ENCODE DLX-2 INTERACTING PROTEINS

Guoying Yu and John Rubenstein*
Nina Ireland Laboratory of Developmental Biology, UCSF, San Francisco, Ca., 94143.

The molecular mechanisms underlying regional specification, morphogenesis, and differentiation of the mouse forebrain are now possible to study through the functional analysis of homeodomain-containing proteins (e.g. DLX, EMX, OTX and NKX) that are expressed in regionally-restricted patterns in the embryonic forebrain. To identify proteins that modulate the function of the DLX proteins, we have used a yeast two-hybrid approach. Two novel proteins were identified that interact with DLX-2, and were named as DIP1 and DIP2 (for Dlx Interacting Proteins). DIP1 and DIP2 genes are expressed nearly ubiquitously within the developing CNS, but at different stages of differentiation. Deletion analysis identified distinct domains within DLX-2 that interact with DIP1 and DIP2. Present studies are aimed at elucidating the functional significance of the DIP-DLX interactions.

G. Yu is supported by Postdoc fellowships from NRSA, NIH

393.2

DLX-1 OR DLX-2 IS NECESSARY FOR THE DEVELOPMENT OF GABA IMMUNOREACTIVITY IN THE MOUSE ISOCORTEX

SA Anderson*, M-S Qiu, D Eisenstat, J Meneses, R Pederson and JLR Rubenstein. Dept. of Psychiatry, UCSF, San Francisco, Ca., 94143.

Dlx-1 and Dlx-2 are homeobox-containing transcription factors with restricted patterns of expression in the embryonic basal forebrain, including the lateral ganglionic eminence (LGE). Null mutant mice for either Dlx-1 or Dlx-2 have an ostensibly normal LGE, but mice lacking both of these genes appear to have an abnormal subventricular zone of the LGE, leading to aberrant striatal differentiation.

Other histological defects are also found in these mice. Interneurons of the olfactory bulb, many of which migrate from the SVZ in the region of the LGE, are undetectable using immunohistochemistry for granule cell and periglomerular cell markers (e.g. GABA).

Analysis of the cortex of Dlx-1/Dlx-2 homozygous mutants, although limited by the death of these animals at P0, also shows almost no GABA immunoreactivity (IR) in cell bodies or processes. This defect, particularly in layer 1, begins abruptly around the boundary of the piriform and isocortex. GABA-IR is also lacking in the subiculum and hippocampus.

Dlx-1, and to a less clear extent, Dlx-2, begin to be expressed in the developing isocortex near the striatopallial angle at about E12.5. By E14.5 Dlx-1-IR and expression is detectable throughout the cortical primordium. At P0, Dlx-1 and Dlx-2-IR cells can be found scattered in all cortical layers except the cortical ventricular zone, with most cells located in the developing cortical plate and layer 1. This pattern of development is similar to that reported for GABA-IR in mice and rats. Moreover, double labeling reveals overlapping populations of Dlx-1 and GABA-IR cells in the cortex and olfactory bulb. Ongoing studies are designed to determine the sources of cortical GABA and Dlx-1-containing cells.

S.A. is supported by a NARSAD Young Investigator Award

393.3

MIGRATION IN THE DORSAL PREOPTIC AREA/ANTERIOR HYPOTHALAMUS. S.A. Tobet* and I.K. Hanna, Prog. Neuroscience, The Shriver Center and Harvard Medical School, Waltham, MA 02254.

The preoptic area/anterior hypothalamus (POA/AH) displays numerous sex differences in neural morphology. We have proposed that there are significant influences on the region that derive from dorsal origins, based on the pattern of radial glial fibers that enter the POA/AH by going around, or through the anterior commissure (AC) during development (Tobet et al., J Neurobiol 26:75, 1995). We conducted video microscopy and immunocytochemical studies to assess the potential roles of the dorsal-ventral (DV) oriented radial glia. We utilized video microscopy to follow the migration of DiI-labeled cells in coronal 250μm brain slices from embryonic day 15 mice maintained in serum-free media. There was approximately 70% more migration along a DV (43.3 ± 8.7%) than a medial-lateral (ML: 25.6 ± 4.4%) orientation as defined by using the percentage of migrating cells per video field (n=11). The average speed of migration was similar in both orientations (approximately 23μm/hr). In the absence of sex steroids there were no significant sex differences. We used immunocytochemistry to examine the distribution of cells containing γ-aminobutyric acid (GABA) in embryonic rats and mice. Prenatally, we found dense accumulations of cells containing GABA within the dorsal POA/AH, and additional cells within the AC. A GABAergic mechanism has previously been shown to alter the differentiation of the sexually dimorphic nucleus of the medial POA. The transient presence of neurons in the AC may be indicative of cells with unique migration histories which may ultimately reside in, and influence, the developing POA/AH. The DV migration of cells provides one avenue for dorsal influences in the POA/AH. Supported by NSF-IBN-9421697.

393.4

DYNAMICS OF CELL MIGRATION FROM THE LATERAL GANGLIONIC EMINENCE IN THE RAT. J.A. De Carlos*, L. López-Mascaraque and F. Valverde, Neuroanatomy, Instituto Cajal (CSIC), 28002 Madrid, Spain.

From previous developmental studies it has been proposed that neurons of the ventro-lateral cortex, including the primary olfactory cortex, differentiate from progenitor cells of the lateral ganglionic eminence (LGE). The objective of the present study was to test this hypothesis. The first-generated cells in the forebrain of the rat migrate to the surface of the telencephalic vesicle E12. Using ^{3}H-T we found that most of these cells contributed to the formation of the deep layer III of the primary olfactory cortex. To study their migratory routes we made localized injections of the carbocyanine fluorescent tracers DiI and DiA into various parts of the LGE in living embryos at E12- E14. The embryos were maintained in a culture device for 17- 48 h. After fixation, most migrating cells were located at the surface of the telencephalic vesicle while others were seen coursing tangentially into the preplate. Injections made at E13 and in fixed tissue at E15, showed that migrating cells follow radial glial fibers extending from the ventricular zone of the LGE to the ventro-lateral surface of the telencephalic vesicle. The spatial distribution of radial glial fibers was studied in Golgi preparations. These observations provided further evidence of the existence of long glial fibers extending from the ventricular zone of the LGE to the ventro-lateral cortex. We conclude that cells of the primary olfactory cortex derive from the LGE and that some early-generated cells migrating from the LGE transgress the cortico-striatal boundary entering the preplate of the neocortical primordium. Supported by Research Project PB91-0066 from Ministerio de Educación y Ciencia of Spain.

393.5

QUANTITATIVE ANALYSIS OF TANGENTIALLY MIGRATING INTERMEDIATE ZONE NEURONS IN EMBRYONIC RAT NEOCORTEX N. Tamamaki*, K. E. Fujimori, R. Takauji Dept. of Anatomy, Fukui Medical School, Matsuoka, Fukui 910-11 Japan

It is well known that neuroblasts produced in the ventricular zone of the neocortex migrate radially toward the neocortical surface and form neocortical cell layers. Recent studies, however, revealed that the tangentially migrating cells in the intermediate zone were not negligible in the embryonic neocortex, little is known about their meanings and functions. To have better understanding on the tangentially migrating cells, we quantified the number of tangentially migrating cell by making focal injections of DiI into various mediolateral levels of the embryonic neocortex *in utero*. Two days after DiI injection, strongly labeled migrating cells with leading processes were found outside of the injection site. In every case of injections, most tangentially migrating cells were found medially to the injection site in the intermediate zone. Tangentially migrating cells were found most when the injection was made into the lateral ganglionic eminence. When injection site was shifted toward medially more less number of migrating cells were found. These migrating cells seemed to correspond to the TuJ1-positive cells (Menexes and Luskin, J. Neurosci. 14:5399,'94), GABAergic cells (Van Eden et al. J Comp. Neurol. 289:213,'89), and MAP2-positive cells (Cobas et al. Neurosci. 40:375,'91) in the lower intermediate zone. These observation suggested that more tangentially migrating cells originated in the lateral ganglionic eminence and directed to the medial structures. On E17, these migrating cells reached the midline structure coinciding with the formation of the corpus callosum.

393.6

COINCIDENCE OF ARRIVAL TIME OF MIGRATING LOWER INTERMEDIATE ZONE NEURONS TO THE MIDLINE STRUCTURE WITH THE CORPUS CALLOSUM FORMATION. K.E.Fujimori*, R.Takauji and N.Tamamaki. Department of Anatomy, Fukui Medical School, Fukui 910-11, Japan.

Although corpus callosum is a conspicuous projection in the cerebral cortex, the mechanism of the formation of corpus callosum is not known. In rats, callosal axons start to cross midline structure at embryonic day (E) 17. Recently it has been shown that a subpopulation of early generated cells migrate tangentially in lower intermediate zone (IZ) and reach the corpus callosum. To know whether the migrating lower IZ neurons are involved in the initiation of callosal formation, we investigated the distribution and migration of the lower IZ neurons in the embryonic rat neocortex.

GABA immunohistochemistry was performed on vibratome sections of E17 rat neocortex. As it was previously reported, many GABAergic neurons were observed in the lower IZ with having leading processes medially. MAP2 immunohistochemistry also revealed a similar population on serial paraffin sections. Both GABA immunoreactive (IR) cells and MAP2 IR cells were found at midline structure at the time of corpus callosum formation. Double labeling of BrdU and MAP2 immunohistochemistry was also carried out to decide the birthdate of MAP2 IR cells in lower IZ and the corpus callosum. In addition, *in vivo* DiI-labeling study revealed that migrating lower IZ neurons reach the midline structure by E18. These findings support that the arrival of the lower IZ neurons into the midline structure coincides with the formation of corpus callosum. The migrating lower IZ neurons may play a certain role in the corpus callosum formation.

393.7

CELL DISPERSION PATTERNS IN THE CEREBRAL CORTEX OF MOUSE CHIMERAS. S-S Tan*[1], K. Sturm[2], P.P.L. Tam[2], B.E.Reese[3] and B. Faulkner-Jones[1]. [1]Dept of Anatomy & Cell Biology,University of Melbourne, Victoria 3052, Australia; [2]CMRI, Westmead NSW, Australia; [3]Neuroscience Research Institute, University of California at Santa Barbara, CA 93106.

Previous studies in our laboratory using transgenic mosaics have shown that neurons and glia in the developing cortex both follow radial and tangential dispersion patterns. Although a significant proportion of migrating neurons follow the radial pathway, it is still unclear whether there might be progenitors that are committed to generating only radially-dispersing progenies. To address this issue, we have made an ES cell line from the H253 transgenic mouse which we used to construct mouse chimeras. The reduced colonization by *lacZ*-expressing cells in the cortices of wild-type mice provided improved resolution of cellular dispersion patterns and their clonal relationships. Adult cortices showed two predominant distributions of *lacZ*-expressing cells. A diffused mosaic of scattered *lacZ*-expressing cells spanning across all cortical layers was detected in a number of cerebral hemispheres. More strikingly , single columns with inverted cone shapes were observed in random locations, although their frequency decreased in the lateral domains of the cortex. These results demonstrate early commitment of certain progenitors to generating radially-dispersing cells only. They also suggest that cells in the deeper layers tended to remain in close register with a point source in the VZ, while upper layer constituents appear to fan out horizontally. It remains to be determined whether the upper layer spreading is due to horizontal movement of progenitor cells in the VZ, or within the cortical plate. Funded by the NH&MRC of Australia.

393.8

MIGRATION OF NEURONAL AND GLIAL PRECURSORS IN CULTURED NEOCORTICAL SLICES. J.M.Edgar * and D.J.Price, Dept. of Physiol., Univ. Med. Sch., Teviot Place, Edinburgh EH8 9AG, U.K.

In vivo, cells destined for the cerebral cortex are generated in the telencephalic ventricular and subventricular zones. They then migrate to their final positions. Transplant studies have suggested that newborn cortical cells receive instructions on how to migrate at the end of S-phase and in G2 of the cell cycle. Cortical precursor cells that are born in vivo still migrate from the ventricular zone if telencephalic slices are then placed in organotypic culture. In these experiments, factors either within or outwith the telencephalon might have instructed cortical precursors to migrate. We asked whether the factor(s) that permit and/or regulate the migration of neuronal and glial precursors the in developing mouse cortex have a cortical and/or subcortical origin.

Telencephalic cortical slices were cultured in serum free medium (i) in isolation or (ii) with thalamic explants. Proliferating cells were labelled with 5-bromo-2-deoxyuridine (BrdU) in vitro and the relative locations of these cells were assessed after two, five or seven days in culture using standard immunohistochemical techniques. Initial results showed that cells in co-cultured slices migrate to their normal laminar position after two days. After five and seven days their distribution is distorted. Cells in isolated cortical slices do not show normal lamination at any of the stages investigated. These results suggest that the thalamus assists normal migration early on but that factors that sustain lamination are absent in the culture environment.

This work was supported by the Wellcome Trust.

393.9

MIGRATIONAL DEFECTS AMONG CORTICAL CELLS FROM SMALL-EYE MICE D.J. Price*, D. Caric[1], K. Gillies, R.E.Hill[1] and S.K.McConnell[2], Dept. Physiology, Univ. Med. Sch., Teviot Place, Edinburgh, U.K., [1] MRC Human Genetics, Crewe Road, Edinburgh, U.K., [2] Dept. Biological Sci., Stanford University, California, USA.

The small-eye (*Sey*) phenotype results from a point mutation in the Pax-6 gene. This gene encodes a transcription factor containing a paired domain and a homeodomain, and is expressed at several sites throughout the developing forebrain, including the cerebral cortex. In the cortex, Pax-6 is expressed mainly in the ventricular and subventricular zones. Homozygous *Sey* mice show defects in the eyes and in the thalamus. Previous studies have suggested that the mutants also have defects of cortical migration. In this study, we investigated the migration of cortical cells by labelling them with the thymidine analogue, bromodeoxyuridine (BrdU). We found that cells born early during cortical neurogenesis (at embryonic day 13) migrate to appropriate positions in the cortical plate, but that most cells born later (at embryonic day 16) fail to migrate. Rather, they remain in the ventricular zone, which expands considerably in thickness. Staining with glial-specific antibodies revealed that the radial glial cells are present in the *Sey* mice. These results raise the interesting question of whether the cortical defect is intrinsic to the migrating cells, or is secondary to a defect in the environment through which they migrate. This could be tested by transplanting cortical cells from +/+ or *Sey/Sey* embryos into the brains of normal rats at embryonic day 16, then examining the positions that they adopt in the normal cortex.

Supported by the MRC

393.10

CORTICAL DEFECTS IN THE SMALL EYE (SEY) MOUSE. D.Caric*, D.J. Gooday[1], D.J. Price and R.E. Hill. MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2UX and [1]Dept of Physiology, University of Edinburgh, Teviot Place, Edinburgh, EH8 9AG.

We are studying the cellular mechanisms involved in the development of the abnormal cortex in the Small eye mouse. In Small eye homozygous mice (which results from a mutation in the Pax-6 gene), the development of the ordered cellular layers in the cortex is disrupted: many cells which fail to migrate to the cortical plate give rise to clusters of cells in the subventricular and intermediate zones. We are interested in finding out the origin and birth dates of these cells, and also in their adhesion properties.

First, we have used BrdU to label cells during various stages of neurogenesis and have examined their fate at embryonic day 19 (E19) in both wild type and mutant. After BrdU injection on E13 and E14, no densely labelled cells were found in the clusters. After injection on E16 and E 17 the clusters contained many labelled cells. We concluded that these clusters contain cells born after E14.

Second, we compared the distribution patterns of the adhesion molecule L1 in the cortices of both Sey homozygotes and wild type embryos using immunocytochemistry. This revealed that although the intermediate zone in both sey homozygotes and wild type was heavily labelled, the clusters of cells in the homozygotes were devoid of L1 staining.

We concluded that the clusters of cells found in Sey homozygotes arise from cells born later in neurogenesis which do not express the L1 adhesion molecule, and although they can be displaced from the ventricular and sub-ventricular zones, they are unable to gain access to the intermediate zone.

This work was supported by the MRC.

393.11

ABNORMAL PROLIFERATIVE PATTERN IN PAX6 MUTANT CEREBRAL CORTEX. J.H. Martin[1], R.L. Maas[2], S. Wawersik[2], R.A. Segal[1]*, C. Walsh[1]. [1]Dept of Neurology, Beth Israel Hosp.; [2]Div. of Genetics, Brigham and Women's Hosp., Boston, MA 02115.

Homozygous Pax6 mutant mice show a severe cortical phenotype but the nature of the malformation is not clear. We have examined the role of Pax6 by using antibody staining for cell type-specific markers, retroviral labelling, and confocal microscopy of the homozygous Pax6 mutant *Small eye (Sey Neu)* mouse strain.

Retroviral labelling experiments showed greater labelling in proliferative regions and less in the developing cortical plate of fetal homozygous mutant mice compared to wild type mice, reflecting the previously described arrest of cell migration into the cortical plate (Schmahl, et al., *Acta Neuropath.* (1993) 86:126). Immuno-staining with Notch1 antiserum in E13 embryos revealed a somewhat broader band of staining in the ventricular zone of homozygous mutants compared to normal littermates. CR-50, an antibody to Cajal-Retzius neurons of Layer 1 revealed the presence of these neurons in E14 mutant embryos, despite previous suggestions that Layer 1 is absent in *Sey* mutants. Confocal microscopic analysis of Syto-11 (nucleic acid stain) stained E14 fetal brain sections showed M-phase cells in the proliferative region of homozygous mutants differed from wild type, since in mutants the mitoses were not localized as precisely to the lining of the ventricles. Frequently, mitoses were located up to 25μm from the ventricular surface. These data suggest that mice mutant for Pax6 show abnormalities in cell proliferation and are defective for the normal interkinetic nuclear migration of proliferating cells in the ventricular epithelium of the cortex.

Funding was provided by NIH and the Klingenstein Foundation.

393.12

DISRUPTION OF CORTICAL HISTOGENESIS IN THE CROOKED TAIL (Cd) MOUSE BRAIN ML Carter*, F. Zhang, C Iadecola, & ME Ross, Dept of Neurology, Univ of Minnesota, Minneapolis, MN 55455.

Cd is a semidominant mutation in which heterozygotes have kinked tails and homozygotes have a high incidence of exencephaly, a rostral neural tube defect related to anencephaly. Approximately 28% of Cd homozygotes escape exencephaly but express a runted phenotype (SMALL) in which Cd/Cd animals weigh ≤40% of age-matched normal siblings. We sought to characterize the morphological effects of the Cd mutation in the SMALL phenotype. In thionin-stained, fresh-frozen sections, the volume of the brain (mm^3) was measured using an image analysis system (JCBF&M 12:962). The whole brain of SMALL mice was 30±7% smaller than that of normal sibs (n=8; $p<0.05$; t-test). However, the cerebral cortex in SMALLs appeared relatively more affected than subcortical structures. To quantitate this difference, the volume of the cerebral cortex and subcortex were measured separately. To correct for differences in whole-brain volume, cortical volumetric data were normalized to those of subcortex and compared between the SMALLs and their normal sibs from postnatal day 4 (P4) to P18. SMALL cortical volumes were reduced by 49±11% in frontal cortex and 52±14% in occipital cortex ($p<0.01$), but were not affected in parietal cortex (-0.75±0.3%; $p>0.05$). Cortical cytoarchitecture was examined by MAP2 immunocytochemistry. Cortical laminae were less well defined in SMALLs than in normal sibs. Medium sized pyramidal neurons were displaced into layer I, extending to the subpial surface. This was most readily apparent in the frontal and occipital regions. The parietal region was less affected while no cytoarchitectural abnormalities were observed in the hippocampus. Thus, SMALL brains reveal preferential volume reduction in the frontal and occipital cortex, and neuronal heterotopia consistent with a cortical migration defect. We conclude that the Cd gene is involved in rostral neural tube closure and the establishment of normal cortical cytoarchitecture as well. (Supported by NINDS and the MMF)

393.13

NEOCORTICAL BIRTHDATING ANALYSIS ON *SCRAMBLER*, A NOVEL MOUSE MUTANT, REVEALS A *REELER*-LIKE PHENOTYPE. J.L. González[1,2], C. Russo[1], H. Sweet[3], M. Davisson[3], D. Goldowitz[4], and C. Walsh*[1,2]. [1]Neurology Dept., Beth Israel Hosp.; [2]Prog. in Neuroscience, Harvard Med. Sch., Boston, MA 02115; [3]The Jackson Lab., Bar Harbor, ME; [4]Univ. of Tenn., Memphis, TN

Mice homozygous for the *scrambler* (*scm*) mutation display unstable gait, trembling and ataxia similar to mice homozygous for the *reeler* (*rl*) mutation. Genetic analysis showed that *rl* and *scm* map to different chromosomes (Sweet et al., submitted). Histological examination of *scm/scm* forebrain shows abnormalities similar to *rl/rl*. Both mutants show abnormal hippocampal cytoarchitectonics, with marked disruption of pyramidal and granule layers. Furthermore, *scm/scm* neocortex shows poor lamination and an absence of the marginal zone, as in *rl/rl* mutants.

In order to test the hypothesis that the *scm* mutant has a *reeler*-like, outside-in gradient of neocortical development, we performed birthdating analysis on *scm/scm* mice. Timed-pregnant, heterozygous or homozygous, dams were injected with 5-bromo-2-deoxyuridine (BrdU) at E10-E19. Animals were sacrificed several weeks after birth and brain sections analyzed to assess the position of BrdU-labeled neurons in the neocortex of *scm/scm* and normal littermates. In general, BrdU-labeled cells in *scm/scm* mice displayed a broader laminar distribution when compared to normal littermates. In addition, the distribution peaks in *scm/scm* and normal neocortex are approximately inverted for E12-, E15-, and E16-labeled neurons. For example, more than 95% of E16-BrdU-labeled neurons occupy the superficial half of a normal cortex, whereas only approximately 30% occupy the same portion in *scm/scm* mutants. These results are strikingly similar to those observed in *rl/rl* neocortex, providing further support for the resemblance of the defect in the two mutants, and suggesting a related role for the two genes in the development of neocortical lamination.

Supported by the NIH, NSF and the Klingenstein Foundation

393.14

SEROTONIN PROMOTES THE SURVIVAL OF RAT CORTICAL NEURONS IN VITRO. A. E. Dooley, I. S. Pappas and J. G. Parnavelas (SPON: Brain Research Association). Dept. of Anatomy and Dev. Biology, University College London, London WC1E 6BT, U.K.

Earlier studies have provided evidence for a role of serotonin (5-HT) in the maturation of the mammalian cerebral cortex. More recent studies have suggested that local environmental signals regulate the growth and differentiation of progenitor cells in the developing cortex. 5-HT is present in the cortex at this early stage, and here we investigated its actions during corticogenesis using a dissociated cell culture preparation. Primary cultures were established from embryonic day 14 (E14), E16, and E18 rats. These cultures were grown in a defined medium containing 1% FCS, or additionally exposed to 5-HT (25 μM) for 1-11 days in vitro (DIV). Using the MTT assay, we found that 5-HT significantly increased the survival of cells in these cultures in a concentration dependent manner. Specifically, there was a 1.3-fold increase in the number of cells at 9 DIV in cultures prepared from E14 embryos, a 2-4-fold increase at 7-9 DIV in E16 cultures, and a 1.6-2.2-fold increase at 2-4 DIV in E18 cultures. This survival was mimicked at all ages by the $5\text{-}HT_{2a/2c}$ agonist a-methyl-5-HT (25 μM), in contrast to the $5\text{-}HT_{1a}$ agonist 8-hydroxy-2(di-n-propylamino) tetralin (25 μM) which showed no effect. Survival was predominantly of postmitotic neurons immunopositive for the microtubule-associated protein-2 (MAP-2). Consistent with increased survival was the observation of reduced cell death in 5-HT treated cultures compared to controls.These results support the importance of 5-HT in the survival of cortical cells and particularly of postmitotic neurons within this environment.

Supported by the Medical Research Council.

393.15

SEROTONIN INCREASES THE PROPORTION OF GLUTAMATE-CONTAINING NEURONS IN ORGANOTYPIC SLICE CULTURES OF EMBRYONIC RAT CORTEX. A. A. Lavdas and J. G. Parnavelas (SPON:ENA) Dept. Anatomy & Dev. Biol., University College London, London WC1E 6BT, UK.

Serotonin (5-HT), present in the developing brain long before it assumes its neurotransmitter function, is regarded as a strong candidate for a role in the maturation of the cerebral cortex. In this study, we sought to investigate the possible effects of 5-HT on the generation and differentiation of cortical neuronal cell types. Slice cultures were prepared from the cortices of E16 rat embryos and kept in vitro for one week, either in defined medium (DM) or in DM plus 250 μM 5-HT. At the end of this period the proportion of cells immunoreactive for Glutamate (Glu) or GABA, the neurotransmitters utilized by cortical pyramidal and nonpyramidal neurons respectively, was estimated for both conditions. We found that the proportion of Glu-positive neurons was significantly increased in the 5-HT-treated group (mean=38.1%) as compared to the control group (22.8%). A number of co-cultures of E16 cortical slices and slices containing the raphe nuclei taken from E18/E19 embryos were subsequently prepared and kept for the same period in DM. After the innervation of the cortical slices by serotonergic fibers had been immunocytochemically demonstrated, the proportion of Glu positive neurons was assessed and found to be also significantly higher (29%) than in the control group, albeit lower than in slices exposed to 5-HT. To investigate whether this effect of 5-HT was the result of changes in cell proliferation, we exposed a number of slices, cultured both in DM and in DM + 5-HT, to 10^{-5} M bromodeoxyuridine (BrdU) for 16 hrs every day. The proportion of BrdU immunoreactive cells was established at each day and was similar for the 5-HT treated and the comntrol groups.These results suggest that 5-HT promotes the survival and/or differentiation of cortical Glu-containing neurons. Supported by the Wellcome Trust.

393.16

GENERATION AND DIFFERENTIATION OF CORTICAL CALRETININ-EXPRESSING NEURONS IN VITRO. I. S. Pappas and J. G. Parnavelas*. Dept. of Anatomy and Developmental Biology, University College London, Gower street, London WC1E 6BT, U.K.

We examined the role of growth factors on the development of the cortical cell types focusing on the subpopulations of neurons defined by the presence of the calcium-binding protein, calretinin (CR). CR-containing neurons in the cortex comprise two groups: the early generated Cajal-Retzius cells of layer I, and the somewhat later generated group found throughout the cortical plate. Treatment of primary cultures prepared from E16 rat cortices with BDNF or NT-3 increased c-fos expression in Cajal-Retzius cells at 1 day *in vitro* (DIV), and promoted their survival and morphological differentiation after longer treatment. We also found that bFGF increased dramatically the number of CR-expressing neurons that are generated in culture and characterized by bipolar morphology. Using BrdU to mark dividing progenitor cells, we observed that CR-expressing neurons are generated during the first 2-3 days in culture and subsequently begin to differentiate. Other growth factors (neurotrophins, EGF, IGF-I) did not appeared to promote the proliferation of the progenitors that give rise to CR-expressing neurons, but EGF sustained the proliferative mode of these progenitors after short exposure to bFGF. Retinoic acid (RA) promoted the differentiation of CR-expressing neurons and, when applied with bFGF, it increased further their frequency. In conclusion, neurotrophins NT-3 and BDNF promote the survival and differentiation of Cajal-Retzius cells, and bFGF and RA enhance the generation and differentiation of CR-expressing neurons in the rat cerebral cortex. Supported by the Wellcome Trust.

CELL DIFFERENTIATION AND MIGRATION VII

394.1

TRANSPLANTATION OF EMBRYONIC STEM CELL-DERIVED NEURAL PRECURSORS.
O. Brüstle, A.C. Spiro, S. Okabe, and R.D.G. McKay*. Lab. of Molecular Biology, NINDS, NIH, Bethesda, MD 20892

The *in vitro* generation of neurons and glia from embryonic stem (ES) cells offers exciting prospects for the study of early neural development and the generation of "designer cells" for transplantation studies. We have recently established a cell culture protocol which allows efficient production of neural precursors from ES cells and their subsequent differentiation *in vitro* (Okabe *et al.*, submitted). To investigate the developmental potential of ES cell-derived neural precursors *in vivo*, cell suspensions at various stages of differentiation were transplanted into the telencephalic vesicles of embryonic rats. The implanted cells were traced using species-specific antibodies and *in situ* hybridization with a probe to mouse satellite DNA. Cells leaving the ventricular system formed clusters within the subependyma and incorporated as single cells into various brain regions. They acquired mature neuronal and glial phenotypes with the donor-derived neurons producing an extensive axonal network throughout the host brain. In addition, cells remaining inside the ventricular system formed neural tube-like epithelial structures when transplanted at an early stage of *in vitro* differentiation. These structures contained abundant radially oriented nestin-positive processes and exhibited a centrifugal gradient of neuronal differentiation. Our data suggest that ES-cell derived neural precursors have the potential to form neural tube-like structures but are recruited to mature glial and neuronal phenotypes when exposed to embryonic brain parenchyma. Incorporation into the developing CNS of neurons and glia derived directly from genetically modified ES cells will allow the *in vivo* analysis of a targeted gene inactivation without generation of a knock out animal.

Supported by the NINDS.

394.2

EARLY SPECIFICATION OF STRIATAL PROJECTION NEURONS AND INTERNEURONAL SUBTYPES IN TRANSPLANTS OF THE LATERAL OR MEDIAL GANGLIONIC EMINENCE. M. Olsson*, K. Campbell and A. Björklund. Wallenberg Neuroscience Center, University of Lund, Lund, Sweden.

Fetal striatal grafts derived from the striatal primordium including both the medial (MGE) and the lateral ganglionic eminence (LGE) of the embryonic day (E) 14-15 rat forebrain display regions which are striatum-like, i.e. contain immunopositivity for striatal projection neurons and interneuronal subtypes. In such grafts, however, the striatum-like regions only constitute 30-50% of the total graft area with the remaining portion of the transplant expressing non-striatal phenotypes. Interestingly, grafts derived from the selectively dissected LGE are highly enriched in striatal projection neurons as detected by DARPP-32 expression (up to 90%) while MGE transplants are only sparsely positive (<10%). These studies, however, have not adressed whether striatal interneuronal subtypes also are contained within the LGE transplants or are derived from the MGE.

The purpose of this study was to adress the contribution of the LGE and MGE, respectively, to striatal projection neurons and interneuronal subtypes at an early- (E12.5) and mid-point (E15) in striatal neurogenesis. Briefly, the LGE or MGE were dissected from E12.5 or E15 rat embryos and dissociated into a single cell suspension, injected into the ibotenic acid lesioned adult striatum. After 6 weeks postgrafting the animals were sacrificed and sections were analyzed using in situ hybridization histochemistry with probes recognizing DARPP-32, choline acetyltransferase (ChAT) or preprosomatostatin (PPSS) mRNA's. As described previously, the E15 LGE grafts were, in contrast to the MGE, highly enriched in DARPP-32 expression. Interestingly, this predominance in DARPP-32 expression was also observed in the LGE grafts derived from E12.5 (early point in striatal neurogenesis) as compared to the MGE grafts. Conversely, ChAT labeling was predominantly observed in the E12.5 MGE grafts with somewhat lower expression in the E15 MGE grafts. Additionally, a few positive cells were detected in the E15 LGE grafts whereas the E12.5 LGE grafts were largely devoid of cells labeled with this probe. Cells expressing PPSS mRNA were observed in both the LGE and MGE grafts from both timepoints.

These data suggests that striatal projection neurons are specified early in the LGE (i.e. E12.5) and that the LGE may not be the single source for all striatal neuronal subtypes since ChAT expressing cells predominanted in the MGE grafts. Supported by The Swedish MRC, The Kock and Wiberg Foundations.

394.3

CLONAL ADULT RAT HIPPOCAMPAL PROGENITORS MIGRATE AND DIFFERENTIATE AFTER IMPLANTATION INTO EMBRYONIC BRAIN J.O. Suhonen*, J. Takahashi, M. Takahashi, T. Palmer, J. Ray and F.H. Gage. Laboratory of Genetics, The Salk Institute, La Jolla, CA 92037

Adult hippocampal progenitors can be cultured for over two year in the presence of basic fibroblast growth factor (FGF-2). When grafted back to adult rat brain, they can migrate to specific structures and undergo site-specific neuronal differentiation. Now we have genetically modified these cells with retrovirus vector to express *E. Coli LacZ* gene in the nucleus or cytoplasm of the cells and isolated clones of normal diploid multipotent progenitors from adult rat hippocampus.

To examine the developmental capacity of these clonal adult progenitor cells *in vivo* the cells were implanted into embryonic (E17) rat brain. At 9 to 40 days postnatal age, brains were fixed and processed for ß-Gal immunoreactivity and neuronal and glial markers. The progenitors implanted into the neuroepithelium of the telencephalon and mesencephalon migrated into the developing hippocampal formation and cerebellar cortex where some cells acquired region-specific morphologies and expressed site-specific neuronal markers, i.e. calbindin and NeuN. Grafted progenitors were found in various other areas undergoing neurogenesis at or after the time of implantation, i.e. striatum, septum, and late developing nuclei of midbrain.

These findings indicate that clonal adult hippocampal progenitors grafted into the fetal brain migrate and integrate into the host brain cytoarchitecture and they can undergo site-specific neurogenesis during embryonic rat brain development.

Supported by grants from NIA, NINDS, the Medical Research Council of the Academy of Finland and Japan Eye Bank.

394.4

ADULT CNS STEM CELL FATE CAN BE MODULATED BY ALTERING THE EXPRESSION OF TROPHIC FACTOR RECEPTORS. J. Takahashi, T.D. Palmer, F.H. Gage. Laboratory of Genetics, The Salk Institute, La Jolla CA, 92037

The ability to propagate central nervous system stem cells in continuous culture provides a valuable resource for examining factors that regulate the early stages of fate determination and differentiation. Once multipotent progenitors exit the cell cycle, the culture environment has a significant effect on differentiation and can be used to influence the ratios of each neuronal or glial cell type generated. We are using adult rat hippocampal stem cell cultures to examine the role of neurotrophins in the commitment and consolidation phases of neuronal differentiation. Proliferative multipotent precursors express trk B and C receptors and the survival and maturation of newly committed neurons are enhanced by treating cultures with the cognate ligands, brain derived neurotrophic factor and neurotrophin-3. Trk A and low affinity nerve growth factor receptor (p75) are not expressed and accordingly, nerve growth factor (NGF) has little effect on differentiation. However, following retinoic acid stimulation, both trkA and p75 are upregulated and NGF is subsequently able to stimulate an immediate early signal transduction cascade as indicated by an activation of c-fos. Ongoing work examines how the sequential application of exogenous agents such as forskolin, glucocorticoids, retinoids and phorbol esters can be used to influence early fate choice events and modulate trophic or growth factor receptors. The ability to alter a progenitor's responses to external cues suggests that the repertoire of adult-derived hippocampal progenitors may be much larger than indicated by the default status of untreated progenitors. This plasticity may have broader significance in cellular replacement strategies if progenitors can be primed to appropriately respond to a particular CNS environment so that specific neuronal differentiation cascades are activated following grafting into the aged or injured CNS. Supported by NIH AG08514 and Kyoto University Medical School, Department of Neurosurgery.

394.5

CHARACTERIZATION OF NEURAL PROGENITOR CELLS ISOLATED AND CULTURED FROM DIFFERENT REGIONS OF ADULT RAT SPINAL CORD. J. Ray*, L. Shihabuddin and F. H. Gage. Laboratory of Genetics, Salk Institute for Biological Studies, 10010 N. Torrey Pines Road, La Jolla, CA 92037

FGF-2 responsive neural progenitor cells have been cultured from different adult brain regions. Here we have explored whether similar progenitor cells can be cultured from different levels (sacral, thoracic, lumber and cervical) of adult rat spinal cord and whether they give rise to neurons and glia as well as spinal cord specific neurons (e.g. motoneurons).

Sacral, thoracic, lumber and cervical areas of adult rat spinal cord (>3 months old) were micro-dissected and neural progenitor cells were isolated and cultured in serum-free medium containing FGF-2 (20 ng/ml) through multiple passages. Although all areas generated rapidly proliferating cells, the cultures were heterogeneous in nature and cell morphology varied within a given area as well as between areas. A large number of cells in cultures from all areas of the spinal cord expressed A2B5, GFAP, MAP2 and GAD K2 as well as p75, CGRP and ChAT, markers for motoneurons. Cells expressing the latter markers are large and have morphology similar to that of motoneurons. The presence of both astrocytes and neurons including potential motoneurons in these cultures may indicate that some cells are undergoing spontaneous differentiation. Comparison of the properties of neural progenitors isolated from different areas of the spinal cord with adult rat hippocampal cells will provide insight as to whether analogous populations of progenitor cells are present within sub-areas of a given region and in different regions of adult CNS. Supported by grants from NIA, APA, ISRT and Hollfelder Foundation.

394.6

RETINOIC ACID AND BDNF INDUCE THE DIFFERENTIATION AND MATURATION OF NEURONS GENERATED FROM EGF RESPONSIVE PRECURSORS. L. Frazer, A.E. Rosser*, M.G. Terborg and C.N. Svendsen MRC Cambridge Centre for Brain Repair, University of Cambridge, UK

EGF promotes the division of a CNS precursor cell which retains the ability to differentiate into either neurons, astrocytes or oligodendrocytes. It has previously been shown that serum, BMP or CNTF can induce the differentiation of these cells into glia. BDNF has been shown to increase the maturation of neurons developing from EGF responsive precursors while not increasing the total number of neurons. In this study EGF responsive precursors from the embryonic rat striatum were isolated and expanded for 14 days *in vitro*. Cells were then plated onto poly-lysine coated coverslips and allowed to differentiate for one week in the absence or presence of various combinations of RA and BDNF. Dose response curves with RA showed that at 0.25 or 0.5μM there was a significant reduction in overall cell survival but at the same time a significant increase in the number of neurons, astrocytes and oligodendrocytes assessed using βtubulin III, GFAP and GAL-C immunocytochemistry suggesting that RA had induced a large proportion of the cells to label with these markers in addition to promoting significant cell death. Surprisingly, at the highest dose of 1.0μM RA there was less cell death than at the lower doses and significantly fewer cells labelled with either of the above markers showing that RA acid is not simply toxic to a proportion of cells. BDNF had no effect on the number of neurons differentiating but did significantly increase neuronal size. BDNF combined with RA was no different to RA alone with regard to cell death and neuronal number and still led to an increase in neuronal size. These results show that RA has powerful differentiation effect on EGF responsive precursor cells in addition to triggering a cell death mechanism, and that BDNF specifically affects the maturation of these neurons without preventing the RA induced cell death. Supported by the Wellcome Trust and MRC.

394.7

CNS PRECURSOR CELLS THAT EXPRESS TRK RECEPTORS: INDUCTION OF DIFFERENT CELL FATES BY NEUROTROPHINS. M.V. Lachyankar, P.J. Condon, P.J. Quesenberry, N.S. Litofsky, L.D. Recht and A.H. Ross*. Worcester Foundation for Biomedical Research, Shrewsbury, MA 01545 and University of Massachusetts Medical Center, Worcester, MA 01655.

Neurosphere cultures grown from murine striatum in the presence of epidermal growth factor (EGF) contain multipotential precursor cells capable of neuronal, astrocytic and oligodendroglial differentiation. In this study, we tested whether neurotrophic factors induce differentiation of these neural precursor cells. We find that these cells express receptors for multiple neurotrophic factors, including TrkA, TrkB, TrkC, the low-affinity nerve growth factor (NGF) receptor and ciliary neurotrophic factor (CNTF) receptor a. In the presence of the mitogen, EGF, treatment of stem cells with neurotrophic factors had no apparent effect. Removal of EGF resulted in cessation of cell proliferation and pronounced astrocytic differentiation. In the absence of EGF, treatment with NGF or brain-derived neurotrophic factor (BDNF) induced neuronal differentiation. Treatment with neurotrophin-3 (NT-3) or CNTF resulted in both neuronal and oligodendroglial fates. These studies suggest that local cues, such as neurotrophic factors, may induce production of different types of neural cells during development or in response to other stimuli, such as injury.

NIH grants NS21716 and NS28760

394.8

ANALYSIS OF MOTILE O-2A PROGENITOR CELLS IN LIVING BRAIN SLICES AND IN CULTURE. C. Schmidt, C. Ohlemeyer, T. Walter and J. Schnitzer*. Max Delbrück Center for Molecular Medicine (MDC), Berlin-Buch, Germany.

O-2A progenitor cells are purported to migrate over long distances[1-4] into the various brain regions where they differentiate into oligodendrocytes and fulfill their appropriate tasks, i.e. myelination of axons. The aim of this study was to visualize and characterize motile cells *in vivo* and *in vitro*. We performed video-time-lapse analysis of cells in living brain slices of the corpus callosum from 1- to 5-day-old rats. Motile cells were analyzed electrophysiologically and filled with Lucifer Yellow. All motile cells have a simple morphology bearing only very few processes. They migrate with a velocity of 10-100 μm/h within the corpus callosum. The main direction of their movement is towards the cortex. However, cells change and even reverse direction of their movement. We also observed dye-coupling between migrating cells and other yet unidentified cells within the corpus callosum. Patch clamp analysis of the motile cells confirmed that they belong to the O-2A lineage: they showed delayed outward rectifying currents ($K+_{DR}$) which is characteristic for these cells[5]. All motile cells examined show inward currents upon perfusion with GABA or kainate at a holding potential of -70 mV.

A combination of video-time-lapse, electrophysiological and morphological analyses of primary cultures of rat neonatal O2-A progenitor cells revealed comparable results: Migration is confined to cells with a simple morphology. Coincidentally, the majority of the motile cells express the A2B5 epitope. Migration ceases, when they are strongly O4-positive. As seen in the living brain slice, they show delayed outward rectifying potassium currents ($K+_{DR}$). When cells are plated on laminin in the presence of the growth factors PDGF and bFGF, both the number and the velocity of the cells is significantly enhanced in comparison to poly-L-lysine.

References: (1) Gumpel et al. (1989) *Dev. Neurosci. 11(2), 132-9*; (2) Gansmüller et al. (1991) *Glia 4(6), 580-90*; (3) Levison and Goldman (1993) *Neuron 10(2), 201-12*; (4) Warrington et al. (1993) *J. Neurosci. Res. 34(1), 1-13*; (5) Sontheimer et al. (1989) *Neuron 2(2), 1135-45*

DFG Schn 346/4-1

MORPHOGENESIS

395.1

TIME-LAPSE ANALYSIS OF CELL DIVISION, MOVEMENT AND SHAPE DURING GASTRULATION AND NEURULATION IN ZEBRAFISH. Miguel L. Concha[1,2], Richard J. Adams[1]* [1]University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K. [2]Facultad de Medicina, Universidad de Chile, Casilla 70005 Correo 7, Santiago, Chile.

In this study we have taken advantage of the optical transparency of zebrafish embryos to investigate the patterns of cell division, movement and shape during early stages of development of the central nervous system. Gastrula and neurula embryos were imaged using time-lapse DIC Nomarski optics and 3-dimensional confocal microscopy. Images were analysed quantitatively using customised routines written for NIH-Image. (1) The onset of gastrulation is accompanied by major changes in cell behaviour within the epiblast. Cells begin to move co-ordinately in a vegeto-dorsal direction, become densely packed and the plane of cell division becomes aligned in the anterior-posterior (AP) plane. These behaviours begin at the blastoderm margin and propagate in a gradient towards the animal pole. (2) As the neural plate differentiates cells undergo extensive convergence movements towards the dorsal midline and a gradual transition to a medio-lateral (ML) plane of division is observed. This becomes the predominant orientation of division during neural keel and nerve rod stages. From late neural keel stage ML divisions concentrate at the dorsal midline and generate bilateral progeny. (3) Surprisingly, ventral (epidermal) cells also orient their divisions in the ML plane. (4) The factors that may determine the plane of division were investigated. The spatial distribution of AP divisions during gastrulation suggests that a common mechanism (e.g. chemical or mechanical) may determine this plane of division at different dorso-ventral positions. Cell movement and shape appear to account for regional differences in this pattern. ML divisions, on the other hand, occur in both epidermal and neural cells. The ML alignment of epidermal divisions is well correlated to cell shape. ML divisions ocurring within the neuroepithelium, however, seem to be dependent upon more factors. Changes in cell movement, shape and the state of polarisation during neurulation have been analysed. *Supported by MRC Research Centre in Brain and Behaviour; CONICYT-British Council*

395.2

PRENATAL STRIATAL DEVELOPMENT IN ORGANOTYPIC SLICE CULTURES. E.I. Barragan* E. Fusco and A.M. Graybiel Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139

The mammalian striatum is divided in two major compartments: the striosomes and the matrix. The mechanisms that regulate the emergence of striosomes during embryonic development are not well understood. To achieve the possibility of studying compartment formation in a system accessible to external manipulations, we have adapted the organotypic slice culture method elaborated by Liu et al. (1993) to the embryonic striatum. Striosomal cells (S cells) and matrisomal cells (M cells) were labeled with the thymidine analog Bromo-deoxyuridine (BrdU) in their birthdate windows, i.e. embryonal days 11.5 (E11.5) for S cells and E15 for M cells, by 1 injection of 100 mg/kg to the mothers. Coronal brain slices 300 microns thick were cut at E16, E17 and at days P1 and P2 as a control to confirm S and M cell birthdate injection times. We stained with the neurodifferentiation-marker MAP-2 to test the viability of the tissue. When we cultured S cell labeled slices harvested at E16-E17 for 0-5 days and afterwards applied immunohistochemistry for BrdU, we observed dispersed nuclei, some forming small clusters in the lateral striatum. In parallel experiments, we cultured M cell labeled slices harvested at E17 for 0-12-24 hr and 2-5 day periods. M cells first appeared in and near the ventricular zone (VZ), and later spread into the striatal primordium. With increasing time *in vitro*, M cells deeply penetrated the striatum, reaching its lateral edge caudally. This shift in position is unlikely to be due simply to the flattening of the cultures, because when we pulse labeled and cut at E17, and then cultured the slices for 5 days, most M cells were still in the VZ, subventricular zone and medial striatum. We conclude (1) that it is feasible to maintain embryonal striatal slices in culture, (2) that in such cultures S and M cells can survive for at least 5 days, and (3) that M cells can successfully migrate from the VZ into the striatum. Supported by NIH 2R01 HD 28341.

395.3

ABNORMAL DEVELOPMENT OF THE OPTIC CUP, OLFACTORY PIT, CRANIAL GANGLIA AND ANTERIOR CARDINAL VEIN IN VITAMIN A-DEFICIENT RAT EMBRYOS. J. C. White & M. Clagett-Dame*. School of Pharmacy, Univ. of Wisconsin-Madison, WI 53706.

Vitamin A is required for reproduction and normal embryonic development. Retinoic acid (RA), a metabolite of vitamin A, is believed to support the functions of the vitamin during early embryogenesis. Experiments were performed to determine how much RA is needed to support normal development from embryonic day 0.5 to 12.5 (E12.5) and to identify structures that are retinoid dependent. Female rats were depleted of stored vitamin A and maintained on 12 µg all-*trans* RA (atRA) until mating. At E0.5, dams were fed control diet (12 µg atRA/g diet + 100 IU/day retinyl palmitate) or one of four experimental diets containing 12, 6, 1.5 or 0 µg atRA/g diet. At E12.5, the percent of dead or resorbed embryos was ~30% for all diet groups with the exception of dams receiving 0 µg atRA/g diet, in which it was ~70%. Embryos from the control group and those receiving 12 µg atRA/g diet were similar in size and morphology. Embryos from the 6 µg atRA/g diet group were similar in size to control embryos, but showed enlargement of the anterior cardinal vein (ACV). Embryos from dams receiving 1.5 µg atRA/g diet were more variable in size and exhibited marked ACV enlargement. Otic vesicles were reduced in size in the 1.5 µg atRA/g diet group and/or absent in the 0 µg group. Embryos in the 0 µg RA/g diet group developed optic vesicles, but showed no evidence of optic cup or neural retina formation. Olfactory pits and cranial ganglia VII/VIII, IX, and X were also not observed. In conclusion, developmental abnormalities were observed in embryos from dams receiving 6 µg atRA/g diet or less. This work supports a role for RA in the development of the ACV, optic cup, olfactory pit, otic vesicle and cranial ganglia VII/VIII, IX and X. Supported by NIH-DK14881.

395.4

ALTERATION OF RADIAL GLIA PATTERN IN SPINAL CORDS OF NOTOCHORDLESS XENOPUS C. E. Maier* and R. H. Miller[1] Depts of Anatomy* and Neurosciences[1], Case Western Reserve Univ., Cleveland OH 44106

Normal neuronal and oligodendrocyte patterns in Xenopus spinal cord are altered when animals are made notochordless as the result of UV irradiation of the uncleaved, fertilized egg. Neuronal numbers are modified while cell bodies are found in atypical locations and axons have unusual patterns. Oligodendrocyte differentiation is delayed and may not occur at all in the spinal cord of notochordless Xenopus.

The influence of the notochord on astrocytes and their radial glia cell precursors has not been determined. To begin to study this impact unfixed frozen sections of experimental and clutchmate control Xenopus at stage 45 were labeled with anti-GFAP antibodies and viewed by indirect immunofluorescence. During normal development continuous GFAP+ processes radiate from the central canal to the pial surface of the spinal cord. Preliminary data suggests that the GFAP+ pattern is variously altered in spinal cords of Xenopus made notochordless by UV irradiation. GFAP+ cells are present but their radial orientation is frequently lost. Labeling of radial processes may be interrupted. Cell bodies are concentrated in the ventral spinal cord in some notochordless animals. The pattern of radial glia/astrocyte development appears to be influenced by an absence of the notochord but in a less predictable form than neurons or oligodendrocytes. Funding: NS25597-08.

395.5

TIME LAPSE PHOTOGRAPHY OF HINDBRAIN EXPLANT CULTURES. P.M. Kulesa* and S.E. Fraser. Div. of Biology, Beckman Institute (139-74), Calif. Inst. of Tech., Pasadena, CA 91125.

The segmented nature of the developing hindbrain shows many parallels to the well studied segmented body pattern seen in the developing *Drosophila*. The correspondence of molecular activity with these segments and the development of specific structure makes it an ideal system for understanding the basic mechanisms of embryonic patterning. However, the *in ovo* and *in utero* nature of chicken and mouse development make it difficult to directly observe the entire process over the course of hindbrain development. To study the dynamic aspects of hindbrain segmentation we utilize an *in vitro* hindbrain explant culture system. Hindbrains can be readily dissected from developing chicken or mouse embryos and cultured for 2-3 days, during which normal cell movement and differentiation continue to occur. Individual cells and small populations of cells in cultured hindbrains can be labeled *in situ* by microinjection with DiI or fluorescent dextrans and visualized with epifluorescence and confocal microscopy. Generation of time lapse recordings of the movements and differentiation of these cells will allow for a better understanding of rhombomere boundary formation, as well as a comparison of such segmented structure with genetic expression domains. (Funding: Sloan Center for Theoretical Neurobiology (PMK) and NIMH Silvio Conte Center (SEF)).

395.6

EFFECT OF N-CADHERIN ANTIBODIES ON HISTOGENESIS OF THE EARLY DEVELOPING CHICKEN BRAIN. S. Gänzler* and C. Redies. Institute for Biology III, Univ. of Freiburg, Schänzlestr. 1, D-79104 Freiburg, Germany.

Cadherins are a family of transmembrane glycoproteins which mediate calcium-dependent cell-cell adhesion by a preferentially homophilic binding mechanism. During development of the brain, N-cadherin, a member of this family, is expressed throughout the neuroepithelium, with strongest expression in the ventricular lining.

We studied the role of N-cadherin during brain morphogenesis by injecting functionally blocking N-cadherin antibodies into the tectal ventricle during early stages of development (embryonic day 3 to 5; E3-E5). To detect mitotic cells, BrdU was injected one hour before fixation at E6. The effect of blocking N-cadherin was analyzed by immunostaining coronal cryostat sections with antibodies against N-cadherin, R-Cadherin, BrdU, Ng-CAM/G4, and as marker for radial glia, with the antibody R5.

Brains treated with N-cadherin antibodies show invaginations of the ventricular lining and the formation of rosettes, most prominently in the tectum and diencephalon. The histological organization of these structures is basically similar to that of the normal neuroepithelium. Their center consists of ventricular lining which is surrounded by an inner ("ventricular") layer of mitotic (BrdU-incorporating) cells and an outer ("mantle") layer of post-mitotic (Ng-CAM/G4-expressing) neurons. In some regions, radial glia extends from the center of the rosettes to the outer layer. In the diencephalon, R-cadherin-expressing early neurons aggregate into nuclear anlagen (see Gänzler & Redies, J. Neurosci., 1995, 15:4157) but their form is distorted. These results show that blocking N-cadherin function disrupts the sheet-like neuroepithelial structure but seems to have little effect on the differentiation of neural cell types.

(Supported by the Max-Planck-Society and the Land Baden-Württemberg.)

395.7

DIFFERENTIAL DORSOVENTRAL DISPERSAL AND CONTRALATERAL MOVEMENT OF NEURAL PRECURSORS IN EARLY CHICK NEURAL TUBE. L. Erskine* and J.D.W. Clarke. Dept. Anatomy & Developmental Biology, University College London, Gower Street, LONDON WC1E 6BT, UK.

Little is known of cell movement within the early neuroepithelium. We have assessed the dorsoventral (DV) dispersal of neural precursor cells in chick hindbrain and spinal cord during the first 48 hrs of neural development. Using iontophoresis, 5-10 neighbouring neuroepithelial cells at identified positions in the neural plate or early neural tube were labelled *in ovo* with the lipophilic dye DiI. The dispersal of these cells and their progeny was examined 48 hrs later in flattened wholemount preparations of the hindbrain or spinal cord.

The position and degree of dispersal of the descendent precursors was dependent on the initial position of the labelled cells. Particularly in the hindbrain, dispersal of cells was not uniform but related to their initial position along the DV axis of the neural tube. Spread of lateral or dorsolateral cells was much more widespread than those labelled ventrally. Thus, not all regions of the neural plate contribute equally to the development of the neural tube. Additionally, considerable overlap and mixing of cells from different DV domains occurred.

Surprisingly, in the spinal cord but not the hindbrain, in 56% (19/34) cases, contralateral movement of neural precursor cells was observed. This produced 2 discrete clusters of labelled cells, usually separated by regions of unlabelled cells, at similar DV positions on either side of the floor plate. Movement of cells through both roof plate and floor plate appears possible. This is direct evidence that precursor cells are able to move large distances within the ventricular zone. Moreover, the symmetrical nature of the bilateral clusters suggests that at least some precursor cells may be specified early for particular regions along the DV axis of the neural tube. Supported by The Wellcome Trust.

395.8

RETINOIC ACID ADMINISTRATION TO EARLY POSTNATAL MICE CAUSES NEOTENIC TRANSFORMATION. J.E. Crandall*, M. Yamamoto, G. A. Schwarting, O. Koul, P. McCaffery, C.K. Deutsch and U. C. Dräger. The Shriver Center, Waltham, MA 02254 & Harvard Med. Sch., Boston.

The transcriptional activator retinoic acid (RA) acts as an endogenous morphogen for the developing organism. In many cases, it terminates growth and initiates differentiation programs. Endogenous RA synthesis occurs at restricted locations, resulting in diffusion gradients of decreasing RA concentration. Systemic flooding of the early embryo, during the formation of organ anlagen causes severe malformations, presumably due to abolishment of morphogenetically significant concentration gradients and spatially inappropriate activation of differentiation. While RA teratogenicity in embryos has been extensively described, our observations indicate that RA-sensitive morphogenesis extends to postnatal development. When early postnatal mice are injected with a single dose of RA and allowed to grow to adulthood, they maintain partially juvenile characteristics in different systems. They have baby-like faces that appear rounder and shorter. The cranium has an overall altered shape, affecting multiple bones and consistently resulting in skulls with a pronounced ventral bend. This malformation reflects an altered shape of the brain; the pontine flexure remains partially curved. Similarly, the postnatal maturation of the olfactory system is disrupted in the RA-treated mice: the epithelial receptor sheet is significantly thinner and the number of glomeruli in the olfactory bulb is decreased. We conclude that a retinoid overdose in neonatal mice interrupts morphogenetic maturation, causing neoteny, a lifelong arrest at partially juvenile features. Supported by NIH grants DC00953, HD04147 & EY01938.

395.9

PATTERNS OF EMBRYONIC DEVELOPMENT OF THE BRAIN AND SENSORY ORGANS STUDIED IN THREE MARINE TELEOST SPECIES

Holmqvist B. I. *, Forsell J. and Helvik J.V. Lab. for Marine Molecular Biology, Univ. of Bergen, Norway, and Dept. Zoology, Univ. of Lund, Sweden.

We have undertaken to study the development of the brain and sensory organs in early life stages of Atlantic halibut (*Hippoglossus hippoglossus*), herring (*Clupea harengus*) and cod (*Gadus morhua*). Here we present data from studies performed from 30 d° (age in days times rearing temp. in C°) to hatching (*Hh* and *Gm* hatching at ca 90d°, and *Ch* at ca 120d°). Immunocytochemical techniques were used for detection of acetylated tubulin (AT), a cytoskeletal protein present in early axons; serotonin (5HT), a neurotransmitter and precursor to melatonin; tyrosine hydroxylase (TH), the rate limiting enzyme for catecholamine synthesis; and opsin (OP) and S-antigen (SA), molecules involved in the phototransduction cascade.

In the brain, the first AT immunoreactive (ir) neuronal populations and major axonal tracts start to form at ca 30d° in herring, 40d° in cod and 60d° in halibut. 5HTir and THir neurons appear around 40d° in herring, 50d° in cod and at 60 and 80d°, respectively, in halibut. In the pineal organ, cells are OPir and SAir at ca 40d° in herring, 60d° in cod, and at 70 and 90d°, respectively, in halibut. Pineal 5HTir cells appear at ca 60d° in herring, and at 90d° in cod and halibut. The retina of both herring and cod displays ATir (ganglion) cells at 40d°, and 5HTir and THir cells at ca 90d°. ATir axons gradually appear coursing in the optic nerve, optic tract and into the optic tectum between 40 and 70d° in herring and cod. In the halibut, no immunoreactivity is present in retina or in the central visual system. Neuromasts and associated axonal projections are strongly ATir in the halibut, appearing at 60d°, however, not detected in cod or herring. In all species, cells and projections in the olfactory placoides and bulb were strongly ATir from about 40-60d° to hatching

The results indicate that the pineal organ is the first differentiated sensory organ in the species studied, possessing putative photoreceptor and light mediator functions already in the embryonic stage. The olfactory system also appears to be relatively well developed in all species at hatching. The visual system, retina and central projections, is relatively differentiated prior to hatching in herring and cod, whereas the mechano-receptor system is well developed prior to hatching only in halibut. Serotonin and catecholamines appear to be among the first neural messengers in the brain, as previously indicated in other teleosts. Variations in the relative timing of differentiated sensory/brain systems are suggested to reflect species specific adaptations to different environmental life conditions.

(Supported by Norwegian Research Council and TMR Large Scale facility)

NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS—BDNF AND NT-4 I

396.1

BDNF DELETION ALTERS GUSTATORY PAPILLA AND TASTE BUD SIZE AND NUMBER IN POSTNATAL MOUSE.

C.M. Mistretta*[1], K. Goosens[1], L. Farinas[2] and L.F. Reichardt[2]. [1]School of Dentistry, Univ. of Michigan, Ann Arbor, MI 48109 and [2]Howard Hughes Med. Center, Univ. of California, San Francisco, CA 94143.

Although gustatory papillae do not depend on sensory innervation for induction and early development, subsequent papilla growth and sustained taste bud development are neurally dependent. We have examined gustatory papillae and taste buds in postnatal BDNF null mutant homozygotes to learn if associated deficits in taste neuron populations affect the peripheral taste system. The geniculate (innervates taste buds in fungiform papillae), trigeminal (innervates nontaste epithelium in fungiform papillae) and petrosal (innervates circumvallate papilla and resident taste buds) ganglia are reduced in volume by 40, 16 and 44%, respectively, in these animals. Tongues were dissected from homozygote and wild type mice at postnatal days 15 and 25. Fungiform and circumvallate papillae were stained with hematoxylin and eosin and measures were made of the number and diameter of papillae and taste buds. Fungiform papillae were reduced in number by about 50% on the anterior quarter of tongues in P25 BDNF -/- mice compared to +/+ tongues. Although each papilla in both null and wild type mice contained a taste bud, the buds were about 36% smaller and papillae were about 40% smaller in -/- tongues. The single circumvallate papilla at P25 was 46% smaller in -/- tongues compared to +/+. Also, total number of taste buds was reduced by about 60% and taste buds were 50% smaller in BDNF null circumvallate papilla. The data demonstrate quantitative relations among the reduction in gustatory ganglia and decreased gustatory organ size and receptor number, and support a role for BDNF in taste organ development. Supported by NIDCD, NIH Grant DC00456 to CMM.

396.2

THE NEUROTROPHIC SUPPORT OF THE DEVELOPING EAR: A COMPARISON OF BDNF/NT-3 WITH TRKB/C KNOCKOUT MICE.

B. Fritzsch*, #I.Silos-Santiago, @L. Bianchi, $I. Farinas, $L. Reichardt, @J. Conover, @R.M. Lindsay and #M. Barbacid. Creighton University, Omaha, NE 68178, # BMS, Princeton, NJ, @Regeneron, Tarrytown, NY, $UCSF, San Francisco, CA.

Recent years showed that the developing sensory cells of the ear produce two neurotrophins, BDNF and NT-3, which support the innervating ganglion cells via two receptors, TrkB and TrkC. Double mutants of both neurotrophins and their receptors show complete loss of all innervation but normal development of hair cells at least until P0. Apparent discrepancies in different studies have prompted us to analyze all four mutants employing the same techniques, DiI labeling, SEM and TEM. BDNF/*trk*B ko mice lose innervation of semicircular canals, reduce utricular and saccular innervation and reduce innevation to outer hair cells in the apex of the cochlea. In contrast, NT-3/*trk*C ko mice show a pronounced qualitative effect on the cochlea with a reduction (*trk*C) or complete loss (NT-3) of ganglia near the base. Quantitative comparison of the cochlea shows the least effect in BDNF ko mice, intermediate effect in *trk*B and *trk*C ko mice and the most pronounced effect in NT-3 ko mice. Surviving cochlear ganglion cells expand to cover a larger area of the cochlea than normal. Electron micrographs show innervation of the outer hair cells by both afferents and efferents in *trk*B ko mice at the base. In contrast, NT-3 ko mice retain this innervation at the apex. We propose a basal (NT-3/*trk*C) to apical (BDNF/*trk*B) gradient of dependency for spiral ganglion cells.

Supported by NIH DC00215-12.

396.3

BDNF-, BUT NOT NT4-KNOCKOUT MICE ARE DEFICIENT IN DOPAMINERGIC VISCERAL SENSORY NEURONS AND DISPLAY SEVERE DEVELOPMENTAL DEFICITS IN CONTROL OF BREATHING.

J.T. Erickson*[1], J.C. Conover[2], V. Borday[3], J. Champagnat[3], and D.M. Katz[1]. [1]Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106, [2]Regeneron Pharmaceuticals, Inc., Tarrytown, NY and [3]Institut Alfred Fessard, 91198 Gif-Sur-Yvette Cedex, France.

Brain-Derived Neurotrophic Factor (BDNF) and Neurotrophin-4 (NT4) support survival of predominantly separate populations of visceral sensory neurons (Conover *et al.*, Nature 375:235.1995). In particular, a subset of dopaminergic afferents thought to be important for controlling ventilation is depleted only in BDNF-deficient animals (Erickson *et al.*, Soc. Neurosci. Abst., 1995). We therefore postulated that loss of these neurons would lead to perturbations of respiratory control in BDNF-deficient (BD) mice. To test this possibility, we compared resting ventilation in newborn wild type (WT) and BD mice, using whole body plethysmography. Minute ventilation (V_E) in BD mice was decreased by 50-70% compared to WT controls. Moreover, BD mice displayed a two-fold increase in breath-to-breath variability of tidal volume and V_E, and long periods of apnea. In contrast, resting ventilation was not depressed in newborn NT4-deficient mice. Hyperoxia (100% O_2), which in normal animals decreases activity in the peripheral chemoreceptors and thereby depresses ventilation, decreased V_E by 30% in WT animals but did not change V_E during resting breathing in BD mice. Hypoxia (12% O_2), on the other hand, elicited a 27% greater ventilatory depression in BD animals than in WT controls. These data indicate that BDNF, but not NT4, is required for expression of normal breathing behavior in newborn animals. The deficits in BD mice appear to reflect diminished chemoafferent drive, consistent with the loss of chemoafferent neurons in these animals. Thus, despite the fact that BDNF and NT4 are equivalent in supporting sensory neuron survival *in vitro*, neuronal dependencies *in vivo* indicate distinct physiologic roles for the two TrkB ligands. Supported by HL-42131 (DMK) and the Francis Families Foundation (JTE).

396.4

LACKING AND MALFORMED GUSTATORY PAPILLAE IN BDNF KNOCKOUTS.

P. Ernfors, C. A. Nosrat, J. Blomlöf, M. Metsis*, S. Lindskog, L. Olson. Dept. of Medical Biochemistry and Biophysics, Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden; Dept. of Oral Diagnostics, Div. of Oral Pathology, Karolinska Institute, S-141 04 Huddinge, Sweden.

In our prior studies, we reported that neurotrophin mRNA species are expressed in the developing and adult lingual sensogustatory system of rats. BDNF mRNA is exclusively present in the developing gustatory epithelium and as the animals become older the labeling is found in a fraction of taste cells within the taste buds. In the present study, we have evaluated the morphological appearance of the tongue in normal and BDNF null mutated mice using scanning electron microscopy (SEM). Further, although still in preparation, the possible innervation changes in the peripheral gustatory apparatus of normal versus knockout mice were evaluated. We observed striking morphological differences in the knockouts compared to the normal animals. The circumvallate papilla showed a much higher keratinization tendency and was barely visible. The fungiform papillae were of smaller size and some of them were lacking a visible taste pore. Remarkably, no foliate papillae could be found in SEM although immunohistochemistry revealed a few taste buds surrounding a short lumen in the area where foliate papillae were to be expected. Protein gene product 9.5 immunoreactive fibers and cells could be observed in taste buds of all the gustatory papillae, although the immunoreactive cells had a somewhat deranged morphology.

Based on the morphological differences between normal and knockout animals we suggest that BDNF, apart from its classical role as a neurotrophic factor, is also involved in the initiation and maintenance of normal morphology in these papillae either by acting directly and/or through attraction and maintenance of proper gustatory innervation. Disappearance of taste pores in some fungiform papillae in BDNF-/- mice is also indicative of loss of function. It is hypothetized that NT3 expression in fungiform papillae of BDNF -/- mice might offer partial papillary and taste bud rescue.

This study was supported by the Dental Faculty of the Karolinska Institute and Svenska Tandläkare-Sällskapet, Stockholm, Sweden.

396.5

NEUROTROPHIN-4 (NT-4) IS REQUIRED FOR THE DEVELOPMENT OF A SUBCLASS OF CUTANEOUS SENSORY NEURONS C.L. Stucky*, M. Koltzenburg, T. Dechiara#, R.M. Lindsay# & G.D. Yancopoulos# *Dept. of Neurology, University of Würzburg, D-97080 Würzburg, Germany & #Regeneron Pharmaceuticals, Tarrytown, NY*

NT-4 is a member of the neurotrophin family which is important for the development, survival and function of sensory neurons. Here, we used standard neurophysiological techniques to study the properties of single afferent fibers innervating hairy skin in adult transgenic mice lacking NT-4 (NT-4 -/-) or wildtype (NT-4 +/+) controls. Neurons were characterized *in vitro* by their conduction velocity and response properties to controlled mechanical or thermal stimuli. Large myelinated fibers were either slowly adapting (SA) mechanoreceptors, which respond tonically to a sustained mechanical stimulus, or rapidly adapting (RA) mechanoreceptors, which respond only at the on- or offset of a stimulus. There was no difference in the percentages of SA and RA fibers in NT-4-/- (SA = 49%; RA = 51%; n = 70) or NT-4 +/+ (SA = 46%, RA = 54%; n = 83) mice. In contrast to transgenic mice lacking BDNF, in which SA fibers display markedly reduced mechanical sensitivity (Koltzenburg et al., *Soc. Neurosci. Abstr.* 21, 1054), there was no difference in the stimulus-response functions of SA or RA receptors in NT-4 -/- and NT-4 +/+ mice. Thin myelinated fibers were either sensitive D-hair receptors, which supply hair follicles, or A-fiber mechanonociceptors (AM). Whereas D-hair receptors make up 34% (n=91) of thin myelinated fibers in NT-4 +/+ mice, they are almost completely absent in mice lacking NT-4 (4%, n=52). Concomittantly, the percentage of AM fibers increased to 96%. The receptive properties of unmyelinated nociceptive afferent fibers (n=39) were not different in either mice strain. Thus, NT-4 is specifically required for the development of D-hair receptors, but not for the survival or function of other types of cutaneous mechanosensitive neurons. (Supported by the DFG, SFB 353).

396.6

BDNF Prevents and Reverses Adult Rat Motor Neuron Degeneration and Induces Axonal Outgrowth. A. Kishino, Y. Ishige, T. Tatsuno , C. Nakayama* and H. Noguchi. Sumitomo Pharmaceuticals Research Center 1-98, Kasugadenaka 3-chome, Konahana-ku, Osaka 554, Japan

To assess and foresee the therapeutic potential of brain-derived neurotrophic factor (BDNF) in clinics, the effects of BDNF on adult motor neuron were extensively investigated using rat spinal root avulsion model. Intrathecal administration of BDNF immediately after the spinal root avulsion greatly protected the motor neuron cell death. BDNF also showed a protective effect on the reduction of transmitter-related enzymes (ChAT and AChE) and on the atrophy of soma size. Very interestingly, BDNF induced axonal outgrowth of severely damaged motor neurons at the avulsion site. The BDNF administration following 2 weeks-treatment with PBS after avulsion terminated the further deterioration of the cell death and relatively restored the cholinergic transmitter-related enzyme deficiency. BDNF was demonstrated to possess a wide variety of biological effects on survival, soma size, cholinergic enzymes and axonal outgrowth of adult motor neurons. These results give a rationale for BDNF treatment against motor neuron diseases such as spinal cord injury and amyotrophic lateral sclerosis.

396.7

Peripheral application of NT-4 increases excitability and decreases somal size of gastrocnemius motoneurons in the adult rat. M. Gonzalez* and W.F. Collins, III. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Recent findings indicate that the muscle-derived neurotrophins, NT-4 and BDNF, are important in the maintenance of adult motoneuron (MN) properties (Gonzalez and Collins, '95; Munson, Shelton and McMahon, '95). The present study was undertaken to characterize further the influence of NT-4 on adult MNs.

Male Sprague-Dawley rats (300-450 g) were treated for 14 days with NT-4 (6.24 µg/day, 6 rats) or bovine serum albumin (BSA, .108 mg/day, 6 rats) applied locally to the left medial gastrocnemius (MG) muscle via miniosmotic pumps (Alzet, model 2002). The rats were then prepared for in vivo intracellular recording and electrical properties of MG MNs in the NT-4 (n=26) and BSA-treated (n=36) rats were measured. In a parallel study, the left MG muscle was injected with cholera toxin B (CTB) on day 11 of NT-4 or BSA treatment. On day 14, the rats were sacrificed. The spinal cords were processed to visualize CTB, and somal cross sectional areas of randomly selected CTB-labeled MG MNs (30 MNs x 6 rats / treatment group) were measured. Data were analyzed using ANOVAs.

MG MN rheobase, total cell capacitance, equalizing time constant and somal cross sectional area were significantly reduced in NT-4-treated, compared to BSA-treated, rats. In addition, an increase in conduction velocity and a decrease in membrane time constant were noted in NT-4-treated rats but were not statistically significant. No significant treatment effect was observed in MN resistance, after-hyperpolarization amplitude and duration, membrane potential sag, action potential height, and electrotonic length.

These results suggest that MN properties, particularly excitability and size, are regulated by muscle-derived NT-4. Furthermore, this regulation likely involves specific effects on MN dendrites and ionic conductances. Supported by grants from NIH and NSF. NT-4 was generously provided by Regeneron Pharmaceuticals, Inc.

396.8

LOCAL APPLICATION OF NT-4/5 TO THE PROXIMAL CUT END OF A NERVE LIMITS THE DEVELOPMENT OF FULL BLOWN AXOTOMY RESPONSE. L.M. Mendell*[1], R.D. Johnson[2] and J.B. Munson[2] Dept Neurobiol. and Behav., SUNY-Stony Brook[1]., Dept. Neurosci., Univ. Florida[2].

In previous studies the decline in conduction velocity of axotomized motoneurons in the adult rat has been shown to be largely attenuated by application of the *trk*B ligand, NT-4/5 (Munson et al., Soc. Neurosci. Abstr., 1995). The present experiments were designed to extend these studies into a different species (the cat) in order to test the generality of the finding and to apply more extensive electrophysiological analysis of the cellular and synaptic changes associated with axotomy (Mendell et al., J. Neurophysiol., 1995). NT-4/5 was applied at a rate of 60µg/day to the cut end of the medial gastrocnemius nerve for 4 weeks in 3 cats. Controls were 2 cats, one treated similarly with saline, the other axotomized for 5 weeks with no treatment. Findings after NT-4/5 include a population of cells (about 15%) whose values of rheobase were higher than any in the axotomized control population and some cells with unusually high axonal conduction velocities. The range of AHP values was less compressed after NT-4/5 than in the control group, which exhibited the compression in AHP range seen normally after axotomy. The amplitude of monosynaptic Ia EPSPs was relatively unchanged after NT-4/5 although many EPSPs exhibited positive amplitude modulation during high frequency stimulation (32 shocks at 167 Hz), not seen in axotomized controls. Overall, these data point to an effect of NT-4/5 in preventing axotomized cells from fully expressing changes characteristic of axotomy although with the current protocol the effect is relatively limited. Supported by NIH. NT-4/5 courtesy of Genentech, Inc.

396.9

RECIPROCAL EFFECTS OF BDNF/*TRK*B AND NT-3/*TRK*C NEUROTROPHIN SYSTEMS ON THE DEVELOPMENT OF THE MONOSYNAPTIC EPSP IN MOTONEURONS OF THE NEONATAL RAT. B. S. Seebach* and L. M. Mendell, Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

From postnatal day (PND) 0-8, motoneurons become larger but monosynaptic EPSPs remain about the same size suggesting an increase in synaptic efficacy. Previously, we demonstrated that systemic administration of BDNF (PND 0,2,4,6) results in reduction of EPSP amplitude at PND 8, using dorsal root- evoked monosynaptic EPSPs recorded intracellularly in motoneurons in the *in vitro* hemisected rat spinal cord preparation (Soc. For Neurosci. Abstr., 1995). In the present studies similar administration of NT-3 had the opposite effect, i.e., increase in EPSP amplitude. Antagonists to both neurotrophins (fusion molecules *trk*B/IgG and *trk*C/IgG) resulted in effects that were opposite to the neurotrophins themselves (increase and decrease in EPSP amplitude, respectively). The same reciprocity was not observed for changes in motoneuron properties. Three of the 4 treatments led to similar relatively small increases in rheobase and decreases in motoneuron resistance; only NT-3 had no effect on motoneuron properties.

These results indicate that neurotrophins have a complex effect on the development of the monosynaptic reflex, more complex than their effect in determining survival of afferent classes such as muscle spindle afferents which depend virtually completely on the availability of NT-3. The BDNF- and *trk*C/IgG-induced EPSP amplitude decreases may have been influenced by increased motoneuron size. However, the increase in EPSP amplitude was uniformly larger than that predicted by the decrease in motoneuron size suggesting a direct effect on synaptic input even in these cases. However, the nature of this influence (structural or functional) remains to be elucidated.

Supported by NIH. Neurotrophins courtesy of Regeneron Pharmaceuticals.

396.10

Neuroprotection of CNS Neurons by Retrograde Transport of Neurotrophic Factor Genes with Adenoviral Vectors

Baumgartner, BJ* and Shine, HD Department of Neurosurgery, Baylor College of Medicine, Houston, Texas 77030

Replication-defective adenoviral vectors were constructed that carried genes for neurotrophic factors (Adv.RSV-*nf*) and included genes for BDNF, CNTF, GDNF, and NGF. Cultured cells transduced with Adv.RSV-*nf* expressed biologically active neurotrophic factors *in vitro*. *In vivo*, the Adv.RSV-*nf* were retrogradely transported to motoneurons of the facial nucleus or spinal motoneurons and expressed the gene products as determined by RT-PCR and immunocytochemical analysis. Several reports have shown that the application of CNTF, BDNF, or GDNF to the transected facial nerve of neonatal rats protects a portion of the facial motoneurons from axotomy-induced death. To test whether the same effects would be produced by retrograde transport and expression of Adv.RSV-*nf* genes, we injected Adv.RSV-*nf* into the muscles of the terminal field of the facial nerve of neonatal (1 day-old) rats. Two days later the facial nerve was cut and 7 days after nerve transection the surviving neurons of the facial nuclei were counted and compared to the untreated nuclei on the contralateral side. Treatment with Adv.RSV-GDNF produced a robust survival effect (53.3 ± 6.5%) and treatment with Adv.RSV-CNTF (18.2 ± 4.4%) or Adv.RSV-BDNF (16.5 ± 3.6%) produced slight but statistically significant survival effects. Protection by Adv.RSV-NGF (11.4 ± 4.7%) was not significantly different from vehicle (5.7 ± 2.1%) or Adv.RSV-βgal (7.9 ± 5.1%) controls.The retrograde transport and expression of neurotrophic factor genes may therefore be a useful strategy to protect neurons of the CNS from trauma. A portion of this work was supported by a grant to H.D.S. from the Texas Higher Education Coordinating Board Advanced Technology Program.

396.11

NEUROTROPHIC FACTOR PROTECTION AGAINST OXIDATIVE STRESS D. Hornfeld, N. Déglon, P. Aebischer, and A.D. Zurn*. Gene Therapy Center and Surgical Research Division, University Medical School, 1011 Lausanne, Switzerland.

Normal cellular metabolism produces reactive oxygen species which are neutralized within cells by antioxidant enzymes (glutathione peroxidase, superoxide dismutase, catalase) and other antioxidants. An imbalance between oxidants and antioxidants may be responsible for neuronal degeneration in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). The purpose of the present work was to evaluate whether brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and ciliary neurotrophic factor (CNTF), which have been shown to promote survival and/or differentiation of motoneurons and dopaminergic neurons *in vitro* and decrease axotomy-induced degeneration of these cells in animal models of ALS and PD, can protect cultured spinal motoneurons and mesencephalic dopaminergic neurons against oxidative stress induced by hydrogen peroxide. We demonstrate that CNTF, but not GDNF, can protect motoneurons against peroxide toxicity. BDNF has only a partial protective effect. In contrast, both BDNF and GDNF protect human neuroblastoma cells against oxidative stress. We are currently evaluating whether this protective action is mediated via an increase in the expression of antioxidant enzymes.

Supported by the Swiss National Science Foundation.

396.12

REGULATION OF QUANTAL SECRETION BY NEUROTROPHIC FACTORS AT DEVELOPING MOTONEURONS. W.M. Fu* and J.C. Liou. Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan.

Neurotrophic factors derived from postsynaptic muscle cells may play important roles in the development and maintenance of presynaptic neuronal functions. In 3-d old *Xenopus* nerve-muscle cultures, embryonic spinal neurons that had made natural contact with co-cultured myocytes exhibited spontaneous release of larger packets of ACh quanta than those released by isolated neurons not in contact with any myocyte. However, neurons which made natural contact with fibroblast did not show such an increase in quantal size. Our previous study has shown that activity-dependently released retrograde factor NT-3 from muscle target enhances the maturation and/or maintenance of the presynaptic nerve terminal in the developing *Xenopus* nerve-muscle co-cultures. We further investigated the effect of other neurotrophic factors. Chronic treatment of native neurons for 2 days with neurotrophin-4, brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF) or glial cell line-derived neurotrophic factor (GDNF) but not basic fibroblast growth factor, insulin-like growth factor-1 increased the average size of quantal ACh packest at newly formed nerve-muscle synapse. BDNF and CNTF, which act via different mechanisms showed additive effect on the increase of quantal size. Furthermore, co-treatment of the culture with d-Tc and neurotrophic factor of BDNF, CNTF or GDNF partially reversed the inhibitory effect of d-Tc on the quantal size. These results suggest that there is target specificity for the maintenance of synaptic function and many kinds of neurotrophic factors may participate in the maintenance of normal transmitter packets in developing neurons.

396.13

AUTOCRINE BDNF SECRETION ENHANCES THE SURVIVAL AND SEROTONERGIC DIFFERENTIATION OF GRAFTED RAPHE NEURONAL PRECURSOR CELLS AND LUMBAR TRANSPLANTS ALLEVIATE CHRONIC NEUROPATHIC PAIN.

M.J. Eaton[*1,2], D.I. Santiago[1], H.A. Dancausse[1] and S.R. Whittemore[1-3].
The Miami Project[1], Departments of Neurological Surgery[2] and Physiology & Biophysics[3], University of Miami School of Medicine, Miami, FL 33136.

We have utilized RN46A cells, an immortalized serotonergic neuronal cell line derived from the E13 raphe (J.Neurosci. 14:6744,1994), as a model for transplant of serotonergic cells. RN46A cells require BDNF for increased survival and serotonin (5HT) synthesis in vitro and in vivo (Dev. Biol. 170:169, 1995). RN46A cells were transfected with the gene for rat BDNF, and the autocrine BDNF-secreting cell line 46A-B14, was subcloned. After transplantation into the cerebral cortex and hippocampus, 46A-B14 cells survived longer than 2 weeks, differentiated with a complex morphology, and were able to synthesize 5HT. Sciatic nerve constriction (SNC) was then used to induce chronic neuropathic pain in adult rats. Mechanical and thermal allodynia and hyperalgesia were assessed after SNC and SNC followed by transplants of 46A-B14 cells into the subarachnoid space of the lumbar spinal cord. 46A-B14 cells survived up to 6 weeks around the spinal cord after transplantation and immunohistochemically stained for 5HT. Transplants of 46A-B14 cells alleviated mechanical and thermal allodynia, and thermal hyperalgesia induced by the SNC. Transplants of RN46A cells transfected with the vector alone, which did not synthesize 5HT, had no effect on chronic neuropathic pain. Collectively, these results suggest autocrine BDNF secretion upregulates differentiation and the serotonergic phenotype in vivo and 5HT delivered near the dorsal horn after peripheral nerve injury can offer a potential treatment modality for chronic neuropathic pain. This work was supported by The Miami Project To Cure Paralysis, General Reinsurance, NS26887 (SRW), and SCRF1524-01 (MJE).

396.14

CNTF INDUCES RAPHE NEURONAL PRECURSORS TO SWITCH FROM A SEROTONERGIC TO A CHOLINERGIC PHENOTYPE IN VITRO.

S.R.Whittemore[*1-3], M.J.Eaton[1,2], P.Mather[4], R.M.Lindsay[4], and J.S.Rudge[4].
The Miami Project[1], Departments of Neurological Surgery[2] and Physiology & Biophysics[3], University of Miami School of Medicine, Miami, FL 33136 and Regeneron Pharmaceuticals[4], Tarrytown,NY 10591

Ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) specifically reduced the number of serotonergic neurons by 90% in E14 raphe culture without affecting the total number of neurons. This effect was reversible, as the number of 5HT-positive neurons increased after removal of CNTF from the culture medium. In the immortalized serotonergic neuronal cell line derived from E13 raphe (RN46A; J.Neurosci. 14:6744, 1994), which is induced to become serotonergic by brain-derived neurotrophic factor (BDNF) (Dev. Biol. 170:169, 1995), the addition of CNTF suppressed tryptophan hydroxylase (TPH) and 5HT synthesis and increased choline acetyltransferase (ChAT) expression by 6-fold and ChAT activity by 20-fold over 12 days. Removal and replacement of CNTF with BDNF after 4 days resulted in a partial restitution of 5HT expression. Other members of the CNTF-cytokine family that use gp130 and/or LIF receptor β as their signal transducing receptors-LIF, oncostatin M, IL6, and IL11, had similar effects on increasing ChAT activity and reducing 5HT expression in RN46A cells. Analysis of 5HT levels in CNTFRα knockout mice at birth showed no difference compared to wild type mice suggesting that the potential to switch phenotype mediated through CNTFRα is a latent property of neuroepithelial precursors in the raphe nucleus. This work was supported, in part, by The Miami Project To Cure Paralysis, General Reinsurance, NS26887 (SRW) and the Spinal Cord Research Foundation of the Paralyzed Veterans of America (MJE).

396.15

THE EFFECTS OF BDNF ON c-FOS AND NOS EXPRESSION IN DORSAL HORN NEURONES OF THE ADULT RAT SPINAL CORD. D.L.H. Bennett*, J.S. French, J.V. Priestley and S.B. McMahon, Dept. Physiol., UMDS, London, UK.

We have investigated the effects of BDNF (which is expressed in primary afferent neurones) on dorsal horn neurones of the spinal cord. To investigate its effects on c-fos expression, saline or BDNF (50 µg, human recombinant, gift of Regeneron) were administered to the spinal cord via intrathecal cannulae and the animals sacrificed after 3 hrs. BDNF produced a significant increase in the number of dorsal horn neurones expressing c-fos (703±97 and 227±48 cells/section for BDNF and saline treatment respectively, $p<0.05$ unpaired t-test) and this difference was most marked in superficial dorsal horn cells.

Another group of animals received injections of untreated or BDNF coated fluorescent latex microsphere into the dorsal horn (1µl), NOS expression was studied 5 days later using immunohistochemistry. Fluorescent microspheres labelled dorsal horn neurones both rostral and caudal to the injection site. BDNF coated bead injections doubled the percentage of labelled cells expressing NOS compared to control bead injections ($p<0.05$, unpaired t-test). The NOS labelled cells also showed an increase in mean cell size following BDNF treatment.

These findings demonstrate that BDNF may have both rapid and more prolonged effects on gene expression in dorsal horn neurones, which could have an impact on neuronal signalling within the spinal cord. This may be of relevance to conditions such as axotomy and inflammation when there is an increase in BDNF expression in DRG cells. Supported by the MRC of Great Britain.

396.16

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) INCREASES OPIOID RECEPTOR mRNA EXPRESSION IN THE PAG. J. A. Siuciak*, D. Lewis, R. M. Lindsay, F. G. Williams[2], Regeneron Pharmaceuticals,Inc., Tarrytown, NY 10591 and [2] Dept. of Vet. Biol., Univ. of Minn., St. Paul, MN 55108

Previous studies have demonstrated that midbrain infusion of BDNF, a member of the NGF/neurotrophin family, produced analgesia and increased beta-endorphin levels within the brain and spinal cord (Siuciak et al., 1995). The present study investigated the regulation of mu, delta and kappa opioid receptors in the rat brain after chronic BDNF administration. Adult rats were infused with BDNF (12 ug/day) or PBS into the midbrain, near the PAG/dorsal raphe (Siuciak et al., 1994). Image analysis was performed on autoradiograms containing either areas of the ventromedial and ventrolateral PAG adjacent to the infusion site or the striatum. Comparison of the levels of mRNA for opioid receptors in PBS and BDNF-treated rats showed BDNF infusion produced significant increases in delta and kappa opioid receptor mRNA levels in the PAG near the infusion site, with delta and kappa riboprobe hybridization signal increased by 39% and 56%, respectively. BDNF administration did not lead to any statistically significant changes in the level of mu opioid receptor mRNA within the PAG. No significant changes in riboprobe hybridization signal were found for either opioid receptor within the striatum. These results suggest that BDNF induces a selective, local upregulation of delta and kappa opioid receptors in the midbrain. Supported by Regeneron Pharm. and NS 28016 (FGW).

396.17

CORRELATION OF BDNF EXPRESSION DEFICIT AND DELAYED GRANULE CELL MIGRATION IN THE CEREBELLUM OF *STARGAZER* MUTANT MOUSE X. Qiao*, F. Hefti, and B. Knusel Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

We have discovered a selective failure of BDNF mRNA expression in the cerebellar granule cells of a spontaneous ataxic mutant mouse, *stargazer* (*stg*, chr. 15). To test the effect of BDNF on the cerebellar granule cell during the development, we examined the migration and synaptic formation of the granule cells in the *stg* mouse. At postnatal day 15 (P15), a clear external layer of granule cells was found in the *stg* cerebellum in comparison with a few scattered cells in the same region of control mouse. Quantitative cell count (n=3 of each genotype) have shown significantly more external granule cells (+126%, $p < 0.01$) in the mutant cerebellum than the corresponding control. This external granule cell layer had completely disappeared in P20 *stg* mouse. Although the migration of granule cells is delayed in the mutants, cerebellar ultrastructure was grossly normal in the adult animal at electron microscopic level. Synapses in the glomeruli and the molecular layer of the granule cells were identified in the *stg* mouse with no significant difference to the wildtype control mice. The only noticeable changes were that some of the granule cells displayed oval shaped nuclei and were studded with clumps of coarse chromatin, resembling migrating immature granule cells. These results indicate that lack of BDNF expression in granule cells may underlie delayed migration of these neurons, but does not affect gross synaptic formation of their input and output connections.

These studies were supported by research grants from NIA AG09793, AG10480, NINDS NS22933, Sankyo Co., Ltd, and NIH F32 NS09985 (XQ).

396.18

NEUROTROPHINS PROTECT AGAINST CYTOSINE ARABINOSIDE-INDUCED APOPTOSIS OF CULTURED NEURONS. P. Leeds, X.-M. Gao*, and D.-M. Chuang. Section on Molecular Neurobiology, Biological Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892.

The mechanism of neurotrophin inhibition of apoptosis was investigated using immature cultured cerebellar granule cells (CGC) from 8-day-old rats and hippocampal cells from 19-gestation-day fetal rats. Cell death was confirmed by morphological inspection and quantified by MTT assays 24 hrs after the induction of apoptosis which was by treatment with cytosine arabinoside (AraC) (500 μM), a pyrimidine metabolite used in the treatment of leukemia. AraC-induced apoptosis was most robust when added to CGC on the day of culture, causing up to 75% cell death. A dose-dependent (50-500 μM) apoptotic induction, up to 50% cell death, was observed when added 24 hrs after plating. Additions made after 48 hrs had no apoptotic effect across the dose range. CGC were also treated with Brain Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3), or Neurotrophin-4/5 (NT-4/5) 2 hrs after plating, followed 24 hrs later with AraC. BDNF and NT-4/5, but not NT-3, reversed AraC-induced apoptosis at 0.5-1 ng/ml concentrations. Only higher concentrations, 50 ng/ml, of NT-4/5 prevented AraC-induced apoptosis in hippocampal cultures. Neither the protein kinase C (PKC) inhibitor calphostin C (10-50 ng/ml), nor the tyrosine kinase inhibitor genestein (1-10 μM) prevented neurotrophin protection. Furthermore phorbol myristate acetate (1 nM-1 μM), a PKC activator, failed to influence either AraC-induced apoptosis or neurotrophin-mediated protection. These results indicate that neither the PKC nor the tyrosine kinase pathway is implicated in neurotrophin protection against AraC-induced apoptosis. Supported by NIMH, NIH.

NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS—BDNF AND NT-4 II

397.1

EXPRESSION OF THE LOW AFFINITY NEUROTROPHIN RECEPTOR BY SUBEPENDYMAL ZONE PROGENITOR CELLS IN ADULT RAT BRAIN: EFFECTS OF NEUROTROPHIN ADMINISTRATION. S.J. Wiegand*, N. Cai, P. Ge, T. Zigova†, K.D. Anderson, M.B. Luskin† and R.M. Lindsay. Regeneron Pharmaceuticals, Inc. Tarrytown, NY 10591 and †Dept. Anat. and Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA 30322

Cells which express the p75 low affinity neurotrophin receptor are distributed within discrete regions of the subependymal zone (SEZ) of adult rat forebrain, in a pattern which corresponds precisely to that of constituitively proliferating neural progenitor cells as determined by BrdU incorporation. The expression of p75 is bilaterally symmetrical and restricted to the cells of the SEZ; neither ependymal cells nor differentiated neurons and glia in the surrounding parenchyma are p75-positive. Infusion of BDNF or NT-4 into the lateral ventricle results in an increase in the number of p75-positive cells within the ipsilateral SEZ. In addition, numerous p75-immunopositive cells become apparent in the immediately adjacent neural parenchyma (septum and striatum). These cells are morphologically heterogeneous, but typically much more complex than those within the SEZ proper, having extensively branched processes and the general appearance of mature neurons or glia. In neurotrophin-treated animals, p75-positive cells with differentiated phenotypes are found most frequently in association with the rostral part of the SEZ. Changes in the pattern of p75 immunostaining are evident as early as 5 days following the initiation of neurotrophin treatment, and are maintained for at least 14 days. These effects of BDNF and NT-4 appear to be mediated via TrkB, as infusions of NGF or NT-3 do not produce similar cytological changes in the SEZ. Double-labeling experiments are in progress to determine definitively the phenotypes of p75 and BrdU-positive cells in BDNF-treated animals.

This work was supported by Regeneron Pharmaceuticals, Inc.

397.2

BDNF INCREASES SURVIVAL OF FETAL MESENCEPHALIC DOPAMINERGIC NEURONS AND ENHANCES THEIR RECOVERY AFTER TREATMENT WITH 6-HYDROXYDOPAMINE M. Cik, A. S. Lesage, M. De Ryck* and J. E. Leysen. Janssen Research Foundation, Turnhoutseweg 33, 2340 Beerse, Belgium.

Mesencephalic dopaminergic neurons (MDNs) have been widely used to study the effects of brain-derived neurotrophic factor (BDNF). MDNs are the target of neuronal degeneration in Parkinson's disease. Primary culture of fetal MDNs are used as an experimental model for neuronal degeneration caused by 6-hydroxydopamine (6-OHDA) and the drug-derived toxin 1-methyl-4-phenylpyridinium (MPP^+). It has been reported that BDNF attenuate toxicity of both, 6-OHDA and MPP^+. It has also been shown that 6-OHDA can not be considered as a selective neurotoxin for catecholaminergic neurons *in vitro*. Here we show that exposure of rat MDNs in primary culture to BDNF (50 ng/ml) for 6 days increases survival of dopaminergic neurons by 1.5 fold, as measured by [^{3}H]DA uptake. Exposure to 6-OHDA at DIV 6 for 24 h destroyed all types of neurons in a concentration dependent manner (EC_{50} 48 μM) as revealed by Calcein-AM measurement. A concentration dependent reduction of [^{3}H]DA uptake was also seen (IC_{50} 27 μM). Pretreatment of MDNs with BDNF for 6 days did not affect 6-OHDA toxicity, nor did it affect the 6-OHDA mediated reduction in [^{3}H]DA uptake (IC_{50} value of 6-OHDA 28 μM). In contrast, when at the end of the toxic treatment the medium was changed to the toxin- and BDNF-free medium and the cells were allowed to recover for 3 days, BDNF treated cultures were protected from continuation of cell death and showed better recovery. Under these conditions, [^{3}H]DA uptake was not different from control level. Our results suggest that BDNF increases survival of dopaminergic neurons in culture and stimulates their recovery after exposure to 6-OHDA. This is in agreement with the recent finding that BDNF protection against MPP^+ toxicity is seen after a recovery period, rather than at the end of the toxic treatment as previously published.

397.3

MIDBRAIN DOPAMINE NEURONS REQUIRE GROWTH FACTORS AND ADDITIONAL EXTRACELLULAR SIGNALS FOR OPTIMAL SURVIVAL. J. Engele* and B. Franke. Dept. Anatomy and Cell Biology, University of Ulm, 89069 Ulm, Germany.

By using growth factor-induced *c-fos* expression as a marker for efficient signal-transcription coupling, our previous studies have demonstrated that a large number of midbrain dopaminergic neurons represent the primary targets for brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and neurotrophin-3 (NT-3). These studies further revealed that the effects of these growth factors on dopaminergic cell survival are rather marginal and not additive. Based on these findings, we have speculated that optimal dopaminergic cell survival depends on the presence of additional extracellular signals. To test this hypothesis, the survival-promoting effects of BDNF, GDNF, and NT-3 on dopaminergic neurons were assessed in serum-free dissociated cell cultures of the E15 rat mesencephalon in which second messenger levels were manipulated with dibutyryl cAMP (dbcAMP) or NMDA. dbcAMP (100 μM) significantly potentiated the survival-promoting effects of BDNF (50 ng/ml), GDNF (5 ng/ml), and NT-3 (10 ng/ml) on dopaminergic neurons after 3 and 6 days *in vitro*. A similar potentiation of the survival-promoting activity of BDNF occured in the presence of NMDA (10 μM). dbcAMP or NMDA alone did not significantly affect the number of surviving dopaminergic neurons. Together these findings suggest that the action of growth factors on midbrain dopaminergic neurons are modulated by other extracellular signals. Supported by Deutsche Forschungsgemeinschaft (En 187/2-3; Kn 200/4-1).

397.4

APOPTOSIS AND DOPAMINERGIC NEUROTROPHIC FACTORS (DNTFs) IN RAT MESENCEPHALIC DOPAMINERGIC NEURONAL CULTURES. T. Takeshima[1]*, J.W. Commissiong[2], K.Mishima[1], K. Nakashima[1]. [1]Div. Neurol., Inst. Neurol. Sci., Tottori Univ. Fac. Med., 36-1 Nishimachi Yonago 683, Japan. [2]LMB-NINDS-NIH, Bldg.36/3D02, Bethesda, MD 20892.

We have previously reported (J. Neurosci. 14:4769-4779, 1994) that serum-deprivation accelerates neuronal death of E14 mesencephalic dopaminergic neurons in vitro. Mesencephalic dopaminergic neuronal death in serum-deprived cultures was characterized using DNA electrophoresis and terminal deoxynucleotide transferase (TdT) DNA-break labeling techniques. Non-random DNA fragmentation (laddering pattern) was detected at 12 and 24 hr in vitro. Using the TdT labeling method (ApopTag, Oncor), DNA breaks were detected in situ. At 8 hours in vitro, 40% of mesencephalic cells were positively stained with ApopTag, while no striatal cell was stained with ApopTag at this stage. We concluded that apoptosis is involved in dopaminergic neuronal death accelerated by serum-deprivation. Apoptosis is observed in cultures from whole forebrain or striatum, however, the apoptotic process in mesencephalic neurons progressed faster than in striatal neurons. Dopaminergic neurotrophic factors (DNTFs), which have been reported to increase neuronal survival, were tested in mesencephalic serum-free cultures, and the number of ApopTag positive cells in cultures were quantified to evaluate the protective effect of trophic factors against neuronal apoptosis. Supplementation with fetal calf serum, brain derived neurotrophic factor (BDNF), platelet derived growth factor (PDGF), and glial cell line-derived neurotrophic factor (GDNF) significantly reduced the number of ApopTag positive cells in mesencephalic cultures at 12 hr. Basic fibroblast growth factor (bFGF) had no protective effect in this culture.

397.5

INTRAVENTRICULAR ADMINISTRATION OF TRKB IMMUNOADHESIN DELAYS KINDLING DEVELOPMENT IN THE RAT. D. K. Binder[1]*, T. E. Ryan[2], G. D. Yancopoulos[2] and J. O. McNamara[1]. [1]Epilepsy Research Laboratory, Departments of Medicine, Neurology, and Neurobiology, Duke University Medical Center, Durham, NC 27710, and [2]Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

Kindling is a model of temporal lobe epilepsy in which repeated initially subconvulsive seizures induced by daily electrical stimulation evolve into more intense partial and generalized convulsive motor seizures. Recent studies have shown that neurotrophic factors are upregulated at both mRNA and protein levels following kindling or chemoconvulsant stimulation. For example, both BDNF and trkB mRNAs are increased severalfold following seizure. However, whether this upregulation is functionally related to kindling development is as yet unclear. In this study, we chose to attempt functional blockade of trkB neurotrophin receptor activation throughout kindling development in the rat via chronic ICV administration of trkB immunoadhesin. Rats were implanted with bipolar electrodes in the right amygdala and left hippocampus and an indwelling cannula in the right lateral ventricle that delivered 50 μg/day trkB-IgG (n=4), 50 μg/day control human IgG (n=5) or saline (n=2) via a subcutaneous osmotic minipump (Alza Corp.). Animals were allowed to recover 4 days following surgery and then were stimulated twice daily via the amygdala electrode. While animals receiving saline or 50 μg/day human IgG ICV showed no difference in number of stimulations to the fully kindled state (8.5 ± 1.5 vs. 10.4 ± 0.7 respectively), animals receiving 50 μg/day trkB-IgG ICV required significantly more stimulations to reach the kindled state (20.2 ± 1.3; $p<0.01$ vs. controls). These results suggest that trkB receptor activation may be causally linked to kindling epileptogenesis.

Supported by NIH Grant NS-32334.

397.6

EFFECTS OF IN VIVO BDNF INFUSION ON AMYGDALA KINDLING, SPROUTING, AND HILAR AREA. P. Osehobo[1]*, B. Adams[2], M. Sazgar[1], J. Verdi[2], R. Racine[2], M. Fahnestock[1]. [1]Department of Biomedical Sciences and [2]Department of Psychology, McMaster University, Hamilton, Ontario, Canada; [3]Amgen Institute, Toronto, Ontario, Canada.

Kindling is an animal model of human temporal lobe epilepsy in which epileptogenic reactivity is permanently enhanced by repeated stimulations. This procedure also increases the expression of NGF, BDNF, and BDNF receptor mRNAs in both the hippocampus and cerebral cortex. We have recently shown that intraventricular NGF infusion enhances the development of kindling and increases kindling-induced mossy fiber sprouting in adult rats. In addition, antibodies to NGF retard amygdaloid kindling and reduce kindling-associated mossy fiber sprouting in the stratum oriens of hippocampal area CA3. The physiological role of BDNF in kindling is not as clear as for NGF. BDNF infusion in rats reportedly slows kindling development, whereas transgenic mice heterozygous for a deletion of the BDNF gene also show a marked suppression of kindling. This study attempts to clarify these apparently contradictory roles of BDNF in kindling. Intrahilar infusion of hrBDNF was performed; rats recieving 1 mg/ml (n=9) and 5 mg/ml (n=4) required 16 and 19 stimulations respectively to reach the fully kindled state, compared to control rats (PBS-infused, n=8) that required 12 stimulations. In addition, unlike NGF, BDNF did not protect against kindling-induced decreases in hilar cell density or increases in hilar area measurement. There was also no significant effect on mossy fiber sprouting as measured by Timm stain densitometry.

We conclude that BDNF inhibits kindling but has no effect on kindling-induced mossy fiber sprouting or changes in hilar area. The mechanisms for kindling inhibition in heterozygous BDNF knockouts and for the opposite effects of BDNF and NGF infusions on kindling are not clear. Various means of blocking BDNF activity are currently being explored, and preliminary results will be reported.

Supported by grants from the MRC of Canada and the NCE NeuroScience Network.

397.7

INCREASED CALCIUM CHANNEL CURRENT IN HIPPOCAMPAL NEURONS FOLLOWING TREATMENT WITH BRAIN-DERIVED NEUROTROPHIC FACTOR. M.M. Rose*, P.W. Landfield and N.M. Porter. Department of Pharmacology, College of Medicine, University of Kentucky, Lexington, KY 40536.

Recent studies have shown that necrotic cell death in serum-maintained cortical cultures is exacerbated by pretreatment with brain-derived neurotrophic factor (BDNF) (Koh *et al.*, 1995). The toxic effect of BDNF is accompanied by an increase in neuronal ^{45}Ca uptake suggesting that Ca^{2+} influx via voltage- or ligand-gated channels may play a role in the toxic effect of BDNF. Whole-cell voltage-gated Ca^{2+} currents in cultured basal forebrain neurons maintained in serum-free conditions are unaffected by pretreatment with BDNF (Levine *et al.*, 1995). However, we report here that in serum-maintained cultures of embryonic rat hippocampus, pretreatment with BDNF (100 ng/ml) significantly increased single voltage-gated L-type Ca^{2+} channel activity as assessed by cell-attached patch recording. We also subjected primary hippocampal cultures chronically pretreated with BDNF to partial medium replacement which appears to induce a moderate glutamate-dependent insult (Rosenberg *et al.*, 1991). Cell death was significantly greater in BDNF-pretreated cultures (100 ng/ml) following medium replacement. Taken together, these results suggest that a BDNF-mediated increase in Ca^{2+} channels may in part contribute to recent observations that growth factors can have deleterious effects on neuronal survival depending on the nature of the traumatic insult. *(Supported by NIH AG10836 and the Kentucky Spinal Cord and Head Injury Research Trust)*

397.8

SEIZURES INDUCE BRAIN-DERIVED NEUROTROPHIC FACTOR IN HIPPOCAMPAL MOSSY FIBERS: POSSIBILITY OF ANTEROGRADE TROPHIC SUPPORT. M. A. Smith*[1], L. X. Zhang[1], and L. A. Mamounas[2]. Biological Psychiatry Branch[1], NIMH/NIH, Bethesda, MD 20892, and Molecular Neurobiology Unit[2], NIA/NIH, Baltimore, MD 21224

Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) are assumed to provide trophic support via a classic, target-derived, retrograde mechanism of action. However, recent studies strongly suggest that neurotrophic factors can act in an autocrine fashion and perhaps even in an anterograde direction similar to neurotransmitters. To further explore this hypothesis, we compared the neuroanatomical pattern of BDNF mRNA and protein in response to electroconvulsive seizures (ECS). Using *in situ* hybridization, we found that ECS, given every other day for 2 wk, induced BDNF mRNA markedly and predominantly in the granule neurons of the dentate gyrus. However, immunohistochemistry with a turkey anti-BDNF antibody revealed that ECS increased endogenous BDNF in the mossy fibers, which are composed of axons projecting from the dentate gyrus to the CA3 pyramidal layer of the hippocampus. This suggests that BDNF was transcribed and translated in granule neuron cell bodies but transported in an anterograde direction to CA3 neurons. Interestingly, following ECS, the mossy fiber pathway appeared to be larger and more dense by Timm staining. The exact role of BDNF in the regulation of CA3 hippocampal neurons remains to be determined. However, our results suggest that BDNF support of CA3 pyramidal neurons may be derived in large part from the dentate gyrus.

(L.A.M supported by National Research Council-NIA/NIH award.)

397.9

SHORT- AND LONG-TERM REGULATION OF HIPPOCAMPAL SYNAPTIC TRANSMISSION BY BDNF. N.J. Tang* and D.C. Lo. Department of Neurobiology, Box 3209, Duke University Medical Center, Durham, NC 27710.

Recently, we and others have observed rapid enhancement of synaptic transmission following acute exposure to neurotrophins. However, the long-term effects of neurotrophins on synaptic transmission, and how these might relate to their short-term, neuromodulatory effects, remain unknown. In this study, we compared the effects of BDNF on excitatory synaptic transmission following short-term (minutes) and long-term (days) treatment using hippocampal CA1 neurons in "microdot" culture. In these cultures, individual neurons are confined to grow on isolated glial islands and thus form synapses only upon themselves ("autapses").

Acute application of BDNF to autaptic CA1 neurons induced a 2- to 6-fold increase in the frequency of TTX-insensitive, miniature EPSCs (mEPSCs), but had no effect on the distribution of mEPSC amplitudes. This enhancement was rapid, occurring within twenty minutes of BDNF application, and was stable for the duration of recording (>1 hr). Interestingly, action potential-evoked EPSCs were not affected by acute application of BDNF. In contrast, CA1 neurons treated with BDNF for 8-16 days showed an average 2.1-fold increase in the amplitude of AMPA-type, evoked EPSCs, with no substantial change in average frequency or amplitudes of mEPSCs. These findings indicate that the acute, modulatory effects of neurotrophins can be quite different from their long-term, regulatory effects on synaptic transmission.

Neurotrophins were generously provided by Regeneron Pharmaceuticals; this research was supported by grants from the Alfred P. Sloan Foundation and the NIH (NS32742 and MH11058).

397.10

BRAIN-DERIVED NEUROTROPHIC FACTOR POTENTIATES DENDRITIC Ca^{2+} TRANSIENTS IN CULTURED HIPPOCAMPAL NEURONS. B. Berninger* & H. Thoenen Dept. Neurochemistry, Max Planck Institute for Psychiatry, D-82152 Martinsried, FRG.

Recently, neurotrophins have been shown to modulate transmission at hippocampal synapses involving both pre- and postsynaptic mechanisms. Using fura-2 fluorescence microscopy we investigated the effect of *brain-derived neurotrophic factor* (BDNF) on the occurrence of spontaneous Ca^{2+} transients in dendritic processes of cultured hippocampal neurons. After 3-8 weeks *in vitro* hippocampal neurons showed spontaneous oscillations in intracellular Ca^{2+} ($[Ca^{2+}]_i$). These oscillations were blocked by tetrodotoxin (1 μM) indicating that they originated from endogenous synaptic activity. Within dendritic processes maximal changes in $[Ca^{2+}]_i$ were localized to varicosities, i.e. to presumable sites of synaptic contacts, suggesting that they resulted from Ca^{2+} influx during synaptic events. Within one minute after bath application of BDNF (40 ng/ml), both the frequency and amplitude of spontaneous Ca^{2+} transients in dendritic varicosities increased markedly. The potentiation of Ca^{2+} signals in dendritic varicosities may directly reflect the synaptic enhancement induced by BDNF.

This work was supported by the Max-Planck-Gesellschaft.

397.11

PROTECTIVE EFFECTS OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN TWO MODELS OF EPILEPSY IN THE RAT.

S. Reibel[1], Y. Larmet[2]*, B.T. Lê[1], J. Carnahan[3], H. Nawa[4], C. Marescaux[1] and A. Depaulis[1]. [1]INSERM U. 398, Université Louis Pasteur, Strasbourg, France, [2]URA 1446 CNRS, Université Louis Pasteur, Strasbourg, France, [3]Amgen Center, Thousand Oaks, Los Angeles, USA, [4]Beckman Neurosciences Center, Cold Spring Harbor Laboratory, New York, USA.

Several data have recently suggested the involvement of neurotrophins in the cascade of events occurring during seizure development. In particular, expression of both brain-derived neurotrophic factor (BDNF) and its high affinity receptor TrkB mRNAs increase in the hippocampus and the amygdala after convulsive seizures. The physiological relevance of this increase was investigated by chronic hippocampal perfusion of BDNF in two models of epilepsy in the rat: kindling and pentylenetetrazole-induced seizures.

A seven-day perfusion of BDNF in the dorsal hippocampus, blocked the kindling process in a dose-dependent manner, as evidenced by a significant reduction of seizure scores and afterdischarges. This effect was observed without any electroencephalographic abnormalities nor motor impairments and was independent of any lesional effect. A seven day perfusion of BDNF in the basolateral amygdala, partially blocked the kindling process, as evidenced by a reduction of amygdala afterdischarges but without effects on seizure scores. Moreover, a seven day bilateral pefusion of BDNF in the dorsal hippocampus prevented in a significant number of rats the occurrence of generalized tonic-clonic seizures induced by an injection of PTZ (50 mg/kg, ip). These results provide *in vivo* evidence for a protective role of BDNF in the control mechanisms of epilepsy and epileptogenesis in adult brain.

This work was supported by a grant from MESR and INSERM.

397.12

BDNF PROTECTS THE NEONATAL RAT BRAIN FROM HYOPXIC-ISCHEMIC INSULTS.

Y. Cheng*, A. Shah, D-H. Xia, J. Gidday and D. M. Holtzman. Dept. of Neurology, Neurosurgery, Mol. Biol. & Pharmacology and CSNSI. Washington Univ. Sch. of Med., St. Louis, MO 63110.

Neurotrophic factors (NTs) such as NGF, BDNF and NT-3 have been shown to protect certain neurons from transient ischemia and excitotoxicity in some animal models. NTs initiate their signaling cascades by activating their respective receptors. Although only a small number of CNS neurons express the NGF receptor, trkA, we have recently demonstrated that intracerebroventricular (ICV) injection of NGF significantly reduces hypoxic-ischemic (H-I) injury in the striatum, hippocampus and cerebral cortex of neonatal rats. In contrast to trkA receptors, the receptor for BDNF, trkB, is widely expressed in the developing brain. Thus, we asked whether BDNF is as or even more protective than NGF against H-I insult in neonatal brain. To approach this question, we first asked whether the exogenous NTs would activate trk receptors following ICV injection. NGF, BDNF and NT-3 were injected into postnatal day (PD) 7 rats, and trk receptor activation was detected by assaying trk phosphorylation 30 minutes after injections. A dose-dependent increase of trk phosphorylation was induced by NGF, BDNF and NT-3 (0-30 μg, peak by 10 μg) in the ispilateral as well as contralateral striatum, hippocampus and cortex. The magnitude of the response was BDNF>NT-3>>NGF. Interestingly, intrahippocampal injection of BDNF failed to induce significant level of trk phosphorylation in PD21 and adult rat hippocampus. Given the widespread expression of the trkB receptor in most CNS neurons and the strong trk phosphorylation response to BDNF in the PD7 brain, we have determined whether BDNF would be protective against H-I injury in the neonatal brain. Using the modified Levine procedure, we found that ICV injections of BDNF (10 μg) either before or after H-I treatment significantly protected against H-I brain injury at PD7 in all brain regions examined. These findings along with the results from the phosphorylation studies suggest that a direct effect of BDNF on most neurons is resulting in significant neuroprotection. (funding: NIH NS32553)

397.13

RECOMBINANT BDNF RESCUES DEFICITS IN HIPPOCAMPAL LTP AND BASAL SYNAPTIC TRANSMISSION IN BDNF KNOCKOUT MICE.

S.L. Patterson*, T. Abel, T.A.S. Deuel, and K.C. Martin. HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

The neurotrophin brain derived neurotrophic factor (BDNF) is expressed at high levels in hippocampal neurons and its expression is modulated by neural activity. The production of mice with targeted disruption ("knockout") of specific genes allows one to study the roles of molecules like BDNF in synaptic plasticity with more molecular specificity than is possible using traditional pharmacological approaches. However, the disrupted gene product is absent in all tissues, throughout the life of the animal; this absence may have developmental consequences that complicate the interpretation of deficits in the adult. One approach to overcoming this limitation is to perform rescue experiments to acutely restore the missing gene product. We have found that treatment of hippocampal slices from BDNF knockout mice with recombinant BDNF significantly improves deficits in basal synaptic transmission and completely reverses deficits in long-term potentiation (LTP) at the Schaffer collateral-CA1 synapse. The physiological rescue of LTP takes time, with full recovery after 5 hrs of treatment. Results obtained using immunocytochemistry suggest that a significant portion of the time required for the rescue of LTP represents the time required for BDNF penetration into the slice. Because the rescue of LTP was complete and much more rapid than the partial rescue of basal synaptic transmission (which takes 8-12 hrs), it is unlikely that the deficit in LTP was simply a reflection of impared basal synaptic transmission. Instead, our results suggest an acute requirement for BDNF in some forms of LTP above and beyond any developmental effect this neurotrophin may have on establishing the number and strength of synapses in the hippocampus.

This work was supported by NIH, NIMH and the Damon Runyon-Walter Winchell Foundation.

397.14

PROGRAMMED CELL DEATH IN THE DEVELOPING RAT STRIATUM AND ATTENUATION BY BDNF BUT NOT NT-3.

Thomas J. Mahalik[1]* and C. Anthony Altar[2]. [1]Dept. of Cellular and Structural Biology, Univ. of Colorado Health Sciences Center, Denver, Colorado. 80262. [2]Regeneron Pharmaceuticals, Tarrytown, N.Y. 10536

In the striatum programmed cell death (PCD) plays an important role during the development of the mammalian nervous system, where up to 25-30% of the generated neurons die during development. Much of this death may be due to the failure of neurons to compete successfully for limiting amounts of target derived neurotrophic factors. In the present study, an in situ method for labeling fragmented DNA (Gavrieli et al., 1992) was used to examine PCD in the striatum on postnatal days 0,4,6,8 and 10 in detail. Separate groups of pups were given intraventricular injections of BDNF and NT-3 during the first postanatal week to determine whether these factors could rescue striatal cells. From birth (P0) to postnatal day 6 (P6) there was a 4-fold increase in the density of apoptotic cells in the striatum. After its peak at day 6, there was a 50-fold decrease in PCD by P10. Multiple injections of BDNF, but not NT-3, attenuated striatal cell death at P6. These results indicate that there is a sharp peak in cell death on P6, and this may be the time when target derived growth factors become limiting.

This work was supported by an NIH Shannon Award (HS DC01916-01A1 R55) to TJM, and NIH grant (NS 09199) to Thomas E. Finger and TJM.

397.15

CNS MONOAMINES AND METABOLITES IN HETERO- AND HOMOZYGOUS BDNF NULL-MUTATED MICE.

K. Trok*, E. Lindqvist, P. Ernfors[§], L. Olson. Dept. of Neuroscience and [§]Dept. of Medical Biochemistry and Biophysics, Karolinska Institute, S-171 77 Stockholm, Sweden

BDNF mRNA is widely expressed by CNS neurons during development. In the adult, levels are generally lower, but expression is rapidly upregulated in response to different forms of neuronal stress. While disturbances in the peripheral nervous system of BNDF-deficient null-mutated mice have been characterized, less is known about the potential CNS effects. Here we have used HPLC with electrochemical detection to measure levels of monoamines and metabolites in the brains of two-week-old BDNF +/– and BDNF –/– animals and in adult BDNF +/– animals. We find a spectrum of disturbances of regional levels of monoamines and metabolites with the most severe changes seen in 15-day-old –/– animals, moderate changes in 15-day-old +/– animals, and only minor changes in adult +/– animals. 5-HT was increased in the 15-day –/– caudate, while 5-HIAA was strongly increased in the olfactory bulb, cerebral cortex, olfactory tubercle, caudate, substantia nigra and accumbens of 15-day –/– mice. 5-HIAA was also increased in the 15-day +/– cortex. NA was markedly increased in the 15-day –/– olfactory tubercle and moderately increased in the caudate. DA was markedly increased in cortex cerebri of both 15-day +/– and –/– mice. DOPAC was markedly decreased in the 15-day –/– accumbens, while we found no significant changes of HVA levels in the nigra or caudate. Taken together, the results demonstrate significant regional changes of both monoamine levels and metabolite levels of a magnitude that should have functional consequences.

Supported by the Swedish MRC and USPHS grants.

397.16

BDNF INFUSION STIMULATES ACTIVITY OF TrkB RECEPTORS AND THE SPROUTING OF SEROTONIN AXONS IN THE RAT BRAIN WITH A SIMILAR DOSE RESPONSE.

W.E. Lyons*[1], D.R. Kaplan[1,2] and L.A. Mamounas[3]. [1]ABL-Basic Research Program, NCI-FCRDC, Frederick, MD 21702; [2]Montreal Neurological Institute, Montreal, Quebec; and [3]Molecular Neurobiology Unit, NIA-NIH, Baltimore, MD.

In a number of *in vitro* systems, the activation of Trk tyrosine kinase receptors by the neurotrophins has been shown to mediate the survival and neurite outgrowth of cultured neurons. Much less is known about the physiologic role of Trk receptor activation in *in vivo* neuronal systems. In rat cortex, brain-derived neurotrophic factor (BDNF) stimulates the sprouting of both intact (uninjured) and chemically lesioned serotonergic (5-HT) axons (Mamounas et al., 1995). The present *in vivo* study examined whether doses of BDNF which stimulate serotonergic axon sprouting can also induce the activity of the TrkB/BDNF receptor as assessed by receptor tyrosine autophosphorylation. To evaluate the effects of BDNF on the 5-HT innervation, BDNF (0.1-12 μg/day) was continuously infused into the cortex of p-chloroamphetamine lesioned rats for two weeks. Serotonergic axon sprouting was first observed after infusion of 400 ng/day of BDNF with a maximal sprouting response found at 4 μg/day of BDNF. The same doses of BDNF were also infused in cortex to assay for levels of tyrosine phosphorylated TrkB at several time points after the start of the BDNF infusion. With continuous infusion of BDNF for 24 hr, TrkB tyrosine phosphorylation was detected at doses of BDNF as low as 400 ng/24 hr and reached maximal levels at 4 μg/24 hr of BDNF. Thus, the dose-response profiles for BDNF in stimulating 5-HT axon sprouting and in activating TrkB are nearly identical in rat cortex. These findings are consistent with a role for TrkB receptor activity mediating the *in vivo* sprouting response of serotonergic axons. Further studies are currently being conducted to examine the role of other components of TrkB signal transduction pathways in the sprouting of 5-HT axons.

Research sponsored by the National Cancer Institute, DHHS, under contract with ABL (WEL, DRK), and a National Research Council-NIA/NIH award (LAM).

397.17

BDNF STIMULATED SPROUTING OF SEROTONERGIC AXONS IS IMPAIRED IN THE AGED RAT BRAIN. L.A. Mamounas*, W.E. Lyons, M.E. Blue and C.A. Altar. Molecular Neurobiology Unit, NIA-NIH, Baltimore, MD 21224; Molecular Ontogenesis, ABL-Basic Research Program, NCI-FCRDC, Frederick, MD; Kennedy-Krieger Research Institute, Baltimore, MD; and Regeneron Pharmaceuticals, Tarrytown, NY.

In normal aging, there is a substantial loss of the serotonergic (5-HT) axon density in rat cortex, concomitant with the emergence of many degenerating, structurally abnormal 5-HT axons. Brain-derived neurotrophic factor (BDNF) stimulates the sprouting of both intact (uninjured) and chemically lesioned serotonergic axons in the young adult rat cortex (Mamounas et al., 1995, J. Neurosci., 15:7929-7939). The present study evaluated whether BDNF could also promote the sprouting of 5-HT axons in the serotonin-compromised aged rat brain. In young adult animals (2-3 months of age), continuous intracortical infusions of BDNF (1-12 µg/day for 2-3 weeks via an osmotic minipump) caused a robust sprouting of both intact and p-chloroamphetamine (PCA; 10 mg/kg, subcutaneous) lesioned 5-HT axons around the BDNF infusion cannula. In contrast, infusions of BDNF (4, 12 or 36 µg/day for 3 weeks) in the cortex of aged rats (21-23 months of age) caused a minimal increase in 5-HT axon density in a few animals, but, in the majority of animals, failed to stimulate any serotonergic axon sprouting. These findings suggest an impaired response to BDNF by serotonergic neurons in the aged brain. Our current studies are directed at evaluating receptor changes or signal transduction mechanisms in response to BDNF in the young versus aged brain.

Research supported by a National Research Council-NIA/NIH award to L.A.M.; the NCI, DHHS, under contract with ABL (W.E.L); and NIH grants #NS29167 and #HD24061 to M.E.B. BDNF was provided by the Amgen/Regeneron partnership.

397.18

DIFFERENTIAL EFFECTS OF NEUROTROPHINS ON THE FUNCTIONAL ORGANIZATION OF THE ADULT BARREL CORTEX *IN VIVO*. N. Prakash*, S. Cohen-Cory[1], & R. D. Frostig. Department of Psychobiology, University of California, Irvine, CA 92717; [1]Mental Retardation Research Center, University of California Los Angeles, School of Medicine, Los Angeles, CA 90024-1737.

Neurotrophins and their receptors are expressed during development and have been implicated in several aspects of developmental plasticity. They are also expressed in the adult cortex, making them good candidate molecules to play additional roles in aspects of adult cortical plasticity. Using quantified intrinsic signal imaging we examined the effects of topical application of different neurotrophins on the functional representation of a single whisker. As we have reported in the past, topical application of BDNF resulted in a rapid (minutes) and long-lasting (hours) decrease in both the size of the functional representation and the amplitude of the activity-dependent intrinsic signal evoked by whisker stimulation (Frostig, *et al.*, Soc. Neurosci. Abstracts, 1995). In contrast, topical application of NGF resulted in a rapid but transient increase in both the size of the functional representation and the amplitude of the activity-dependent intrinsic signal (Prakash, *et al.*, Nature, in press, 1996). We are now examining the effects of NT3 and NT4/5 in the same system. We found that topical application of NT4/5 did not significantly change either the size of the whisker representation or the amplitude of the activity-dependent intrinsic signal. These findings suggest that although BDNF and NT4/5 share the same high-affinity receptor, they differ in the mechanisms by which they exert their modulatory effects. The mechanisms underlying these differential effects, and the effects of NT3 application are being investigated.

Supported by the Markey-UCI Program in Human Neurobiology, NSF IBN-9507936, NSF IBN-9405146, and the UCI Medical Scientist Training Program. We thank Genentech, Inc. for the generous gifts of NT3 and NT4/5.

397.19

A MODEL OF OCULAR DOMINANCE COLUMN DEVELOPMENT BY COMPETITION FOR A TROPHIC FACTOR. A.E. Harris[1,2,3], G.B. Ermentrout[2,4], and S. L. Small[1,2,3]*, Department of [1]Neurology, [2]Neural Processes in Cognition Program, [3]Intelligent Systems Program, and [4]Department of Mathematics and Statistics; University of Pittsburgh, Pittsburgh, PA 15261.

Several lines of experimental evidence have suggested that the segregation of lateral geniculate nucleus (LGN) afferent axons to form ocular dominance (OD) columns occurs by an activity dependent competition for a post-synaptic trophic factor. Recent evidence has shown that application of excess amounts of certain neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and NT-4/5, prevents OD column development in a local area. One possible interpretation of these results is that excess trophic factor eliminates the competition between the incoming afferents, permitting connections from both eyes to innervate the cortical cell and thereby preventing OD column development in that area.

We present a computational model with three main features that accounts for these findings: 1) activity dependent changes in connection strength via long-term potentiation (LTP) and long-term depression (LTD), 2) positive feedback between the rate at which individual axons take up trophic factor and the rate at which connection strengths increase due to LTP, and 3) stabilization of this positive feedback by competition for a limiting supply of trophic factor. Center-surround lateral connectivity in the cortex is sufficient for self-organization of developing cells into OD columns. All terms in our equations are supported by specific neurobiological evidence. OD columns form by spontaneous symmetry-breaking with positive inter-eye correlation and without ad-hoc weight normalization mechanisms. Application of excess trophic factor prevents OD column development in a local area. Finally, several experimentally testable predictions arise from the model.

(Supported by NIH DC00054)

397.20

NITRIC OXIDE MEDIATES POTENTIATION OF NEUROTOXICITY BY NEUROTROPHINS.
A. Samdani[1], C. Newcamp[1], A. Resink[1], F. Facchinetti[1], V. L. Dawson[1,2,3], T.M. Dawson[1,2,3]. Depts. Of Neurol.[1], Phys.[2], and Neurosc.[3] Johns Hopkins Sch. Of Med., Baltimore, MD 21287.

Neurotrophins participate in multiple developmental processes including neuronal survival, axonal growth, and reduction of neuronal cell death following ischemia. Recently in primary neuronal/glial cultures it has been shown that 24 hour pre-treatment with neurotrophins results in potentiation of neuronal cell death induced by oxygen-glucose deprivation or NMDA exposure. In this study using primary cortical cultures, a role for nitric oxide (NO) in neurotrophin mediated neurotoxicity is established. Rat and mouse cortical neurons from embryonic day 15 were cultured either on a glial feeder layer or polyornithine plates. Cultures were treated on day *in vitro* 13 with BDNF (100 ng/ml) and experiments conducted day *in vitro* 14. In pure neuronal cultures BDNF attenuates NMDA mediated cell death which is primarily a nitric oxide (NO) mediated process. In neuronal/glial cultures BDNF markedly induces neuronal nitric oxide synthase (nNOS) as measured by NADPH diaphorase staining and western blot analysis. NT-3, NT-4/5, GDNF, but not NGF, also induce nNOS expression. BDNF potentiates NMDA neurotoxicity in neuronal/glial cultures and this cell death is reversed by the NOS inhibitor L-NAME. Confirming that NO mediates the increased neuronal death associated with neurotrophins, cultures from transgenic neuronal NO synthase (NOS) null (nNOS-) mice exhibit no potentiation of NMDA neurotoxicity after BDNF treatment. These data support the role of NO in the increased neuronal death caused by neurotrophins in primary neuronal/glial cultures.

A. Samdani recipient of Harold Lamport Research Grant.

397.21

BRAIN DERIVED NEUROTROPHIC FACTOR INDUCES A TRANSIENT CALCIUM INCREASE IN CORTICAL NEURONS. B. Yang, R. Remington, L. Kojic, M. Cynader*, Q. Gu. Dept. of Ophthalmology, University of British Columbia, 2550 Willow St., Vancouver, BC, Canada V5Z 3N9.

Brain derived neurotrophic factor (BDNF) is now well established as crucial to the survival of several neuronal populations. Recently, BDNF has also been implicated in synaptic plasticity. In this study, we have examined the acute effects of BDNF on intracellular calcium concentrations in cultured cortical neurons derived from embryonic rats. The calcium sensitive dye fluo-3 was employed in conjunction with a laser based confocal microscope to image calcium concentrations. Acute intracellular calcium increases induced by BDNF were observed. The response was found to be largely dependent on interneuronal interactions, because the presence of TTX in the medium greatly reduced the number of cells responding to BDNF. Nevertheless, in the presence of TTX, a small percentage of cells responding to BDNF was consistently observed, suggesting that BDNF can induce calcium increase through more than a single mechanism. When calcium was removed from the perfusion medium, the intracellular calcium increase was no longer observed in either the absence or presence of TTX, suggesting calcium entered from the extracellular space. To further assess the mechanisms responsible for these acute responses to BDNF, we examined the effects of blocking NMDA receptors. APV was found to be able to block the calcium response in most neurons, while the voltage-dependent calcium channel blocker nifedipine could not block the BDNF induced calcium increase. However, when TTX was included in the medium, the APV blockade effect was less obvious than in the TTX-free situation. In addition, when magnesium was deleted from the TTX-containing perfusion medium, no significant change in calcium response to BDNF was noted. These data suggest that BDNF can induce acute increases of intracellular calcium in cortical neurons, and that the mechanism may involve glutamergic receptors of both NMDA and non-NMDA classes.

Supported by BCHRF and NCE of Canada.

397.22

BDNF (BRAIN-DERIVED NEUROTROPHIC FACTOR) REDUCES RETINAL CELL DAMAGE CAUSED BY CHEMICALLY INDUCED EX VIVO HYPOXIA AND HYPOGLYCEMIA IN RATS.

Kazuhito Ikeda, Tohru Tatsuno*, Chikao Nakayama and Hiroshi Noguchi. Sumitomo Pharmaceuticals Research Center, 1-98, Kasugadenaka 3-chome, Konohana-ku, Osaka 554, Japan.

Retinal ganglion cells and inner nuclear cells (INL) have been shown to express high affinity receptors for BDNF, Trk B, and BDNF has been reported to protect these cells from either ischemic or traumatic damage. In this study, we examined whether BDNF can reduce hypoxic and hypoglycemic damage of INL . Acute necrotic cell death of INL was observed after incubation of isolated eyeballs of rats (Wistar, male, 8 weeks) with 5 mM potassium cyanide (KCN) for 30 min in Krebs-Ringer solution. BDNF (0.1, 1,and 10 µg), injected intravitreally two days before KCN-exposure, spared the inner nuclear cells from cell death in a dose-dependent manner. The number of surviving cells was 283 ± 9, 193 ± 5, and 252 ± 8 (mean ± S.E.M. cells / 880 µm) in control, KCN-exposed, and BDNF(10 µg) pretreated KCN-exposed eyeballs, respectively. The glutamate (Glu) level in the vitreous body was elevated by KCN exposure. Glu antagonist-treatment or the removal of external Ca^{2+} partially prevented KCN-induced cell death. These results suggested that the Glu receptor-activation and Ca^{2+}-influx were involved in the mechanism of cell death. BDNF pretreatment increased calretinin positive cells (200%) in INL, and reduced the elevation of Glu by KCN-exposure (62%). These data demonstrated that BDNF protected on INL against chemically induced hypoxia and hypoglycemia via up-regulation of calretinin and / or reduction of Glu content in the retina.

397.23

ABERRANT MYELINATION IN THE CNS OF BDNF -/- MICE. P. Carroll"Ψ, A. Cellerino#, G. Kreutzberg, H. Thoenen and Y.-A. Barde. Max-Planck Inst. for Psychiatry, 82152 Martinsried, Germany.Ψ, INSERM U. 382, IBDM, Case 907 Luminy, 13288 Marseille 09, France. # Dept. Neuroophthalmol., Uni. Eye Clinic, 72076 Tuebingen, Germany.

The neurotrophins (NGF, BDNF, NT-3, NT-4/5) have been well-characterised with respect to their effects on the survival and differentiation of PNS neurons and neuronal precursors. However, the expression patterns of neurotrophins in the CNS, their effects on processes which involve synaptic plasticity and the apparent absence of morphological defects or cell loss in the CNS of null mutant mice suggests that they may play other roles in this tissue. We examined a defined population of CNS neurons (retinal ganglion cells, RGCs) in BDNF mutant mice. BDNF is a survival factor for RGCs *in vitro* and BDNF can rescue such cells after axotomy. Axon counting in the optic nerve at P13 and P21 shows that BDNF -/- mice have no loss of RGCs compared with +/+ littermates. However, the axons in the optic nerve of mutants are reduced in diameter and the proportion of myelinated axons is reduced by 40 %. This effect was CNS-specific in that the same parameters were unchanged in the PNS of mutant mice. Hypomyelination was confirmed in other CNS structures by Northern analysis using probes for the myelin proteins proteolipid protein (PLP) and myelin basic protein (MBP). PLP and MBP mRNA levels were dramatically reduced in the hippocampus and cortex, but hardly at all in the cerebellum, of BDNF -/- mice. Markers for other CNS cell-types; NF-L for neurons and GFAP for astrocytes, were not altered in the mutant mice. Whether these effects are due to direct or indirect effects of BDNF on oligodendrocytes remains to be established. Funded by the Max-Planck Society.

397.24

BDNF PROMOTES SURVIVAL OF CULTURED RETINAL BIPOLAR CELLS VIA A MULTI-RECEPTOR PATHWAY. O. Berkovich, E. Wexler and S. Nawy*. Depts of Ophthalmology & Neuroscience, Albert Einstein College of Med., Bronx NY 10461.

Retinas from P10 rats were dissociated, plated onto coverslips coated with poly-d-lysine and cultured in media containing 5% FCS and supplemented with mito$^+$ serum extender. Bipolar cells were identified by selective labeling with an antibody to PKC, by their morphology and electrophysiological properties. BDNF (100 ng/ml) increased the number of PKC-positive cells by nearly 4-fold after 1 week in culture. Since rodent retinas are fully differentiated at P10, with few if any uncommitted progenitor cells remaining, we believe that the increase in the number of bipolar cells in the presence of BDNF is due to an increase in survival rather than further proliferation or differentiation of uncommitted cells.

The ability of BDNF to promote survival of bipolar cells was reduced in the presence of NGF. Furthermore, incubation of cultures with BDNF and anti-rex, an antisera which blocks function of the low affinity neurotrophin receptor (p75), reduced survival of bipolar cells to control levels. This block was dose-dependent with 50% block at approximately 300 ng/ml, and complete block at 36 µg/ml of the antisera. An antisera that specifically blocks function of the trkA receptor had no effect. Bipolar cell survival was not increased by p75 receptor activation in the absence of trkB activation, since ligands that bind p75 but not the trkB receptor (NT-3 and NGF) did not augment survival. Our data suggest that BDNF promotes survival of bipolar cells through coordinated activation of both the trkB and p75 receptors, and that activation of either receptor type alone is insufficient. Supported by Alcon Laboratories and the NEI.

397.25

TREATMENT WITH BDNF DOES NOT PREVENT NORMAL CHICK RETINAL GANGLION CELL DEATH *IN OVO* K. Drum*, M.E. Forbes, S.W. Wang and J.E. Johnson Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, N.C. 27157

Like many other neuronal populations, retinal ganglion cells (rgc) appear to depend upon target-derived factors for survival during development. Results from studies by several investigators have demonstrated that brain-derived neurotrophic factor (BDNF) can substitute for retinal targets by directly supporting the survival of isolated rat or chick rgc *in vitro*. Most (70%) of these cells are BDNF dependent when isolated during target innervation. We have shown than postmitotic chick rgc express BDNF receptor, trkB, throughout development (E6-E21). BDNF is expressed in both the target optic tectum and the retina. Tectal production appears to increase with retinal ganglion cell innervation during normal cell death and then subsequently decrease.

BDNF was, therefore, applied to chick embryos to determine if elevated levels of exogenous factor can prevent physiological cell death during development *in ovo*. BDNF (1-10μg/day, Amgen) was applied systemically to the chorio-allantoic membrane prior to and during the cell death period (E6-E16). This treatment has been shown to prevent normal cell death in other CNS neurons (ION) during a similar time period. No significant differences were observed in either the rate of cell death (density of pyknotic nuclei) or the total number of rgc with this BDNF treatment. We conclude that the majority of chick rgc either enjoy access to excess endogenous factor or instead do not normally depend upon limited amounts of BDNF for survival. These results are in sharp contrast to rgc axotomy or target-deprivation experiments where exogenous BDNF and/or NT-4 have marked effects on survival. BDNF may normally be expressed to regulate other developmental events in the chick visual system (ie. differentiation and connectivity). Sponsored by 5R01 EY11127 (JJ)

397.26

NEUROTROPHINS ENHANCE NEURITE OUTGROWTH FROM ADULT RAT RETINA *IN VITRO*. M. Takano*, Y. Iijima, M. Sato, H. Horie, T. Takenaka, S. Ohno, Departments of Ophthalmology and Physiology, Yokohama City Univ. Sch. of Med., Yokohama 236, JAPAN.

Recent studies have clarified that neurotrophins promote axonal regeneration from adult rat retina *in vitro*. In their culture systems, retina was dissected from an animal one week after transection of the optic nerve, and then cultured, because the pre-treatment activates neurite outgrowth from adult retinal explants. In the present study, we employed retinal explants from adult rat without the pre-treatment. The dissected retina was cultured in a collagen gel with a serum free medium, and the effects of neurotrophins on neurite regeneration were examined. Neurites stared to appear at day 3 in the culture either with or without neurotrophins. The relative increase of neurite numbers with neurotrophins were 78% (NGF), 59% (BDNF), 12% (NT-3) and 105% (NT-4) compared to the culture without neurotrophin at day 3. At day 9, these became 15, 88, 31 and 101%, respectively. BDNF and NT-4 kept high levels of the increase during the culture, but the enhancement by NGF decreased with culture days. In conclusion, adult rat retina exhibits a capability of neurite regeneration without pre-treatment *in vitro*. BDNF and NT-4 could enhance this ability continuously, but NGF showed the enhancement only initial period of the culture.

NEUROTROPHIC FACTORS: BIOLOGIC EFFECTS—NT-3

398.1

PROTECTIVE EFFECTS OF NEUROTROPHINS FROM APOPTOSIS OF MERKEL CELLS IN MONOLAYER CULTURE. Jun Fukuda*, Shingo Tsukada and Taku Iwamoto. Laboratory of Molecular and Cellular Physiology, Department of Physiology, National Defense Medical School, Tokorozawa, Saitama 359, Japan.

Merkel Cells are sensory cells in the skin that transmit signal of pressure to the primary sensory nerve fibers. Culture of these cells has not been successful. We report here that an almost pure culture of Merkel cells was achieved in a serum-free medium by dissociating them from whiskers of newborn rats. We also report that these Merkel cells underwent apoptosis and that this apoptosis was inhibited by neurotrophins, actinomycin D or sera added to the medium. Merkel cells were isolated from cores of follicles of whiskers dissected from newborn rats by pippeting, and were then grown on coverglass coated with poly-*L*-lysine. Merkel cells were identified by uptake of quinacrine that emits fluorescence by UV light. Quinacrine-fluorescence-positive Merkel cells occupied more than 85 % of the cells that were platted in a dish at the start of culture. During incubation in the serum-free medium, population of Merkel cells was decreased in time. Staining of nuclei with TUNEL method represented that Merkel cells underwent apoptosis during culture. Addition of actinomycin D or sera to the culture reduced the population of Merkel cells that were stained with TUNEL method. NT-3 reduced number of TUNEL-stained Merkel cells, while NGF, BDNF, CNTF, bFGF or EGF did not. NT-4 appeared to have a small protective effect against apoptosis of Merkel cells. Altogether, it is likely that Merkel cells that transmit sensory signals to the nerve fibers are also regulated by them by means of releasing neurotrophins.

398.2

FUNCTION OF NEUROTROPHINS IN SUBPOPULATIONS OF DEVELOPING DRG NEURONS. D. J. Liebl*, L. Klesse, S. M. Colvin and L.F. Parada. Center for Developmental Biology, UTSW Med. School, Dallas, TX 75235

Analysis of neurotrophin gene-targeted knockout (KO) mice deficient in NGF, BDNF and NT-3 has indicated losses in the number of dorsal root ganglia (DRG) neurons. Experiments were conducted to examine neurotrophic dependencies in DRG neurons during embryonic development by employing individual neurotrophin KO mice and mice deficient in two neurotrophins (i. e. BDNF and NT-3, BDNF and NT-5, & NT-3 and NT-5). Our cell count studies indicate that DRG neurons acquire neurotrophin dependence for BDNF and NT-3 at about E-11.5. Trk (A, B & C) expression studies, primary culture assays, and cell losses in neurotrophin(s) KO mice are consistent with the model that the DRG contains subpopulations of neurons that are dependent on either individual or multiple neurotrophins. In addition our studies support and elaborate previous observations (Tesserollo et al., 1994) which indicate that initial neurotrophic dependencies occur prior to the period of programmed cell death as originally proposed in the neurotrophic theory.

NT-3 binds and activates the high affinity receptor, TrkC, which mediates neuronal survival. However, NT-3 can also bind and activate TrkA in cultured PC-12 cells, and, whether this occurs in vivo remains unclear. We observe losses of TrkA expressing neurons in NT-3 KO mice, suggesting that in some cases NT-3 may support survival of developing DRG neurons via TrkA receptor. Supported by TRK NIH Grant # 1-R01-NS33199-02.

398.3

Local actions of neurotrophin-3 prevent the death of proliferating sensory neuron precursor cells.

W. M. ElShamy and P. Ernfors* Dept. of Medical Biochemistry and Biophysics, Laboratory of Molecular Neurobiology, Doktorsringen 12A, Karolinska Institute, 171 77 Stockholm, Sweden.

The role of neurotrophin-3 (NT3) in early development of the dorsal root ganglion was investigated. Excessive cell death in the dorsal root ganglion of mice that carry a deleted NT3 gene ($NT3^{-/-}$ mice) preceded the period of programmed cell death, detected by the TUNEL method, and caused a reduction in the number of proliferating precursor cells without altering the proportion of proliferating cells to total number of neurons, indicating that NT3 prevents the cell death of proliferating sensory precursor cells. Furthermore, the majority of proliferating cells detected by bromodeoxyuridine incorporation also stained with the TUNEL method. NT3 mRNA was expressed locally in the embryonic, but not the postnatal dorsal root ganglion. In contrast to neurons with an intact NT3 gene, most cultured early embryonic $NT3^{-/-}$ neurons as well as wild-type neurons when cultured with NT3 neutralising antibodies died in the absence of exogenously added NT3. Thus our results suggest that NT-3 acts locally to prevent the death of proliferating sensory precursor cells during neurogenesis. These findings raise the possibility that NT3 can inflict constraints on the number of proliferating precursor cells and thereby affect the number of neurons generated during development of the peripheral nervous system. (Supported by the Swedish Medical Research Council, The Swedish Cancer Society and Petrus, Augusta Hedlunds Foundation).

398.4

NT-3 STIMULATES THE ADRENERGIC PHENOTYPE OF SYMPATHETIC NEURONS IN VIVO BUT UNLIKE NGF DOES NOT PRODUCE HYPERALGESIA. V. Wong*, C. Jackson, J.A. Siuciak, D. Lewis, T. Do, P.S. DiStefano, and R.M. Lindsay. Regeneron Pharmaceuticals, Tarrytown, NY 10591.

NGF is an essential survival factor for developing sympathetic neurons and is required for their maintenance in the adult. Although exogenous NGF attenuates damage to sympathetic (and sensory) neurons produced by nerve injury or toxic agents, the potential therapeutic benefit of NGF may be confounded by the finding that systemic NGF administration produces hyperalgesia in both rodents and man. Recent evidence has shown that neurotrophin-3 (NT-3) also plays an important role in developing sympathetic neurons, e.g., mice bearing an NT-3 null mutation show a 50% reduction in neurons in the superior cervical ganglion (SCG). Here we have compared the effects of exogenous NT-3 versus NGF on phenotypic markers of sympathetic neurons in the adult rat. Vehicle, NGF or NT-3 was administered to adult rats by s.c. injection daily for 4-7 days. Animals were evaluated for pain sensitivity by a paw thermal stimulation test. Target catecholamine levels were unaffected by either NGF or NT-3. NT-3 administration stimulated TH activity in the SCG and celiac ganglia to the same extent as did NGF. Only NT-3 increased TH levels in the superior mesenteric ganglion (SMG). At doses which produced equivalent increases in TH, NGF, but not NT-3, decreased paw withdrawal latency, suggesting that NT-3 did not induce hyperalgesia. NGF-, but not NT-3-treated rats showed swelling and redness of face and limbs, frequent scratching, and increased tissue serotonin levels, indicative of mast cells degranulation. Thus, NT-3 may be as effective as NGF in attenuating sympathetic neuron dysfunction, but benefit from the absence of hyperalgesia. Differences in the molecular signalling of NGF and NT-3 in sympathetic neurons are being investigated. Research supported by Regeneron Pharmaceuticals, Inc.

398.5

INCREASED NUMBERS OF MYENTERIC NEURONS ARISE IN TRANSGENIC MICE THAT OVEREXPRESS NEUROTROPHIN-3 (NT-3) DIRECTED TO THE ENTERIC NERVOUS SYSTEM (ENS) BY THE DOPAMINE β-HYDROXYLASE PROMOTER. T. Pham[1], P. R. Wade[1], A. Chalazonitis*[1] S.L. Skirboll[2], M. Bothwell[2], & M.D. Gershon[1]. 1. Dept. of Anat. & Cell Biol., Columbia Univ. New York, NY.10032. 2. Dept. Physiol. Biophys., Univ. Washington, Seattle, WA

NT-3 promotes the development of enteric neurons *in vitro*. An ENS is present, however, in mice lacking NT-3. The current study was thus carried out to determine whether NT-3 affects enteric neuronal development *in situ*. Neural crest-derived precursors of a subset of enteric neurons are transiently catecholaminergic (TC). These cells express DBH; the DBH promoter thus directs the expression of genes to the ENS. Transgenic DBH-NT-3 mice overexpress NT-3 in the gut, but survive and gain weight normally. Laminar preparations of myenteric and submucosal plexuses were dissected from the small intestine. Total numbers of neurons were counted in tissue stained with cuprolinic blue. The numbers of neurons in the myenteric plexus were significantly greater in DBH-NT-3 than in control mice. In contrast, the numbers of submucosal neurons were only slightly elevated. Calcitonin gene related peptide (CGRP)-containing and other neurons that develop from a precursor lineage that is non-TC, *mash-1*-independent, and born late were present in the same numbers in DBH-NT-3 and control mice. These observations confirm that NT-3 promotes neuronal development *in situ* and suggest that NT-3 may preferentially affect the TC, mash-1-independent, precursor lineage. Grants NS12969 and NS15547.

398.6

NEUROTROPHIN- 3 INCREASES THE PROPORTION OF NEURONS IN DISSOCIATED CULTURES OF POSTNATAL RAT MYENTERIC PLEXUS M.J. SAFFREY*, J.N. DAISLEY, T.M. WALKER, T. WARDHAUGH Dept. of Biology, The Open University, Milton Keynes, MK7 6AA, UK.

Neurotrophin-3 has previously been found to stimulate the differentiation of enteric neurons and glial cells from precursors isolated from the embryonic gut *in vitro*. Since NT-3 transcripts have also been detected in the gut postnatally, we examined the effects of NT-3 on myenteric ganglia isolated from the postnatal rat gut and grown in dissociated cell culture. Segments of the muscularis externa from 6-8 day Wistar rats were incubated in 1mg/ml collagenase. Myenteric ganglia were then separated from the smooth muscle by mechanical agitation. After rinsing, freed segments of plexus were dissociated in trypsin. Cells were seeded in medium 199 containing 10% FCS at a density of 0.75 -1.5 x 10^4 cells/200µl on to 16mm poly -L lysine coated coverslips. After 1 hour, cultures were supplied with serum-free hormone supplemented 199 (N1) containing 100pg, 1ng or 10ng/ml NT-3. Cells were fixed after 48 hours and immunolabelled using an antiserum to PGP 9.5. The numbers of neurons and non-neuronal (glial) cells were counted. The proportion of neurons:non-neuronal cells was elevated in the NT-3- treated cultures at all three concentrations compared to untreated controls (n=4). The increase was significant in 3 out of 4 experiments ($p<0.05$; ANOVA). In general, the increase appeared to be due to an increase in the number of neurons, which was consistently seen in these experiments but which was not significant in all cases. These observations indicate that NT-3 has actions in the postnatal enteric nervous system; this may be a direct effect on myenteric neurons, but may also involve enteric glial cells, or precursors which may also be present in enteric ganglia at this stage of development.

This work was supported by the Medical Research Council of Great Britain

398.7

RAPID EFFECTS OF NEUROTROPHIN-3 (NT-3) ON THE ELECTRICAL PROPERTIES OF NEURONS OF THE MESENCEPHALIC TRIGEMINAL NUCLEUS (MES-V). Pose, I., Yamuy*, J. Morales, F.R. and Chase, M.H. *Departamento de Fisiología, Facultad de Medicina, Montevideo, Uruguay, and Department of Physiology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA, 90024.*

Mes-V neurons have been shown to express high-affinity receptors for NT-3 (*trk*C) (1); they also receive trophic support from NT-3-producing proprioceptors (2). We have recently reported that nerve growth factor is capable of rapidly increasing the excitability and enhancing the membrane potential oscillatory activity of Mes-V neurons (3). Accordingly, we were interested in examining whether the application of NT-3 results in a change in the electrical properties of Mes-V neurons.

Intracellular recordings from Mes-V neurons were obtained utilizing brainstem slices of adult rats. Microdroplets of NT-3, at a concentration of 0.5-1.0 µg/µl were applied locally in the vicinity of the recording electrode.

Within 4 to 20 sec. of NT-3 application, there was an increase in the amplitude of the depolarization-induced membrane potential oscillations (4) and a decrease in the rheobase. Increased oscillations and spikes appeared at the break of the hyperpolarizing current pulses. The resting membrane potential was depolarized by 1-4 mV. The spike configuration and the AHP potential remained unchanged. The duration of these NT-3-induced effects was approximately 3 minutes.

The present data suggest that NT-3, in addition to its known long term trophic effects, is capable of rapidly modulating the electrophysiological activity of Mes-V neurons.

1. Wetmore, C. and Olson, L. J. (1995) J. Comp. Neurol., 353: 143-159.
2. Ernfors, P., Lee, K-F., Kucera, J. and Jaenisch, R. (1994) Cell, 77: 503-512.
3. Yamuy, J., Pedroarena, C., Pose, I., Morales, F.R. and Chase, M.H. (1995) Soc. Neurosc. Abstr. Vol. 21: 1048.
4. Pedroarena, C., Pose, I., Yamuy, J., Morales, F.R. and M. H. Chase. (1994) Soc. Neurosc. Abstr. Vol. 20: 1756.

Supported by USPHS grants MH 43362, NS 23426 and NS 09999.

398.8

EFFECTS OF NT-3 OVEREXPRESSION IN MUSCLE ON THE TRIGEMINAL MESENCEPHALIC NUCLEUS. T.A. Henderson*, D.E. Wright, W.D. Snider & M.F. Jacquin. Neurology and the Center for the Study of Nervous System Injury, Washington University School of Medicine, St. Louis, MO 63110.

An accompanying paper (Wright et al. '96) describes the effects of NT-3 overexpression in skeletal muscle (Myo/NT-3 transgenic mice) upon proprioceptive DRG neurons that innervate muscle spindles. These results confirm that the survival of proprioceptive neurons in the DRG is powerfully regulated by the levels of target derived-NT-3, since the absence of NT-3 results in the loss of proprioceptive neurons and NT-3 overexpression increases proprioceptive neuron survival. This is in striking contrast to findings in the trigeminal (V) system, where proprioceptive neurons in the mesencephalic nucleus (MesV) that innervate jaw muscle spindles seem only partially affected by the absence of NT-3 or its receptor *trk*C (Ernfors et al., '94; Arends et al., '96). To investigate potential differences in the trophic requirements of spinal and V proprioceptive neurons, we examined MesV proprioceptive neurons in Myo/NT-3 mice. Nissl-stained profiles of MesV cell bodies with nucleoli were counted in complete series of paraffin-embedded "thin" sections from 3 Myo/NT-3 (15 day-old) and 6 wild-type mice. NT-3 overexpressors had 1,166, 1,150, and 1,107 Mes V cells vs. an average of 638 Mes V cells (range: 529-813) in wild-type controls. Our results reveal that muscle-derived NT-3 is capable of rescuing MesV neurons from naturally occurring cell death to a similar extent as proprioceptive DRG neurons. Whether increasing the number of surviving MesV proprioceptive neurons has secondary effects on cell numbers in the principal and spinal V nuclei will also be reported, as well as the status of whisker-related patterns in the brainstem. Supported by NIH DE07734, NS17763.

398.9

ACTIVITY-DEPENDENT EXPRESSION OF NT-3 IN MUSCLE CELLS MEDIATES SYNAPTIC POTENTIATION AT THE DEVELOPING NEUROMUSCULAR JUNCTION. K. Xie, T. Wang, P. Olafsson, and B. Lu* Roche Inst. of Mol. Biol., Nutley, NJ, 07110 and Lab. of Devel. Neurobiol., NICHD, NIH, Bethesda, MD 28920-4480.

While traditionally viewed as trophic factors regulating neuronal survival and differentiation, neurotrophins have recently been shown to acutely enhance transmitter release and to chronically facilitate synaptic maturation at the neuromuscular junction. The neurotrophins BDNF, NT-3 and NT-4/5 are expressed in the postsynaptic muscle cells, while their receptors TrkB and TrkC are expressed in the presynaptic motor neurons. We have investigated activity-dependent neurotrophin expression in muscle cells, and its role in developing neuromuscular synapses. Membrane depolarization, elicited either by depolarizing agents or repetitive electric stimulation, rapidly and specifically increased the levels of neurotrophin-3 (NT-3) mRNA in developing Xenopus muscle cells in culture. NT-3 gene expression was also enhanced by ACh, the neurotransmitter that causes muscle membrane depolarization. The effects of depolarization were mediated by increasing intracellular calcium concentration. Moreover, the depolarization-induced NT-3 potentiated synaptic activity at the developing neuromuscular synapses. The frequency of spontaneous synaptic currents recorded from neuromuscular synapses was markedly increased after treatment with conditioned medium from depolarized muscle cultures. This effect of conditioned medium was blocked by TrkC-IgG fusion protein, a specific scavenger of NT-3. These results suggest that muscle-derived NT-3 may serve as a retrograde message for activity-dependent synaptic strengthening at the developing neuromuscular junction.
We also thank Dr. D. L. Shelton from Genentech for the gift of TrkC-IgG fusion protein. P. O. is supported by a Swiss NSF postdoctoral fellowship, and a grant from Ciba-Geigy Jubiläums-Stiftung.

398.10

SUPPRESSED KINDLING EPILEPTOGENESIS AND PERTURBED BDNF AND TrkB GENE REGULATION IN NT-3 MUTANT MICE.
M. Kokaia*, E. Elmér, P. Ernfors†, I. Ferencz, Z. Kokaia, and O. Lindvall. Section of Restorative Neurology, Department of Clinical Neuroscience, Wallenberg Neuroscience Center, S-221 85 Lund, Sweden; †Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-171 77 Stockholm, Sweden.

In the kindling model of epilepsy, repeated electrical stimulations lead to progressive and permanent intensification of seizure activity. We find that the development of amygdala kindling is markedly retarded in mice heterozygous for a deletion of the neurotrophin-3 (NT-3) gene (NT-3+/- mice). These mice did not reach the fully kindled state (third grade 5 seizure) until after 28±4 days of stimulation as compared to 17±2 days in the wild-type animals. The deficit in the NT-3+/- mice reflected dampening of the progression from focal to generalized seizures. The number of stimulations required to evoke focal (grade 1 and 2) seizures did not differ between the groups, but the NT-3 mutants spent considerably longer time (13±3 days) than wild-type mice (2±1 days) in grade 2 seizures. As assessed by test stimulation 4-12 weeks after the tenth grade 5 seizure, kindling was maintained in the NT-3 mutants. *In situ* hybridization showed 30% reduction of basal NT-3 mRNA levels, and lack of upregulation of TrkC mRNA expression at 2 h after a generalized seizure in dentate granule cells of the NT-3+/- mice, whereas the seizure-evoked increase of brain-derived neurotrophic factor (BDNF) and TrkB mRNA levels was enhanced (by 130 and 38%, respectively). These results indicate that NT-3 might be involved in kindling epileptogenesis, and suggest a link between NT-3 and BDNF gene regulation in dentate granule cells.
Supported by a grant from the Swedish MRC.

398.11

EFFECTS OF NT-3 TREATMENT ON GALACTOSE-INDUCED NEUROPATHY. A.P. Mizisin*, K.C. Dines, M.W. Kalichman, M. Bache, R.M. Lindsay, P.S. DiStefano. University of California, San Diego, La Jolla, CA 92093 and Regeneron Inc., Tarrytown, NY 10591.

In galactose intoxication, Schwann cell injury and axonal dwindling are ameliorated by inhibiting aldose reductase, suggesting that these abnormalities are linked to polyol pathway metabolism. How inhibition of aldose reductase, localized to Schwann cells and skeletal muscle, can restore axonal caliber is puzzling unless polyol accumulation disrupts the synthesis and/or release of trophic substances capable of influencing axonal structure and function. The present study investigated the effect of NT-3, a neurotrophic factor expressed in sciatic nerve and skeletal muscle, on myelinated fiber disorders of galactose-fed rats. Adult, female Sprague-Dawley rats were fed diets containing complete micronutrient supplements and either 0% D-galactose (control) or 40% D-galactose. One group of galactose-fed rats received NT-3 (15 mg/kg) by thrice weekly subcutaneous injections. After two months, sciatic sensory and motor nerve conduction velocity (SNCV and MNCV) were measured and the sciatic nerves and dorsal and ventral roots processed for light microscopy. The contralateral sciatic nerves were analyzed for polyol content. Compared to controls, there was marked accumulation of dulcitol and water in the sciatic nerves of galactose-fed animals that was not affected by NT-3 treatment. Galactose feeding reduced SNCV by 23% and MNCV by 10% compared to control values. NT-3 treatment attenuated the sensory conduction deficit seen in untreated galactose-fed rats such that SNCV was increased to 86% of control values, but had no effect on MNCV in galactose animals. In galactose-fed animals, myelin sheaths of fibers in the dorsal and ventral roots were characterized by splitting that occurred at the intraperiod line. Treatment with NT-3 did not attenuate myelin splitting in either the dorsal or ventral roots. These observations indicate that NT-3 can attenuate sensory, but not motor, conduction deficits in galactose neuropathy. Determination of whether the effect of NT-3 on sensory function is related to the restoration of axonal caliber depends on completion of ongoing morphometric studies. Supported by NIH Grant NS32339.

398.12

ACTIVATION OF NEUROTROPHIN-3 RECEPTOR INDUCES APOPTOSIS IN A MEDULLOBLASTOMA CELL LINE. S.L. Pomeroy*, T.A. Cho, S. Khoxayo, C.D. Stiles, R.A. Segal, L.C. Goumnerova, M.A. Sutton. Depts of Neurol and Neurosurg, Children's Hosp, Dept of Neurol, Beth Israel Hosp, Dept of Cell Mol Biol, Dana-Farber Cancer Inst, Harvard Med Sch, Boston MA 02115.

We demonstrated previously that patients with tumors expressing high levels of the neurotrophin-3 (NT-3) receptor, TrkC, have a significantly better survival than those with low levels of expression (Segal et al., Proc Natl Acad Sci, USA, 1994; 91:12867-71). Here, we address whether TrkC activation alters the growth of medulloblastomas.

TrkC expression was upregulated in the medulloblastoma cell line Daoy by transfection with an expression plasmid encoding the full length TrkC receptor. A higher proportion of cells expressing high levels of TrkC were found to undergo apoptosis (0.52 ± .04; mean ± S.D.), defined by nuclear condensation and fragmentation, when grown in the presence of 50 ng/ml NT-3 than when they were grown under identical conditions but without NT-3 (.05 ± .01; $P < .0001$, Student's t test). Neither BDNF nor NGF increased apoptosis rate. These results are consistent with the view that endogenous TrkC activation influences medulloblastoma outcome by induction of apoptosis in cells expressing the receptor.
Support by the NIH, the Eliz O'Brien Found. and the Brain Tumor Soc.

398.13

DIFFERENTIAL EFFECT OF NT-3 ON GAP-43 AND TUBULIN α1 mRNA LEVELS IN AXOTOMIZED RETINAL GANGLION CELLS. P. Kittlerova, G. M. Bray and A.J. Aguayo*. Centre for Research in Neuroscience, McGill University and Montreal General Hospital Research Institute, Montréal, Québec, H3G 1A4.

We have previously shown that the effects of NT-3 on axotomized retinal ganglion cells (RGCs) in adult rats include stimulation of intraretinal axonal growth and a dramatic upregulation of GAP-43 in certain RGCs (Kittlerova et al., Soc. Neurosci. Abstr. 20: 1098, 1994). These effects presumably involve a subset of RGCs that express the high-affinity receptor TrkC (Kittlerova et al., Soc. Neurosci. Abstr. 21: 1550, 1994).

We have extended these observations on GAP-43 by investigating the expression pattern of another growth-associated protein, tubulin. We performed quantitative *in situ* hybridization using a tubulin α1 (Tα1) radiolabelled cRNA probe on retinal sections 1, 2 and 4 weeks after optic nerve (ON) transection and a single injection of NT-3 or PBS. We observed a progressive reduction of Tα1 hybridization signal in both NT-3- and PBS-treated RGCs. At 2 weeks after ON cut and NT-3 injection, the Tα1 mRNA levels were about one-half those of the contralateral intact eyes. This result is different from the previous finding of a 3-4 fold upregulation of GAP-43 message by NT-3 treatment, suggesting that the expression of these two molecules in injured retinas is differentially regulated by NT-3. We are currently investigating the possible role of GAP-43 in the modulation of specific features of RGC axon branching patterns in axotomized retina. (Supported by the Canadian NeuroScience Network)

398.14

INFLUENCES OF NT-3 ON PROPRIOCEPTIVE AXON PROJECTIONS TO SPINAL MOTONEURON POOLS. S. Ozaki*, D. E. Wright, L. Zhou and W. D. Snider. Department of Neurology, CSNSI, Washington University School of Medicine, St. Louis, MO 63110.

Work with the prototypical neurotrophin, NGF, suggests that neurotrophic factors can regulate axon arbors. A variety of lines of evidence extend this concept to other neurotrophins and to axon projections in the CNS (see Thoenen, 1995, for a review). Proprioceptive sensory neurons that project to motoneuron pools in the ventral spinal cord express TrkC and require NT-3/TrkC signaling for survival. NT-3 is known to be intensely expressed in the ventral horn prior to the onset of the proprioceptive axon collateral branching in the spinal cord, suggesting that NT-3 may have tropic and/or trophic effects on proprioceptive axons. To address the functions of NT-3 related to the development of proprioceptive axon arbors, NT-3 levels in the spinal cord were altered by generating two kinds of transgenic mice: Tα1-NT-3 mice in which an NT-3 transgene is overexpressed by all spinal and DRG neurons under control of the α-tubulin promoter (Gloster et al., 1994) and myogenin-NT-3 x NT-3(-/-) mice in which NT-3 is expressed in muscle but not in the spinal cord. Primary afferent projections were visualized using DiI placed in the thoracic nerves of the transgenic animals on postnatal day 0. In Tα1-NT-3 mice, overexpression of NT-3 has striking effects on spinal cord development: dorsal columns are larger than the controls and there is a large increase in axons projecting to the Clark's column, suggesting that there are many more proprioceptive sensory axons. Surprisingly, the basic pattern of the afferent projections to the ventral horn is similar to the wild type, despite the diffuse availability of NT-3 in the spinal cord. In myogenin-NT-3 x NT-3(-/-) mice, proprioceptive sensory neurons are "rescued" by the NT-3 transgene overexpressed in muscle. Again, the basic pattern of proprioceptive afferent projections is similar to the controls. These results indicate that NT-3 is unlikely to have tropic effects on proprioceptive afferent projections (see Oakley et al., 1995, for a similar result in the chick). Whether NT-3 has trophic effects on axon arborizations and bouton formation should be amenable to analysis in these animals. Supported by NIH.

398.15

NEUROTROPHIN-3 TREATMENT OF DIABETIC RATS SELECTIVELY PREVENTS REDUCED SENSORY NERVE CONDUCTION VELOCITY.

N.E. Garrett, P. Fernyhough, L.T. Diemel and D.R. Tomlinson *.
Department of Pharmacology, St. Bartholomew's and The London School of Medicine and Dentistry, QMW, Mile End, London E1 4NS, U.K.

Reduced sensorimotor conduction velocity and distal axonal shrinkage are hallmarks of diabetic neuropathy. In animal models the latter derives from deficient production of the neurofilament proteins with breakdown of the axonal endoskeleton. In myelinated sensory fibres this may be controlled by target cell-derived neurotrophin-3 (NT-3) and the present study examined the effect of administration of its human recombinant form in streptozotocin-diabetic rats (65mg/kg i.p.). NT-3 (1mg/kg s.c. 3x/week) was given for the last 4 weeks of 12 weeks of diabetes; another diabetic and a non-diabetic (control) group received vehicle. Finally motor and sensory nerve conduction velocities (MNCV/SNCV) were measured (under halothane anaesthesia) and lumbar dorsal root ganglia removed for measurement of expression of two neurofilament protein (NF-M, NF-H) mRNA and the NT-3 receptor (trkC) mRNA.

In vehicle-diabetic rats MNCV and SNCV were reduced (respectively 76% and 84% controls; both $p<0.01$); NT-3 did not affect MNCV (79% control; $p<0.01$), but increased SNCV to 110% non-diabetic rats ($p<0.01$). In vehicle-diabetic rats there were reductions in mRNA (% control) for NF-H (62), NF-M (45) and trkC (43; all $p<0.05$).

These deficits were unaffected by NT-3 treatment. These data implicate NT-3 in deficient sensory conduction, with therapeutic possibilities for correction, but not in neurofilament impairment in diabetic rats.

Thanks to Regeneron for NT-3 and British Diabetic Association for support.

398.16

THE ROLE OF NT-3 IN THE REGULATION OF GAP-43 AND tα1-TUBULIN IN INTACT AND INJURED PRIMARY SENSORY NEURONS

K.A. Gratto & V.M.K.Verge*Dept of Anatomy and Cell Biology & Cameco MS Neuroscience Research Center, University of Saskatchewan, Saskatoon, Canada S7N 5E5

Exogenous NT-3 has been shown to modulate the injury-induced expression of its receptors (trk C, p75), NFM, cJUN, and a number of neuropeptides towards normal levels, thus implicating a role for this neurotrophin in the maintenance of aspects of the intact, differentiated states of responsive adult primary sensory neurons (Gratto et al., 1994, 1995). As a continuation of these studies, NT-3's ability to mitigate the expression of two regeneration-associated genes, GAP-43 and tα1-tubulin was examined. Adult rat right sciatic nerves were proximally cut. 14d after injury NT-3 was intrathecally infused for 7d in half of the rats. Serial cryostat sections of intact and injured DRG with and without NT-3 infusion were processed for *in situ* hybridization to detect mRNAs encoding trkC, GAP-43, and tα1-tubulin. Computer-assisted image-analysis was used to determine relative hybridization signal intensities. Results indicate that NT-3 infusion is, at least partially, effective in mitigating the axotomy-induced increases in GAP-43 and tubulin mRNA levels. Preliminary quantitative data show that while only ~20% of trkC-positive neurons coexpress GAP-43 in the intact state, virtually all of this population strongly expresses mRNA for this marker following injury (~70% express signal > 35X background). NT-3 infusion appears to modulate GAP-43 mRNA expression in trkC-positive neurons such that <20% strongly express signal, with the remainder exhibiting levels < 35X background. That NT-3 appears to downregulate the expression of both GAP-43 and tα1-tubulin (as well as cJUN) mRNA *in vivo* implies that this trophic factor may function in depressing the regeneration process. The influence of this neurotrophin on the numbers and growth rates of regenerating axons is being determined. *NT-3 generously supplied by Regeneron Pharmaceuticals, Tarrytown, N.Y. Research supported by MRC Canada.*

398.17

RESPONSE OF PRIMARY SENSORY NEURONS TO PERIPHERAL NERVE INJURY IN P75 DEFICIENT MICE,

L.A. Karchewski* & V.M.K. Verge
Dept. of Anatomy and Cell Biology & Cameco MS/Neuroscience Research Center, University of Saskatchewan, Saskatoon, Canada S7N 5E5.

The functional significance of the low-affinity neurotrophin receptor is not clear. p75 may modulate neurotrophin sensitivity, mediate retrograde transport of select neurotrophins, play a role in apoptosis and/or be involved in neurite extension. Peripheral nerve injury invokes a number of cell body responses believed influenced by alterations in trophic support and/or altered expression of p75 and trk receptors. As a first step in elucidating the role of p75 in intact and injured sensory neurons, unilateral sciatic nerve transection time course studies were performed on mice carrying a null mutation in the p75 locus and compared to host strain mice. *In situ* hybridization to detect p75, trk receptors, various neuropeptides, cytoskeletal and immediate early gene mRNAs were performed on pairs of experimental and control DRG cryostat sections. Preliminary results show that, although these DRG contain ~ 50% fewer neurons (a lack of primarily small size neurons), there is not a proportionate loss of trkA, trkB, trkC, BDNF or CGRP expressing neurons. Time course analysis of injury responses indicate that at 4d &7d, reduced expression of trkA, trkC, CGRP, SP and elevated expression of GAL, NPY and GAP43 are much more dramatic in the p75 deficient mice than in controls. However by 5 weeks, p75 deficient mice show enhanced levels of hybridization signal detecting trkA, SP, CGRP and BDNF mRNAs as compared to control mice. These results suggest that availability and/or response to NGF may be altered in these mice, perhaps through altered p75/trk ratios, different endoneurial trophic support and/or retrograde transport of NGF following injury. Whether axonal regeneration is altered in p75 knock-out mice is being determined. *Supported by the Neuroscience Network for the Centers of Excellence, Canada.*

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS III

399.1

TRUNCATED TrkB VARIANTS ARE DEVELOPMENTALLY REGULATED NEGATIVE MODULATORS OF BDNF SIGNALLING IN SENSORY NEURONS

Natalia Ninkina, Jimi Adu, Anne Fischer, Luzia G. P. Pinon, Vladimir L. Buchman and Alun M. Davies* Bute Medical Buildings, University of St. Andrews, St. Andrews, Fife KY16 9AJ, Scotland, UK.

Mouse trigeminal neurons survive independently of neurotrophins when their axons are growing to their targets and are transiently supported by BDNF during the early stages of target field innervation before becoming NGF dependent. During the stage of neurotrophin independence, *trkB* transcripts are expressed at very low levels. During the stage of BDNF responsiveness, a 9 kb *trkB* transcript encoding the kinase-containing, catalytic receptor required for BDNF signalling is expressed at high levels. As the neurons lose responsiveness to BDNF, there is a concomitant increase in the expression of 8.2 and 2.5 kb *trkB* transcripts which encode truncated TrkB variants that lack the tyrosine kinase domain, suggesting that the increased expression of these variants may account for the loss of the BDNF survival response. To test directly whether truncated TrkB modulates BDNF signalling via catalytic TrkB, we microinjected TrkB expression plasmids into NGF-dependent postnatal sympathetic neurons. Whereas expression of catalytic TrkB alone conferred a survival response to BDNF, co-expression of truncated TrkB substantially reduced the BDNF survival response but had no effect on the NGF survival response. Our results suggest that BDNF responsiveness in developing sensory neurons is modulated by the relative levels of catalytic and truncated TrkB.

GRANT FUNDED: WELLCOME TRUST

399.2

LIGAND-INDUCED DOWNREGULATION OF TRKB IN CULTURES OF CEREBELLAR GRANULE CELLS

E. Hoppe, M.T. Serejo, B. D. Carter, and Y.-A. Barde.*
Max-Planck-Institute for Psychiatry, Department of Neurobiochemistry, D-82152 Martinsried, Germany.

The mechanism of desensitization of TrkB receptors by neurotrophic factors was investigated in cultures of cerebellar granule cells. Exposure of cells to BDNF resulted in a loss of binding of radiolabeled BDNF to TrkB. This reduction of binding correlated with a downregulation of $gp145^{TrkB}$ protein as shown by Western blotting of total cell lysates. In contrast to the full length TrkB, no regulation of the truncated $gp95^{TrkB}$ isoform could be observed. Consistent with an effect induced by direct receptor activation, the regulation of TrkB could be induced by BDNF and NT4/5 but not by NGF or NT3. The addition of BDNF resulted in a rapid reduction in the amount of TrkB with a half life time of 2-3 hours suggesting an involvement of cellular proteases. Accordingly, the ligand-induced downregulation was prevented by pretreatment of the cells with different protease inhibitors, with acetyl-leu-leu-norleucinal and lactacystin being the most potent ones. Therefore, the data suggest that proteases associated with the proteasome could be important for promoting the efficient degradation of TrkB.

399.3

BDNF DEFICIT IN THE CEREBELLUM OF *STARGAZER* MUTANT MICE: EFFECTS ON TRKB SIGNAL TRANSDUCTION. B. Knusel*, K. Rice and X. Qiao. University of Southern California, Los Angeles, CA 90089.

The BDNF deficit in the cerebellum of the mutant mouse *stargazer* (*stg*) presents a spontaneous model to study the effects of chronic BDNF deprivation on signal transduction of its transducing receptor, TrkB. We have earlier found TrkB mRNA in *stg* to be expressed at levels identical to control mice. In the present study we have analyzed TrkB protein levels and ligand-induced (NT-4/5) tyrosine phosphorylation of TrkB and some of its signal transducing molecules. By wheat germ lectin precipitation of glycoproteins and TrkB probing of western blots we have found normal levels of full-length and truncated TrkB protein in adult *stg* cerebellum. However, treatment of brain slices from *stg* cerebellum *in vitro* under physiologic conditions with NT-4/5 revealed a consistent deficit of TrkB signal transduction. Whereas the protein levels of the TrkB tyrosine kinase target phospholipase-Cγ1 (PLC) was elevated in *stg* cerebellum, PLC tyrosine phosphorylation after NT-4/5 stimulation was reduced when compared to slices from cerebellum of matched control mice. Similarly, NT-4/5-induced tyrosine phosphorylation of Erk1 and Erk2 were also reduced. Our data indicate a regulatory effect of BDNF on the effectiveness of TrkB signal transduction without changes in the level of BDNF receptor protein.

Supported by research grants from NIA (AG09793, AG10480), NINDS (NS22933) and Sankyo Co., Ltd.

399.4

BDNF AND NT-4/5 ACTIVATION OF *TRKB* RECEPTORS AND INITIATION OF INTRACELLULAR SIGNALING CASCADES *IN VIVO*. J. Blahos, II* and L.F. Kromer. Dept. of Cell Biology, Georgetown University Medical Center, Washington, DC 20007

The function of the *trkB* receptor ligands, BDNF and NT4/5, in adult rat brain remains disputable. Previous *in vitro* experiments using slice preparations indicated that there is little or no BDNF-induced *trkB* phosphorylation in CNS tissue obtained from rats after postnatal day 14, despite similar levels of full-length *trkB* receptors. This reduced phosphorylation response correlates with increased expression of truncated *trkB* (*trkB*-tr). Thus, it was hypothesized, that the presence of *trkB*-tr receptors results in the inhibition of the ligand effects. In the present study both a developmental time course and dose dependence for BDNF and NT-4/5 activation of *trkB* signaling pathways *in vivo* were evaluated. Intraparenchymal injections of BDNF or NT-4/5 into the neocortex and striatum induce *trkB* receptor phosphorylation and activate intracellular signaling cascades. Activation of *trkB* receptors is associated with a rapid phosphorylation of ERK1/2 that correlates with the degree of *trkB* receptor phosphorylation. The magnitude of these responses is dose dependent (10ng < 100ng) and varies with age. Our findings demonstrate that despite high levels of *trkB-tr* receptors in the adult CNS, even low amounts of *trkB* ligands (e.g. 10 ng BDNF/1µl CSF) result in the induction of intracellular signaling events associated with *trkB* receptor activation. Supported by NIH grant NS-31445.

399.5

INHIBITION OF GABA$_A$ SYNAPTIC RESPONSES BY BDNF IN RAT HIPPOCAMPUS. T. Tanaka*, H. Saito and N. Matsuki. Dept. Chemical Pharmacology, Fac. of Pharmaceutical Science, The Univ. of Tokyo, Tokyo 113, JAPAN.

Brain-derived neurotrophic factor (BDNF) is one of neurotrophins involved in the development and maintenance of both the peripheral and central nervous systems (CNS). Although the expression of BDNF and its receptor Trk B still occur in adult stage, their physiological role in the mature CNS is still not fully understood. In the present study we examined in detail the possibility that BDNF can modulate synaptic neurotransmissions using patch clamp technique in rat hippocampal CA1 region. BDNF (20-100 ng/ml) did not show any apparent effect on evoked EPSCs, but reduced evoked IPSCs amplitudes markedly within 5 min (55.6±9.9 % of control) and this reduction was persisted while BDNF was present. BDNF also attenuated GABA$_A$ receptor-mediated response to applied GABA (64.6±8.8 % of control). However, BDNF failed to attenuate IPSCs when the postsynaptic pyramidal neuron was loaded intracellularlly with K252a by the patch pipette containing 200 nM K252a, an alkaloid that inhibits the kinase activity of Trk receptor family. Intracellular application of 200nM K252b, a weaker inhibitor of Trk type kinase, did not affect the inhibition. The attenuating effect was also prevented by postsynaptic injection of U73122 (5 µM), a broad spectrum PLC inhibitor, and by strong chelation of intracellular Ca^{2+} with 10 mM BAPTA. These data suggest that BDNF modulates GABA$_A$ synaptic responses by postsynaptic activation of Trk type receptor and subsequent Ca^{2+} mobilization in the mature CNS. (Supported partly by Grant-in-Aid 05558095 from the Ministry of Education, Culture and Science of Japan)

399.6

Activation of Phosphatidylinositol (PI-3) Kinase by Brain Derived Neurotrophic Factor (BDNF) Gene Transfection

Y.H.Qiu, K.Yang*, X.Zhao, G.L.Clifton, R.L.Hayes, Neurosurgery, U of TX Health Science Ctr., Houston, TX 77030

Brain derived neurotrophic factor (BDNF) has therapeutic potential for treatment of the injured central nervous system (CNS). Gene transfer is an alternative method of introducing BDNF into the CNS. The transient expression produced by cationic lipid-mediated gene transfection may limit its application in diseases caused by genetic defects. However, cationic lipid transfection of neurotrophins may prove useful for treatment of CNS injury. Our laboratory is conducting systematic studies of the potential of cationic lipids for gene transfection in CNS (Yang, et al., Neurosci.Lett. 182:287;290, 1994). We recently reported reduced neurofilament loss in traumatized septo-hippocampal primary cell cultures by lipofectin-mediated BDNF cDNA transfer (Hayes, et al., Neurosci. Lett. 191:121, 1995).However, the mechanism of this effect is unknown. Recent studies have shown that the ability of NGF to prevent apoptosis is mediated by a PI-3 kinase signaling pathway in PC-12 cells (Yao, et al., Science 267:2003, 1995). We examined the consequence of cationic lipid-mediated gene transfection of BDNF in primary septo-hippocampal cell cultures (Whitson,J.S., et al., Brain Res. In press). Two days after BDNF gene transfection in primary cell cultures, PI-3 kinase activity assayed by thin-layer chromatograph (TLC) was significantly increased. The increased PI-3 kinase activity could also be inhibited by Wortmanin. Our studies suggest cationic lipid-mediated BDNF cDNA transfection involves PI-3 kinase signaling pathways in primary septo-hippocampal cell cultures. Further experiments are needed to more rigorously examine the possible role of PI-3 kinase activation in BDNF mediated effects on recovery from neuronal injury. (Supported by NIH grant PO1 NS 31998 and RO1 NS 21458).

399.7

IDENTIFICATION OF THE RECEPTORS WHICH BIND THE NEUROTROPHINS BDNF AND NT-3 DURING RETROGRADE AND ANTEROGRADE AXONAL TRANSPORT. C.S. von Bartheld*. Department of Physiology, University of Washington, Seattle, WA 98195

Retrograde and anterograde axonal transport of neurotrophins is a receptor-mediated process. BDNF and NT-3 are retrogradely transported to the isthmo-optic nucleus (ION), and NT-3 is anterogradely transported by retinal ganglion cells to the optic tectum in chick embryos (*Nature* 379:830-833; *J. Neurosci.*, in press). To determine which receptors (p75, trkB or trkC) bind BDNF and NT-3 before, during, and after axonal transport in the developing visual system, neurotrophins were iodinated and injected into the eyes of E15-16 chick embryos. After 20 hours, the retina, chiasm, ION and optic tectum were dissected, lysed, and the neurotrophins crosslinked to their receptors with EDC or DSS, and immunoprecipitated with antibodies specific for chicken p75, trkB or trkC. For examination of only retrograde transport (10-15% of total transport), anterograde transport (85-90%) was abolished with monensin. Retrograde transport: NT-3 bound to p75:trkC with a ratio of 1:1 in the chiasm during retrograde transport to the ION, and 2:1 in the ION. BDNF bound to p75:trkB with a ratio of 3:1 in the retina, 2:1 in the chiasm, and 1:1 in the ION. These data indicate that BDNF transfers from p75 to trkB in the ION or that the p75-bound BDNF dissociates or is degraded faster than the trkB-bound BDNF. Anterograde transport: NT-3 bound to p75:trkC with a ratio of 2:1 in the retina, 3:2 in the chiasm, and 1:2 in the optic tectum. These data indicate that NT-3 is transported by both p75 and trkC to the terminals of retinal axons in the tectum, and that NT-3 is preferentially released from p75 and/or binds predominantly to trkC after axodendritic transfer to tectal neurons. I am currently examining if full-length or truncated trkC receptors bind NT-3 in the chiasm, ION and optic tectum.

Neurotrophins: courtesy of R. Lindsay, Regeneron. Antibodies: courtesy of G. Weskamp, L. Reichardt, F. Lefcort, D. Clary. Supported by NIH grant HD 29177.

399.8

BDNF LOCALIZATION IN SYNAPTOTAGMIN-CONTAINING VESICLES OF PC12 CELLS AND IN SYNAPTOSOMAL FRACTIONS OF RAT BRAIN

James P. Fawcett, Peter S. McPherson, Raquel Aloyz, Freda Miller and Richard Murphy* Montreal Neurological Institute, McGill University, Montreal, Canada

NGF, BDNF, NT-3 and NT-4 play an important role in the development and survival of both CNS and PNS neurons. Neurotrophins produced in non-neuronal target tissues exert their action on innervating neurons, a mechanism described in the PNS. In the CNS, neurotrophins are produced and are exchanged by neurons. As well, some neurons can synthesize more than one neurotrophin. Neurotrophins can be released in an activity dependent fashion (Thoenen, H. (1995) Science 270:593-597) and radiolabelled neurotrophins can be anterogradely transported down CNS axons and released trans-synaptically (von Bartheld, C.S. et al. (1996) Nature 370:830-832). We have now begun to investigate possible biochemical mechanisms for this anterograde transport. Using a vaccinia virus system, we have expressed human recombinant BDNF in undifferentiated PC12 cells and detected mature BDNF and BDNF precursor by western blotting. Using well characterized methods of sucrose density centrifugation, we detected mature BDNF and BDNF precursor in fractions containing synaptotagmin, a known secretory vesicle marker; only a small amount was detectable within ER-containing fractions, as identified by calnexin antibodies. BDNF precursor containing samples in cell homogenates were digested by proteinase K in the presence but not absence of Triton X 100. Further, in mature rat brain, BDNF immuno-reactivity was enriched in a microsomal fraction isolated from lysed synaptosomes. In the brains of animals treated with kainic acid, a sharp increase occurred in BDNF immuno-reactivity within this fraction. These results are consistent with a model in which BDNF and its precursor are transported within a population of intracellular vesicles, the precise nature of these vesicles is yet to be characterized.

Work supported by NCE, Canada. We thank Dr. Nabil Seidah for providing the BNDF vaccinia virus.

399.9

PHARMACOLOGICAL ACTIVATION OF TRKB RECEPTOR IN THE CORTEX OF MICE. Carrie G. Causing*, Raquel S. Aloyz #, James P. Fawcett, Shernaz Bamji, John H. McLean, Tomas Reader#, Serge Rossignol# and Freda Miller. Montreal Neurological Institute, McGill University, Montreal & #Department of Physiology, University of Montreal, Montreal, Quebec.

Neurotrophic factors play a role in activity-dependent plasticity and survival. The NGF family of neurotrophins, BDNF, NT-4 and NT-3 act via preferential activation of the trk family of tyrosine kinase receptors. NGF binds to trkA, BDNF and NT-4 to trkB and NT-3 to trk. It is well established that cortical neurons depend on BDNF to develop and survive, and that they express both BDNF and the trkB receptor. Studies of embryonic cortical neurons in vitro suggest that BDNF acts via an autocrine loop in an activity-dependent fashion. In vivo studies have shown that the organization of visual cortex depends on visual input, and that BDNF is involved in this process. In these studies we determined whether activation of afferent input to adult cortical neurons can stimulate trkB activation in vivo. We injected adult mice with reserpine (5mg/kg) or yohimbine (2mg/kg) and 20 to 30 minute later sacrificed the mice , removed the brains, and dissected out the cortex on ice. The tissue was then homogenized, immunoprecipitated with pan-trk antibodies and the precipitates were analyzed on western blots with antibodies specific for phosphotyrosine or different domains of trkB. Our results suggests that pharmacologically-induced depletion of noradrenergic terminals induces trkB phosphorylation in vivo. Whether the BDNF responsible for the trkB activation originates from the noradrenergic afferents or indirectly from cortical neurons is not known. However, the presence of BDNF in axons immunoreactive for DBH suggest that anterograde secretion of BDNF could account for these results.

Work supported by NCE, Canada.

399.10

DISTRIBUTION OF BDNF IMMUNOREACTIVE PROCESSES IN THE MOUSE FOREBRAIN. J.H. McLean[#1], J.P. Fawcett [2], R.S. Aloyz[2], C.G. Causing [2], S.X. Bamji[2] and F.D. Miller [2]. [1]Div. of Basic Med. Sci., Memorial Univ. of Nfld., St. John's, Nfld., A1B 3V6 and [2] Centre for Neuronal Survival, McGill Univ., Montreal, P.Q., Canada H3A 2B4.

The presence of brain derived neurotrophic factor (BDNF) in the CNS has been described mainly in cell bodies and dendrites. However, there is evidence that BDNF is trafficked anterogradely in axons (von Bartheld et al., 1996). In this study, we examined the distribution of BDNF-like immunoreactive (IR) processes in the mouse brain using a sensitive polyclonal antibody to BDNF. Specificity of the IR label was determined by competition with the BDNF peptide immunogen which totally eliminated label, or by preincubation with NT-4, a similar neurotrophin to BDNF, which resulted in no change in IR label. Similar results of competition were observed on western blots. The immunoreactivity showed a distribution of BDNF similar to that described by others but, in contrast to previous reports, the BDNF was only observed in processes (axons and possibly dendrites). Using confocal microscopy, densities were observed within many processes, suggestive of vesicles within axons. Especially dense label was observed in tracts such as the lateral olfactory tract, corpus callosum, external and internal capsule. Process label was heterogeneous in the cortex with most intense label observed consistently in the deeper layers. Thus, the results are consistent with the possibility that BDNF is trafficked in axons and secreted anterogradely. Supported by the MRC and NCE of Canada.

399.11

MICE OVEREXPRESSING AN ISOFORM OF THE TRUNCATED TRKC RECEPTOR DEVELOP A NEURONAL PHENOTYPE SIMILAR TO THAT OF NT-3 DEFICIENT MICE. M.E. Palko, J. Blair-Flynn, S.W. Reid and L. Tessarollo*. Molecular Ontogenesis Group, ABL-Basic Research Program, NCI-FCRFDC, Frederick, MD 21702.

Recent knockout experiments in mice have confirmed a direct role for the Trk family tyrosine kinase receptors in mediating the function of the Nerve Growth Factor family of neurotrophins during development of the nervous system. This family of receptors includes three known members; the prototype trkA, trkB and trkC. These three loci encode for receptors with a catalytic tyrosine kinase domain. The trkB and trkC loci also encode for other isoforms some of which lack the catalytic domain. Whereas the function of the kinase active isoforms has been widely investigated and their role in signalling is relatively well understood, almost nothing is known about the in vivo role of the receptors lacking the tyrosine kinase domain. It has been proposed that this truncated receptor may act in vivo as a dominant-negative inhibitor of the full length isoform or by sequestering soluble neurotrophic factor therefore regulating the amount of neurotrophin interacting with the tyrosine kinase receptor. In order to address the mechanism by which this truncated receptor might exert its function we generated a transgenic mouse line overexpressing an isoform of the trunkated trkC receptor. Surprisingly, mice expressing high levels of the truncated trkC die within the first 2-3 days after birth and some of them exhibited the behavioral phenotype attributed to defects in proprioception. To discriminate between a dominant-negative or a neurotrophin-sequestering effect, we counted populations of neurons from sensory and sympathetic ganglia. These counts revealed severe sensory and sympathetic neuronal deficits similar to those observed in the NT-3 knockout mice. This result suggests that, physiologically, the truncated form of this receptor may act by sequestering soluble neurotrophic factor, therefore regulating the amount of neurotrophin available to interact with the receptor with the tyrosine kinase domain.

Research sponsored by the National Cancer Institute, DHHS, under contract with ABL

399.12

TARGET DEPENDENT AND INDEPENDENT EXPRESSION OF *trk*C TRANSCRIPTS IN DEVELOPING SENSORY NEURONS R. A. Oakley*, A.S. Garner, T. H. Large and E. Frank.
Dept. of Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261 and Dept. of Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

We are analyzing the developmental expression of *trk*C transcripts during the interaction of chick sensory neurons with their peripheral targets. Prior to target contact, most sensory neurons express *trk*C transcripts as revealed by *in situ* labeling with a probe to the extracellular domain of the receptor. After two days of target interaction, two distinct classes of *trk*C+ neurons can be distinguished on the basis of cell size and position; small dorsomedial (DM) and larger ventolateral (VL) neurons. By the end of the cell death period, *trk*C transcripts are only detected in the large VL neurons. These two populations can be further distinguished on the basis of target dependence. Early removal of the limb bud leads to a selective loss of the VL population. These results suggest that most sensory neurons express trkC during early phases of differentiation, before becoming dependent on peripheral targets.

To determine if both populations of *trk*C+ neurons could potentially respond to NT3, we also examined *trk*C expression using a probe specific for transcipts encoding catalytic forms of the receptor. Both DM and VL populations express catalytic forms of *trk*C as do sensory neurons that have yet to contact their peripheral targets. In addition, RT-PCR was used to examine the other splice variants of *trk*C. These experiments show that by the end of the cell death period, DRG neurons express transcripts encoding full length, kinase-insert, kinase-truncated and kinase-deleted forms of the *trk*C message, suggesting that all these splice variants are expressed in the *trk*C+ VL neurons that survive the cell death period.

Supported by grants from NINDS and the MDA.

399.13

FUNCTIONAL trkB NEUROTROPHIN RECEPTORS ARE COMPONENTS OF THE ADULT BRAIN POSTSYNAPTIC DENSITY. K. Wu*, H.T.J. Mount, E.S. Levine, J.L. Xu, P.C. Suen, S.Y. Lin, Y.Y. Huang[‡] and I.B. Black. Dept. of Neuroscience and Cell Biology, Robert Wood Johnson Med. Sch., Univ. of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854; [‡]Dept. of Neuroscience, New York State Psychiatric Inst. and Dept. of Psychiatry, Coll. of Physicians and Surgeons, Columbia Univ., New York, NY 10032

Neurotrophins have long been thought to act as target-derived factors that regulate the survival and differentiation of afferent neurons. Recently, brain-derived neurotrophic factor (BDNF) was shown to elicit a rapid increase in synaptic activity of cultured rat hippocampal neurons via enhanced responsiveness to excitatory input (Levine et al., 1995, Proc. Natl. Acad. Sci. USA 92: 8074-8077). These findings suggest a postsynaptic localization of neurotrophin receptors. In this study, we examined expression of trkB, a high affinity receptor for BDNF, in the postsynaptic density (PSD), a proteinaceous specialization of the postsynaptic membrane. Western blot analyses with antibodies to trkB revealed localization to the PSD in adult rat cerebral cortex and hippocampus. Only the full-length, active form of trkB was detected in PSD samples. BDNF treatment of cortical PSD resulted in a 5-fold increase in trkB autophosphorylation, supporting the contention that the PSD contains functional trkB. Truncated trkB, which does not contain the tyrosine kinase signaling domain, though present in membrane fractions, was undetectable in the PSD. In addition, there was a selective developmental increase in trkB in the PSD relative to the synaptic membrane fraction. The presence of trkB in the PSD during development and maturity is consistent with a role for neurotrophins in the regulation of synaptic activity through direct postsynaptic mechanisms.

(Supported by NIH grant HD 23315 and the Medical Research Council of Canada)

399.14

REGULATION OF TRKB ACTIVATION BY ALTERNATIVE SPLICING IN THE EXTRACELLULAR DOMAIN. K.L. Boeshore , A.S. Garner, and T.H. Large* Dept. of Neurosciences, CWRU, Cleveland, OH 44106-4975.

The generation of multiple trkB isoforms by alternative splicing suggests that receptor function is regulated, in part, at the level of receptor structure. An extracellular deletion (ED) isoform of trkB lacks 11 amino acids near the ligand binding region (Garner et al. 1996 J. Neurosci. 16: 1740), suggesting that it may exhibit altered neurotrophin binding properties. To test this hypothesis, ED or full-length (FL) isoforms were expressed in chicken embryonic fibroblasts (CEFs). NT-4/5 and BDNF were equally effective (maximum response at 25ng/ml) in activating the FL and ED isoforms, as determined by anti-phosphotyrosine Western blot analysis. In contrast, the maximum phosphotyrosine response to NT-3 was 10-fold lower for the ED receptor, compared to the FL receptor. The diminished response to NT-3 could be due to either a decrease in the number of phosphotyrosine residues on activated receptors or a decrease in the activation rate of the ED isoform. Although BDNF, NT-3, and NT-4/5 activated FL trkB receptors expressed in fibroblasts, only BDNF stimulated process outgrowth from PC12 cells expressing the FL isoform. Since PC12 cells, but not CEF cells, express endogenous p75 low-affinity neurotrophin receptor, we are now testing the ability of p75 to restrict ligand binding/activation of trkB isoforms. Thus, NT-3 activation of trkB receptors can be regulated by two distinct mechanisms: co-expression with neuronal elements, such as p75, or alternative splicing of the extracellular domain.

(Supported by NIH EY08885).

399.15

TRUNCATED *TRKB* RECEPTORS ARE INHIBITORY TO NEURITE OUTGROWTH *IN VITRO*. L.F. Kromer*, R.H. Fryer, and D.R. Kaplan[1]. Dept. of Cell Biology, Georgetown University Medical Center, Washington, DC 20007 and [1]The Montreal Neurological Institute, Montreal, Quebec, Canada H3A 2B4

Currently the function of truncated *trkB* receptors (*trkB*-T1) on glia in the adult CNS and their role in promoting or inhibiting axonal regeneration are unknown. Our data indicate that mature CNS glia in myelinated pathways up-regulate *trkB*-T1 receptors in response to axonal degeneration, whereas PNS Schwann Cells down-regulate this receptor. Given the failure of CNS regeneration, these observations suggest that *trkB*-T1 receptors on non-neuronal cells may hinder axonal regeneration. To further evaluate this hypothesis, *trkB* expressing SY5Y neuroblastoma cells were seeded on monolayer substrates of 3T3 fibroblasts or stably transfected 3T3 cells expressing *trkB*-T1 receptors. Phosphorylation of *trkB* receptors and neurite outgrowth (at 48hr) were evaluated at various concentrations of BDNF (0, 5, 25, and 100ng/ml). In the co-culture experiments, *trkB*-T1 on 3T3 cells reduced the percentage of SY5Y cells developing neurites and greatly inhibited both BDNF-induced neurite extension and *trkB* receptor phosphorylation on SY5Y cells. These results suggest that *trkB*-T1 on non-neuronal cells can block activation of neuronal *trkB* receptors and inhibit neurite outgrowth by a transcellular mechanism. Thus, truncated *trkB* receptors may contribute to the lack of axonal regeneration from BDNF responsive neurons that is observed following CNS lesions in adults. Supported by NIH grant NS-31445.

399.16

TRANSCRIPTIONAL REGULATION BY BDNF AND PACAP INVOLVED IN THE CONTROL OF APOPTOSIS IN CEREBELLAR GRANULAR NEURONS.

Y. Larmet, P. Kienlen-Campard, C. Gaiddon, C. Crochemore, A.L. Boutillier*, J.P. Loeffler, URA 1446 CNRS, IPCB, Université Louis Pasteur, 21, rue René Descartes, 67084 Strasbourg Cedex, France.

The contribution of BDNF and PACAP as well as the transcriptional regulations involved in programmed cell death were investigated in primary cultures of cerebellar neurons. TUNEL histochemistry revealed that K+ deprivation induces extensive neuronal apoptosis, and BDNF or PACAP reverse this effect. Since apoptotic processes require de novo protein synthesis, the regulatory pathways controling gene expression were investigated. We show that both PACAP and BDNF stimulates *c-fos* and *c-jun* mRNA accumulation in cerebellar neurons. Detailed promoter deletion analysis by gene transfer revealed that both ligands operate through the same cis-acting elements. Our results indicate that the SRE, FAP element and the CRE present in the *c-fos* promoter are able to respond independently to BDNF and PACAP. By using the Gal4-CREB fusion protein to specificaly study CRE-driven transcription, we show that BDNF and PACAP stimulates CREB dependent transcription through phosphorylation of Serine 133. In addition, unlike the other activators of CREB in neurons, BDNF neither stimulates the cAMP/PKA pathway (e.g. PACAP, CRH etc...) nor induce detectable variation of intracellular free Calcium(e.g. K+, Glutamate, etc...).Collectively these data indicate that under specific experimental conditions, second messenger-coupled receptors (PACAP) and neurotrophins (BDNF) stimulate a common set of genes although they transduce through distinct regulatory pathways.

399.17

FUNCTIONAL ANALYSIS OF THE TRKB RECEPTOR TYROSINE KINASE. J. H. McCarty and S. C. Feinstein*. Neuroscience Research Institute and Department of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, CA 93106.

The TrkB protein tyrosine kinase is a high affinity receptor for the neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5). Neurotrophin binding leads to activation of the receptor's cytoplasmic tyrosine kinase domain, leading to the autophosphorylation on five intracellular TrkB tyrosines: Y484, Y670, Y674, Y675, and Y785. Phosphorylation of these residues initiates a series of poorly understood cytoplasmic and nuclear signal transduction events, which lead to differentiative or proliferative responses, depending on the cellular context.

To better understand the role of individual autophosphorylation sites in mediating BDNF and NT-4/5 action, we have generated a panel of stably transfected 3T3 and PC12 cells expressing trkB receptors containing a variety of tyrosine to phenylalanine substitutions. These mutant receptors have been assessed for their ability to engage in a number of wild type trkB activities, such as promoting cellular differentiation or proliferation, autophosphorylation, association with SHC, SYP, and PLC-γ and c-fos induction. A comparative analysis of the signaling pathways used in these two cellular contexts will be presented. Supported by American Heart Association #94-421 and PHS grant EY10739.

399.18

FUNCTION OF trkB NEUROTROPHIN RECEPTOR SPLICE VARIANTS. T. Saarelainen*, M. Mochniakow+, M. Saarma+ and E. Castrén . A.I.Virtanen Institute, University of Kuopio, P.O.Box 1627, 70211 Kuopio, Finland, +Institute of Biotechnology, University of Helsinki, 00014 Helsinki, Finland.

Alternative splicing of trkB mRNA generates different trkB isoforms, each possessing the same extracellular and transmembrane domains but differing in their intracellular regions. The full length trk-TK(+) form contains the catalytically active tyrosine kinase (TK) domain but the truncated trk-TK(-) forms lack this domain. Both variants bind BDNF, but only trkB-TK(+) activates intracellular signal transduction. Trk-TK(-) forms may prevent the trophic actions of BDNF by mediating internalization of BDNF into non-neuronal cells. In addition, if the two isoforms are expressed in the same neurons, trkB-TK(-) may act as a dominant negative receptor.

We are investigating the ability of trk-TK(-) to act as a physiological dominant-negative isoform in neurons. We have created expression constructs of trkB splice variants under CMV or elongation factor 1 promoter. The constructs were mutated to contain an octapeptide tag sequence, which is recognized by a monoclonal antibody (IBI Flag®). These plasmids will be transiently transfected into cell lines and cultured primary hippocampal neurons and the ability of BDNF to induce signal transduction or support survival of transfected cells will be monitored.

Supported by Sigrid Juselius Foundation and the Academy of Finland.

399.19

Signal transduction of TrkB in septal neurons in primary culture. K. Wada, M. Ishikawa, N. Okada, Y. Iwasaki, and S. Koizumi*, International Research Laboratories, Ciba-Geigy Japan Ltd., Takarazuka 665, Japan

Brain-derived neurotrophic factor (BDNF), one of the neurotrophin, promote the survival and differentiation of neurons both in the PNS and CNS. BDNF stimulates its receptor, TrkB, and the autophosphorylation of TrkB initiates the intracellular signal transduction.

In the present investigation, we have examined the presence of TrkB receptors in septal neurons by immunostaining and western blot analysis. In addition, the association and tyrosine phosphorylation of different intracellular signaling molecules induced by TrkB stimulation have been studied in primary neurons.

Septal neurons were prepared from fetal rats (E17), plated at the initial cell density of 1.0×10^5 / cm^2 and maintained for 3 days. When the neurons were treated with BDNF, but not NGF, the tyrosine phosphorylation of a Trk family protein(s) was clearly stimulated. In addition, BDNF was more potent than NGF in promoting the survival of septal neurons. In the down stream signal transduction of the receptor, the tyrosine phosphorylation of SHC proteins, PLC γ and MAP kinase were stimulated with BDNF-treatment.

To confirm that the BDNF activity is mediated through TrkB activation in septal neurons, an immunohistochemical study was performed using specific antibody against the tyrosine kinase domain of TrkB. The staining result clearly demonstrated the presence of TrkB in most of the neuronal population. In conclution, our data supports the importance of TrkB in mediating the action of neurotrophin in septal neurons under this condition.

399.20

EXPRESSION OF *trk*B AND *trk*C mRNAS BY DOPAMINERGIC NEURONS OF THE ADULT RAT VENTRAL MIDBRAIN S. Numan* and K.B. Seroogy. Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536.

Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are two members of the neurotrophin family that have been shown to increase the survival and differentiation of ventral midbrain dopamine neurons *in vitro*. Since BDNF and NT-3 mRNAs are expressed by midbrain dopamine neurons, these neurons may be able to derive trophic support locally. In order to respond to BDNF or NT-3, the neurons must express the appropriate high-affinity receptors, the tyrosine kinase receptors *trk*B or *trk*C, respectively. Although both *trk*B and *trk*C mRNAs have been detected in the ventral midbrain, their precise distribution within this region remains to be determined. In the present study, adjacent sections through the adult rat mesencephalon were processed for the *in situ* hybridization localization of *trk*B and *trk*C mRNAs. Double-labeling *in situ* hybridization was also performed to determine the proportion of tyrosine hydroxylase (TH, a dopamine cell marker) mRNA-expressing cells that also express *trk*B or *trk*C mRNA. *trk*B cRNA-hybridizing cells were densely labeled medially in the substantia nigra pars compacta (SNc) and moderately labeled laterally in the SNc, the substantia nigra pars lateralis, the substantia nigra pars reticulata and the ventral tegmental area. A more uniform and moderate expression of *trk*C mRNA was observed in all of the above nuclei. The double-labeling results demonstrated that virtually all of the TH mRNA-expressing cells in the ventral midbrain also expressed *trk*B or *trk*C mRNAs. In contrast, there were additional cells within the above nuclei that expressed the neurotrophin receptor mRNAs but were not dopaminergic. These results provide evidence that midbrain dopaminergic neurons may be able to derive trophic support *in vivo* via paracrine and/or autocrine interactions. Supported by NIMH fellowship MH10806 (SN) and the National Parkinson Foundation (KBS).

400.1

ACTIVATION OF SHP-2 BY NEUROTROPHIN B.A. Goldsmith*, K. Wada, N. Okada and S. Koizumi. Bio-organics Res.Dept., Int'l Research Laboratories, Ciba Geigy (Japan) Ltd., Takarazuka 665, Japan.

Upon ligand binding, the Trk family of receptor tyrosine kinases become autophosphorylated leading to recuitment of proteins to the receptor via *Src* homology 2 domains (SH2) interactions. Protein tyrosine phosphatases (PTPases) with SH2 domains may play a role in regulating receptor tyrosine kinases. These phosphatases, SHP-1 and SHP-2, may bind to activated receptors or other phosphoproteins to dephosphorylate the targets. This localization may also activate the PTPases by phosphorylation and/or occupation of the SH2 domains. To examine the role of SHP-2 in neurotrophin signalling, we asked whether Trk activation regulates SHP-2 in cortical neurons and PC12 cells.

We found that application of BDNF to cortical neurons caused a significant increase in PTPase activity in SHP-2 immunoprecipitates. In addition, BDNF induced the formation of a complex of SHP-2 with tyrosine phosphorylated proteins. In PC12 cells, application of NGF similarly stimulated PTPase activity in SHP-2 immunoprecipitates and induced a complex of tyrosine phosphorylated proteins with SHP-2. In cortical neurons and PC12 cells, Trk receptors activation also increased PTPase activity associated with the receptors. To further characterize the SHP-2/Trk interaction, we are now utilizing an in gel PTPase assay (Burridge and Nelson (1996) *Anal. Biochem.* **232**, 56-64.) to assess whether SHP-2 associates with Trk and Trk-associated proteins.

These results show that Trk stimulation regulates SHP-2 activity and localization, suggesting that SHP-2 plays an important role in regulating the Trk signal. No tyrosine phosphorylation of SHP-2 was detected after neurotrophin treatment, suugesting that the enhancement of PTPase activity may be a result of occupation of SHP-2's SH2 domains by the coprecipitated phosphoproteins.

400.2

PROTEIN-TYROSINE-PHOSPHATASE 2C IS NECESSARY FOR MAXIMAL ACTIVATION OF THE MITOGEN ACTIVATED PROTEIN KINASE BY THE LEUKEMIA INHIBITORY FACTOR RECEPTOR AND GP130. J.L. Bartoe, S.E. Hamilton*, and N.M. Nathanson. Department of Pharmacology, Univ. of Washington, Seattle, WA 98195.

The neurokines ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) initiate transmembrane signaling through heterodimerization of two shared receptor subunits, the low affinity LIF receptor α (LIFRα) and gp130 polypeptides. The protein-tyrosine-phosphatase 2C (PTP2C) has been shown to associate with gp130, and the domain necessary for binding of PTP2C by gp130 lies within a region recently identified by our lab as necessary for activation of mitogen activated protein kinase (MAPK). Using chimeric receptors composed of the extracellular domains of the granulocyte colony stimulating factor receptor (GR) and the cytoplasmic domains of either the LIFRα and gp130 (GR/LIFRα and GR/gp130), we have shown that PTP2C is necessary for maximal activation of MAPK by both the LIFRα and gp130 polypeptides. Overexpression of a dominant negative PTP2C construct reduces MAPK activation by GR/LIFRα and GR/gp130 by as much as 70%, while overexpression of a wild type PTP2C construct had no effect on MAPK activation. Our results suggest that both gp130 and the LIFRα can associate with and activate PTP2C, leading to the activation of MAPK. (Supported by the National Institutes of Health).

400.3

Generation of various major and minor RPTPζ/β isoforms and regulation of their mRNA expression

J. Li*, S. E. Snyder, P. E. Schauwecker#, T. H. McNeill# and S. R. J. Salton. *Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY, 10029. #Andrus Gerentology Center, University of Southern California, Los Angeles, CA, 90089.*

The receptor protein tyrosine phosphatase RPTPζ/β is distributed throughout the rat nervous system, abundantly in the embryonic and early postnatal brain, primarily in astroglia, but also in certain neuronal populations. RPTPζ/β, expressed as chondroitin sulfate proteoglycans (CSPGs), binds to cell adhesion molecules and the extracellular matrix protein tenascin. Previously three major (e.g. phosphacan) and at least one minor isoform of rat RPTPζ/β have been identified. In this study, we have used genomic southern analysis and genomic library screening to demonstrate that these various rat RPTPζ/β isoforms are encoded by the same gene and are generated by alternative mRNA splicing. Genomic sequences around exon/intron splicing junctions of all the major RPTPζ/β isoforms have also been determined. Furthermore, we have found that mRNA expression of these RPTPζ/β isoforms is differentially regulated during neural development and is induced in regions of axonal sprouting and glial scarring in response to CNS lesions. Supported by grants from the NIH, Pew Foundation and American Paralysis Association.

400.4

DISTRIBUTION OF RECEPTOR PROTEIN TYROSINE PHOSPHATASE KAPPA IN A TRANSGENIC MOUSE BRAIN. P. Shen, P. Canoll, J. Sap, G.I. Botchkina and J.M. Musacchio*. Dept. of Pharmacology, NYU Medical Center, New York, NY 10016

RPTP-k is expressed at high levels in brain, kidney and liver, and contains an ectodomain that engages in homophilic cell-cell interactions (Sap et al., 1994, Mol. Cell. Biol., 14:1). In a transgenic mouse model, which has a LacZ insertion upstream of the phosphatase domains of the RPTP-k gene (Skarnes et al., 1995, PNAS, 92:6592), visualization of RPTP-k expression by ß-galactosidase activity revealed a widespread, but non-uniform, cellular distribution throughout the brain and spinal cord. The majority of labeled cells occurred in the superficial layers of the cerebral cortex. In the preoptic/hypothalamic area, the suprachiasmatic n., anterior medial preoptic n. and arcuate hypothalamic n. expressed RPTP-k. The medial amygdaloid n. was intensely labeled. Mesencephalic areas with prominent RPTP-k expression included the colliculi, central gray and raphe. RPTP-k was found in the spinal trigeminal n. and nucleus of the solitary tract in the medulla. In the spinal cord, labeled cells appeared in the dorsal horn and area X. Labeled cells also were found in the trigeminal and dorsal root ganglia. Most labeled cells in the cerebellum occurred in the granule cell layer. Ependymal cells were labeled throughout the CNS. Because these data are consistent with the wildtype RPTP-k immunostaining pattern, this transgenic model may be useful in determining the role of tyrosine phosphatases in the brain.

Supported by USPHS grant 1RO1 MH51634 and RSA MH17785.

400.5

CLONING AND CHARACTERIZATION OF A NOVEL RECEPTOR PROTEIN TYROSINE PHOSPHATASE EXPRESSED IN RAT BRAIN AND OLFACTORY TISSUE. L. Rydelek Fitzgerald*, K.M. Walton^, and B.L. Largent. Dupont Merck Research Labs, CNS Molecular Biology Research, Wilmington, DE 19880 and Univ. of Maryland, Dept. of Anatomy, Baltimore, MD 21201 ^Cephalon Inc., West Chester, PA, 19380.

Protein Tyrosine Phosphatases (PTPs) regulate the level of tyrosine phosphorylation within a cell and as a result play an important role in both neuronal function and development. Here we report the cloning of a novel protein tyrosine phosphatase expressed in rat brain and olfactory tissue. PCR was performed on cDNA from rat olfactory tissue using degenerate primers designed to the conserved catalytic regions of PTPs. A novel PCR fragment showed approximately 73% sequence homology with other PTPs. Northern blot analysis using this fragment as a probe showed expression of a 5.4 kb mRNA in rat brain and to a lesser extent in olfactory tissue. A 5.2 kb clone as well as several other overlapping clones were isolated by screening a rat whole brain cDNA library. Sequence analysis demonstrated that this clone encodes a receptor phosphatase and appears to be homologous to a newly developing subclass of receptor PTPs. Our clone, as well as other previously published clones, contains a single catalytic domain which diverges from the consensus sequence at a critical amino acid previously shown to be important for phosphatase activity on a variety of substrates. The role of this class of putative phosphatases in regulating tyrosine phophorylation has yet to be determined. (NIH Grant DC01946)

400.6

STEP$_{61}$ AND STEP$_{38}$: NEW MEMBERS OF A FAMILY OF BRAIN-ENRICHED PTPS. A. Bult*[1], F. Zhao[1], R.A. Dirkx[2], Jr., E. Sharma[3], E. Lukacsi[4], M. Solimena[2], J.R. Naegele[1,4] and P.J. Lombroso.[1] [1]Child Study Ctr., and [2]Dept. Med., Yale Univ. Sch. Med., New Haven, CT 06520; [3]Ctr. Mol. Behav. Neurosci., Rutgers Univ., NJ 07102; and [4]Dept. of Biol., Wesleyan Univ., Middletown, CT 06459.

Tyrosine phosphorylation appears to be an essential component of many signal transduction pathways involved in neuronal development and differentiation. Studies of protein tyrosine kinases and protein tyrosine phosphatases (PTPs) in the CNS indicate that many members of these classes of enzymes are likely to exist. We have previously identified and characterized a family of brain-enriched PTPs designated STEP. Members of this family of PTPs arise through alternative splicing, with the result that cytosolic, transmembrane, and truncated forms are expressed in the nervous system. Here we report findings concerning two novel members of the STEP family, STEP$_{61}$ and STEP$_{38}$. Their NH$_2$-terminal regions contain several amino acid motifs not present in a cytosolic form, STEP$_{46}$. These motifs include two putative transmembrane domains, two PEST sequences, and two polyproline-rich domains. Transfection experiments in fibroblasts, as well as subcellular fractionation, sucrose density gradient, immunofluorescence staining, and immunogold EM studies have shown that STEP$_{61}$ and STEP$_{38}$ are targeted to the endoplasmic reticulum. Enzymatic assays indicate that STEP$_{61}$ has approximately 8-fold less phosphatase activity than STEP$_{46}$. As STEP$_{46}$ is contained in its entirety within STEP$_{61}$, our data suggest that the NH$_2$-terminus of STEP$_{61}$ is responsible not only for targeting STEP isoforms, but also for regulating their enzymatic activity.

Support: NARSAD, NIH, NIMH, Juvenile Diabetes Foundation.

400.7

LAR TRANSCRIPTS WITH A CAG-RICH ALTERNATIVE 3' UNTRANSLATED REGION RESULT FROM *TRANS*-SPLICING. C. Zhang*, J.S. Zhang, L.A. Martignetti, S.M. Massa and F.M. Longo. Dept. of Neurology, UCSF/VAMC, San Francisco, CA 94121 and Dept. of Pediatrics, Mt. Sinai Med Ctr, NY, NY.

Trans-splicing of RNA transcripts derived from separate genes has been found in trypanosomes but its existence in mammalian cells is not widely established. The Leukocyte Common Antigen-Related (**LAR**) gene codes for a tyrosine-phosphatase receptor which may regulate mammalian neural development (Longo et al., JBC 1993). We isolated an LAR cDNA clone containing an alternative 3' untranslated region (3'UTR) with extensive tandem CAG repeats (JS Zhang and FM Longo, JCB 1995). RT-PCR of rat brain mRNA using primers flanking the junction between the known LAR sequence and the alternative sequence verified that these alternative transcripts exist *in vivo*. Northern analysis of rat cerebellum mRNA with probes corresponding to the constitutive and alternative 3'UTRs further confirmed their presence. Unexpectedly, Southern and PCR analysis of P1 genomic clones (> 80kb inserts) derived with either constitutive (2 P1 clones) or alternative (4 P1 clones) 3'UTR PCR primers showed no overlap. The absence of overlap suggested that the alternative 3'UTR exon was separated from the remainder of the LAR gene by an unconventionally large segment of genomic DNA. Fluorescent in-situ hybridization (FISH) with LAR P1 and alternative 3'UTR P1 probes demonstrated that these loci are in fact on different chromosomes. Thus, LAR transcripts with the alternative 3'UTR are derived by *trans*-splicing. The existance of *trans*-splicing in mammalian cells may have important implications for gene mapping and for defining the concept of a mammalian gene. Supported by the ACS (FL), NIA (FL) and V A (FL).

400.8

LAR TYROSINE PHOSPHATASE RECEPTOR: EXPRESSION AND FUNCTION DURING SCIATIC NERVE REGENERATION. Youmei Xie*, Tao Yang, Tracy T. Yeo, Cheng Zhang and Frank M. Longo. Dept. of Neurology, UCSF/ VAMC, San Francisco, CA 94121 .

Developmentally-regulated expression of the Leukocyte Common Antigen-Related (**LAR**) tyrosine phosphatase receptor and neuron-preferential alternative splicing suggested that LAR regulates mammalian neural development (Longo et al. SNS, 1991; Longo et al, JBC, 1993; Zhang and Longo, JCB, 1995). In previous studies we found aberrant cholinergic innervation of the dentate gyrus in LAR-deficient transgenic mice (Yang et al SNS 1995 and Yeo et al, submitted). Since LAR is expressed by spinal cord motor and dorsal root ganglia (DRG) sensory neurons (Zhang et al, SNS, 1995) we tested the hypothesis that LAR also functions during peripheral nerve regeneration. We evaluated alternative splicing of LAR in DRG and lumbar spinal cord in response to sciatic nerve transection. RT-PCR demonstrated that following transection: i) the proportion of LAR transcripts containing LAR alternatively spliced element-a (LASE-a) decreased in spinal cord; ii) the proportion of LAR transcripts containing LASE-c decreased in spinal cord and increased in DRG; iii) LAR levels increased in spinal cord and DRG. LAR-deficient transgenic mice were used to evaluate neurite outgrowth by the pinch method (performed in a blinded manner) in sciatic nerves 14 days following crush injury. LAR +/- mice (n=5) demonstrated a 27% decrease in outgrowth distance compared to +/+ mice (n=6), (p= 0.001). These studies demonstrate the first peripheral nervous system phenotype for a tyrosine phosphatase transgenic mouse and introduce a major new gene family playing an important role in mammalian nerve regeneration. Supported by: NIA (FL) and VA (FL).

400.9

LAR RECEPTOR-DEFICIENT TRANSGENIC MICE: ALTERED EXPRESSION OF NEUROTROPHIN RECEPTORS. T. Yang*, Y. M. Xie, T. T. Yeo, and F. M. Longo. Dept. of Neurology, UCSF/VAMC, San Francisco, CA 94121 .

Developmentally regulated expression and alternative splicing of the Leukocyte Common Antigen-Related (**LAR**) tyrosine phosphatase receptor in mammalian neurons suggests a role in neural development (Longo et al, SNS Abst, 1991; Longo et al, JBC, 1993; Zhang and Longo, JCB, 1995). Our previous Northern analyses of LAR-deficient transgenic mice demonstrated decreased LAR transcripts in +/- mice and trace LAR transcripts in -/- mice (Yang et al SNS Abst, 1995). In this study, Western analysis confirmed similar decrease in LAR protein. The tyrosine phosphatases regulating Trk receptor activity are unknown. To analyze potential functional interactions between LAR and neurotrophin receptors, mRNA and protein levels of TrkA, TrkB, TrkC and p75 were examined in LAR +/+, +/- and -/- mice using RT-PCR and Western blots. In -/- mice, there was a significant decrease of TrkA mRNA expression in whole brain at postnatal day 0 (PO) and in the adult hippocampus. TrkA mRNA expression was increased in the adult septum and spinal cord. TrkB mRNA was increased only in the adult hippocampus. No significant change was found for TrkC. The ratio of TrkA/p75 mRNA was decreased in adult cortex and hippocampus and increased in septum. On the protein level, there was a marked reduction of TrkA in whole brain at PO. p75 protein level was unchanged in whole brain at PO but was significantly decreased in the adult. In all cases, intermediate levels of mRNA and protein were observed in +/- mice and in all cases (except expression of p75 protein in the adult) changes in mRNA and protein were correlated. Current experiments will confirm whether neurons derived from +/+ and -/- mice have differential responses to neurotrophins. These observations support our hypothesis (Longo et al, JBC, 1993) that LAR-type tyrosine phosphatases regulate neurotrophin function through direct or indirect interaction with neurotrophin receptors. Supported by an AFAR Beeson Award (FL),NIA R01 (FL), VA (FL).

400.10

SPATIAL-TEMPORAL EXPRESSION PATTERNS OF THE PROTEIN TYROSINE PHOSPHATASE, PC12-PTP1, IN RAT BRAIN. T.M. Perney* and E. Sharma. Center for Molecular and Behavioral Neuroscience, Rutgers, Newark, NJ 07102.

In the nervous system, protein tyrosine phosphatases play important role in neuronal differentiation, axon guidance and signal transduction. Recently, a novel protein tyrosine phosphatase (PC12-PTP1) was isolated from nerve growth factor treated PC12 cells (JBC 270: 49-53, 1995). We have begun to examine the expression pattern of PC12-PTP1 mRNA in the rat brain. Two major transcripts of 2.6 and 3 kb are detected on Northern blots of total rat brain RNA (only the 3 kb transcript is present in PC12 cells). Regional analysis of PC12-PTP1 expression revealed that the 3 kB transcript is present in all brain regions, whereas the 2.6 kb transcript is detectable only in the cerebellum and brainstem. *In situ* hybridization histochemistry showed that the subpopulation of neurons with highest levels PC12-PTP1 message are the Purkinje cells of the cerebellum and granule cells of the dentate gyrus

The expression levels of the two transcripts were found to be differentially regulated during early postnatal development. The 3 kb transcript is detected as early as embryonic day 17 and expression levels decrease dramatically between PD14 and PD17. The expression of the 2.8 kb transcript, on the other hand, does not appear until approximately PD14 and is followed by a sharp increase in the expression level that is maintained into adulthood. The switch in the expression levels of the two transcripts occurs at a time when many neurons are becoming both morphologically and functionally mature. It is possible that PC12-PTP1 may play an important role in these developmental changes. (Supported by a Hoechst Celanese/Rutgers Innovative research award)

400.11

THE ROLE OF PROTEIN TYROSINE DEPHOSPHORYLATION IN ACTIVATION OF VOLUME-SENSITIVE CHLORIDE CHANNELS. P. Doroshenko*. Loeb Research Institute, Univ. of Ottawa, Ottawa, Ontario K1Y 4E9, Canada

Involvement of protein tyrosine kinases (PTK) and phosphatases (PTP) in activation of volume-sensitive Cl channels (J. Physiol. 449: 197, 1992) has been studied in bovine chromaffin cells using whole-cell patch clamp techniques. PTK inhibitor, tyrphostin B46 (5-20 μM), applied either extra- or intracellularly, did not prevent the current activation by GTPγS or cell swelling. However, sodium pervanadate, an inhibitor of PTP, effectively inhibited the current when added to the patch pipette solution (100-200 μM). To see whether stimulation of protein tyrosine dephosphorylation may result in activation of the Cl channels in the absence of other stimuli, we used a recombinant enzyme, a murine analog of the human T-cell protein tyrosine phosphatase (Mol. Biol. Cell 14: 3030,1994). In preliminary experiments, a slow developing inward current was observed in 13 out of 17 cells perfused with the GST-PTP fusion protein. The current reversed at close to E_{Cl} (0 mV). No current was observed in cells perfused with the PTP inactivated by boiling (4 cells) or by addition of 200 μM sodium pervanadate (4 cells). The 34-kDa catalytic subunit of PTP from *Yersinia enterocolitica* (Calbiochem) failed to elicit the current when applied in similar conditions. These findings suggest that protein tyrosine dephosphorylation may mediate activation of the volume-sensitive ion channels. *Supported by the Loeb Research Institute.*

400.12

SOMATOSTATIN (SRIF) INHIBITION OF CELL PROLIFERATION IS IMPAIRED BY E1A ONCOGENE EXPRESSION VIA THE UNCOUPLING OF SRIF RECEPTORS TO PHOSPHOTYROSINE PHOSPHATASES T. Florio*, A. Scorziello and G. Schettini. Dept. Clinical and Experimental Oncology, Univ. of Genoa, IST, Unit of Neuroscience Advanced Biotechnology Center, Largo R.Benzi 10, 16132 Genova, Italy.

The effects of SRIF on the proliferation of PC Cl3 thyroid cell line, the intracellular mechanisms involved and the alterations induced by the malignant transformation of these cells, by means of stable expression of the E1A oncogene, are reported. In PC Cl3 SRIF inhibited insulin- and insulin+TSH-dependent cell proliferation, blocking the G1-S progression in the cell cycle. These effects were completely reverted by vanadate, suggesting that SRIF may modulate phosphotyrosine phosphatases (PTP) activity. Indeed, SRIF increased by 100% PTP activity. In the E1A-transformed cell line (PC E1A), SRIF was completely ineffective in inhibiting cell proliferation although SRIF receptors were regularly expressed and coupled to adenylyl cyclase activity. Conversely, in PC E1A cells, an alteration in the coupling of SRIF receptors to the phosphotyrosine phosphatase occurred. In conclusion, SRIF activates PTP in PC Cl3 thyroid cells to inhibit cell proliferation, and that the stable expression of E1A oncogene in these cells completely abolishes this antiproliferative effect. (AIRC 1994 to GS and CNR 95.02266 to TF).

401.1

EFFECTS OF p75NGFR ANTIBODY ON SURVIVAL OF CULTURED SYMPATHETIC NEURONS. M. Freidin* and M. V. Chao, Dept. Cell Biol. and Anat., Cornell Univ. Coll. of Medicine., New York, NY 10021

Sympathetic neurons become dependent on different neurotrophins during development in direct correlation with the expression of the corresponding high affinity Trk receptor. The low affinity p75NGFR, which binds all neurotrophins with similar affinity, is expressed at increasing levels across development. Neonatal rat sympathetic neurons are dependent on NGF for maturation and survival and express high levels of both Trk A and p75NGFR. To examine the role of p75NGFR on survival of these cells, we treated dissociated cultures of rat superior cervical ganglion cells (SCG) with an antibody to the extracellular domain of the rat p75NGFR (α-pREX). 36-48H cultures were treated with purified α-pREX in the presence of NGF (100ng/ml) and examined after 12-18H. Exposure to the antibody caused a significant decrease in cell survival. The effect was target specific, dose dependent and reversed by cycloheximide and ActinomycinC1. αpREX treatment affected both neurons and nonneuronal cells,while withdrawl of NGF affected only neuronal survival. Antibody-mediated cell death was partially blocked by forskolin and unaffected by ceramide. By contrast, NGF withdrawl was partially reversed by ceramide. To examine the cellular mechanism of α-pREX, nuclear extracts from cultured SCG were examined for NFκB and AP1 binding by EMSA. Changes in NFκB and AP1 binding were observed with time following treatment. These effects were attenuated by pretreatment with forskolin and dexamethasone. A different shift pattern and time course for NFκB binding were observed following treatment with TNFα. These results suggest α-pREX stimulates cell death in cultured sympathetic neurons through apoptosis. Moreover, these observations suggest a role for p75NGFR in neuron survival.

401.2

NGF-INSENSITIVE APOPTOSIS INDUCED BY NFκB INHIBITION IN PC12 CELLS. G.Taglialatela* & J.R.Perez-Polo, Dept. of HBC&G, UTMB Galveston, TX.

The mechanism(s) underlying NGF-mediated rescue of neurons from apoptosis is poorly understood. The report that the transfection of the low-affinity NGF receptor ($p75^{NGFR}$) induces apoptosis prompted us to analyze $p75^{NGFR}$ signal transduction in the modulation of apoptosis in PC12 cells. Since $p75^{NGFR}$ and the tumor necrosis factor α (TNFα) receptor (TNFR) belong to the same receptor protein superfamily, the nuclear factor kappa B (NFκB) transcription factor mediating TNFα-induced apoptosis in lymphoid cells could also mediate $p75^{NGFR}$ signal transduction. Here we report that inhibition of NFκB function by treatment with a proteasome inhibitor (PSI) or a mild free-radical scavenger (PDTC) results in apoptosis of rat PC12 cells. Furthermore, NGF did not protect PC12 cells from cell death induced by the inhibition of NFκB. These results indicate that NFκB function is essential to PC12 cell survival and to NGF rescue, consistent with the idea that $p75^{NGFR}$- and TNFR-mediated signal transductions are differentially regulated in apoptosis. In addition these data support the idea that NFκB may be a common downstream signaling element for both $p75^{NGFR}$ and TrkA, the NGF tyrosine kinase receptor that is essential to mediate NGF effects.

Supported by AFAR, UTMB Small Grant Program, and UTMB Center on Aging (GT). This is publication No. 54a supported by USPHS grant PO1 AG10514 awarded by the National Institute of Aging (JRP-P).

401.3

FEATURES OF RECEPTOR ULTRASTRUCTURE REQUIRED FOR NERVE GROWTH FACTOR SIGNALING. S. Maliartchouk, A. C. Cuello* and H. U. Saragovi, Dept of Pharmacology & Therapeutics, McGill Univ., Montréal, Québec, CANADA.

Nerve Growth Factor (NGF) actions are mediated via distinct TrkA and p75 receptors, but the structure of the functional receptor remains undefined. Although expression of TrkA alone is sufficient for cellular responses, studies have shown that p75 can regulate TrkA-NGF interaction and signal transduction.

Alternative models of functional NGF receptors propose either heteromeric, homodimeric or homooligomeric complexes of TrkA and p75. Models of monovalent, bi-valent, homodimeric and/or heteromeric binding of NGF receptors were experimentally tested using bivalent anti-TrkA or anti-p75 monoclonal antibodies, and their monovalent fragments as artificial ligands. Trophic effects upon neuronal and receptor transfected cell lines were studied after receptor ligation.

Results demonstrate that (i) trophic signals mediated by p75 require simultaneous expression and ligation of TrkA; (ii) p75 homodimerization is absolutely necessary for the trophic effect mediated by this receptor; (iii) TrkA homodimerization significantly enhances its biological activity, and also potentiates the effect of p75 ligation; (iv) TrkA homodimers and p75 homodimers produce more robust cellular responses than TrkA-p75 heterodimers. These data suggest that the functional NGF receptor consists of at least p75 and TrkA homodimers, or higher order oligomers of p75 and TrkA.

This work is supported by the Medical Research Council of Canada.

401.4

REGULATION OF P75 NGF RECEPTOR EXPRESSION IN BASAL FOREBRAIN NEURONS AND THEIR AFFERENT PROJECTIONS TO HIPPOCAMPUS AFTER KAINATE-INDUCED SEIZURES.

S. Koh, T. Torgerson and A. J. Cole*, Pediatric Neurology, New England Medical Center, Boston, MA and Epilepsy Research Laboratory, Neurology Service, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Basal forebrain neurons express low affinity NGF receptors (p^{75NGFR}), which interact with both BDNF and NGF, and are dependent on these neurotrophins for survival and differentiation. Systemic administration of kainate (KA) not only induces prolonged seizures, but causes a marked, transient increase in NGF and BDNF mRNAs in hippocampus. Using 192 IgG immunocytochemistry, we studied the changes in the expression of p^{75NGFR} in rats following KA (15mg/kg i.p.) induced seizures. Animals were perfused 12h, 24h, 48h, 72h and 10days after KA.. Horizontal sections from control (n=3) and from different time points (n=4 each) were processed together for immunocytochemistry. Adjacent sections were stained with cresyl violet. Dramatic increases in immunoreactive neurons and in immunoreactivity of dendritic plexi are observed in basal forebrain nuclei 12 hr after KA. In addition, intensely immunoreactive axons are clearly visible as they course through fimbria/fornix to form diffusely laminated terminals in hippocampus. By 24-48h, profuse dendritic plexi or axon terminals are no longer visible, and immunoreactive soma appear shrunken, vacuolated and pyknotic. At 72h, remarkable recovery of basal forebrain cell integrity and neurite staining is observed along with reappearance of terminal staining in hippocampus. By 10 days, only a few atrophic, pyknotic immunoreactive profiles remain and severe neuronal loss is seen in CA1-CA3 pyramidal neurons. These studies shows biphasic regulation of P^{75NGFR} expression in basal forebrain projection neurons after KA, likely in response to activity dependent induction of neurotrophins at their terminals. This regulation may underlie synaptic reorganization after repeated seizures *Support: Whitehall Foundation.*

401.5

NEUROTROPHINS ACTIVATE JNK IN CELLS EXPRESSING THE LOW AFFINITY RECEPTOR $P75^{LNTR}$. Christian Lachance, Christine Zeindler, Philip A. Barker*. Center for Neuronal Survival. Montreal Neurological Institute. McGill University, Montreal, Quebec. H3A 2B4

p75LNTR was the first identified member of a superfamily of related receptors which includes CD27, CD30, CD40, 4-1BB, OX40, the fas antigen and the two tumor necrosis factor receptors (TNF-R1 and TNF-R2). The shared homology that defines this group resides in conserved protein modules, termed Cys repeats. In addition to this extracellular homology, the fas, TNF-R1 and p75LNTR receptors contain an intracellular domain, termed the "death domain", which, in the case of the TNF-R1 and fas, has been implicated in signal transduction events culminating in apoptosis. TNF-R1 signals in part by activating jnk/sapk, possibly via a ceramide-dependent pathway, and this activation appears to play a key role in activating the cell death pathway. Using a fibroblast co-transformation paradigm, we have previously shown that p75LNTR overexpression may be linked to cell death. To test if p75LNTR ligation activates jnk, we measured neurotrophin-dependent changes in jnk activation and jun mRNA levels in cell lines. In fibroblasts expressing p75LNTR, NGF produces a modest increases in jnk activity and jun mRNA levels. Differentiated PC12 cells tested in a NGF-deprivation paradigm showed BDNF-mediated increases in jnk activity. To determine the functional consequences of the neurotrophin-mediated activation observed in fibroblasts, MTT assays were performed to determine if neurotrophins affect cell viability. The results show that NGF at high concentrations causes a marked decrease in cell survival. Together, these results suggest that neurotrophins may activate the jnk pathway via p75LNTR and that such activation may affect cell survival. This work is supported by the Medical Research Council of Canada.

401.6

DEVELOPMENTAL AND INJURY-INDUCED ALTERATIONS IN THE EXPRESSION OF THE LOW-AFFINITY NEUROTROPHIN RECEPTOR (p75) IN THE THALAMIC VENTRAL BASAL COMPLEX (VB) IN RAT. D.P. Crockett*, E. Kim, L. Wang, R.X. Zhang and M.D. Egger. Dept. Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854-5635.

We report on the transient, patterned expression of p75 in the VB of the thalamus, the major thalamic relay for somatosensation. We immunostained the brains of neonates on postnatal days (pd) 0 - 3, 7 - 9 and 14 with an antibody against p75. To compare p75 expression with the developing synaptic organization within VB, we localized the synaptic-vesicle-associated protein, synaptophysin (SYN), on alternate sections. Dense uniform p75 immunoreactivity (IR) was observed on pd 0 - 3. On pd 7 - 9, p75 IR was patterned, reminiscent of cytochrome-oxidase-stained barreloids, a characteristic feature of the VB in rodents. By pd 14, p75 IR was not detectable in VB. Light, but clearly patterned SYN IR was first visible on pd 3, increasing in intensity with age and through pd 14. Infraorbital nerve sectioning on pd 0 disrupted patterned p75 and SYN IR within the ventroposterior medial nucleus in pd 7 - 8 neonates. Lesioning the somatosensory cortex on pd 0 resulted in a complete loss of p75 IR as well as a greatly reduced SYN IR within VB. Intense p75 IR observed in VB during the first two weeks postpartum suggests that p75 plays a significant role during periods of active synaptogenesis and pattern formation.

401.7

DEPENDENCE OF BASAL FOREBRAIN CHOLINERGIC INNERVATION OF THE LIMBIC SYSTEM ON THE p75 NGF RECEPTOR. T. T. Yeo*[1,2], F. M. Longo[1], D. E. Bredesen[3], L. L. Butcher[2]. [1]Dept of Neurology, UCSF-VAMC, San Francisco, CA 94121; [2]Dept of Psychology, UCLA, Los Angeles, CA 90024; [3]Program on Aging, La Jolla Cancer Research Foundation, CA92037.

The role of p75 in the development of the basal forebrain cholinergic system was examined using transgenic mice carrying a non-functional p75 gene (p75-knockout mice; K-F Lee et al, 1992). Cholinergic innervation was revealed by use of AChE-histochemistry. Lack of p75 caused a reduction of AChE-positive fibers in several areas of the limbic telencephalon that are heavily innervated by p75-positive basal forebrain cholinergic neurons, including the hippocampus and retrosplenial cortex. In the hippocampus, the most prominent reduction of AChE-fibers was found in areas where cholinergic and p75-positive fibers are colocalized, including the suprapyramidal layer in all CA regions and the supragranular layer in the dentate gyrus. Reduction of AChE-fibers was also observed in cortical layers of the retrosplenial cortex, including layer 1, which is heavily innervated by p75-positive cholinergic fibers in normal animals, and to a lesser extent in layers 2/3 and 5. No apparent difference in the staining intensity of cholinergic cell bodies and patterns of AChE-fibers in the fimbria-fornix was observed between wildtype and p75-knockout mice, indicating that the reduction of AChE-fibers in target areas is not due to defects in cholinergic phenotype or a generalized deficiency in neurite outgrowth, but rather a loss of innervation for specific cholinergic targets. These data suggest that p75 is crucial for basal forebrain cholinergic innervation of certain target areas of the limbic system. [Support: French Fdn Alzheimers Res (TY), NIA-AG09873 (FL), VA Merit Rev (FL), Ame Health Assist Fdn (DB), UCLA Retirement Res Fdn & Ctr on Aging (LB)].

401.8

ROLE OF p75 NGF RECEPTOR IN RETROGRADE TRANSPORT OF NEUROTROPHINS BY CHOLINERGIC SEPTOHIPPOCAMPAL NEURONS

T. Hagg*, B.M.R. Kramer, C.E.E.M. Van der Zee

Dept. Anatomy & Neurobiology, Dalhousie Univ., Halifax, B3H 4H7, Canada.

The neurotrophins NGF (nerve growth factor), BDNF (brain-derived neurotrophic factor), NT-3 (neurotrophin-3) and NT-4/5 (neurotrophin-4/5) are retrogradely transported from their innervation territories to the neuronal cell bodies of the peripheral and central nervous system. All neurotrophins bind to the low-affinity NGF receptor (p75) whose functions are just beginning to be identified. Besides its role in receptor signaling, p75 appears to be involved in retrograde transport of BDNF and NT-4/5 (and to a lesser extent of NGF) to cell bodies of peripheral sensory neurons (Curtis, Neuron 14:1201). We have shown that adult transgenic mice with a null mutation for p75 have approximately 50% more septohippocampal neurons than control mice (C. Van der Zee, this Volume) and that they have an apparently normal cholinergic innervation pattern in the hippocampal formation. Thus, these p75-deficient transgenic mice provide an excellent opportunity to investigate the role of p75 in retrograde transport of neurotrophins in the central nervous system. The adult transgenic mice and their parental wild type controls (Jackson Labs) were injected into the right hippocampal formation with 0.6 µl ^{125}I-labeled HPLC-purified mouse ß NGF (0.3 pmol; 7 ng; Cedar Lane Inc.). After 24 h, the animals were sacrificed and the percentages of cholinergic septohippocampal basal forebrain neurons that retrogradely transported ^{125}I-labeled neurotrophin will be determined by combined ChAT-immunostaining and emulsion autoradiography. Similar experiments will be performed for recombinant human BDNF, NT-3 and NT-4/5 (a gift from Regeneron Pharmaceuticals Inc.).

Supported by the Network of Centres of Excellence, Canada.

401.9

p75 NGF RECEPTOR BLOCKING PREVENTS DEVELOPMENTAL DEATH OF CHOLINERGIC BASAL FOREBRAIN NEURONS IN VIVO

C.E.E.M. Van der Zee*, G.M. Ross#, R.J. Riopelle#, T. Hagg

Dept. Anatomy & Neurobiology, Dalhousie Univ., Halifax, B3H 4H7, Canada ; # Dept. Medicine (Neurology), Queen's University, Kingston, K7L 2V7 Canada

Nerve growth factor (NGF), through activation of TrkA receptor, promotes survival of neurons. Low-affinity NGF receptor (p75) binds all neurotrophins but its functions are just beginning to be identified. p75 can signal independent of TrkA by formation of ceramide and can cause apoptosis in vitro. Here we show in transgenic mice with a null mutation for p75 (p75-KO) that the same number of cholinergic medial septum neurons are present early postnatal and in adults. However, in control mice (wild type parental and two other strains), 25% of these neurons undergo cell death (apoptosis) between postnatal day 6 and 15, suggesting that p75 causes their death. At P6, all cholinergic septal neurons in control mice were p75 immunoreactive (anti-REX antibody) but only 75% had detectable levels of TrkA in control and p75-KO mice (both antibodies: gift from L.F. Reichardt). Beyond P6, the TrkA-positive number remained constant in control and p75-KO mice, suggesting that only neurons that lack TrkA (but with p75) undergo the developmental death, i.e., that p75 induces apoptosis in the absence of TrkA. We have synthesized a peptide, based on the putative p75-interacting NGF residues 28-36, which inhibits neurite outgrowth in vitro and inhibits binding and crosslinking of NGF to p75 receptor but not to TrkA. Systemic injection with p75-inhibiting peptide from P0 to P15 in control mice prevented the developmental death of cholinergic septal neurons. Thus, p75 plays a role in apoptosis of developing cholinergic basal forebrain neurons and its pharmacological manipulation can promote neuronal survival. Support: Network of Centres of Excellence, Canada.

401.10

The p75 and Trk Receptors Are Expressed in Reciprocal Patterns in a Wide Variety of Non-Neural Tissues During Rat Embryonic Development, Indicating Independent Receptor Functions. **Wheeler, E.F.*#; Gong, H.#; Bothwell, M.+** #The University of Texas at San Antonio, San Antonio, TX. +The University of Washington, Seattle, WA.

The p75 neurotrophin receptor has been detected in a number of non-neural tissues, especially during development. Reports of *Trk* receptor transcripts in non-neural tissues raises the possibility that the sites of p75 receptor expression may correlate with *Trk* receptor expression in developing non-neural tissues. Co-expression of p75 and the *Trk* receptors would indicate a cooperative function between the two receptor subclasses. To address these questions, the p75 receptor was localized relative to the three known *trk* receptors during the later stages of embryonic rat development when the highest levels of p75 receptor have been observed in the muscle, maxillary pad, kidney and lung. Using *in situ* hybridization and immunhistochemical analyses, we show here that the *Trk* receptors are expressed extensively in these and other non-neural tissues during cell differentiation and tissue morphogenesis but in patterns that are generally reciprocal to p75 receptor expression patterns. The results indicate that the p75 receptor most likely functions independently of the *Trk* receptors in most developing non-neural tissues. All of the neurotrophin receptors are expressed in patterns that suggest they cooperate in integrating innervation with the development of non-neural tissues. Supported by San Antonio Area Foundation, UTSA Faculty Research Awards, H. F. Kerr Foundation, NIH-MBRS-GM08194-16 and NIH-HL43397.

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS VI

402.1

NERVE GROWTH FACTOR (NGF) INDUCES C-FOS PROTEIN IN TRKA IMMUNOREACTIVE DORSAL ROOT GANGLION CELLS *IN VIVO*. G.J. Michael*, R.K. Joshi, D.L.H. Bennett, S.B. McMahon and J.V. Priestley. UMDS, St. Thomas' Hospital, London, SE1 7EH, UK.

Systemic injection of NGF elicits a delayed, but long lasting, centrally-mediated hyperalgesia which has been proposed to involve changes in the expression of neurotransmitters or neuromodulators by dorsal root ganglion cells. Neuropeptide regulation by NGF has been well documented in a number of *in vitro* and *in vivo* models. Immediate early genes, which may affect these later alterations in neuropeptide expression, are also known to be induced by NGF *in vitro*. Whether NGF has similar effects *in vivo* has not been established. In this study, we examine the effects of *in vivo* administration of NGF on the expression of the immediate early gene product, c-fos, in lumbar dorsal root ganglion cells. Anaesthetized, male Wistar rats were implanted with intrathecal cannulae which were inserted at the level of the L6 and S1 vertebrae and then advanced beneath the dura to the level of the lumbar enlargement. After recovery of three days, NGF (50 µg in 100 µl saline) or vehicle was administered intrathecally via the cannulae. Animals were sacrificed by perfusion and dorsal root ganglia were processed for c-fos immunohistochemisty. Three hours after NGF administration a dramatic induction of c-fos immunoreactivity was observed in ganglia extending from L1 to S1. Up to 40% of DRG cells showed c-fos immunoreactivity. Intrathecal adminisration of vehicle or BDNF using the same protocol had little effect on c-fos immunoreactivity in DRG cells. Intraperitoneal injection of NGF (3mg/kg) elicited a similar c-fos expression pattern, however the staining was less intense. Using dual colour immunofluorescence, c-fos protein was found to be confined exclusively to the trkA immunoreactive subpopulation of DRG cells. These results indicate that NGF is capable of activating c-fos via the trkA receptor *in vivo*. The induction of immediate early gene expression in dorsal root ganglia is hypothesized to mediate further alterations in gene expression which might underlie the hyperalgesic effects of NGF. *Supported by Medical Research Council, UK.*

402.2

DELINEATING THE MECHANISM OF ACTION OF RETINOIC ACID ON CNS NEURONAL DIFFERENTIATION. W. M. W. Cheung*, A. H. Chu, M. F. Leung and N. Y. Ip. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.

Retinoic acid (RA) induces human embryonal carcinoma cells (NT2/D1) to undergo terminal differentiation and become postmitotic CNS neurons. The underlying molecular mechanism by which RA induces neuronal differentiation, however, is not known. As part of an effort to investigate the potential involvement of the neurotrophic factors in the actions of RA, we have examined the expression of the Trk receptors in NT2/D1 cells after RA treatment. Our findings demonstrated that the mRNA expression of all three human trk receptors (trkA, trkB and trkC) was upregulated by RA in NT2/D1 cells, raising the possibility that the co-ordinated upregulation of these receptors may be involved in the RA-induced differentiation of the neuronal progenitor cells into postmitotic human CNS neurons. This is consistent with our finding that treatment of NT2/D1 cells with multiple neurotrophic factors was able to drive a proportion of these cells into differentiated CNS neurons, in a manner similar to that observed with RA, albeit with a faster time course. To further elucidate the mechanism of action of retinoic acid, RNA fingerprinting by arbitrarily primed PCR was used to identify transcripts that are differentially expressed upon RA treatment. Our findings revealed that RA treatment of NT2/D1 cells induced the expression of clathrin as well as several novel genes. Similar studies with neurotrophic factors should provide insight into the molecular mechanism underlying the effects of RA on CNS neuronal differentiation.

402.3

A MUTANT V-CRK ADAPTOR PROTEIN DISSOCIATES NGF-INDUCED SIGNAL TRANSDUCTION FROM NEURITE ELONGATION IN PC12 CELLS. J.C. Courtney[1], K.K. Teng[2], R.B. Birge[3], J.A. Wagner[1*], and B.L. Hempstead[1,2]. Program in Neuroscience[1], Division of Hematology[2], Department of Medicine, Cornell University Medical College, and Department of Molecular Oncology[3], The Rockefeller University, New York, NY 10021.

Ectopic expression of the adaptor protein v-Crk in PC12 cells augments NGF-induced neurite outgrowth while stable expression of a SH2 mutant v-Crk protein v-Crk(R273N) delays NGF-induced neuritogenesis. Although a sustained activation of NGF-induced signaling pathways correlates with augmented neuritogenesis in v-CrkPC12 cells, the inhibitory effect of the v-Crk(R273N) on neurite elongation does not result from impaired Trk A dependent signaling and gene induction; these responses to NGF are either prolonged or unchanged in the v-CrkPC12 or v-Crk(R273N)PC12 cells as compared to native PC12 cells. Immunofluorescence studies and Triton X-100 extraction experiments establish that v-Crk co-localizes with the cytoskeletal protein paxillin in the actin cytoskeleton whereas the v-Crk SH2 mutant disrupts such interactions at the growth cones. The expression of the SH2 mutant v-Crk serves to uncouple signaling events from the cytoskeletal interactions necessary for neurite elongation. Furthermore, the neurotrophin receptor p75 in v-Crk PC12 cells displays enhanced localization to the cytoskeleton and these cells have an increased rate of NGF internalization, implicating a direct action of v-Crk on NGF receptor trafficking. Taken together, the data suggest that a major cellular target of v-Crk is the neuronal cytoskeleton and that v-Crk targets NGF-activated receptor signaling complexes to this subcellular compartment; thereby directly potentiating the neuritogenic action of NGF at the level of the growth cone. (Supported by Public Health Service awards to BLH and RBB, and the American Health Assistance Foundation award to BLH. JCC is supported by T32 EYO7138).

402.4

Expression of Human trkA Receptors in trkA-deficient PC12/Endothelial Hybrid Cells (PC12EN) Results in Nerve Growth Factor (NGF)-induced DNA Synthesis. H. Jiang*, P. Lazarovici, V. Movsesyan, M. Fasler, M. Whalin, Y. Katagiri, M. Monshipouri, G. Dickens, P.I. Lelkes¶, and G. Guroff, Section on Growth Factors, NICHD, NIH, Bethesda, MD 20892 and ¶ Laboratory of Cell Biology, Dept. of Medicine, Univ. of Wisconsin, Milwaukee, WI 53201-0342

NGF, an important neurotrophin, regulates proliferation, differentiation and survival in sympathetic and sensory neurons using trkA receptor tyrosine kinase activity. These different biological effects of NGF signalling depend on trkA substrates which probably differ between neurons. To better define trkA receptor substrates and NGF proliferative signalling, we have stably transfected PC12EN with an expression vector encoding the full-length human trkA cDNA. Two stably transfected clones overexpressing trkA with high affinity (PC12EN-trk3, K_d-57.4 pM, B_{max}-9.7 fmole/mg) and low affinity (PC12ENtrk1, K_d-392.4 pM, Bmax-5.7 fmole/mg) binding were generated. RT-PCR and immunoblot analysis indicate the absence of trkA in parental cells and stable expression of trkA transcripts and protein in PC12EN transfectants. Radioreceptor assays indicate that transfected trkA receptors show slow dissociation kinetics, are resistant towards trypsin or Triton X-100 treatment and internalize or downregulate similarly to the native PC12 trkA receptors. Furthmore, in both clones, NGF stimulates trk and erk tyrosine phosphorylation in a dose- and time-dependent fashion, a process inhibited by 200-1000 nM K 252a (a selective trk inhibitor). NGF treatment in low serum media stimulated 1.3- and 2.3-fold increase of DNA synthesis measured by ^{3}H-thymidine incorporation in PC12EN-trk1 and PC12EN-trk3, respectively. This effect is comparable to the mitogenic stimulation of bFGF and serum under identical conditions. These data emphasize the functionality of the transfected receptors and indicate these transfectant cells can serve as a novel cellular model to study the mitogenic properties of NGF and substrates of trkA receptors.

402.5

NGF/TRKA SIGNALING IN THE SN56 MURINE SEPTAL CELL LINE . B. Berse*[1] and J.K. Blusztajn[1,2]. Departments of [1]Pathology and [2]Psychiatry, Boston University School of Medicine, Boston, MA 02118.

TrkA tyrosine kinase is the high affinity receptor for nerve growth factor (NGF), and is essential *in vivo* for the survival, differentiation, and growth of sympathetic neurons, subpopulations of neural crest-derived sensory neurons, and striatal and basal forebrain cholinergic neurons. However, the majority of the data on NGF/TrkA signal transduction *in vitro* have been obtained from a single model system, rat phaeochromocytoma PC12 cells. In order to study TrkA-mediated signaling in cholinergic cells, we transfected *trk*A cDNA, under the control of the murine sarcoma virus long terminal repeat promoter, into a mouse septal cholinergic cell line SN56. Immunobloting with an anti-TrkA antibody indicated that the transfected clones express varying amounts of TrkA protein. In one TrkA-positive clone, T17, NGF treatment (100 ng/ml) induced better attachment to the substratum and prominent neurite formation. The growth rate of two other clones was markedly increased in a medium containing NGF (200 ng/ml), whereas the growth rates of untransfected SN56 cells and a TrkA-negative transfectant, T1, were not affected by NGF. These data indicate that NGF and TrkA can modulate both cell growth and differentiation. On the molecular level, we showed that stimulation of the TrkA-positive transfectants with NGF induced a rapid increase in tyrosine phosphorylation of many protein species, including the adaptor protein SHC. We also assayed the effect of NGF on cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) by Fura-2 fluorescence. Basal $[Ca^{2+}]_i$ in the trasfectants was approximately 100 nM. Application of NGF caused a small but sustained elevation of $[Ca^{2+}]_i$ in clone T22, whereas the response was transient in clone T17, which expresses a lower level of TrkA. The $[Ca^{2+}]_i$ response appears to be mediated by TrkA, as no measurable changes were observed in the TrkA-negative clone T1. Thus, both major signalling pathways initiated by NGF, one involving SHC, and one mediating the calcium response, are functional in SN56 cells expressing TrkA.
(Supported by NIH grant AG09525).

402.6

EXPRESSION, PURIFICATION, AND CHARACTERIZATION OF THE EXTRACELLULAR DOMAIN OF TRKA RECEPTOR. Sang B. Woo* and Kenneth E. Neet. Dept. of Biological Chemistry, Finch UHS/The Chicago Medical School, N. Chicago, IL 60064

The 373 amino acid extracellular domain of human TrkA receptor for nerve growth factor (NGF) was overexpressed in Sf21 insect cells and purified to homogeneity. NH_2-terminal sequencing and analysis of the amino acid composition verified the authenticity of the recombinant TrkA-RED. TrkA-RED also inhibited the neuritogenic activity of NGF on PC12 cells. Reduction of the molecular weight on SDS-PAGE from 70 kDa to 52 kDa upon treatment with an endoglycosidase suggested a high degree of glycosylation of the polypeptide. *In vitro* binding assays were performed with NGF to confirm the receptor capability of TrkA-RED. The formation of the NGF-TrkA-RED complex was shown qualitatively by retarded migration of TrkA-RED on a native gel in the presence of NGF at neutral pH, crosslinking studies with ^{125}I-labeled NGF to TrkA-RED with disuccinimidyl suberate (DSS), and Superose 12 FPLC gel filtration chromatography from a mixture of NGF and TrkA-RED. Quantitative kinetic analysis was conducted with the Pharmacia Bialite biosensor. When NGF was immobilized onto a carboxymethyl sensor chip and TrkA-RED was used as analyte, or *vice versa*, the association rate was determined to be 10^4 - 10^5 $M^{-1}s^{-1}$, while the dissociation rate was in the range of 10^{-2} - 10^{-3} s^{-1}. These results suggest that the recombinant TrkA-RED adequately mimics TrkA receptor binding to NGF, but with a somewhat lower equilibrium association constant, and is an appropriate system in which to study the details of NGF-TrkA interactions. (Supported by NIH grant NS24380)

402.7

AG879 BLOCKS NGF MODULATION OF iCGRP RELEASE AND PHOSPHORYLATION OF *trk A*. WR Bowles*, RM DeCamp, CM Flores & KM Hargreaves. Dept Rest Sci, Univ of MN School of Dentistry, Minneapolis, MN.

NGF has been shown to involved in hyperalgesia (Lewin et al; J Neurosci. 13:2136, 1992; Kehl et al; IASP abst, 1996). NGF interaction with its high affinity receptor, *trk A*, is thought to initiate signal transduction through tyrosine phosphorylation (Kaplan et al, J. Neurobiol 25:1404, 1994). We have shown previously that high concentrations of NGF stimulate release of iCGRP from bovine dental pulp in an *in vitro* superfusion model. Pretreatment of tissue with the tyrphostin AG879 (which blocks autophosphorylation of *trk A)* inhibits this release by >90% of the control response ($p< .01$). To examine the phosphorylation event, NGF(7.4 nM), a combination of AG879(40μM) and NGF(7.4 nM), or Krebs buffer alone, were applied to bovine dental pulp during in vitro superfusion. Tissue was removed, homogenized, and proteins separated by SDS-PAGE and transferred to nitrocellulose. Blots were probed with anti-*trk A* (Santa Cruz, CA) or anti-phosphotyrosine (UBI) and immunoreactivity visualized and quantitated using an imaging densitometer (BioRad). Anti-*trk A* and anti-phosphotyrosine antibodies both identified a 140 kD band. Administration of NGF to dental pulp evoked > 50% increase in the anti-phosphotyrosine band density. Further, co-administration of AG879 and NGF to dental pulp blocked NGF effects on peptide release and phosphorylation of the 140 kD band. Collectively, these data indicate that autophosphorylation of *trk A* is necessary for modulation of neuropeptide secretion. Further, evaluation of *trk A* phosphorylation may be a useful method for determining activation of this pathway during tissue inflammation, injury, or development. Supported in part by DE07014, DE09860, STRC and Genentech.

402.8

DIFFERENTIAL LOCALIZATION OF trkA & p75 NEUROTROPHIN RECEPTORS IN POSTNATAL & ADULT SYMPATHETIC NEURONS. J.Kohn*, J. Toma, G.A. Kuchel and F.D. Miller. Centre for Neuronal Survival, Montreal Neurological Institute, Montreal, PQ, Canada.

Biological responses of sympathetic neurons to NGF may be partially regulated by the spatial distribution of TrkA and p75 receptors along the neuronal surface. Previous work in our lab has shown that NGF-inducible gene expression (tyrosine hydroxylase and p75 mRNAs) in cultured postnatal sympathetic neurons is regulated as a function of the cellular localization of the activated ligand-receptor complex. To visualize distribution of trkA and p75 along the neuronal surface, we performed immunocytochemistry with antibodies against these receptors. Immunocytochemical analysis revealed very little trkA staining on neuritic processes, but a high relative density on cell bodies, while p75 density was similar on both neurites and cell bodies. To obtain a more quantitative measure of relative receptor levels on cell bodies versus neurites, results were analyzed densitometrically using confocal microscopy on neurons cultured in 20 ng/ml or 50 ng/ml NGF. Regardless of NGF concentration, there was 3-4 times more trkA and approximately 1.6-fold more p75 on cell bodies than on neurites. To confirm that distribution observed *in vitro* was representative *in vivo* distribution, immunocytochemistry with the same antibodies was also carried out on tissue sections of adult rat SCG and one of its targets, the pineal gland. TrkA and p75 were both distributed diffusely throughout the SCG. In the pineal gland, however, p75 antibody stained the entire axonal arbor, while trkA was distributed in a punctate pattern, suggestive of varicosities of nerve terminals. These data suggest that biological responses of sympathetic neurons to the trkA ligands NGF and NT-3 may be regulated as a function of the spatial source of the neurotrophin. Supported by the Network for Centres of Excellence, and the Jeanne Timmins Costello and FCAR-FRSQ-Santé Studentships.

402.9

TRKA IMMUNOREACTIVITY INCREASES IN THE DRG NEURONS AFTER PERIPHERAL NERVE INJURY IN THE RAT. H. Shen, K. Chung and J. M. Chung*. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77555.

A neurotrophic receptor trkA is responsible for signal transduction triggered by nerve growth factor, which is critical for the normal metabolism and survival of sensory neurons. There have been conflicting reports about the effects of peripheral nerve injury on trkA mRNA expression in dorsal root ganglion (DRG). The aim of the present study was to determine the trkA receptor regulation in the DRG after peripheral nerve injury in the rat.

Young adult male rats were subjected to a unilateral spinal nerve ligation. Rats were sacrificed by perfusion at various time points after the surgery. TrkA expression was identified by staining for immunoreactivity (IR) with polyclonal antibodies to trkA (Santa cruse, 1:1,000) by the ABC method.

Under normal conditions, some small sized neurons were labeled with trkA-IR. After spinal nerve ligation, the staining intensity and the number of neurons expressing trkA-IR were increased, suggesting an increase in the level of trkA receptor in the DRG after peripheral nerve injury. (Supported by NIH grants NS 31680 and NS 11255)

402.10

CHARACTERIZATION OF THE HUMAN TRKA PROMOTER IN NEUROBLASTOMA CELLS B. Chang, D. Martin-Zanca† D.L. Kilpatrick*, Worcester Foundation for Biomedical Research, 222 Maple Ave. Shrewsbury, MA 01545, USA and †Dept. of Microbiol. and Genetics, Univ. De Salamanca, Salamanca, Spain 37007

Up-regulation of the NGF receptor TrkA plays a critical role in peripheral sympathetic and sensory neuronal development and in regulating cholinergic neurons in the CNS. In addition, elevated TrkA expression is associated with a favorable prognosis for neuroblastoma. Investigating the mechanisms underlying TrkA gene regulation is thus of great importance in understanding nervous system development and in the possible management of neuroblastoma. To this end, we have analyzed the human TrkA promoter in neuroblastoma cell lines that express this protein. A 2.6 kb genomic fragment encompassing the TrkA promoter region was inserted into the pGL3 luciferase reporter and tested by transient transfection. After normalizing luciferase activities using CMV-βgal, SMS-KCN cells (high TrkA expression) exhibited 5- to 10-fold higher luciferase activity than SH-SY5Y cells, which express TrkA at low to modest levels. Expression of the 2.6 kb promoter construct in TrkA-negative human kidney 293 cells was only 5-10% of that in SH-SY5Y cells. Analysis of a series of deleted reporter constructs in SH-SY5Y cells revealed that 130-bp surrounding the presumptive initiation region is sufficient for activity. Further analysis of the proximal promoter region in human neuroblastoma cells is now underway, including transfection and DNA mapping studies. This work should ultimately help to reveal the factors specifically mediating TrkA transcription in human neuroblastoma tumors. (This was supported by PHS Grant DK-36468)

402.11

DEVELOPMENT AND CHARACTERIZATION OF NOVEL TRK-A SPECIFIC MONOCLONAL ANTIBODIES, J.S. Hongo, D.L. Shelton*, H. Phillips, L. O'Connell, R. Urfer, M. Sadick, A. Galloway and B.M. Fendly. Depts. of BioAnaltyical Technology, Neuroscience and Immunology, Genentech, Inc., South San Francisco, CA USA

A panel of 5 murine monoclonal antibodies (MAbs) specific to the extracellular domain of the trkA receptor have been developed and characterized. Epitope mapping using domain deletion and trkC domain substituted mutants have revealed both unique and overlapping MAb epitope specificities. Two MAbs (1488 and 1503) bind a conformational epitope spanning domains 1-4 (cysteine rich I, leucine rich II, cysteine rich II, Immunoglobulin I domains); MAb 1486 binds an epitope formed by domains 4 and 5 (Immunoglobulin I and II domains); MAb 1487 shows domain 4 specificity (Immunoglobulin I domain), and MAb 1502 is domain 5 specific (Immunoglobulin II domain). Two of the MAbs (1488 and 1503) bind trkA in formalin-fixed chimpanzee, monkey and human tissue. Additionally, MAb 1503 is a potent inhibitor of trkA-NGF binding as determined by a kinase-induced-receptor-activation (KIRA) bioassay. MAb 1487, in conjunction with an anti-NGF specific MAb, has been used to develop a sensitive, quantitative ELISA for the analysis of trkA variants prepared by site-directed mutagenesis. Novel, specific MAbs with varying epitope specificities and functional characteristics should be valuable reagents for both *in vitro* and *in vivo* characterization of the trkA receptor and its relationship to NGF.
(Genenctech, Inc.)

402.12

NEUROTROPHIN-3 AND NGF DIRECTLY ACTIVATE TrkA BUT DIFFERENTIALLY MEDIATE BIOLOGICAL RESPONSES IN SYMPATHETIC NEURONS. D.J. Belliveau*, C.F. Ibanez and F.D. Miller. Centre for Neuronal Survival, Montreal Neurological Institute, Montreal, PQ, Canada, H3A 2B4.

We have been examining the role of NGF and NT-3 in mediating survival and neuritogenesis in cultured sympathetic neurons. Previous data from our lab has shown that NT-3 can mediate neuritogenesis of sympathetic neurons as well as NGF but cannot mediate survival. Biochemical investigations revealed that induction with NT-3 lead to the autophosphorylation of trkA in addition to trkC in these neurons. In the present experiments we examined whether other neurotrophins had similar effects in sympathetic neurons and, in addition, if NT-3 directly activated the trkA receptor. Cultures of sympathetic neurons induced for 10 minutes with various neurotrophins revealed that NGF, NT-3 and NT-4/5 resulted in the autophosphorylation of trkA, whereas BDNF had no effect. Survival assays for each of the neurotrophins demonstrated that although the degree of trkA phosphorylation was similar, NT-3 mediated 2-4 fold greater survival than NT-4/5. NGF, conversely, induced greater trkA autophosphorylation and survival than any other neurotrophin. In attempting to determine if NT-3 mediates its effects directly through trkA, we used a mutant NT-3 (Ryden and Ibanez, J. Biol. Chem. **271**: 5623, 1996) which could bind only trkC, not trkA. Induction assays on trk-transfected fibroblasts and sympathetic neurons confirmed the effectiveness of the mutant NT-3. Survival assays with 50-100 ng/ml wild type NT-3 mediated the survival of 50-65% of sympathetic neurons respectively whereas the mutant NT-3 mediated less than 5% survival. These data provide strong evidence indicating that multiple neurotrophins may act through one receptor to mediate a variety of biological responses. Supported by MRC-Genentech Fellowship, MRC Canada, and Network for Centres of Excellence.

402.13

TrkA EXPRESSION LEVELS OF SYMPATHETIC NEURONS CORRELATE WITH NGF RESPONSIVENESS DURING DEVELOPMENT AND AFTER TREATMENT WITH RETINOIC ACID. A. von Holst*, F.B. Lefcort# and H. Rohrer. Max-Planck-Institute f Brain Research, Dept. Neurochem., 60528 Frankfurt, Germany; #Dept. Biol., Montana State Univ., Bozeman, MT 59717, USA.

Sympathetic neurons depend on the classical neurotrophin nerve growth factor (NGF) for survival by the time they innervate their targets, but not before. The acquisition of NGF responsiveness is thought to be controlled by environmental cues in sympathetic neurons. In cultures of immature chick sympathetic neurons, which do not respond to NGF *in vitro*, we only detected low mRNA expressionlevels for the NGF receptor trkA. Treatment with retinoic acid (RA) lead to a strong and selective increase in trkA mRNA levels and the induction of NGF-dependent survival. Concommitantly, trkA mRNA expressed at low levels in sympathetic ganglia at embryonic day 6.5 (E6.5) *in vivo*, increases more than 5-fold between E6.5 and E10 of chick embryo development. In parallel, the number of sympathetic neurons responding to NGF with survival *in vitro* increases from 0% at E6.5 to almost 100% at E10. A single systemic addition of RA into the egg at E6 lead to an increase in trkA mRNA levels at E6.5, showing that sympathetic neurons can also respond to RA in the *in vivo* situation. This increase however, is less strong (2-fold instead of 6-fold) and not sufficient to induce NGF-responsiveness in cultures of sympathetic neurons from treated embryos.

To study the developmental expression of trkA protein, immunohistochemical labelling of tissue sections and confocal microscopical analysis were carried out, using a polyclonal antibody specific for chick trkA. Interestingly, trkA immunoreactivity was detected as early as E4 (Hamburger Hamilton stage 23). Expression decreased to undetectable levels around stage 27 (E5) but reappeared at stage 29 (E6.5) and strongly increased up to E10. Thus, trkA expression increases between E6.5 and E10 both at the RNA and protein level. The role of trkA during the first expression phase is under investigation.

Supported by the Deutsche Krebshilfe W 52/94/Ro1

402.14

THEORETICAL STUDIES ON THE BIOACTIVE CONFORMATION OF NERVE GROWTH FACTOR FOR TRKA ACTIVATION. I.L. Shamovsky, G.M.Ross, R.J.Riopelle and D.F.Weaver. Departments of Chemistry and Medicine, Queen's University, Kingston, Ontario, Canada K7L 3N6.

While the amino and carboxyl termini of NGF are endowed with trkA receptor activating properties, their conformations have not been resolved in X-ray studies. Monte Carlo simulated annealing algorithm is utilized to determine the most stable conformations of the termini of different NGF analogues in order to identify their bioactive conformation. Stereospecific interaction of the termini of dimeric (1-118)NGF results in the formation of two distinct moieties of the complex: a rigid region (residues 9-11 and 112′-118′) and a flexible loop (residues 1-8), with residues 6-9 adopting a β pleated sheet motif. The geometry of the rigid region, which is mainly maintained by electrostatic interaction between Glu^{11} and $Arg^{118'}$, is conserved in active molecules only. Currently available experimental data on 21 molecules (117-, 118- and 120-residue NGF analogues and their deletion and point mutations) are interpreted using this structural model.

Supported by Allelix Biopharmaceuticals, Inc.

402.15

A $p75^{NTR}$-trkA HETERODIMER IN PC12 CELLS IS AN NGF RECEPTOR COMPLEX WHICH DISSOCIATES IN THE PRESENCE OF LIGAND. G.M. Ross*, G. Lawrance, M. Solc, and R.J. Riopelle. Apps Research Centre, Queen's University, Kingston, Ontario, Canada K7L 2V7.

The ability of the low affinity neurotrophin receptor $p75^{NTR}$ to influence a variety of high affinity trkA receptor mediated biochemical and biological outcomes has been well documented. In order to explain this phenomenon, several models have been proposed to suggest a mechanism whereby the low affinity receptor could alter the binding and signal transduction properties of the high affinity receptor. Fundamental differences between these models include the existence of a $p75^{NTR}$-trkA heterodimer (eg. a "conformation" model) versus an indirect interaction of these proteins (eg. a "presentation" model). We have used BDNF as a $p75^{NTR}$ specific ligand to probe the effects of the low affinity receptor on trkA binding and phosphorylation. In the presence of saturating concentrations of BDNF, we demonstrate a significant reduction of trkA affinity for NGF as well as reduced trkA phosphorylation. These data are consistent with a direct interaction of the receptors, which has prompted us to develop an affinity cross-linking system to demonstrate directly a heterodimeric complex of trkA and $p75^{NTR}$. The receptor complex is consistent with a high affinity binding species which appears to dissociate upon binding to ligand.

Supported by Allelix Biopharmaceuticals Inc.

CEREBRAL CORTEX AND LIMBIC SYSTEM

403.1

EARLY RESTRICTION OF DEVELOPMENTAL POTENTIAL AND LATER REGULATION OF DIFFERENTIATION IN CORTICAL PRECURSOR CELLS FOR NEURONAL EXPRESSION OF LATEXIN. Y. Arimatsu*, M. Ishida, K. Takiguchi-Hayashi, Y. Uratani, M-H. Jin and M. Sato. Mitsubishi Kasei Inst. Life Sci., Machida-shi, Tokyo 194, Japan.

Latexin is expressed in neurons in infragranular layers of lateral (*L*), but not dorsal (*D*), neocortex in the rat (PNAS **89**,8879,1992). To understand the region-specific differentiation of latexin(+) neurons, we monitored latexin expression in fetal cortical cells in vitro. BrdU-labeled cells from a *L* or *D* portion of the cerebral wall of E13-20 rat fetuses were maintained in a reaggregated-cell culture with an excess of unlabeled cells from either *L* or *D* cortex of the same age. Substantial E13-20 *L* cells, but far less *D* cells, became latexin(+) in vitro with either *L* or *D* cells. E13-18 *L* cells becoming latexin(+) were significantly fewer with *D* than *L* cells while E20 *L* cells becoming latexin(+) were almost the same with *D* and *L* cells. These results suggest 1) that E13-20 *D* cells are restricted in their ability to differentiate to latexin(+) neurons, 2) that E13-20 *L* cortex contains precursor cells with developmental potential to become latexin(+) neurons, 3) that the fate of *L* cells has not been determined ultimately at E18, and 4) that, at least until E18, there is a regional difference between *D* and *L* cortex in the ability to influence latexin expression in *L* cells. To address whether the apparent difference between the results with E13-18 cells and that with E20 cells were due to 1) completion of terminal differentiation of *L* cells at around E20, or due to 2) loss of regional difference at around E20 in the ability to influence cell differentiation, we performed a heterochronic mixed-region reaggregated-cell culture experiment. *L* cells, either from E18 or E20 rats, that became latexin(+) were fewer with E18 *D* cells than with E18 *L* cells, but were almost the same with E20 *D* and E20 *L* cells, showing that the second possibility is more conceivable. Taken together, we propose that the neocortical regional specificity represented by latexin(+) neurons is substantiated early by restriction of developmental potential in certain cortical progenitors but that terminal differentiation of competent precursors can be regulated by environmental cues even after their final mitosis. Supported by Mitsubishi Kasei Inst. Life Sci.

403.2

VENTRICULAR ZONE GENE-1 (VZG-1), EXPRESSED WITHIN THE EMBRYONIC VENTRICULAR ZONE OF THE CEREBRAL CORTEX, ENCODES A LYSOPHOSPHATIDIC ACID RECEPTOR. J. H. Hecht, J. A. Weiner, and J. Chun*. Dept. of Pharmacology and Program in Neurosciences, School of Medicine, University of California, San Diego, La Jolla, CA 92093-0636.

Molecular studies of signaling pathways regulating cortical neurogenesis within the proliferative ventricular zone (vz) have implicated receptor tyrosine kinases (RTKs). However, properties of G-protein coupled receptors (GPCRs) suggest that they could also regulate cortical neurogenesis. This question was addressed by using a novel murine telencephalic cell line (line TSM, Chun and Jaenisch, Molecular and Cellular Neuroscience, in press) to isolate GPCRs via degenerate oligonucleotide PCR. One PCR product, which had homology to GPCRs and was expressed in the vz, was used to isolate a novel GPCR, termed *vzg-1*. Northern blot analysis with *vzg-1* detected a 3.8 kb transcript in the brain by embryonic day 13 (E13). *In situ* hybridization to E12 to E18 embryos demonstrated that *vzg-1* expression within the vz follows cortical neurogenesis. Transient overexpression of *vzg-1* in TSM cells induced serum-dependent neurite retraction and sustained cell rounding. Lysophosphatidic acid (LPA), a bioactive lipid present in high concentrations in serum, reproduced the effect of serum on transfected cells. Stable TSM transfectants overexpressing *vzg-1* in the sense and antisense orientations were generated. Compared to controls, sense transfected lines had enhanced responses to LPA, and antisense lines had reduced responses. LPA, but not four related phospholipids, induced *vzg-1* dependent sustained cell rounding. Ligand binding indicated sense overexpression was associated with an increase in specific LPA binding. Western analysis with anti-Vzg-1 antiserum detected a protein of 41-42 kDa, consistent with previous studies using photolabeling. The adult tissue distribution of *vzg-1* is consistent with previous studies of functional LPA receptors. In addition to describing the isolation of an LPA receptor, this study suggests that bioactive lipid signaling, mediated by pathways distinct from RTKs, may be an important regulator of cortical neurogenesis. [supported by the March of Dimes, the NIMH and NIH/NIGMS GM07198]

403.3

TARGETED MUTATION OF THE TBR-1 GENE CAUSES ABNORMAL DEVELOPMENT OF THE CEREBRAL CORTEX AND OLFACTORY BULB. R. F. Hevner*, S. Smiga, A. Bulfone, J. J. Meneses, R. A. Pedersen, and J. L. R. Rubenstein. Dept. of Psychiatry, Nina Ireland Lab., and Lab. of Radiobiol. and Environ. Health, UCSF, San Francisco, CA 94143-0984.

The Tbr-1 gene encodes a putative transcription factor that is expressed in postmitotic neurons of the cerebral cortex and olfactory bulb almost exclusively (Bulfone et al., Neuron 15:63-78). To investigate the role of Tbr-1 in brain development, we used gene targeting in ES cells to disrupt Tbr-1 and generate mutant mice. Animals heterozygous for the mutation are phenotypically normal. Homozygotes generally die on the first day of postnatal life, apparently due to poor feeding; with assisted feeding, some animals survive to adulthood. Mutant homozygotes have severely atrophic, disorganized olfactory bulbs with some well-formed glomeruli but no mitral cell layer. A few large neurons do persist in the bulbs, but it is unclear as yet whether they represent mitral or tufted cells. The lateral olfactory tracts and primary olfactory (piriform) cortex are severly atrophic or absent. The isocortex in neonatal animals is thin, lacks a well-formed subplate, and has few or no Cajal-Retzius neurons. The somatosensory barrels, which form characteristic modules in the normal isocortex, are not detectable by cytochrome oxidase histochemistry in adult homozygotes. Posterior cortical regions (including the occipital pole, entorhinal area, and subicular complex) contain abnormally large clusters of neurons. The hippocampus proper and dentate gyrus display a normal cytoarchitecture.

These findings indicate that Tbr-1 expression is essential for normal development of the olfactory bulb and cerebral cortex. Further studies are in progress to determine which steps in development (e.g., cell differentiation, migration, or survival) are disrupted by the mutation.

Supported by grants from the March of Dimes, NARSAD, and NIMH (RO1 MH49428-01 and KO2 MH01046-01) to JLRR.

403.4

FUNCTIONAL IPSILATERAL CORTICOSPINAL PROJECTIONS IN THE PRESENCE OF EITHER HOMOTOPIC OR HETEROTOPIC E17 CORTICAL TRANSPLANTS. S. Barbay*, B. Shi & B. B. Stanfield. Lab. of Neurophysiology, NIMH, NIH Animal Ctr., Poolesville, MD 20837.

An E17 homotopic transplant placed into a lesion cavity in the rostral cortex of a newborn rat has been shown to spare sensorimotor behaviors (vibrissae tactile placing and forelimb tactile placing) contralateral to the transplants. A similar lesion, but without a transplant, has been shown to induce an aberrant ipsilateral corticospinal projection from the intact hemisphere. This aberrant projection has been implicated in the sparing of vibrissae tactile placing found in these animals. To explore what role any aberrant ipsilateral corticospinal fibers may play in rat pups receiving transplants anatomical and behavioral experiments were used to test for the presence and function of these fibers following transplantation of fetal tissue in the rostral cortex of newborn rats. Homotopic or heterotopic (occipital) E17 or E15 fetal tissue was transplanted into a lesion cavity placed in the rostral cortex of newborn rats. Similar lesions were placed in control rats with no transplants. Five weeks later, biotinylated dextran was injected into the homotopic contralateral hemisphere of several of these rats and into an additional control group of normal rats. The remaining rats from each group were assayed for tactile placing behaviors 41 days after birth. One week later the cortical origin of any ipsilateral corticospinal projections was destroyed. Behaviors were assayed again five days later. The results demonstrate that the aberrant ipsilateral projection fibers are present following E17 rostral or occipital transplants. Behavioral tests suggest that these fibers contribute to the vibrissae tactile placing, but not to forelimb tactile behavior seen after E17 homotopic transplants. Thus the behavior of these animals is influenced by both the transplanted tissue and the aberrant projection of the unlesioned hemisphere. The behavioral results from the E15 homotopic and heterotopic transplant groups were similar to the E17 homotopic group. Supported by NIMH.

403.5

EFFECTS OF INFRAORBITAL NERVE SECTION ON THE DEVELOPMENT OF INTRACORTICAL CONNECTIONS IN MOUSE BARREL CORTEX. N.M.J. Blake*, B. Miller and T.A. Woolsey. Department of Neurology and Neurosurgery, Washington University School of Medicine, St. Louis MO 63110.

Intracortical axons in rodent barrel cortex connect barrels in the same row and barrels of adjacent rows. Section of the infraorbital nerve (ION) at postnatal day 7 (P7) arrests development of the intracortical connections, resulting in few if any connections with adjacent barrels (PNAS 89: 1832). This pattern is reminiscent of the extent of axon growth in P7 brain (Soc. Neuro. Abs. 21: 44). We sought to examine the developmental window in which disruption of peripheral input affects the pattern of intracortical projections. The right ION was sectioned on P7 or P10, biotinylated dextran amine injected into barrel subfield on P30 and mice sacrificed 5 days later. Tangential sections 40μm thick were processed using the ABC procedure with DAB and enhanced with silver/gold. The position of axons in the supragranular layers was plotted relative to the barrel pattern. Axonal projections were measured in the tangential plane as the average distance from the center of the injection. Labeled axons in the deafferented cortex of animals lesioned at P7 extended only to the limits of the barrel of origin. Axonal projections in animals with ION lesion at P10 however extended into adjacent barrels, a pattern similar to that observed in normal adults. This contrasts with the axonal projections in normal P10 pups, which extend approximately half the adult distance. Additionally, the projections in the P10 ION lesioned animals were significantly more abundant than in normal P10 pups, indicating that additional axon growth occurred in the lesioned animals. These results show that unlike ION section at P7, ION section at P10 does not grossly disrupt normal development of intracortical projections. Thus, after P10, intracortical connections continue to grow in the absence of peripheral input.

Supported by NIH grants NS 17763, DE 07734, the McDonnell Center for Studies of Higher Brain Function, and the Spastic Paralysis Foundation of Illinois Eastern-Iowa District of the Kiwanis International.

403.6

THE DEVELOPMENT OF CORTICOTHALAMIC PROJECTIONS IN THE MOUSE SOMATOSENSORY SYSTEM. B. Miller* and T.A. Woolsey. Dept. of Neurosurgery, Washington University School of Medicine, St. Louis, MO 63110.

Reciprocal connections between the thalamus and cerebral cortex are topographically precise. In rodent barrel cortex, corticothalamic cells in layer 6 are aligned in barrel-associated clusters in register with thalamic arbors in layer 4, and project back to the homotopic barreloid in thalamus and to an arc of barreloids in other rows at the same row position (Hoogland et al. 1987, Exp. Brain Res., 68:73-87). In rodent visual system (Miller et al. 1993, J. Comp. Neurol., 335: 16-41) the arrival of corticothalamic axons is relatively late and immediately topographic. We investigated the development of somatosensory corticothalamic projections by placing crystals of the lipophilic tracer DiI in the ventrobasal (VB) thalamic complex of aldehyde fixed brain. Between postnatal days 2-4 (P2-4), the few DiI retrogradely labeled layer 6 cells in the cortex are widely dispersed and not associated with barrel columns, while thalamic arbors form clear barrel-like clusters in layer 4. In contrast, at P9 large numbers of layer 6 cells are retrogradely labeled and the position of these cells is in register with the columnar position of anterogradely labeled thalamic afferents. Thus, the major contingent of layer 6 corticothalamic projections arrives at the thalamus after thalamic afferents have entered and arborized in layer 4.

Supported by NIH grants NS 17763, DE 07734, the McDonnell Center for Studies of Higher Brain Function, and the Spastic Paralysis Foundation of the Illinois Eastern-Iowa District of the Kiwanis International.

403.7

NEONATAL INFRAORBITAL (IO) NERVE TRANSECTION RESULTS IN AN INCREASE IN THE NUMBER OF CORTICOTRIGEMINAL NEURONS WITH PROJECTIONS TO IPSILATERAL AND CONTRALATERAL BRAINSTEM. W.E. Renehan* and N. Hadzijahic. Lab. of Gastrointestinal, Gustatory and Somatic Sensation, Henry Ford Health Sciences Center, Detroit, MI 48202.

We have previously shown that neonatal IO transection results in an increase in the density of the cortical projection to the ipsilateral trigeminal brainstem nuclear complex (TBNC). In the present study, we sought to determine whether these ipsilateral corticotrigeminal (CT) axons are collaterals of the CT neurons that project to the contralateral TBNC. Fast Blue (FB) was injected into the right or left TBNC and fluorogold (FG) was injected into the contralateral brainstem of normal adult rats and adults that had sustained transection of the left IO nerve on the day of birth. In normal animals, an average of 1.04 % (SE = 0.12) of the labeled neurons in the left (ipsilateral) somatosensory cortex contained both FB and FG. In IO-lesioned animals, however, 9.2 $\pm$ 3.0 % of the CT neurons in the left cortex were double-labeled. The difference between the number of double-labeled neurons in normal cortex and the number of double-labeled neurons ipsilateral to IO transection was statistically significant (t-test, $p < 0.01$). We suggest that the results provide evidence that the neonatal IO lesion has caused a significant number of CT neurons to retain a normally transient collateral to the ipsilateral TBNC. Supported by DE 07734.

403.8

GRADIENTS AND TIMING OF THE ARRIVAL OF THALAMIC AXONS IN THE MOUSE NEOCORTEX. F. Polleux*, C. Dehay and H. Kennedy. INSERM U371,18 Av Doyen Lépine 69675 Cedex BRON (FRANCE).

Thalamic afferents (TA) are known to exert a major organizing influence on the developing cortex (Killackey, 1990). The stage of development at which the environmental influence begins is determined by the date of arrival of the TA in the vicinity of the cortex (Kennedy and Dehay, 1993). In this context we have investigated the date of arrival of TA in the vicinity of the developing cortical plate since numerous studies have shown that afferents axons can modulate the proliferative activity of target tissues. We have used multiple injections of the carbocyanine DiI in order to label virtually all the developing thalamic cells on paraformaldehyde fixed mouse embryos ranging from E14.5 to E18.5 (reference day of vaginal plug E1). Using this approach we have found a pronounced gradient in the date of arrival of TA. On E14.5 the first TA exit the thalamus and are located in the internal capsule. On E15.5, TA arrive at the most lateral part of the middle third of the cerebral cortex which roughly correspond to the future primary somato-sensory areas. At E16.5, TA in the middle third are mid-way to the medial wall. At the anterior and posterior thirds, TA have only just contacted the most lateral part of the neocortex. At E17.5, TA have fully innervated the most medial extent of the middle third and are midway to the medial wall in the anterior and posterior thirds. At E18.5 the anterior and posterior thirds of the cortex are fully innervated. This implies that cortical areas originating from the most rostral and caudal thirds of the developing telencephalon are contacted by thalamic afferents at least 24 hours later than the somatosensory field located at the middle third of the cortex.

Bibliography: Kennedy and Dehay (1993) Cerebral Cortex 3:27-35, Killackey (1990) J.Cognit.Neurosci. 2:1-17.

This work was supported by a MRE grant 93006 and EEC grant # SCI CT91-0622

403.9

SUBPLATE DELETION RESULTS IN ABNORMAL FORMATION OF LAYERS IN FERRET SOMATOSENSORY CORTEX. Sharon L. Juliano*, Stephen C. Noctor, Sidney L. Palmer. Department of Anatomy & Cell Biology and Program in Neuroscience, USUHS, Bethesda, MD 20814.

The significance of the subplate as an important factor in development of the neocortex has emerged over the past few years. This early-generated cortical layer does not persist into maturity, but plays an important role in forming subsequent cortical architecture. In order to more clearly understand the effect of earlier born cortical layers on the maturation of cerebral cortex, we conducted experiments in which layers of the neocortex were deleted *in utero*. This was achieved by injections of methylazoxymethanol (MAM) at appropriate gestational ages into pregnant ferrets. MAM prevents cells from dividing during a specific period of time, therefore blocks generation of a specific population of cells. When layer 3 or 4 of the somatosensory cortex was deleted by MAM injection at E34 or 35, most aspects of cortical organization were relatively normal in neonatal and adult ferrets, except for the absence of the target layer. When the subplate underlying the somatosensory cortex was deleted by MAM injection on E24 or E25, the resulting cortex was markedly disorganized. Although neurons continued to be born, and attempted to migrate, as evidenced by BRDU label, cortical layers did not form properly, a distinct laminar pattern was not seen, and the cortex was very thin (103 μm compared with 376 μm in normal neonatal somatosensory cortex). In normal P1 ferrets, injections of dextrans label precise radial columns consisting of radial glia and neurons migrating to the neocortex. In animals treated with MAM to delete the subplate, the normal radial columnar pattern after dextran injections is highly distorted. Immunoreactivity for antibodies directed against vimentin verify that the radial glia form fan-like arrays, rather than their normal spoke-like pattern. The appearance of the disrupted pattern of vimentin immunoreactivity suggests that the absence of the subplate may result in early differentiation of radial glia into astrocytes. Supported by PHS RO1 NS24014.

403.10

TRANSPLANTATION OF VENTRICULAR ZONE CELLS INTO LAYER DELETED FERRET SOMATOSENSORY CORTEX. S.C. Noctor*, S.L. Palmer, S.L. Juliano. Neuroscience and Anatomy, USUHS, Bethesda, MD 20814

Our earlier studies determined that timed administration of the anti-mitotic methylazoxymethanol (MAM) to pregnant ferrets effectively deletes a specific layer of the somatosensory cortex (SSC). After injections timed to delete layer 4, many properties of cortical maturation appear relatively normal, but layer 4 is absent. The current study investigates the possibility that appropriately timed migrating cells can repopulate a missing cortical layer when transplanted into hosts treated with MAM. To test this prospect, we injected MAM into pregnant ferrets at E34 or E36, which deletes layer 3 or 4 of the SSC. On P0 static interface organotypic cultures were prepared from the layer deleted or normal kits. After 1-2 days in culture (DIC), we prepared a cell suspension comprised of cells from the ventricular zone (VZ) underlying the SSC of E34 or P0 normal ferrets. The VZ cells obtained from P0 animals would normally be migrating to layer 2 of the SSC at the time of transplantation. The E34 VZ cells would not normally be migrating at the time of transplantation but would populate layer 4, the missing layer. The cell suspensions were labeled with fluorescent microspheres and transplanted into the ventricular zone or deep intermediate zone of the organotypic slices. Slices were removed from culture at regular intervals up to 7 DIC. Only about 15% of the transplanted cells migrated from the injection site. P0 VZ cell suspensions were more likely to migrate to upper cortical plate in layer-deleted host organotypic cultures. The suspensions prepared from the E34 VZ were more likely to migrate to lower layers in the layer-deleted hosts. The cells were less likely to migrate from the injection site in normal host cultures. These data suggest that layer-deleted host organotypic cultures are a more permissive substrate than normal host cortical cultures and that transplanted VZ cells tend to migrate close to cortical sites they would normally populate according to their date of birth. Supported by PHS RO1 NS24014.

403.11

THALAMOCORTICAL DEVELOPMENT IN ORGANOTYPIC COCULTURES OF NORMAL AND MAM-LESIONED FERRET SOMATOSENSORY CORTEX.
S.L. Palmer*, S.C. Noctor, and S.L. Juliano. Anatomy and Cell Biology & Neuroscience, USUHS, Bethesda, MD 20814.

To understand factors involved in the growth of thalamic axons into somatosensory cortex, we examined the effect of maintaining organotypic cultures of thalamus with either normal or layer-deleted cortical slices. Cortical layers were deleted by injections of methylazoxymethanol acetate (MAM) into pregnant ferrets on appropriate gestational days. In ferret, the thalamic recipient layers (3&4) of somatosensory cortex are deleted by *in utero* injections of MAM at E34 or E36. On PND 0, cortical slices prepared from normal and E34-36 MAM-treated ferrets were placed in an incubation chamber as static interface cultures. Thalamic slices obtained from PND 0 ferrets were added to the cortical slices after 0-3 days in culture. At various days of coculture (DCC), slices were removed and placed in an oxygenated chamber perfused with artificial CSF; injections of fluororuby were made into the thalamic pieces. In layer-deleted cortex after 3-8 DCC, thalamic fibers terminated in layers 5-6 of the cortex as well as subplate and white matter regions, but did not invade upper cortical plate. Later, at 8-10 DCC, thalamocortical fibers grew into the upper cortical plate. The cocultures of normal cortex and thalamus developed in a similar manner. Thalamic invasion of upper cortical layers in the normal cocultures occurs slightly earlier than in the MAM-lesioned slices however. Even after 3 DCC axons from thalamic cultures grew long distances to terminate near layer 1. In some cases, subplate cells in the cortical cultures established reciprocal connections with the thalamic pieces. Evidence from DiI injections into thalamus of layer 4 deleted brains also demonstrate that thalamic axons do not enter superficial cortical layers at early ages. As reported for *in vivo* thalamocortical development in the somatosensory system, permissive cues are present in lower layers prior to their appearance in the upper layers. The deletion of layers 3 or 4 delays the invasion of thalamocortical fibers into the cortical plate.
Supported by PHS RO1NS24014.

403.12

TOPOGRAPHIC ORGANIZATION OF SOMATOSENSORY CONNECTIONS IN A NOVEL MUTANT RAT WITH A 'DOUBLE' CORTEX.
F. Schottler*, A. Rao, D. Couture, H. Fabiato, and K.S. Lee. Dept. of Neurological Surgery, University of Virginia, Charlottesville, VA, 22908

The present study examined the topography of somatosensory connections in a novel mutant rat having what appears to be a 'double cortex' located below the cortical mantle. Injections of anterograde tracers (biotinylated dextran amine, or *Phaseolus vulgaris* leucoagglutinin) into the ventral posterolateral nucleus (VPL) resulted in terminal labeling in layers IV and VI of medial portions of the overlying somatosensory cortex (SSCtx) and columnar-like labeling in medial portions of the underlying Telencephalic Internal Structural Heterotopia (Tish). In contrast, injections into the ventral posteromedial nucleus (VPM) labeled barrel-like clusters of terminals in layer IV of more lateral portions of SSCtx; patches of terminal labeling were observed in the Tish region of animals in which the structure extended laterally at the same level. Terminal labeling of SII was sparse following injections into VPL and VPM; caudal portions of SII were predominately labeled following injections into caudal portions of VPM/ posterior nucleus. These latter injections led to sparse labeling in the Tish region; the extent of labeling appeared to depend on the presence or absence of the Tish structure at caudolateral levels. A virtually identical topographic pattern of retrogradely labeled neurons was obtained following injections of fluorogold into various parts of the ventral basal complex. Labelled neurons were observed mainly in layer VI of medial and lateral portions of SSCtx and scattered neurons were observed dorsoventrally in the immediately underlying Tish region following injections into VPL and VPM, respectively. The present results demonstrate a topographic organization of reciprocal connections between specific thalamic somatosensory relay nuclei and both the overlying SSCtx and Tish region in these mutant animals.
Supported by NS34124 to KSL.

403.13

Cortical Neurogenesis in the Tish Mutant Rat: Evidence for Dual Proliferative Zones.
J.L. Collins and K.S. Lee*. Dept. of Neurological Surgery, Univ. of Virginia, Charlottesville, VA 22908.

The Tish rat is a neurological mutant exhibiting a bilateral cortical heterotopia that resembles the human syndrome of double cortex. Previous findings indicate that cells comprising the normal and heterotopic cortices are generated over the entire period of normal neocortical neurogenesis (Collins *et al.*, Soc. Neurosci. Abs., '95). However, the cells in the heterotopic cortex fail to exhibit typical lamination and orientation. The present study examined the birth sites and early migratory paths of cells in the developing cortex of the Tish mutant.

Brain cells undergoing DNA synthesis were labeled between embryonic days 13 and 20 by injecting pregnant Tish dams intraperitoneally with 5-Bromo-2'-deoxyuridine (Brdu). Pups were removed by hysterotomy and sacrificed at 2h, 8h, or 24h post-injection.

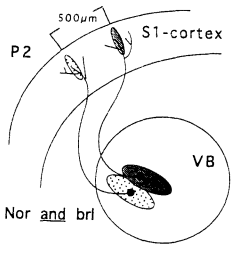
Brains from normal rats exhibited typical DNA synthesis near the ventricular zone followed by migration toward the pial surface. In contrast, brains from Tish animals exhibited dual proliferative zones. One zone was in the typical position while a second was displaced radially to a point between the ventricular and pial surfaces. These results indicate that some cortical heterotopias may derive from an early error in the formation of the germinal matrix, rather than resulting from a strict error in the process of post-mitotic neuronal migration.
Supported by NIH grant NS34124 to KSL.

403.14

INITIAL TOPOLOGICAL ORDER OF THALAMOCORTICAL PROJECTIONS IN THE *BARRELLESS* MUTANT MOUSE
Zoltán Molnár[1]*, Gilles Bronchti[2], Colin Blakemore[1], Egbert Welker[2] [1]University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K. and [2]Institut d'anatomie, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne, Switzerland

Barrelless (brl) mice lack the morphological whisker representation in SI cortex. Whisker-related patterns exist in brainstem nuclei, but are less clear in the ventrobasal nucleus of the thalamus (VB) than in normal mice (Welker et al. *Science*, **271**: 1864, 1996). We tested the hypothesis that the cortical phenotype is due to disorganized thalamocortical projections early in development.

Single crystals of two carbocyanine dyes (DiI and DiAsp) were placed 500 µm apart, in both putative somatosensory and visual cortex of normal (Nor) and brl-mice fixed at postnatal day 2. Results were indistinguishable in the two phenotypes both in VB and LGN. In VB the cells back-labelled by the two different dyes formed two clear, just overlapping clusters. In LGN the number of labelled cells was 3-5 times lower and their scatter somewhat greater than in VB. In both phenotypes, and in both nuclei, very few cells were double-labelled (VB: Nor, 0.6%, brl, 0.7%; LGN: Nor, 0.5%, brl, 0.4%).



Anterograde labelling from the thalamus revealed similar laminar distributions of thalamic fibres in both groups of animals. A cortical defect of pattern formation after correct ingrowth of thalamic axons may cause the barrelless abnormality, rather than initial spatial disorganization of the thalamocortical projection (see figure).
Supported by Swiss NSF 31-39184.93, McDonnell-Pew and MRC G9320738

403.15

STEREOLOGICAL QUANTITATION OF THE CENTRAL NERVOUS SYSTEM.
B. Pakkenberg*, M.J. Bundgaard, C. Fisher, L. Regeur, L. Korbo, B. Bo Andersen, M. West, S. Oster, J. Troncoso, G.B. Samuelsen, U.J. Weber, C.R. Cabello and H.J.G. Gundersen. Neurological Research Laboratory, Bartholin Institute, Kommunehospitalet, 1399 Copenhagen K, and Stereological Research Laboratory, Bartholin Building, University of Århus, 8000 Århus C, Denmark.

New stereological techniques, superior to conventional methods in quantitating macroscopic and microscopic structures in the central nervous system, have been developed within the last decade. Particles of any size and shape can only be sampled with equal probability by the use of a three-dimensional probe. With a combination of the disector and the Cavalieri method, it is possible to obtain an efficient and yet precise estimate of the total number of neurons in any specific region, where a delineation of the region can be made, and the cells identified. Using uniform sampling designs, the efficiency is highly increased: e.g. a subset of approximately 200 uniformly selected disectors from the human neocortex (taken with a known, fixed, but arbitrary probability) is sufficient for the estimation of the total number of nerve cells. The method has been applied to brains from controls, schizophrenics, Alzheimer and AIDS patients and to brains from chronic alcoholics.

403.16

FORMATION AND FOLDING OF CEREBRAL AND CEREBELLAR CORTEX: A TENSION-BASED THEORY OF MORPHOGENESIS AND COMPACT WIRING.
D. C. Van Essen* Anatomy and Neurobiology Dept., Washington Univ. Sch. of Med., St. Louis, MO, 63110.

Why is the cerebral cortex a thin sheet that in many species folds into characteristic convolutions? I propose that mechanical tension, acting upon anisotropically oriented and asymmetrically connected neuronal and glial processes, accounts for many aspects of cortical development. Neuronal processes in vitro generate substantial tension. Likewise, many CNS neurons and glia are likely to be under tension that counterbalances hydrostatic pressure generated across cell membranes and from elevated intraventricular pressure. During development, radially oriented neural and glial processes should be associated with greater compliance (lower stiffness) tangential to the surface, contributing to the sheet-like expansion of the embryonic neuroepithelium and cerebral cortex. Folding of the cortical surface can be explained by characteristic projection asymmetries. In general, tension along axons that enter and leave the cortex should tend to "shrink-wrap" the cortex around a compact subcortical interior, even when this necessitates folding. Tension along major pathways between neighboring cortical regions should tend to draw them together, leading to "outward folds" (crowns of gyri) such as those along the border between areas V1 and V2 in many species. "Inward folds" (fundi of sulci) should separate regions that are only weakly interconnected (e.g., areas 3 and 4 along the central sulcus).

Tension, interacting with cellular architecture and connectivity patterns characteristic of other structures, can also account for the distinctive structure of the hippocampus (including the perforant pathway), cerebellar cortex (with its highly anisotropic elongation), retina (including the foveal pit), and olfactory bulb (with its glomeruli). Altogether, tension-based morphogenesis provides an efficient developmental mechanism for achieving compact wiring of the adult CNS.
Supported by NIH EY02091.

403.17

DIFFERENT ORIGINS AND DEVELOPMENTAL HISTORIES OF TRANSIENT NEURONS IN THE MARGINAL ZONE OF THE FETAL RAT CORTEX. G. Meyer*[1] and A. Fairén[2]. [1]Depto. Anatomía, Univ. La Laguna, Tenerife, [2]Inst. Neurociencias Alicante, Spain

Several populations of transient neurons, here collectively referred to as Cajal-Retzius (CR) cells, lead an ephemeral life in the marginal zone (MZ) of the fetal rat cortex. We used Calbindin (CB), Calretinin (CalR), GABA and BrdU immunohistochemistry to assess their major developmental steps. (1) The primary CR cells appear at E11 in the outer aspect of the neuroepithelium of the ventrolateral telencephalic vesicle (TV), prior to the derivatives of the olfactory placode. At E12, pioneer cells in the ganglion terminale, closely apposed to the medioventral TV, innervate CR cells and ventricular zone (VZ) in the rostral TV, and also seem to enter the MZ. At E12.5, terminal mitoses in the MZ and at different levels of the VZ throughout the TV increase the number of the primary CR cells. A CR subset sends an axonal projection into the subplate. All primary CR cells disappear after E16. (2) From E12.5 to E14, the neuroepithelium of the retrobulbar TV generates huge numbers of small neurons, the secondary CR cells, possibly the rodent equivalent of the human subpial granular layer.They invade the MZ of the entire cortex in successive, neurochemically different waves through tangential, subpial migration. The first waves express CB and GABA, the later ones CalR. The secondary CR cells disappear from the MZ at different moments of pre and postnatal life.

Grants PB94-0582 (GM) and PB94-0219-CO2-01(AF)

403.18

PRENATAL DEVELOPMENT OF LAYER I IN THE HUMAN PREFRONTAL CORTEX. R. Spreafico*, P. Arcelli, M. Selvaggio, P. Canetti, G. Giaccone and C. Frassoni. Depts. Neurophysiology and Neuropathology, Ist. Naz. Neurologico "C. Besta", via Celoria, 11, 20133 Milano, Italy.

Histochemical and immunocytochemical studies were performed on 13 brains from premature infants, ranging from 21 gestational weeks (GW) and 2 months postnatally, died for non neurological diseases. Autopsies were performed, according to the Italian law, after 24 hours from death. Brains were fixed by immersion in either 4% paraformaldehyde buffered solution or in Carnois. 5-10 mm thick slabs from the prefrontal cortex were embedded in paraffin, subsequently cut in 5-10 μm thick sections and processed for Thionine, Bodian stains and for immunocytochemistry (ICC) using antibodies against MAP_2 , GFAP, Vimentine (VIM) and Calretinine (CR). Some sections were also processed using Tunel method for the detection of apoptosis. Data obtained from histochemical study and from ICC using GFAP and MAP_2 were similar to those reported by Sims et al. (1988) and by Gadisseux et al (1992). In addition, CR ICC revealed that this calcium binding protein is already present in layer I (LI) as early as 21 GW in a large number of neurons. They decrease progressively so that by 34 GW only few large CR positive neurons, with a morphology similar to the Cajal-Retzius cells, are present. The progressive reduction of CR positive neurons during the second gestational semester does not seem to be due to cellular death. In fact, during this period, although some apoptotic nuclei are present in LI, their number is lower than the amount of the disappeared CR positive cells. The reduction of the CR positive neurons could be due to either a transient expression of CR during LI development or to an incorporation of some CR positive neurons in the superficial part of the cortical plate.

This work was supported by Mariani Foundation.

403.19

A DISTINCTIVE AChE STAINING PATTERN IN THE RETROSPLENIAL CORTEX OF REELER MUTANT MICE. B. B. Stanfield*[1] and J. M. Wyss[2]. [1]Lab. of Neurophysiology, NIMH, NIH Animal Center, Poolesville, MD 20837 & [2]Dept. of Cell Biology and Medicine, Univ. of Alabama, Birmingham, AL 35294.

Acetylcholinesterase (AChE) histochemistry reveals an elaborate staining pattern near the midline of the posterior cerebral cortex of weanling and mature reeler (rl/rl) mice. This AChE staining pattern is obvious even at low levels of magnification and is characterized by a complex trabecular network of dense AChE staining that is largely surrounded by zones of very sparse AChE staining. Some patches of moderate staining, comparable to the levels seen in nearby cortical fields, are also present in this cortical field. Comparison with adjacent Nissl-stained sections reveals that the dense AChE staining corresponds, at least in part, to clusters of neuronal cell bodies, while the zones of very low AChE staining contain relatively few neuronal somata. No similar configuration of AChE staining is seen in any other region of reeler cerebral cortex, or in any region of the cortex of phenotypically normal (rl/+ or +/+) littermates. This complex of AChE staining appears to be within the retrosplenial cortex. This is confirmed by retrograde and anterograde tracing experiments examining the thalamocortical projections to this cortex. Injections of the retrograde tracer Fast Blue into the region of reeler cortex that contains the elaborate AChE staining pattern label thalamocortical neurons primarily in the anterior thalamic nuclei. Injections of the anterograde tracer biotinylated dextran into the anterior thalamic nuclei of the reeler label individual thalamocortical axons and terminals that are within the region of cortex that contains the elaborate AChE staining pattern. These observations indicate that the elaborate and striking network of AChE staining in the posterior-medial cortex of reeler mice is contained within and confined to the retrosplenial cortex. The connectivity patterns presumably underlying this AChE staining pattern remain to be clarified. Further, these observations do not support a previous description of a similar AChE-staining pattern in the visual cortex of reeler mice. Supported by NIMH and AG11958.

403.20

POSTNATAL DEVELOPMENT OF THE HUMAN ENTORHINAL CORTEX. L. Graterón, L.M. Gonzalo and R. Insausti* . Dept. Anatomy, Univ. Navarra, Apdo. 273, 31080 Pamplona, SPAIN.

Studies on the development of the human cerebral cortex are increasingly important for the understanding of pathology such as schizophrenia. We present here a study based on 12 cases ranging in age from newborn to 5 years. Some preterm infant brains were also available for study.The brains were fixed by immersion in a solution of 4% paraformaldehyde and 0.02% picric acid, or 10% formalin.When possible, the brain was blocked orthogonal to the anterior-posterior commissures axis. Serial sections were obtained, and thionin- and myelin stained series were analyzed, focusing on the laminar and areal differentiation of the entorhinal cortex and comparing it to the adult pattern. Our results show that at the time of birth, the overall laminar structure of the entorhinal cortex is present. Compared to adults, the most relevant difference was in the upper layers (II-III), where dense clumps of small, immature cells were separated by acellular intervening spaces. These clumps (prospective cell islands of layer II) were fused with layer III at the time of birth. At 5 months postnatally, only a fraction of the cells in the clumps had the appearance of stellate cells, and the clumps were still separated by wide, acellular spaces. By one year of age, clearly defined cell islands of neurons resembling the morphology of stellate cells were present in layer II, and the spaces between clumps decreased in size. At 2½ and 5 years the maturation affected mainly the morphology of the neurons. The deep layers display less variation than the upper layers.

DEVELOPMENT OF VISUAL CORTEX I

404.1

CORTICAL SPECIFICATION: THALAMIC PROJECTIONS TO LAYER I DEVELOP WEEKS AHEAD OF PROJECTIONS TO LAYER IV IN FERRETS. Francisco Clascá* and Mriganka Sur. Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139 and Dept. of Morphology, Sch. of Med., Autónoma Univ., Madrid, SPAIN 28029.

We investigated the timing of thalamocortical axon ingrowth in the cerebral cortex using the anterograde transport of DiI and the highly sensitive tracer Cholera Toxin B subunit (CTB). CTB was stereotaxically iontophoresized in discrete thalamic nuclei in living pups (n=19), subsequently perfused at 43 to 59 days postconception (PC). DiI crystals were implanted the thalamus of fixed brains aged PC36-60 (n=31). We analyzed the cortical labeling using optical and confocal microscopy. Thalamocortical axons start branching in the subplate of the topographically appropriate cortical zone by PC36. Density of the subplate plexus peaks around PC42, remaining high for the rest of the period examined. Axons destined for layer I, which in the adult are known to arise from a subclass of small-sized thalamic relay neurons, grow though the cortical plate by PC40-42 but do not branch within the plate. CTB labeling demonstrates that upon arrival in the marginal zone (prospective layer I) thalamic axons give off a number of varicose branches which extend horizontally, rapidly establishing a dense subpial plexus. This layer I projection is topographically precise, and by PC49-50 has reached adult-like density and complexity. At this same age, however, axons destined for layer IV, which in the adult arise from a different subclass of thalamic relay neurons, have not yet entered their target cortical layer. The ingrowth and branching of layer 4 axons begins after E50-51, and proceeds slowly, so that even by the last age examined with CTB (PC59), the layer IV plexus is still less dense than in the adult. As a whole, our data indicate that the layer I projecting thalamocortical system reacts to cortical cues regulating ingrowth and branching in a way completely different from that of the axons projecting to layer IV. Furthermore, these findings raise the possibility that the early growing layer I projection system plays a role in the precise mapping of the later-developing layer IV projection.

Support: NIH grants EY 07023 and Fogarty FO5-TWO4881.

404.2

HOMEOBOX GENE EXPRESSION DURING THE CRITICAL PERIOD OF THE DEVELOPING KITTEN VISUAL CORTEX. Sarven Sabunciyan* and Max Cynader. Dept. of Ophthalmology, UBC, Vancouver, BC, Canada, V5Z 3N9.

Homeobox genes are an extensively studied family of helix-turn-helix type transcription factors which function as developmental regulators. Although much of the published data about the homeobox genes suggest that their role during development is limited to embryonic stages, homeobox gene involvement in developmental events occurring postnatally has been reported. For example, homeobox genes have been discovered in the retinae of adult goldfish which maintain visual plasticity throughout life. In addition, experiments examining the role of homeobox genes in neuronal tissue suggest that they may act as neurotrophic factors and may also be involved in synapse formation. Furthermore, genes encoding glutamate receptors have homeobox-binding-domains in their promoter regions and thus, may be under the regulation of homeobox genes. Given this evidence, we believe that homeobox genes are likely to play a general role in the postnatal development of the brain and hence, we decided to investigate their potential expression in the developing visual cortex of the cat. Degenerate primers targeting the conserved homeodomain region of this gene family were designed and PCR was performed on 30 day old cat visual cortex (VC) cDNA. The PCR product was electrophoresed and the appropriate size band was excised from the gel and cloned. Since the degenerate primers had the potential to match and amplify all of the members of the homeobox gene family, the single excised band represented a heterogeneous population of homeobox genes and thus, the clones derived from this band contained different homeobox sequences. Hence, several clones were picked at random and sequenced. Database searches of these clones revealed that they had >90% homology to homeodomain sequences found in other species and furthermore, confirmed the existence of more than one type of homeobox gene in the 30 day old kitten visual cortex. At present, we are trying to determine the different types of homeobox genes expressed in the 30 day VC by sequencing more of these homeobox clones. We are also trying to determine the expression pattern of these genes throughout the critical period using *insitu* hybridization and quantitative PCR.

404.3

DEVELOPMENTAL STUDY OF CYCLIC NUCLEOTIDE -GATED CHANNELS IN RAT VISUAL CORTEX

D.Samanta Roy*, P.A. Kingston, and C.J. Barnstable, Interdepartmental Neuroscience Program, Dept. of Ophthalmology and Visual Sciences, Yale University School of Medicine, New Haven, CT 06510

To begin to study how cGMP might affect development and plasticity in mammalian visual cortex, we previously described the developmental expression of a series of molecules involved in cGMP metabolism and cGMP effector molecules (Soc. Neurosci. Abstr. 650.6, 1995). Using RT-PCR, we detected expression of the rod cGMP-gated channel, cGMP kinase, cGMP stimulated phosphodiesterase, and the 61 and 63 kD forms of the calcium-calmodulin dependent phosphodiesterase. Furthermore, each molecule had a unique pattern of expression during postnatal development.

These data led us to ask the question of whether the three known forms of the cyclic nucleotide-gated (CNG) channel- rod, olfactory, and cone/testis- may also be differentially expressed in rat visual cortex. Preliminary PCR data suggests that they do have different developmental time courses, with the rod and olfactory RNA levels peaking earlier in visual cortical development, and the cone/testis peaking later in development. In situ hybridization, using digoxigenin-labeled RNA probes specific to each form of CNG channel, was also carried out to determine the layer-specific pattern of each in the visual cortex.

In summary, we have found the three known forms of cyclic nucleotide-gated channel to be differentially expressed in developing rat visual cortex, and have found each to be localized to discrete layers of the visual cortex. These findings suggest that different forms of the CNG channel may play an important role at different stages of rat visual cortical development.

Supported by grants from the NIH.

404.4

THE DEVELOPMENT OF TAURINE-LIKE IMMUNOREACTIVITY IN THE VISUAL CORTEX OF FERRETS. K. Herrmann*, Lab. Neurophysiol., NIMH, NIH Animal Center, Poolesville, MD 20837, USA.

The sulfur containing amino acid taurine is the second most abundant amino acid after glutamate in brain and can be found in millimolar concentrations throughout the CNS. Functions of taurine include osmoregulation, regulation of Ca^{++} fluxes and regulation of protein phosphorylation. The role of taurine as a true neurotransmitter is still controversial, despite the fact that taurine interacts with both excitatory and inhibitory systems in the CNS. Nevertheless, during development taurine might play a crucial role, and is necessary for the development and survival of certain cell types. The present study employed immunohistochemical methods using an polyclonal antibody against taurine (a gift from Drs. D.V. Pow and D.I. Vaney, VTHRC, Brisbane, Australia), to determine the distribution of taurine-positive cells in the developing visual cortex of ferrets between postnatal day (P)1 and adulthood. In the adult, taurine-positive neurons could be found in all cortical layers, but pyramidal cells in the upper cortical layers 2/3 showed the strongest immunoreactivity, while neurons in layers 1 and 5 contained only few taurine-positive neurons. At P1, only a small population of cells in the cortical plate was immunopositive for taurine, however, neuropil staining was obvious in the marginal zone, the subplate, the lower intermediate zone and the ventricular zone. Subplate cells were also immunoreactive. Over the course of the following three weeks, the number of taurine positive cells increased in the cortical plate. By postnatal day 42, the pattern of immunostaining was indistinguishable from that seen in adults. These data show that taurine is present during early cortical development, and needs therefore to be included among the several transmitter-like molecules that are candidate agents to influence cortical development. Supported by NIMH.

404.5

TRANSMITTERS USED BY SUBPLATE NEURONS WITH AXONAL PROJECTIONS INTO VISUAL CORTEX. E. M. Finney*, J. R. Stone, and C. J. Shatz. Howard Hughes Medical Institute and Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720

Subplate neurons send axonal projections into the cortical plate during development, and are involved in the formation of ocular dominance columns (ODC's) (Allendoerfer and Shatz, 1994). To understand further how subplate neurons interact with neurons of the cortical plate, we have begun to identify the neurotransmitters and peptides expressed by those subplate neurons that send axons into the overlying visual cortex during the time ODC's are forming in ferret. Fluorescent latex microspheres were injected into the primary visual cortex (with the exception of layer 6) of postnatal day 28 ferrets (P28) in order to label subplate neurons retrogradely. Brains were processed for immunohistochemistry using antibodies raised against glutamate, gamma-aminobutyric acid (GABA), glutamic acid decarboxylase 67 (GAD-67), neuropeptide Y (NPY), somatostatin (SRIF), and nitric oxide synthase (NOS). Results indicate: (1) the majority of retrogradely labeled subplate neurons are located in the region of the subplate just below layer 6--in the upper subplate; (2) while many subplate neurons can be immunostained for GABA, GAD-67, NPY, SRIF, or NOS, few if any of the retrogradely labeled population are immunoreactive for these transmitters or enzymes; (3) in contrast, subplate neurons that project to cortex can be glutamatergic, since they can be both retrogradely labeled with microspheres and immunostained for glutamate; (4) as in cat, so too in ferret, the SRIF positive subplate neurons are located primarily in the upper subplate. These results indicate that only a subset of all subplate neurons--those located primarily in the upper subplate--project into the cortical plate. The fact that very few GABAergic subplate neurons have cortical projections suggests that the input from the subplate is not likely to be directly inhibitory. Thus, subplate neurons may provide excitation required for the activity-dependent modification of synaptic connections during visual cortical development.

Supported by NIH EY02858 to CJS and a HHMI fellowship to EMF

404.6

NMDAR-1 GENE EXPRESSION IN THE RAT VISUAL CORTEX DURING POSTNATAL DEVELOPMENT AND THE EFFECT OF DARK REARING. Z. Cao*, B. Gordon, M. E. Lickey, and M.H. Kim. Inst. of Neuroscience., Univ. of Oregon, Eugene, OR 97403.

NMDA receptors may mediate activity dependent plasticity in the visual cortex during development (for review see Fox and Zahs, 1994). All functional NMDA receptors contain the NMDAR-1 subunit (Nakanishi, 1992). In rats, maximal visual plasticity probably occurs at approximately 24-34days of age (Fagiolini et al., 1994). To study the relationship between NMDA receptors and plasticity in the visual cortex of Long-Evans rats, we studied NMDAR-1 gene expression with *in situ* hybridization. For normally reared (NR) rats (P14, P22, P30, P45, and P90, n=5 per age), the level of NMDAR-1 mRNA was decreased gradually from P14 to P90. For dark-reared (DR) rats (P14, P22, P30, P45, and P90, n=2 per age), the message declined markedly from P14 to P22, and then remained approximately constant until P90. By P22 it was already substantially lower than it was at P14. In both NR and DR rats, the pattern of decline was similar in all cortical layers. The decline of NR1 mRNA reported here occurs distinctly earlier than the decline of plasticity reported by Fagiolini et al. Therefore the abundance of NMDAR-1 mRNA is probably not a limiting factor controlling visual plasticity. The next step in studying the role of NMDA receptors in plasticity is to examine other subunits. Supported by NEI grant EY04050 to Barbara Gordon.

404.7

NMDA-R1 enriched neurons in layers 6B and the white matter of neonatal visual cortices: Are they the "peptidergic subplate neurons"? Chive AOKI* and Alev ERIŞIR, Center for Neural Science, New York Univ., New York, NY 10003.

In a previous study, we showed that the laminar and subcellular distributions of NMDA-R1 in the visual cortex of rats and cats undergo dramatic changes during early postnatal period (Aoki et al., J. Neurosci. 14: 5202). Most notably, we observed prominent immunolabeling of large multipolar neurons in the infragranular layers and of fusiform neurons in the white matter. Since these are detectable only during the earliest postnatal period, we thought that these might be the transiently present peptidergic "subplate" neurons reported to be involved in guiding the formation synaptic connections between the cerebral cortex and subcortical structures. Since many of the "subplate neurons" contain neuropeptide Y (NPY) and these, in turn, stain positively by the NADPH-diaphorase (NADPHd) histochemical procedure and nitric oxide synthase (NOS) immunocytochemistry, this hypothesis was tested by determining whether the intensely NMDA-R1-immunoreactive neurons label dually for NOS or NADPHd. Our results indicate that few, if any, of the NMDA-R1 enriched infragranular neurons at stages PND3, PND 12 or 25 in the kitten visual cortex are positive for NADPHd or NOS. These results indicate that neurons residing in the subplate consist of a heterogeneous population with distinct modes of neurotransmitter receptivity. Whether or not those neurons specialized for NMDA-type synaptic transmission undergo apoptosis as do the other subplate neurons or merely cease to express high levels of the receptor within matured cortices remains to be determined. Supported by NIH Grants EY08055 and NS30944 (Shannon Award), the NSF Presidential Faculty Fellowship RCD 92-53750 and the Human Frontiers Science Program RG-16/93 to C.A.

404.8

NMDA AND AMPA RECEPTOR SUBUNIT EXPRESSION IN DEVELOPING FERRET PRIMARY VISUAL CORTEX. A.L.Smith[*1,2], I.D.Thompson[1] and H.Monyer[2]. [1]University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K. [2]ZMBH, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.

In the primary visual cortex of ferrets NMDA receptor protein levels increase by up to 9-fold during the first two postnatal months while AMPA receptor levels remain relatively constant. Here we examine the developmental patterns of gene expression for NMDA and AMPA receptor subunits in this region. Sequences spanning the TM1 to TM3 region of ferret NR2A-D and GluRA-D were obtained by RT-PCR and mRNA expression was measured by radioactive *in situ* hybridization using ^{35}S-labelled oligonucleotide probes. The NR2 subunits probably provide the basis for NMDA channel diversity and transcripts encoding each of the NR2 subunits in developing primary visual cortex exhibit different spatiotemporal patterns of expression. NR2A mRNA levels increase greatly throughout development and by adulthood are highest in layer IV and lowest in layers I and V. NR2B transcripts maintain a constant level during postnatal development and are highest in layers VI and II/III and lowest in layers I and V. NR2C and NR2D mRNAs are prominent at the superficial edge of the cortical plate at birth, but levels in cortex rapidly decrease from the first and second postnatal weeks respectively. Thus only the changes in the levels of transcripts for NR2A parallel the changes seen in NMDA receptor protein levels during postnatal development. While GluRD levels within cortex are negligible, the levels of mRNAs encoding GluRA-C are high at birth and exhibit little change during development. GluRA-C transcripts are most concentrated in layer VI and the superficial edge of the cortical plate, later to become layers II/III, and while GluRA and GluRB mRNA expression levels are equally low in layers IV and V, GluRC mRNA levels in layer V exceed those in layer IV. *Supported by the EMBO and by The Wellcome Trust, UK.*

404.9

Spontaneous neuronal coactivation correlates with dye coupling in developing neocortical slices A. Peinado* Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461.

Injections of the gap junction tracer Neurobiotin into single neurons in developing neocortical slices result in labeling of many neurons in the immediate vicinity of the injected cell. Compared to the injected neuron most coupled cells are faintly stained, suggesting weak coupling. Occasionally, however, two or three neurons show strong dye-coupling. I combined calcium imaging and fluorescent dye-coupling techniques to directly test the prediction that strongly coupled neurons would exhibit highly correlated patterns of activity.

Transverse slices of neocortex were made from neonatal and early postnatal rat and mouse pups (P0-P7) and stained with Fura-2 AM. Neuronal activity, as reflected by changes in Fura-2 fluorescence, was monitored at a single excitation wavelength (380 nm) with a fast cooled integrating CCD camera. Post-aquisition image processing was used to identify sets of neurons having correlated patterns of spontaneous activity among dozens of active neurons found within the imaged area (approx. 200 μm X 200 μm) in one plane of focus.

Several pairs of neurons were found whose activity showed a consistent pattern of correlation over successive 20-second recording episodes. In 5 such cases, one of the two neurons was subsequently targeted with a patch pipette containing a fluorescent gap junction tracer, a technique that is useful for revealing instances of strong coupling. In 4 of these, the dye spread to the other coactive neuron but not to non-coactive neurons in the vicinity of the patched cell. In the fifth pair no dye coupling was detectable.

These results are consistent with the idea that coupling in developing neocortex can synchronize electrical activity between neurons.

Supported by grants from NIMH and NINDS.

404.10

DEVELOPMENT OF HORIZONTAL CONNECTIONS IN LAYER 2/3 OF TREE SHREW STRIATE CORTEX: RELATION TO MAPS OF ORIENTATION PREFERENCE. J. C. Crowley, W. H. Bosking, M. Foster, and D. Fitzpatrick*, Dept. Neurobiol., Duke Univ. Medical Center, Durham NC 27710

Neurons in layer 2/3 of tree shrew striate cortex give rise to horizontal connections that exhibit modular specificity, contacting other neurons with similar preferred orientations, and axial specificity, contacting other neurons whose receptive fields are aligned collinearly in visual space. We combined optical imaging techniques with injections of biocytin to explore the normal development of these connections and their relation to maps of orientation preference. Maps of orientation preference could be visualized within the first week following eye opening. The size, shape, and spacing of the orientation domains in these maps were similar to those found in the adult. Injections of biocytin made into layer 2/3 of animals with 1-3 weeks of visual experience revealed a patchy network of horizontal connections that extended up to 5 mm from the injection site. Comparisons of the labeled bouton distributions with maps of orientation preference revealed a modular and axial specificity similar to that seen in the adult. Injections of biocytin in other animals with as little as 2 days of visual experience also resulted in a patchy distribution of boutons that was aligned along an axis, consistent with the pattern seen in the older animals. These results suggest that a small amount of visual experience is sufficient for establishing the modular and axial specificity of horizontal connections, as well as the functional map of orientation preference. Supported by EY06821.

404.11

MECHANISMS INVOLVED IN THE NORMAL AND ABNORMAL DEVELOPMENT OF GENICULO-CORTICAL TOPOGRAPHY IN THE SYRIAN HAMSTER. K.Krug and I.D.Thompson (SPON: Brain Research Association). Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, UK.

Previous work has shown that adult geniculo-cortical topography is sculpted postnatally from an initially unordered projection. In newborn hamsters, a discrete injection of a retrograde fluorescent tracer into area 17 fills cells throughout the lateral geniculate nucleus (LGN). A comparable adult injection labels a discrete column of cells. We were interested in the extent to which terminal reordering and cell death shape the geniculo-cortical map and what role neuronal activity might play in this process.

We have examined the contribution of selective cell death to emerging topography by heterochronic tracing techniques. A single injection at P2 results in a similar pattern of labelled geniculate cells after both long-term (4-10d) and short-term (24h) survival: more than 75% of the LGN is labelled in both cases and the numbers of filled cells are comparable. Superimposing a second injection at P6 or P12 - at a time when the projection is adult-like - gives a maximum 35% of double-labelled neurons. These results suggest terminal rearrangement rather than selective cell death as the mechanism underlying development of topography. Making paired injections at different ages and using different survival times we are also able to quantify changes in topographic order.

Monocular enucleation at birth leaves an altered geniculo-cortical topography ipsilateral to the remaining eye. Preliminary results following intra-ocular injections of TTX into monocularly enucleated animals reveal no dramatic change on the side which retains the majority of its input, whereas topography appears grossly disturbed on the side ipsilateral to the remaining eye. This suggests a role for retinal activity in the reorganization of projections.

This work was supported by the Wellcome Trust, UK.

404.12

DEVELOPMENT OF HORIZONTAL AND VERTICAL MOTION SYMMETRIES IN MONKEYS: VEP ASSESSMENTS. A. Aiyer, J.R. Wilson, and R.G. Boothe*. Division of Visual Science, Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

The present study compares the development of horizontal and vertical directions of motion symmetries in the rhesus macaque. Ten normal rhesus macaques, ages 6 mths to 5 yrs were tested. An additional 2 normals were tested biweekly for a period of six months. To facilitate testing, younger animals were given butorphanol. Older monkeys were given propofol. Stimulus generation and VEP response analysis were carried out using the sweep VEP system of Norcia et al. (IEEE. Vol. 4, 1985). For horizontal motion, stimuli consisted of vertical grating that was "jittered" horizontally in both directions on a video monitor. Two different spatial (0.25 and 1.0 cpd) and temporal (6 and 10 Hz.) frequencies were tested. For vertical motion, the stimulus monitor was rotated by ninety degrees. Horizontal and vertical motion data were obtained within a two-day span for each monkey. At birth, both directions of motion show a large asymmetry. By six months of age, animals exhibited motion symmetry for both horizontal and vertical motion at 6 Hz. but some residual asymmetry at 10 Hz. Horizontal and vertical asymmetries were found to be highly correlated. Results for development of horizontal symmetries are similar to those observed in human infants (Norcia et al. (IOVS Vol. 32, 1991). Higher spatial and temporal frequencies take longer to mature in comparison to lower frequencies. The fact that the response to vertical and horizontal motion systems show a similar developmental time course suggests that they share a common cortical mechanism. Supported by NIH grants RR00165 and EY05975.

404.13

LAMINAR REMODELLING OF CORTICO-CORTICAL PROJECTIONS IN THE CAT, A. Batardière*, P. Barone, C. Dehay, H. Kennedy, INSERM U371, 18 Ave du Doyen Lépine, 69675 BRON (France).

In the development of cortical pathways in the primate we have shown that there is a massive reduction of neurons in the supragranular layers which project back to area 17 (Kennedy et al 1989; Barone et al., 1995). This remodeling of connections is a major feature in the development of the primate where it occurs exclusively in the feedback pathways. We have investigated if a similar developmental process occurs in the cat. Retrograde tracers were injected separately into areas 17, 18 and 19 in kittens. For each case the laminar distribution of retrogradely labeled neurons was determined in extrastriate areas. These results showed that during development there was a small but significant reduction in the numbers of supragranular neurons projecting to striate cortex.

These resuts show that the laminar remodelling of the feedback cortical pathways is very weak in the cat and that the extensive remodelling in the primate is a charactersitic feature of this order.

Bibliography: Kennedy et al., PNAS (1989) 86:8093-8097; Barone et al., Cerebral Cortex (1995) 5:22-38

404.14

EFFECTS OF EARLY PRENATAL ENUCLEATION ON CORTICAL CONNECTIVITY IN MONKEY. P. Barone*, A. Batardière, C. Dehay, M. Berland and H. Kennedy. INSERM U371, Cerveau et Vision, 69675 BRON, France.

In monkey, early prenatal enucleation induces a large reduction in the dimensions of the striate cortex, and the cortex which was destined to acquire area 17 features becomes indistinguishable from area 18. We refer to this as DEC (default extrastriate cortex, Dehay et al, J. Comp. Neurol. 367, 1996). A recent study in the visual cortex of blind humans showed an activation from other sensory modalities (Sadato et al. Nature 380,1996). We investigated if DEC could be invaded by projections from other sensory modalities. Bilateral enucleation was performed prenatally around E58. At birth, retrograde tracers were injected in the ventro-lateral part of the DEC.

The analysis of the distribution of retrogradely labeled cells shows that there are no projections from auditory or somatosensory primary areas to DEC. Likewise, there are no retrogradely labeled neurons in thalamic nuclei other than those dealing with visual inputs to the cortex. All the visual extrastriate areas that normally send feedback projections to area V1, are connected to DEC. Feedback projections arise mostly from infragranular neurons. This adult pattern emerges during development by the elimination of a large number of feedback projections from supragranular neurons (Barone et al Cerebral Cortex 4, 1994). We analysed the effect of the enucleation on this laminar remodelling of feedback projections that normally occurs during development. In the enucleate, the laminar distribution of labeled cells in the visual associative areas buried in the intraparietal and superior temporal sulci (IPS and STS) suggests that in both loci there is a greater proportion of labeled neurons located in the upper layers than it is the case in the normal neonate.

These results suggest that following prenatal enucleation, the areal pattern of feedback projections is unchanged. However, the lack of retinal inputs reduces the process of laminar reorganization that characterized the establishment of feedback pathways in the developing monkey. Supported by the FRM, HFSP# RG-55/94B, CEE #SCI*CT91-0622.

404.15

LOCAL LEARNING RULE FOR SELF-ORGANIZATION AND NON-PARAMETRIC MODELING. Marc M. Van Hulle* Laboratorium voor Neuro- en Psychofysiologie, K.U.Leuven, Faculteit Geneeskunde, Campus Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium.

We show that topographic map formation can be achieved in a self-organizing manner, using a competitive learning rule which maximizes the information-theoretic entropy of the map's neural output. The key difference between this learning rule, called the Maximum Entropy learning Rule (MER), and the most widely-used rule, called the Self-Organizing (feature) Map (SOM) algorithm (Kohonen, *Biol. Cybern.*, 1982), and the many variants of the latter (Kohonen, *Self-organizing maps*, Springer Verlag, 1995), can be summarized as follows. In the SOM algorithm non-overlapping receptive fields (RFs) are defined (Voronoi tessellation). Hence, in order to introduce neighborhood relationships, the weight update rule needs a neighborhood function. In MER, the RFs of neighboring neurons mutually overlap so that, by the overlap, neighborhood relations are already present at the neural activation stage. Hence, no neighborhood function is required *per se* in our weight update rule: purely local weight updates suffice to achieve correct topographic maps.

We show that our local learning rule can be used for non-parametric modeling in essentially two distinct ways: probability density estimation and regression. In the first case, we show that MER yields an optimal map in terms of resource usage and an areal magnification factor range in accordance with the biological evidence available for area V1 of primates (Wässle *et al.*, *Nature*, 1989). Hence, entropy maximization is a plausible computational principle for topographic map formation. In the second case, we show that MER can be used for identifying unknown input functions and for unsupervised classification and pattern recognition purposes. Hence, entropy maximization is a viable default learning strategy for modeling the input flow in a self-organizing way, using only locally-available information.

The author is a research associate of the National Fund for Scientific Research (Belgium) and is supported by research grants received from the National Fund (G.0185.96) and the European Commission (ECVnet EP8212).

401.16

EFFECT OF NEONATAL CORTICAL INJECTION OF KAINIC ACID ON THE DEVELOPMENT OF FUNCTIONAL ARCHITECTURE OF THE VISUAL CORTEX. K. Imamura*[1,2], T. Shiomitsu[1,2], Y. Yoshimura[1], and Y. Watanabe[1,2], [1]Dept. of Neurosci., Osaka Biosci. Inst., [2]Subfemtomole Biorecognition Project, JRDC, Osaka 565, Japan.

Neuronal activity and function of subplate cells have been proposed to play important roles in development of the functional architecture of cat visual cortex. We have studied effects of a neurotoxin, kainic acid, injected locally into the visual cortex of newborn cats to destroy early generated neurons in deeper layers of the cortex. The affected neurons were evaluated by recording electrophysiological responses to kainic acid in slice preparation. Electrophysiological and immunohistochemical examinations showed that the synaptic connections for normal visuocortical function was prevented to develop by this pharmacological manipulation. The anterograde tracing experiments using PHA-L revealed that axons of the lateral genicualte (LGN) neurons extended to layer II-III, outside the normal termination layer IV in the visual cortex treated with kainic acid. This abnormal termination pattern of LGN axon is probably responsible for the lack of vigorous visual response in the cortex injected with kainic acid. We also examined the effects of kainic acid injection on the development of the visual callosal projection. Retrograde tracing experiments showed that the number of the callosal projection to the visual cortex injected with kainic acid was markedly reduced. These results for the first time demonstrated that cortical injection of kainic acid in the first week of age affected not only thalamocortical projection but also the callosal projection in kitten visual cortex. It is also suggested that interaction of axons with deep-layer cortical neurons, including subplate cells, plays an important role for establishing appropriate connections to the postsynaptic cells and maintaining them in the visual cortex.

404.17

MONOCLONAL ANTIBODIES TO NGF AFFECT DEVELOPMENT OF VISUAL CORTICAL NEURONS FUNCTIONAL PROPERTIES IN RATS N. Berardi, R. Siciliano, M.C. Cenni, F. Ruberti, A. Cellerino, A. Cattaneo, L. Maffei and L. Domenici *. Istituto di Neurofisiologia del CNR, I-56127 Pisa, Scuola Normale Superiore, Pisa, Dept. Physiol. Biochem., Pisa, and SISSA, Trieste (Italy)

Recent results suggest an important role for neurotrophins in visual cortex (VC) development and plasticity. In particular, we have shown that antagonizing endogenous NGF action by intraventricular (i.v.) implant of αD11 hybridoma cells secreting neutralizing antibodies (Mabs) to NGF at the beginning of the critical period (day P15) affects ocular dominance distribution (ODD) of rat visual cortical (vc) neurons. We have now examined, by means of electrophysiological recordings, orientation selectivity and receptive field (RF) size of vc neurons in 5 rats i.v. implanted with αD11 (10^6 cells) at P15 and recorded (urethane anaesthesia) at the end of the critical period (P45). Mabs titre in the CSF was assessed (ELISA). Five littermates implanted at P15 with parental myeloma cells and 5 normal P45 rats were recorded as controls. We confirm that in αD11 rats ODD was strongly shifted towards the contralateral eye dominance, with reduction of binocular cells (Binocular Index=0.28±0.08) with respect to controls (BI=0.5±0.1). RFs were larger in αD11 rats than in controls (mean size 21±4 and 9±4 deg, respectively) and the number of orientation selective cells was reduced (50% vs 67%). None of these effects was present in a parallel group of P45 rats (N=5) deprived (by excitotoxic lesion) of the cholinergic input to VC at P15. This points out to a direct role of endogenous NGF in regulating development of vc neurons functional properties which is not ascribable to a possible cholinergic hypofunction brought about by anti-NGF Mabs.

Supported by HFSP grant RG93/93 and BIOMED (BMH1-C794-1368)

404.18

ROLE OF BDNF IN THE ACTIVITY-DEPENDENT REGULATION OF CORTICAL FIRING RATES AND GABA EXPRESSION. L.C. Rutherford, A. DeWan, and G.G. Turrigiano*, Dept. of Biology and Volen Center, Brandeis University, Waltham, MA 02254.

Maintaining the proper balance of excitation and inhibition is important for normal cortical function. Here we show that activity regulates the firing rates of cultured cortical neurons, in part through the brain-derived neurotrophic factor (BDNF)-mediated regulation of GABA expression.

Whole-cell recordings were obtained from pyramidal neurons from cultures of P5-6 rat visual cortex after 7-9 DIV. Recordings were made from control cultures grown in normal medium or sister cultures treated for 48 hrs with TTX to block all spikes, then washed extensively prior to recordings. Pyramidal neurons from TTX treated cultures had 9-fold higher firing rates (20.7±7.8 spikes/min) than from control cultures (2.3±0.8 spikes/min , $p < 0.05$, Student's t test). Resting potentials and input resistances were not different. To determine whether a decrease in inhibition could contribute to such hyperexcitability, cultures were treated with TTX for 48 hrs, fixed, and labeled against GABA and the neuronal marker MAP2. TTX treated cultures had a lower percentage of GABA positive neurons than control cultures (20.2±1.6% and 28.2±1.0%, respectively; $p < 0.01$, Student's t test). Coapplication of BDNF prevented this decrease (28.4±4.0 %), and the trk receptor blocker K252a produced a decrease similar to that produced by TTX (18.0 ± 1.9 %), suggesting that activity regulates GABA levels through a BDNF-dependent mechanism. These data indicate that activity can regulate cortical firing rates in culture, and part of this regulation may be through the expression of the inhibitory neurotransmitter GABA.

Supported by NSF and the Whitehall Foundation (GGT).

REGENERATION: ALTERED GENE EXPRESSION—CNS

405.1

ALPHA$_1$-TUBULIN mRNA LEVELS ARE UNCHANGED BY PROXIMAL AXOTOMY IN MOUSE CORTICAL NEURONS. E.J. Elliott*, D.A. Parks, P.S. Fishman. Research Service, VA Medical Center, Baltimore, MD 21201.

Our previous work with transcallosal cortical neurons in the mouse showed that proximal axotomy did not significantly alter their levels of GAP-43 mRNA, suggesting that the neurons failed to mount any regenerative response. The cytoskeletal protein α_1-tubulin, like GAP-43, is strongly upregulated in developing and regenerating neurons, but may be regulated independently from GAP-43. We have examined brain sections from the same animals used in the GAP-43 study, this time looking for changes in α_1-tubulin mRNA, to determine whether the response seen with GAP-43 (which was variable) would be reproduced with α_1-tubulin.

In situ hybridization with a ^{33}P-labeled oligonucleotide probe for mouse α_1-tubulin showed no consistent changes in α_1-tubulin mRNA in axotomized cortical neurons at 1 day, 3 days or 7 days after injury. (This contrasted with regenerating spinal motor neuron controls, which showed a consistent large increase in α_1-tubulin expression.) Half the animals showed no significant differences in silver grain density over axotomized compared to unaxotomized cortical neurons. Four animals out of 17 showed small increases in grain density (35-63%) that were statistically significant, and 5 out of 17 showed decreases (26%-60%). However, of those animals which showed increases in α_1-tubulin, only one was an animal which had shown slight increases in GAP-43 mRNA. The other three had shown either no change, or a slight decrease in GAP-43. Similarly, none of the animals which showed decreases in α_1-tubulin had shown decreases in GAP-43, but rather had been unchanged, or shown slight increases. These results confirm our earlier conclusions that transcallosal cortical neurons in the mouse give little regenerative response even to proximal axotomy. *Supported by Dept. of Veterans Affairs Merit Award.*

405.2

INDUCTION OF A PHOSPHORYLATED MAP1B IN TARGET-DEPRIVED AFFERENT FIBERS AFTER NEURODEGENERATION PRODUCED LESION. S. Soares, I. Fischer[1], J.-D. Vincent and F. Nothias*, Inst. A. Fessard, CNRS, Gif-Sur-Yvette, France, 1. Med. Coll. of Penn., Philadelphia, PA 19129.

Microtubule associated protein 1B (MAP1B) is the first MAP to be detected during the development of the CNS and becomes markedly down regulated postnatally. Its expression, particularly of its phosphorylated forms, is associated with axonal growth. We previously showed that a phosphorylated MAP1B (MAP1B-**P**) was restricted to axons, while total MAP1B was present in cell bodies and processes. In the adult MAP1B-**P** levels decreased dramatically and remained detectable in only a few areas that undergo morphological plasticity. To confirm these observations, the present study was undertaken to determine whether MAP1B-**P** expression is induced in axonal remodeling after neurodegenerative lesion. The right ventrobasal thalamus of adult rats was depleted of neurons by in situ injection of kainic acid. Rats were allowed to survive 4 days to 3 months. Somatosensory and cortical afferents were identified by anterograde tracing with dextran biotin injected in dorsal column nuclei, trigeminal nuclei, and somatosensory cortex.

MAP1B-**P** immunostaining is absent in the unlesioned control adult thalamus. Four days after kainic acid injection, MAP1B-**P** staining is detected in the afferent fibers within the lesion, increased during the two following weeks and remained high for several weeks. The double staining showed that MAP1B-**P** is induced in both somatosensory and cortical afferents. Deprived of their specific neuron-target, these fibers undergo morphological changes that resemble regenerating growth cones and are able to regenerate synapses when new target is provided by neuronal transplant.

405.3

TUBULIN ISOTYPE REGULATION IN INJURED, REGENERATING AND BDNF TREATED RETINAL GANGLION CELLS A.E. Fournier, L. McKerracher* Center for Research in Neuroscience, McGill University; Université de Montréal, Departement de pathologie, Québec, H3C 3J7.

We are interested in the factors which regulate gene expression during axon regeneration in the CNS. Axon injury is known to affect the expression of tubulin, and here we investiage how growth permissive peripheral nerve (PN) grafts or the application of BDNF modulates the neuronal response to injury. Five tubulin isotypes in adult rat retinal ganglion cells (RGC) were examined after intraorbital axotomy, after applying a PN graft, and after RGC injury accompanied by an intraretinal injection of 5µg of BDNF. The mRNA levels were monitored by quantitative *in situ* hybridization with tubulin isotype-specific RNA probes. Treated and control sections were hybridized on the same slide and silver grains over individual RGCs were counted. RGCs with regenerated axons were detected by retrograde labelling from the graft. Following axotomy, mRNA levels for the ßI, ßII, ßIII, and ßIV ß-tubulin isotypes and for the Tα1 α-tubulin isotype decreased to approximately 70% of control levels by two weeks. RGCs which regenerated their axons into PN grafts, showed dramatically enhanced mRNA levels for the ßII, ßIII and Tα1 isotypes (approximately 300%). This enhancement was specific for these isotypes whose expression levels were also enhanced during development, and was not observed for the ßI, and ßIV isotypes. A single injection of the neurotrophin BDNF, which is known to enhance survival of injured RGCs, had a specific effect on the ßII isotype 3 days following treatment. The effect of BDNF on the ßII isotype was modest, but significant, and was not observed for the ßIII, ßIV or Tα1 isotypes. The inability of BDNF to upregulate the full complement of mRNAs that increase with a PN graft suggest that multiple factors present in PN grafts help modulate tubulin expression. (Supported by NSERC and by an FCAR scholarship to A.E.F.)

405.4

CHONDROITIN SULFATE PROTEOGLYCAN EXPRESSION FOLLOWING SPINAL CORD INJURY

M.L. Lemons*, D.R. Howland and D.K. Anderson. VAMC and Dept. of Neuroscience, University of Florida, Gainesville, Fl 32610.

Extrinsic factors appear to be primarily responsible for the lack of regeneration in the adult CNS. To ultimately enhance axonal growth, these factors must be more fully understood. We are examining *in vivo*, the role of an extracellular matrix molecule, chondroitin sulfate proteoglycan (CSPG), which inhibits neurite outgrowth *in vitro*. Our studies are designed to answer two main questions: 1) Does CSPG expression increase *in vivo* after spinal cord injury? and, if so, 2) can CSPG be enzymatically cleaved and its inhibitory function disrupted *in vivo*? Four groups of adult rats are being studied; normal, sham injured, spinal cord contused and contused + fetal spinal cord graft. CSPG immunocytochemical staining in shams is similar to normals. In cross-section it outlines axons in the white matter and shows a peri-neuronal pattern in the gray matter. By one week post injury, CSPG immunoreactivity is intensified around axons in the white matter and in the gray matter particularly at the lesion cavity edge. This host pattern persists for at least 30 days and is not altered by a fetal graft. By 30 days, the graft also becomes CSPG positive. To disrupt this increase in CSPG expression, gelfoam soaked in chondroitinase ABC (chase) is laid on the dorsal spinal surface of sham and spinal cord injured rats. Chase is known to cleave and disrupt CSPG's inhibitory function *in vitro*. Within 4 days, chase cleaves CSPG in the superficial white matter. CSPG is not cleaved in a saline soaked gelfoam control group. These results suggest that a) CSPG increases after spinal cord contusion injury and correlates with the lack of axonal growth and b) chase can cleave CSPG *in vivo*. Currently, CSPG expression is being characterized in other spinal cord lesion models and the application of chase is being modified to enhance its penetration and prolong its exposure to the site of injury as well as to intraspinal grafts. Supported by CM & KE Overstreet Foundation, UF Medical Guild & Department of Veterans Affairs.

405.5

EXPRESSION OF c-JUN IN MATURE CNS AFTER SPINAL CORD INJURY E.Broude, M.McAtee, H.Bernstein-Goral*, B.S.Bregman, Georgetown University, Washington, DC 20007

Protein product of the immediate early gene *c-jun* is highly expressed in axotomized peripheral neurons during regeneration and in central neurons after cervical, but not thoracic spinal cord injury. Fetal spinal cord transplant induced and application of exogenous neurotrophic factors enhanced more growth of host axons in the adult rat after midthoracic overhemisection. The aim of this study was to determine if interventions shown to increase the amount of axonal growth after injury upregulate expression of c-Jun and alter the extent of neuronal atrophy and/or cell loss of the axotomized neurons. The following experimental interventions were used: hemisection only; hemisection plus transplant; hemisection plus neurotrophins (BDNF or NT-3) with or without transplant. Animals were sacrificed at 7 and 30 days after surgery. By 7 days after lesion immunohistochemistry and quantitative image analysis showed increased c-Jun expression in axotomized red nucleus and locus coeruleus of animals from all experimental groups, as compared to normal and hemisected-only rats. Fetal spinal cord transplants, exogenous neurotrophins and the combination of both significantly affected axotomized neurons, inducing high expression of c-Jun protein. Both cell number and cell size were greater than in lesion only animals. We also compared expression of c-Jun in dorsal root ganglion neurons (DRG) after central or peripheral axotomy. The number of c-Jun-positive cells was increased when embryonic spinal cord transplant was present at the site of central dorsal root axotomy, as compared to central axotomy-only animals. Small and medium diameter DRG neurons were affected preferentially. Our results suggest that expression of c-Jun protein in axotomized mature CNS neurons is elicited by interventions which increase the extent of axonal growth after injury and plays an important role in induction of genes specific for growth of the mature CNS. Supported by NIH NS 19259; neurotrophins generously provided by Regeneron.

405.6

B-50/GAP-43 OVER-EXPRESSION IN TRANSGENIC MOUSE PURKINJE CELLS IS NOT SUFFICIENT TO INDUCE AXON REGENERATION. A. Buffo[1], A.J.G.D. Holtmaat[2], T. Savio[1], B. Oestreicher[2], W.H. Gispen[2], J. Verhaagen[2], P. Strata[1]*, F. Rossi.[1] Dept. Neurosci., Univ. of Turin, Turin, I-10125; [2]Neth. Inst. Brain Res., 1105 AZ Amsterdam., NL.

It has been shown that the growth permissive/promoting conditions created by grafts of embryonic nervous tissue and Schwann cell suspensions induce the regeneration of several populations of severed axons in the adult CNS. By contrast, Purkinje cell (PC) axons never regenerate into such grafts. The failure of PC axon regeneration may be due to their inability to express the gene programme necessary to sustain neurite outgrowth. Indeed, both intact and lesioned PCs never express B-50/GAP-43, a protein associated to axonal outgrowth during development and involved in neuronal response to axotomy and regeneration. According to this hypothesis, PC axon regeneration might be obtained by inducing the expression of growth associated genes in these neurones. We have investigated the regenerative capabilities of PC axons in a strain of mice carrying a transgene in which the rat coding sequence of B-50/GAP-43 was cloned downstream to the mouse L7 protein promoter to specifically direct the expression of B-50/GAP-43 in PCs. High levels of B-50/GAP-43 were expressed in all PC somata and axons from intact mice and this expression was maintained after axotomy obtained by passing a microscalpel through the cerebellar parenchyma. However, by anti-calbindin or anti-B-50/GAP-43 immunolabelling no PC axon regeneration was observed either after axotomy alone, or when embryonic cerebellar grafts or Schwann cell suspensions were placed into the lesion track. Thus, B-50/GAP-43 expression alone does not seem to be sufficient to obtain PC axon regeneration into growth permissive territories. Alternatively, it is possible that neurite outgrowth is prevented by the strong hypertrophic reaction which affects transected PC axons. (Supported by A.I.S.M.).

405.7

REPRESSION OF GAP-43 EXPRESSION BY CYCLIC AMP IN THE CNS NEURONAL CELL LINE RN46A D.J. Schreyer*[1], P.L. Andersen[1], and S.R. Whittemore[2] [1]Dept. Anatomy and Cell Biology, Univ. Saskatchewan School of Medicine, Saskatoon, SK, Canada S7K 0M7, and [2]Dept. Neurological Surgery, Univ. Miami School of Medicine, Miami, FL 33136

Neurons of the adult mammalian central nervous system (CNS) display poor regenerative growth following axon injury. Regenerative failure has been attributed to growth inhibitors associated with CNS myelin. It is not known whether CNS myelin growth inhibitors act only at the level of the growth cone, or additionally influence neuronal gene expression which supports growth. Using a cell-ELISA assay, we show that expression of the growth-associated protein GAP-43 is normally detectable in cultured RN46A cells, a cell line derived from E13 rat raphe nucleus neurons. RN46A cell expression of GAP-43 is markedly suppressed by chronic exposure to cholera toxin, forskolin, cAMP analogues and phosphodiesterase inhibitors. DNA assays performed on sister cultures indicate that the changes in GAP-43 are not due to changes in cell survival. These findings suggest that extracellular influences may inhibit GAP-43 expression in CNS neurons via a G-protein linked receptor and adenyl cyclase. Supported by MRC Canada and NS26887.

405.8

ELEVATED LEVELS OF THE CELL ADHESION MOLECULE, L1, FOLLOWING SPINAL CORD INJURY IN THE ADULT RAT. C. Roonprapunt*, A. Hooper, W. Huang, D. Friedlander, M. Grumet and W. Young. Departs. of Physiology and Pharmacology, NYU Medical Center, New York, NY 10016.

As a mediator of cell to cell interactions in the nervous system, L1 functions in cell adhesion, neuronal migration, and neurite outgrowth. In an effort to understand the effects of spinal cord injury on L1 expression, we conducted an immunohistochemical analysis of L1 at the site of injury. With the NYU weight drop device, spinal cord contusions were induced in male and female adult Long-Evan's hooded rats. A 10 gm rod was dropped from a height of 2.5 cm directly above the spinal cord exposed by laminectomy at T-10. The spinal cords were analyzed by immunostaining and quantitative Western blotting at one day, one week, and six weeks following impact.

Immunostaining of normal non-injured cords revealed weak L1 signal. In the white matter L1 was present on axonal fiber tracts seen on horizontal section, and in the gray matter there was some L1 primarily at the dorsal root entry zone seen in cross-section. At one week after injury, staining at the injury site appeared more intense when compared to age-matched, non-injured control animals. The results of the biochemical quantitation confirmed that the observed increase in L1 immunoreactivity represented actual increases in concentrations of the cell adhesion molecule, and not simply a staining artifact (eg. unmasking of antigenic components in uninjured spinal cords). By six weeks, the injury site had developed a post-traumatic cyst filled with a loose non-neuronal matrix of fibers. This new growth of neurofilament-positive fibers expressed intense L1 immunoreactivity. The origin of these regenerating fibers, peripheral or central, is currently under investigation. *Supported by Acorda Therapeutics*

405.9

EXPRESSION CHANGE OF F3/CONTACTIN IN GERBIL HIPPOCAMPUS AFTER TRANSIENT FOREBRAIN ISCHEMIA

K. Shimazaki*[1], H. Cho[1], K. Takeuchi[2] and K. Watanabe[3]
[1]Depts. Physiol. Neurosurg Jichi Medical School, Tochigi-ken 329-04, Japan. [2]Dept. of Bio. Sci. Nara Adv. Inst. Sci. Tech. Nara-ken 630-01, Japan. [3]Dept. Exp. Biol. Tokyo Metropolitan Inst. of Gerontology. Tokyo 173, Japan.

The neural cell specific adhesion molecule F3(contactin), a member of the immunoglobulin(Ig) superfamily, plays important roles of neurite outgrowth and axonal guidance during development. However, the role of F3 in adult brain has been poorly understood. We have studied changes in the expression in gerbil hippocampus after transient forebrain ischemia for 5 min. Immunoblotting study revealed that F3 expression decreased from 1 to 7 days after ischemia, while it increased later than 21 days after ischemia. By immunohistochemical study , biphasic change in F3 was seen selectively in CA1 sector. The initial decrease of F3 may reflect on going cell death of CA1 pyramidal neurons. Late increase in F3, which showed similar time course with increased immunoreactivity of 145 kDa neurofilament . That may be caused by sprouting of the neurites of the input fibers to CA1, since damage to CA3 neurons failed to increase in F3. Our results suggest that F3 plays an important role in regenerative processes after ischemic cell death, including sprouting of the Schaffer collateral/commissural axons in CA1 subfield.

This work is partly supported by Grants-in Aid from the Ministry of Education Science and Culture of Japan(No. 07680852).

405.10

SEPTOHIPPOCAMPAL NEURONS TRANSIENTLY UP-REGULATE CNTF RECEPTOR α EXPRESSION FOLLOWING BILATERAL FIMBRIA-FORNIX TRANSECTION.

T. Naumann*, Mun-Yong Lee, M. Kirsch, M. Frotscher and H.-D. Hofmann, Institute of Anatomy, University of Freiburg, P.O. Box 111, Germany.

Most cholinergic as well as parvalbumin-containing GABAergic septohippocampal neurons survive bilateral fimbria-fornix transection (ff-t). Moreover, they are able to restore the synthesis of their marker proteins choline acetyltransferase and parvalbumin after transient downregulation following axotomy (Naumann et al.,'94). It has been shown that exogenously applied ciliary neurotrophic factor (CNTF) can prevent degeneration and loss of most injured medial septum neurons whereas the effects of nerve growth factor are restricted to the cholinergic neurons (Hagg et al.,'92). To investigate, if CNTF could act directly on septal neurons to influence their response to axotomy, we have studied the expression of CNTF receptor α in the medial septum / diagonal band complex (MSDB) in normal and lesioned rats.

Adult Sprague-Dawley rats received bilateral ff-t and survived for 3, 7, 10 days and 3, 10, 30 weeks. In some of the animals septohippocampal projection neurons were prelabeled by intrahippocampal Fluoro-Gold injections prior to axotomy. Expression of CNTF receptor α mRNA was determined by in situ hybridization using a digoxigenin-labeled RNA probe.

CNTF receptor α mRNA was undetectable in the MSDB of unlesioned animals, but was upregulated following ff-t. Labeling of MSDB neurons was first observed at 7 days postlesion, reached a maximum after 10 days, and was close to normal levels after 3 weeks. CNTF receptor α-expressing septohippocampal neurons could be identified by the presence of Fluoro-Gold. The dramatic upregulation of the CNTF receptor α following ff-t suggests that locally supplied CNTF may contribute to the survival of axotomized MSDB neurons. (Supported by the DFG: SFB 505 (A1, A4), and Leibniz Program)

405.11

VESTIBULAR NEURONS INCREASE EXPRESSION OF GAP-43 mRNA AFTER CERVICAL SPINAL CORD HEMISECTION.

B.C. Tryon*[1], J.L. Seeburger[1], T.M. Connors[1], A. Tessler[1,2], M. Murray[1], J.E. Springer[2] [1]Dept. Neurobiology and Anatomy, Medical College of Pennsylvania Hahnemann University, Philadelphia, PA 19129, [2]The VA Medical Center, Philadelphia, PA, 19104 [3]Dept. Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536-0084.

Because vestibular neurons play a critical role in maintaining balance and posture, promoting regeneration of vestibulospinal axons may improve functional outcome after spinal cord injury. To examine the capacity for regeneration of vestibulospinal neurons, we studied the expression of GAP-43 mRNA in axotomized neurons of the lateral vestibular and adjacent spinal vestibular nuclei. 24 adult female Sprague-Dawley rats underwent hemisection of the spinal cord at the C5 segment. At 3 days and at 1, 3, and 8 weeks postoperative (n=6, each group) brainstem tissue was processed for in situ hybridization using a full length antisense riboprobe for GAP-43 mRNA. Since the vestibulospinal tract is primarily an ipsilateral projection, the vestibular nuclei contralateral to the hemisection served as an internal control for GAP-43 mRNA expression. GAP-43 mRNA levels did not change from baseline in lateral vestibular or adjacent spinal vestibular neurons 3 days following cervical hemisection, but were significantly increased in the nuclei ipsilateral to surgery at 1, 3 and 8 weeks postoperatively. This result suggests that lateral vestibular and adjacent spinal vestibular neurons retain the capacity for growth for at least 2 months following cervical spinal cord hemisection. This propensity may account for the regeneration of vestibulospinal axons into intraspinal grafts (Ye and Houle, '95; Xu et al., '95). Supported by grants from the Dept. of Veterans Affairs, NIH NS 24707, PVA, APA and International Research in Paraplegia.

405.12

COMPARISON OF THE EXPRESSION OF A NOVEL FABP IN DEVELOPING AND ADULT RAT CENTRAL NERVOUS SYSTEM.

Y Liu*, C.A. Molina*, M. Kirby*, L D Longo* and M. De León*. *Center for Molecular Biology and Gene Therapy and Department of Physiology, *Dept. of Pathology and Human Anatomy; *Center for Perinatal Biology and Department of Physiology, Loma Linda Univ., Loma Linda, CA, 92350.

DA11 is a novel fatty acid lipid-binding protein isolated from a rat cDNA library made from dorsal root ganglia ipsilateral to a sciatic nerve crush. DA11 mRNA and protein are induced in neurons in the DRG ipsilateral to a sciatic nerve cut as compared with contralateral and naive DRG (Molina et al., Soc Neurosci. Abstr 21:1073; De León et al., J. Neurosci Res., In press), and in neonatal brain tissue homogenates as compared with adult brain (Liu et al., Soc Neurosci. Abstr 21:1073). The present study expands our earlier observations, and identifies which cell types express DA11 in the rat developing brain. *In situ* hybridization and immunocytochemical experiments showed that DA11 protein and mRNA were localized in neurons. In the cerebral cortex, those neurons undergoing neurogenesis, migration, and differentiation exhibited higher levels of DA11 expression than differentiated cells. The cerebral cortex neurons expressing higher levels of DA11 mRNA and protein were part of the group of neurons forming the characteristic "column" structures throughout the brain cortex. The Purkinje cells in the cerebellum contained very high levels of DA11 mRNA and protein. This expression was observed as early as E21, sustained during the first 10 days of postnatal development, and was significantly down-regulated in adult cells. Neurons in the hippocampus, brain stem, and spinal cord also exhibited a similar developmental pattern of DA11 expression to that observed in the Purkinje cells. High levels of DA11 were also found in the olfactory bulb and the retina. In the retina, DA11 was localized in bipolar and ganglion cell layers. In the olfactory bulb, DA11 was expressed in the mitral cell layer, but there was also a moderate level of expression in glomerular and granule cell layer. Our data show that the observed transient but significant induction of DA11 during early and postnatal development of CNS is associated with the process of neuronal cell differentiation. This work was supported by NIH grant HD3807-Supplement.

405.13

IDENTIFICATION OF GROWTH-ASSOCIATED GENES IN RAT RETINAL GANGLION CELLS DURING REGENERATION AND EMBRYOGENESIS.

C. V. Esguerra*[1,2], E. Guenther[3], A. Cellerino[3], E. Hoffmann[1,2], and M. Bähr[1,2]. [1]Max Planck Institute for Developmental Biology, [2]Department of Neurology, University of Tübingen, and [3]Research Center for Experimental Opthalmology, D-72076 Tübingen, Germany

We recently devised an approach involving patch-clamp technology, global PCR amplification of mRNA from single cells, and subtractive hybridization for the rapid identification of genes potentially involved in the regeneration of adult retinal ganglion cell axons into a peripheral nerve graft after lesion. An efficient multiple-parameter selection strategy involving Boolean hybridization steps enabled us to significantly enrich for genes upregulated in both regenerating and embryonic retinal ganglion cells.

Initial screening of this library resulted in the isolation of a series of genes, both known and novel. Aside from highly enriching for and recovering the GAP-43 gene, we isolated genes that code for putative transcription factors (GATA-1 and a novel zinc finger protein), a growth factor and its corresponding receptor (a novel IGF-1 isolog and IGFIIR), signal transduction proteins (calmodulin and a novel G-protein), cytoskeletal proteins (α- and β-tubulin), axoplasmic transport proteins (myosin light-chain and dynein), an extracellular matrix glycoprotein (fibronectin), and a synaptogenesis protein (synaptotagmin IV). Initial characterization of the zinc finger gene, a novel member of the Krüppel family of transcription factors, revealed a significant upregulation of expression in embryonic and regenerating retinal ganglion cells in comparison with the levels of expression in normal adult and degenerating ganglion cells. Final results of the library screen will be presented, in addition to a thorough characterization of the zinc finger gene.

This work is supported by the Deutsche Forschungsgemeinschaft (DFG).

405.14

EXPRESSION OF GROWTH ASSOCIATED PROTEINS AFTER LESION AND DURING RETINAL AXON REGENERATION IN RATS.

B. Petrausch[1], M. Jung[1], D. Karagogeos[2] and C.A.O. Stuermer[1]*. [1]Faculty of Biology, University of Konstanz, D-78434 Konstanz, F.R.G., [2]Dept. of Medical Science, University of Crete, Iraklion, 71110 Crete, Greece.

After optic nerve transection (ONS) and during axon regeneration in fish, all retinal ganglion cells (RGCs) re-express GAP-43 and cell adhesion molecules (CAM), which they originally displayed during axon outgrowth. In rats, RGCs express related proteins in the embryo. In adults, a limited number of rat RGCs re-expresses GAP-43 after ONS, as well as during axon regeneration, when a peripheral nerve graft (PN) is provided.

Using immunocytochemistry and in situ hybridisation, we examined in rats whether regenerating axons in PN grafts carry the CAMs ε587 antigen, L1/NILE, DM-GRASP/SC-1, TAG-1 and whether this correlates with mRNA upregulation in RGCs. In embryos axons expressed all these proteins, and mRNA of GAP-43 and TAG-1 (those tested so far) were present in all RGCs. RGC axons in normal adults and upon ONS expressed all these proteins in their intraretinal path but lost TAG-1 some days after ONS. Accordingly, DM-GRASP/SC-1 and TAG-1 mRNAs were present in normal adult RGCs. Upon ONS, DM-GRASP/SC-1 mRNA remained in some RGCs but TAG-1 mRNA was lost. Regenerating axons in PN grafts carried GAP-43, ε587 antigen, L1, DM-GRASP, but not TAG-1. Accordingly, DM-GRASP/SC-1 mRNA was present in some RGCs of PN grafted rats (much like GAP-43 mRNA), whereas TAG-1 mRNA never reappeared. Expression of L1/NILE and ε587 antigen mRNAs is currently studied. Thus, axon regeneration is paralleled by re-expression of GAP-43 and upregulation of certain CAMs, but TAG-1 which interacts with other CAMs is lost and not re-expressed.

Probes were kindly provided by L.I. Benowitz, A.J.W. Furley, T.M. Jessell, G.E. Pollerberg, F.G. Rathjen, J.H.P. Skene and W.B. Stallcup. Supp. by BMBF, ISRT, and Dt. Stiftung Querschnittslähmung.

405.15

gRICH68 AND gRICH70 ARE GOLDFISH 2',3'-CYCLIC-NUCLEOTIDE 3'-PHOSPHODIESTERASES UPREGULATED DURING OPTIC NERVE REGENERATION. R. P. Ballestero, G. R. Wilmot, B. W. Agranoff and M. D. Uhler*. Department of Biological Chemistry and Mental Health Research Institute, University of Michigan, MI48109.

The teleost optic nerve is a model system for the study of axonal regeneration in the CNS. p68/70 is a doublet of acidic proteins induced in retinal ganglion cells upon optic nerve crush. We previously reported the cloning of a cDNA encoding RICH, a protein homologous to mammalian CNPases (2',3'-cyclic nucleotide 3'-phosphodiesterases; 36.3% identical to human CNPase1) and with several characteristics that relate it to the p68/70 doublet (Proc. Natl. Acad. Sci. USA, 1995, Vol 92, pp 8621-8625). We have now isolated a cDNA encoding a second isoform of RICH. Using recombinant technology we have expressed the encoded proteins both in prokaryotic and eukaryotic systems. We have obtained additional support documenting their relation to the components of the p68/70 protein doublet, and thus we have renamed them gRICH68 and gRICH70. We have additionally found and characterized the enzymatic properties of the bacterially expressed and purified gRICH proteins versus recombinant mCNP1, confirming that gRICH proteins are 2',3'-cyclic-nucleotide 3'-phophodiesterases. The physiological role of these enzymes remains unknown, but the induction of these novel members of the family during goldfish optic nerve regeneration provides a new model to elucidate their biochemical role in nervous tissues.

This work was supported by a grant from the Markey Charitable trust to M.D.U. and B.W.A. and by the Ralph and Elsie Colton MHRI Frontiers of Neuroscience Fund.

405.16

ISOLATION OF A PARTIAL cDNA ENCODING THE ZEBRAFISH PROTEIN zRICH AND ITS EXPRESSION AFTER OPTIC NERVE CRUSH. M. D. Uhler, R. P. Ballestero, J. A. Dybowski and B. W. Agranoff*. Department of Biological Chemistry and Mental Health Research Institute, University of Michigan, MI48109.

Teleosts are used as models for nerve regeneration in the central nervous system. We have previously isolated cDNAs encoding gRICH, a protein induced during goldfish optic nerve regeneration that shows homology with mammalian CNPases (2',3'-cyclic nucleotide 3'-phosphodiesterases). We also showed the presence of cross-reacting proteins in other fish species including zebrafish by immunoblot analysis. Using goldfish-derived probes, we have isolated a cDNA clone from zebrafish that shows a partial ORF encoding zRICH. The zebrafish represents a model genetic system that offers experimental approaches not easily achieved in the goldfish. We are currently examining the expression of zRICH mRNA after optic nerve crush by *in situ* hybridization and RNase protection assays. The presence of a close homolog in this second species is suggestive of a conserved role for RICH in optic nerve regeneration. Studying the role of zRICH in nerve regeneration in zebrafish CNS is a starting point for the genetic and developmental study of as yet unidentified proteins related to this family from other organisms.

This work was supported by a grant from the Markey Charitable trust to M.D.U. and B.W.A. and by the Ralph and Elsie Colton MHRI Frontiers of Neuroscience Fund.

405.17

IDENTIFICATION OF GENES DIFFERENTIALLY EXPRESSED AFTER OPTIC NERVE LESION IN ZEBRAFISH. P. Bormann, A. Bonnet and E. Reinhard*. Dept. Pharmacology, Biozentrum of the University of Basel, 4056 Basel, Switzerland.

Retinal ganglion cells (RGCs) in the adult zebrafish retina are easily accessible for studies of regeneration in the adult central nervous system. Following optic nerve lesion, RGCs re-express genes that were selectively silenced after neuronal development. Two well characterised genes, whose mRNA expression is strongly increased after lesion, are GAP-43 and α-tubulin. Comparison of their temporal expression pattern by in situ hybridization and RT-PCR studies revealed that both genes are induced at 24-48 hours after lesion and mRNA levels reach a maximum between 5 and 7 days after lesion. At 15 days after lesion, when abundant re-innervation of the optic tectum occurs, the GAP-43 mRNA level is drastically reduced. In contrast, α-tubulin declines more slowly and its mRNA level is still elevated 4 weeks after lesion. This indicates that a common pathway upregulates both genes, whereas distinct pathways are used in their downregulation. To learn more about the molecular requirements for regeneration, we started to identify additional genes that are regulated in response to nerve injury. To do this, we compared gene expression in regenerating and non-regenerating zebrafish retinas by differential display. We have so far isolated eleven partial cDNA clones, of which eight are upregulated and three are downregulated in response to lesion. One clone is homologous to the 60S ribosomal protein L24, another to the vesicular fusion protein NSF. All other clones, including a 2.2 kb clone isolated from a cDNA library made from regenerating retinas showed no homologies to sequences in GenEMBL. Isolation of full-length cDNA clones will allow study of the function of these potentially novel growth-associated genes during regeneration. Supported by a grant from the SNF # 31-45925.95.

405.18

ELECTROPHORETIC ANALYSIS OF AXONALLY TRANSPORTED PROTEOGLYCANS OF REGENERATING GOLDFISH OPTIC NERVE, J.S. Elam* and C.E. Hudson Program in Neuroscience, Florida State University, Tallahassee, Florida 32306.

Our previous studies on proteoglycans in nerve regeneration have focused primarily on the characteristics of chondroitin 4 sulfate (C4S) and heparan sulfate (HS) that undergo 6-7 fold enhanced axonal transport in regenerating goldfish optic system (Challacombe and Elam, Neurochem Res 20 253 (1995). We have extended these studies to the intact transported proteoglycans (PGs) that contain the HS and C4S chains. Control and 21 day regenerating optic tracts were isolated 10h after intraocular injection of $^{35}SO_4$. Following homogenization and extraction with 1% Triton-1M NaCl, proteoglycans were purified by DEAE. Results show a regeneration related increase in the proportion of extracted $^{35}SO_4$ eluting as proteoglycans (from 49% to 68%) and a corresponding increase in the amount of protein per tract in the proteoglycan fraction. Proteoglycan fractions were then subjected to electrophoresis on 6% SDS gels. Regions of the gel containing axonally transported chondroitin-4-sulfate proteoglycans (CSPGs) and heparan sulfate proteoglycans (HSPGs) were identified by treatment of proteoglycan samples with chondroitinase AC and heparitinase. Autoradiographic results from both control and regenerating tracts indicate that CSPGs are distributed in the size range of 120--260kDa. However, the greatest regeneration correlated increase was in molecules with apparent sizes of 150 and 180 kDa. Both control and regenerating tracts showed a size range of HSPGs ranging from 190-270 kDa. Labeling with ^{35}S-methionine revealed the presence of core proteins of 65kDa and 59 kDa following treatment with chondroitinase and heparitinase respectively. These results support the conclusion that there is enhanced axonal transport of proteoglycans in regenerating nerve. The present results also indicate that the proportionately large regeneration related increase in transported C4S is concentrated in particular sized CSPGs. (Supported by NIH grant NS 20502)

405.19

CNS AXON REGENERATION *IN VIVO* AND *IN VITRO* IN TRANSGENIC MICE EXPRESSING A PROTO-ONCOGENE *BCL-2*. Dong Feng Chen[1,2]*, Jean-Claude Martinou[3], Gerald E. Schneider[2] & Susumu Tonegawa[1,2]. [1]Howard Hughes Med. Institute, Center for Cancer Research., [2]Department. of Brain and Cognitive Sciences., Massachusetts Institute of Technology, Cambridge, MA 02139; [3]Glaxo Institute for Molecular Biology, 14 Chemin des Aulx, plan-les-Ouates, 1228 Geneva, Switzerland.

Most neurons of mammalian central nervous system (CNS) lose the ability to regenerate severed axons after a certain point in development. This lose is controlled by a genetic program in the neurons of origin (Chen *et al., PNAS*, 1995). We have been studying the molecular mechanisms that underlie this process. We report here that the proto-oncogene *bcl-2* is critical in this developmental switch. First, during development the level of expression of *bcl-2* in retinal ganglion cells correlates well with their ability to regenerate axons. Second, neurons from mice genetically deficient in *bcl-2* exhibit a great reduction in their ability to grow axons in embryo. Third, in mice that express transgenic *bcl-2* constitutively, the ability of CNS neurons to regenerate their severed axons remains intact throughout life as demonstrated in our retinotectal explant coculture model. Finally, extensive nerve regeneration has been achieved *in vivo* in young transgenic mice after postnatal transection of the optic tract.

The axonal regeneration observed in *bcl-2* transgenic mice does not seem to be an indirect consequence of the well known anti-apoptotic activity of *bcl-2*: Another anti-apoptotic agent -- ZVAD -- does not promote axonal regeneration; in addition, quantitative studies of ganglion cell survival and their axonal growth in our *in vivo* and *in vitro* experiments have shown a dissociation between these two properties. We conclude that *bcl-2* plays a key role in regulating the growth ability of CNS axons during neuronal development and regeneration. (Supported by NIH Grants R35-CA53874 and EY00126).

406.1

ANTINOCICEPTIVE EFFECTS OF SPINALLY TRANSPLANTED SYNTHETIC FOAM SCAFFOLDS IMPREGNATED WITH BOVINE ADRENAL CHROMAFFIN CELLS IN THE RAT Aδ/C FOOT WITHDRAWAL TEST D.C. Yeomans, T. M. Sioussat†, P. Michalewicz, Y. Lu and G. D. Pappas* Dept. of Anatomy and Cell Biology, University of Illinois at Chicago. Chicago, IL 60612; †Tissue Engineering, Inc., 451 D St., Boston, MA 02210.

The purpose of this study was to determine whether spinal transplantation of bovine chromaffin cells which had been seeded into NATPOL™ collagen foam cell scaffolds would attenuate Aδ or C fiber mediated nociception. Three different collagen foam preparations were seeded with freshly isolated chromaffin cells. After 48 hr in culture, the foams were transplanted onto the dorsal surface of the spinal cord of rats who were allowed 1 week to recover. Other animals received sham operations. After induction of light anesthesia with urethane, foot withdrawal latencies as evoked by high rate (Aδ nociceptor mediated) or low rate (C nociceptor mediated) skin heating were measured at 10 min intervals for 1 hr. Sham-operated rats demonstrated latencies similar to those measured previously for normal animals. Transplants of chromaffin cells in NATPOL™ foam scaffolds produced antinociception for both Aδ and C nociceptor-mediated responses. The antinociception varied in degree depending on the preparation of NATPOL™ used for the foam. These results contrast with results from non-scaffolded cells, with which systemic administration of nicotine is critical to their antinociceptive function. These data, taken with light and electron microscopic findings indicating better uptake of cells in foams of certain types indicate that the preparation of scaffolding foam may play a role in determining the extent to which adrenal chromaffin cell transplants can inhibit nociceptive responsivity. In addition, these results may indicate that foam scaffolding upregulates secretion of antinociceptive effectors from chromaffin cells.

406.2

OLFACTORY NEUROEPITHELIUM SPINAL CORD OVERLAYS. U. Vaidya,* R.M. Galler, B.H. Hallas, and M.R. Wells. New York College of Osteopathic Medicine, Dept. Of Neuroscience, Old Westbury, NY 11568-8000.

Olfactory neuroepithelium of young post natal rats was isolated, labeled with DiI, and overlaid onto the spinal cord of ten adult hosts (Vaidya and Wells, 1990). The sheets of neuroepithelium was dissected under high magnification, labelled with DiI for 30 mins. at 37°C, washed several times before the neuroepithelium was drawn into a needle for implantation. Using standard surgical technique and dissection, a lower thoracic laminectomy was performed and the spinal cord was exposed. The neuroepithelium was then placed under the dura onto the host spinal cord. Transplants were examined using standard histofluorescence techniques, and semi-thin plastic sections 14 and 30 days after implantation. All the transplants survived, grew and differentiated. In histological preparations, all transplants were seen to contain normal looking, well differentiated neurons, integrated with the host tissue. There was no excessive gap or scar between the grafts and host tissue surrounding them. Neither transplants nor the host spinal cord showed any pathological reaction or evidence of neoplastic growth. Olfactory neuroepithelium has the potential as a suitable donor material for neural grafting into the spinal cord.

Supported by the New York College of Osteopathic Medicine

406.3

A MET-ENKEPHALIN PRODUCING PC12 CELL LINE AS AN ALTERNATIVE DONOR SOURCE FOR NEURAL TRANSPLANTS IN THE REDUCTION OF CHRONIC PAIN. P. H. Kim, X.-T. Wang*, A. F. Seasholtz, J. Sagen and J. R. Unnerstall Dept. of Anatomy and Cell Biology, University of Illinois-Chicago, Chicago, Illinois 60612.

Many studies in our laboratory have demonstrated the efficacy of using adrenal medullary chromaffin cell transplants in the reduction of chronic pain. It is hypothesized that the production and release of catecholamines and opioid peptides (met-enkephalin) by adrenal medullary chromaffin cells seem be contribute to the mechanism of action for pain relief. Unfortunately, the ready availability and consistent preparation of these cells are some of the limiting factors in the clinical setting. The generation of a cell line that can produce both catecholamines and met-enkephalin may allow us to circumvent this obstacle of donor tissue availability and consistency. In this study, PC12 cells - a tumor cell derivative of rat adrenal medullary chromaffin cells - were transfected with a human proenkephalin gene to increase met-enkephalin production.. Although these cells produce high levels of catecholamines, they only produce low levels of met-enkephalin. PC12 cells were co-transfected with two plasmids (pCON II and pRSVneo) using the calcium-phosphate precipitation method. After transfection and selection, surviving colonies were transferred to separate plates and grown out for analysis. Radioimmunoassay revealed a four fold increase in both cellular content and release of met-enkephalin in transfected PC12 cells compared to non-transfected PC12 cells. Immunohisochemistry demonstrated the conservation of catecholamine production and morphological alterations resembling chormaffin cells following transfection. Due to the many similarities with chromaffin cells, these newly transfected PC12 cells may prove to be a viable alternative donor source for neural transplantation in chronic pain therapy. Supported by NS25054 and Campus Research Board of the University of Illinois-Chicago.

406.4

TRANSPLANTATION STRATEGIES TO REPLACE DEGENERATED SPINAL MOTONEURONS AND REINNERVATE MUSCLE S.M. Onifer*[1,3], A.B. Cannon[1], L.A. White[1], M.B. Bunge[1-3], R.P. Bunge[1-3], S.R. Whittemore[1,3,4]. [1]The Miami Project to Cure Paralysis, Departments of [2]Cell Biology & Anatomy, [3]Neurological Surgery, and [4]Physiology & Biophysics, University of Miami School of Medicine, Miami, FL 33136.

Spinal motoneuron degeneration occurs in motoneuron diseases and after spinal cord injury. Experimental evidence indicates that transplanted embryonic spinal motoneurons have the ability to replace degenerated motoneurons and reinnervate muscles. The therapeutic potential of this approach has been hindered by 1) limitations in obtaining sufficient numbers of embryonic motoneurons, 2) poor survival of transplanted motoneurons, and 3) the inability of transplanted motoneurons to extend axons out of the spinal cord via the ventral root to reinnervate muscles. It is possible to produce unlimited numbers of neurons for transplantation through the generation of non-tumorigenic neural cell lines. Schwann cells transplanted in a linear array in the CNS or PNS act as a bridge and provide a permissive substrate for axon elongation by CNS neurons, including adult motoneurons (*J. Neurosci.*, 1992, 12: 3310-3320; *J. Comp. Neurol.*, 1995, 351: 145-160). To enhance the therapeutic potential of motoneuron transplantation, the present study examines 1) various neuronal cell lines for replacing lost spinal motoneurons and 2) Schwann cells for enhancing axon elongation into the ventral root by co-transplanted embryonic spinal cord cells. The cell lines that are being investigated include: SC10, a conditionally immortalized neural cell line derived from embryonic rat spinal cord, and 46Ch1, a mutant of RN46A (*J. Neurosci.*, 1994, 14: 6744-6753; *Molec. Cell. Neurosci.*, 1996, in press) stably transfected with the secreted-ciliary neurotrophic factor gene. Preliminary data suggests that Schwann cells transplanted into the ventral white matter of the motoneuron-depleted lumbar spinal cord promote axon elongation into the L4 and L5 ventral roots by co-transplanted embryonic spinal cord cells. Supported by The Miami Project, General Reinsurance, and NS26887.

406.5

AXONAL REGROWTH INTO DISTAL HOST SPINAL CORD THROUGH A SCHWANN CELL BRIDGE IN A PARTIAL LESION MODEL IN THE ADULT RAT. S.X. Zhang, H. Li, N. Kabeer, and X. M.Xu*. Dept. Anat. & Neurobiol. Saint Louis Univ. Sch. of Med., St. Louis, MO 63104.

We previously demonstrated that a mid-thoracic spinal cord hemisection and transplantation of a small diameter semipermeable channel (O.D.=1.25 mm) filled with Schwann cells (SCs) effectively promoted axonal regeneration in adult rats (Soc. Neurosci. Abstr., 21:820,1995). To study if axons that regenerated into the graft were able to reenter the host spinal cord through the distal graft-cord interface, an anterograde tracer, PHA-L, was employed. SCs were purified in culture from adult rat sciatic nerves, suspended in a 30:70 solution of Matrigel:DMEM, and seeded into channels (1.25 x 3 mm, MWCO=50K Da, 120 x 10^6 cells/ml). After the spinal cords of adult Fischer rats were hemisected at T8 on the right, SC-channels were introduced into the gaps with both ends of the channels contacting the adjacent cord stumps. Two months later, the intact left hemicords were completely transected to prevent axons from entering the distal cords via that route and PHA-L was injected iontophoretically into the rostral gray matter 6-9 mm from the grafts. After appropriate processing, PHA-L labeled axons from the rostral propriospinal neurons were observed in the graft and, more significantly, in the distal host spinal cord. These labeled axons exhibited terminal boutons, suggesting that they innervated the appropriate gray matter. In a second set of experiments, Fast Blue, a retrograde tracer, was injected into the middle of the graft 30 days after grafting. Labeled propriospinal neurons were found in both rostral and caudal cord segments. Surprisingly, a group of supraspinal nuclei were retrogradely labeled as well. Whether supraspinal axons are able to reenter the distal spinal cord through the graft remains to be determined. (Supported by Daniel Heumann Fund for Spinal Cord Research and Leda Sears Trust Fund)

406.6

AXONAL GROWTH AFTER CO-IMPLANTATION OF CARBON FILAMENTS AND FETAL TISSUE IN THE CONTUSED RAT SPINAL CORD. L. S. Liu*, T. Khan, and S. Sayers Rehabilitation R & D Center, Hines VA Hospital, Hines, IL. 60141.

We have recently reported that the co-implantation of carbon filaments and fetal spinal cord tissue play an important role in promoting electrophysiological recovery after spinal cord injury (Neurosci. Lett. 200, 1996). This study was designed to determine whether culturing fetal spinal cord on carbon filaments, and then subsequently implanting these filaments into the injured spinal cord, would enhance axonal regrowth across the lesion site. The rats were divided into five groups: Group One consisted of normal rats (n=7), Group Two consisted of rats which sustained contusion injuries and the lesion sites were subsequently filled with a bundle of approximately 10,000 carbon filaments of 5um in diameter (AMOCO Thornel ™) cultured with 15 day old rat fetal spinal cord explants (n=5), Group Three consisted of rats which received fetal spinal cord tissue implants after contusion injury (n=4), Group Four consisted of rats which received carbon filament implants after contusion injury (n=4), and Group Five consisted of rats which sustained a contusion injury only (n=4). The implantation was performed one hour after the contusion injury. After a ten-week survival period, all animals received injections of Fast Blue in the lumbar spinal cord. The results showed more retrograde labelling across the lesion in the group which received carbon filament implants cultured with fetal spinal cord tissue as compared to the other three injury groups. Further studies are underway to determine the relation between the host and the grafted explant. Supported by Rehabilitation R&D Service (B742-RA), DVA.

406.7

SPONTANEOUS NEURAL ACTIVITIES RECORDED FROM REGENERATING AXONS IN PERIPHERAL NERVE AUTOGRAFTS INTO SPINAL DESCENDING PATHWAYS IN ADULT RATS. I. Kohama and M. Aoki*. Dept. of Physiology, Sch. of Med., Sapporo Medical Univ., Sapporo 060, Japan

We implanted autologous segments of the peroneal nerve into the upper cervical cord of adult rats to induce axonal regeneration from spinal descending pathways. The distal part of each graft was left unconnected. Five to 18 wk after implantation, we recorded the neural activities from regenerated fibers in the graft to examine if brainstem and upper cervical cord neurons regenerate their axons into the nerve graft. Spontaneous multi-unit discharges were present in all of 18 grafts examined. In 11 (61%) of these grafts, we found units with respiratory-related activity. In 7 other grafts (39%) we found units with non-respiratory related activity. Respiratory discharge patterns were similar to those of intrinsic respiratory neurons in the normal rat. After the recordings were completed, we bathed the proximal end of each graft in HRP solution for 2 h to examine the location of neurons regenrating axons. Many HRP-labeled neurons were observed in the region of medullary ventral respiratory group (VRG), especially at the obex level. These results demonstrate that: (1) some spinal descending axons from brainstem respiratory and non-respiratory neurons regenerate into the peripheral nerve grafts, (2) normal respiratory discharge patterns are maintained in respiratory neurons with regenerating axons.

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

406.8

SCHWANN CELLS GENETICALLY ENGINEERED TO PRODUCE BDNF PROMOTE AXONAL REGENERATION OF BRAINSTEM NEURONS ACROSS TRANSECTED ADULT RAT SPINAL CORD. **P. Menei, C. Montero-Menei, S.R. Whittemore, M.B. Bunge*, R.P. Bunge. The Miami Project, University of Miami School of Medicine, Miami, FL 33136**

The infusion of BDNF and NT-3 into Schwann cell (SC) grafts promotes regeneration of brainstem neurons into the grafts placed in adult rat spinal cord transected at T8 (Exp. Neurol. 134:261-272, 1995). Here, we compared normal SCs with SCs genetically engineered to produce human BDNF, grafted as trails 5 mm long in the cord distal to a transection site and also deposited in the transection site, in their ability to stimulate supraspinal axonal regeneration beyond the injury. SCs purified from adult rat sciatic nerve were infected with the replication-deficient retroviral vector (pL(hBDNF)RNL encoding the human preproBDNF cDNA. The amount of BDNF secreted (ELISA) was 23 and 5 ng/ml/10^6 cells/24h for infected and normal SCs, respectively. Activity of the secreted BDNF was confirmed by retinal ganglion cell bioassay. The spinal cord was transected at T8. The use of Hoechst prelabelled SCs demonstrated that trails were maintained for a month. In controls, no SCs were grafted. One month after grafting, axons were present in SC trails. More 5-HT-positive and some DBH-positive fibers were observed in the infected vs. normal SC trails. When Fast Blue was injected 5 mm below the transection site (at the end of the trail), an average of 57 retrogradely labelled neurons was found in the brainstem, mostly in the reticular and raphe nuclei (normal SCs, 10, mostly in vestibular nuclei). Numerous neurons were labelled in the ventral hypothalamus (normal, 0). A mean of 138 labelled cells was present in rostral dorsal root ganglia (normal, 46) and rostral spinal cord (39 vs. 32). No labelled neurons rostral to the transection were seen when SCs were not transplanted. Thus, the transplantation of SCs secreting increased amounts of BDNF led to the best regenerative response across a transection site in the thoracic cord. The regeneration we observe with infected SCs appears specific; the largest response was from neurons known to express *trk*ß. (Supported by NIH NS28059, IRME, the Heumann and Rudin Foundations, and The Miami Project.)

406.9

A MOUSE SCHWANN CELL LINE INDUCES AXON GROWTH IN ADULT RAT BRAIN. A. Soekarno* and G. Raisman. Division of Neurobiology, NIMR, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

We wanted to investigate the extent to which the IMS32 cell line (derived from spontaneously immortalized adult mouse Schwann cells; Watabe *et al.*, 1995, J. Neurosci. Res., 41, 279-290) could be used to mimic the known axogenic effects of primary Schwann cells on cut CNS axons.

Cells were extrusion grafted as vertical columns through the thalamus and hippocampus of adult rat hosts. Primary Schwann cells from neonatal rat sciatic nerve and the immortalized cells formed similar elongated vertical columns that were maintained for up to at least 4 weeks. Neurofilament staining showed that both cell types were able to recruit comparable outgrowth of host axons. Although both cell types were S100 positive, the immortalized cells were less intensely labelled for the low affinity neurotrophin receptor, p75.

Supported by ROPA #PG36/G84.

406.10

COMPENSATORY EFFECT OF EMBRYONIC RAPHE NEURONS GRAFTING ON THE REGULATION OF THE GAD_{67} ISOFORM mRNA IN TRANSECTED ADULT RAT SPINAL CORD. A. Dumoulin, M. Giménez y Ribotta, M. Anoal, A. Privat and S. Feldblum*. INSERM U336, ENSCM, 34053 Montpellier, France.

We have demonstrated in recent studies an activation of the GABAergic spinal system following rat spinal cord injury. Since the graft of embryonic raphe neurons within the transected adult rat spinal cord is known to improve functional recovery, we studied the effect of such a graft onto the regulation of the GABAergic system. In a first group of animals the spinal cord was transected at thoracic level. In a second group the cord was similarly transected and a cell suspension of embryonic (E14) caudal raphe was injected at lumbar level, one week following the transection. Detection of GAD_{65} and GAD_{67} mRNAs was assessed by non-radioactive *in situ* hybridization, 21 and 42 days after lesion. In the grafted cords numerous cells containing GAD_{65} and GAD_{67} mRNAs were observed within the graft itself, indicating the expression of the GABAergic phenotype *in vivo* in the transplanted immature tissue. In transected and host cords minor modifications in the number of cells expressing GAD_{65} mRNA were observed at either 21 or 42 days. The number of cells containing GAD_{67} mRNA appeared to be 1.6 fold increased in sectioned cords at 21 days as compared to intact animals. In contrast, the number of GAD_{67} mRNA positive cells in the grafted host cords at 21 days had returned close to the intact animals level. In conclusion, differential mRNA regulation of the two GADs is thus observed, suggesting that mainly GAD_{67} is involved in the restructuration of the adult lesioned cord. Moreover, since the graft is enriched in GABAergic cells, it may act as a GABA supplier for the host transected cord.

Supported by AFM, FRMF, IRME and Association Verticale.

406.11

CHARACTERIZATION OF HUMAN FETAL SPINAL CORD GRAFTS IN ACUTE AND CHRONIC INTRASPINAL LESIONS IN THE RAT.

M.A. Giovanini, T.A. Eskin, P.J. Reier, and D.K. Anderson*. Departments of Neurosurgery, Neuropathology, and Neuroscience, Univsity of Florida College of Medicine, Gainesville FL 32610.

Previous reports of homotopic fetal intraspinal allografts in animal models of spinal cord injury have demonstrated graft survival, integration and degrees of improved functional recovery. The aim of this study is to evaluate the growth potential and integration of human fetal spinal cord (HFSC) grafts in acute and chronic lesions of the rat spinal cord using whole pieces or suspensions of dissociated tissue. Six to 9 week HFSC tissue was collected from elective abortions. Acute thoracic lesions were created by gentle aspiration of the intramedullary portion of the spinal cord. Chronic lesions were created by contusion of the thoracic cord with the NYU weight drop device followed by grafting 10-30 days after injury. The intraspinal xenografts were analyzed 2 months after grafting using either 2µ plastic sections or paraffin immunocytochemistry. Preliminary results show excellent survival of HFSC grafts in all lesion types. Intraspinal grafts (ISG) of whole pieces into both acute and chronic lesions show poor integration with large residual areas of cavitation. Suspension grafts show excellent integration with the host and typically fill the cavity. ISG in acute lesions are largely GFAP (glial fibillary acidic protein) positive and sparsely positive for NFP (neurofilament protein) and MAP2 (microtubule associated protein). In contrast ISG in chronic lesions show large areas of MAP2 and NFP positivity. The MAP2 shows a greater affinity for immature cells and, therefore, delineates the graft by robustly labeling graft but not host neurons. In addition, MAP2 positive neurons in host white matter suggest that grafted neurons can migrate from suspension grafts into the host spinal cord. Supported by the C.M. and K.E. Overstreet Endowment for Spinal Cord Regeneration and The University of Florida Brain Institute.

407.1

ASTROCYTIC CONNEXIN43 IN RAT BRAIN AFTER FOCAL ISCHEMIA. W.E.I. Li*[1], E.L. Hertzberg[2] and J.I. Nagy[1]. [1]Dept. of Physiol., Univ. of Manitoba, Winnipeg, MB R3E 0W3 Canada; [2]Dept. of Neurosci., Albert Einstein College of Med., Bronx, NY10461.

In order to study the role of astrocytic gap junctions and Cx43 in CNS homeostasis, we utilized three anti-Cx43 antibodies (designated 18A-8, 16A-8 and 13-8300) to examine Cx43 responses in a rat cerebral focal ischemia model after different degrees of ischemic injury and after various survival times following reperfusion. After 15 min ischemia, a reduction of immunostaining was seen with 18A-8 and 16A-8 in hypothalamic preoptic areas at zero survival time, but no loss was evident at 7 days survival, suggesting a transient, reversible effect of mild ischemia on Cx43. After 1 hr ischemia with 3-48 hr survival, a near total loss of staining with 18A-8 at the ischemic center was surrounded by intense labeling, while increased labeling was seen with 16A-8 at the ischemic center. Under these conditions, a rim of intense labeling at the ischemic penumbra was seen with 13-8300. Similar results were obtained in striatum and cerebral cortex except that changes in labeling in these areas were delayed in a manner depending on ischemia severity. These results were reproduced in brain slices under in vitro conditions mimicking ischemia and this is expected to allow correlation of Cx43 detectability with the functional state of astrocytic gap junctions and with changes that occur in astrocytic intercellular communication during ischemic pathology. Supported by a grant to J.I.N. from the MRC of Canada, studentship to W.L. from the Man. Health Res. Council.

407.2

DETECTION OF DEPHOSPHORYLATED Cx43 IN BRAIN, HEART AND IN SPINAL CORD AFTER NERVE STIMULATION. J.I. Nagy*[1], W.E.I. Li[1], B.W. Doble[1,2], S. Hochman[1], E.L. Hertzberg[3] and E. Kardami[1,2]. Dept. of Physiol[1] & Anat.[2], Univ. of Manitoba, Winnipeg, R3E 0W3 Canada; Dept. of Neurosci.[3], Albert Einstein College of Med., Bronx, NY 10461.

Cells that express connexin43 (Cx43) contain various levels of phosphorylated or dephosphorylated forms of this protein. We have identified a monoclonal anti-Cx43 antibody (designated 13-8300; Zymed Laboratories Inc.) that appears to recognize specifically a dephosphorylated form of Cx43. Immuno-histochemically, it did not detect Cx43 in heart, cultured cardiac myocytes or in brain, but produced staining in brain after Cx43 dephosphorylation. By Western blotting, only a 41 kDa form of Cx43 was detected in control brain and in homogenate from neonatal cardiac myocyte cultures after alkaline phosphatase treatment, while no bands were recognized in a gap junction-enriched membrane preparation from adult hearts. In contrast to intense staining seen in dorsal horn with other anti-Cx43 antibodies, only faint staining was seen with 13-8300. After nerve cut, intense labeling appeared in lateral regions of lamina I-III. After 1 hr sciatic nerve stimulation, it gave increased punctate labeling in the dorsal horn. These results suggest that neuronal activity increases Cx43 dephosphorylation, which may alter coupling efficiency of astrocytic gap junctions.

Supported by grants to J.I.N. from the MRC of Canada and to EK from the Heart & Stroke Fundation of Canada (HSFC), studentships to W.L. from Man. Health Res. Council and to BWD from HSFC.

407.3

KINETICS OF CELL CYCLE INHIBITION IN PRIMARY ASTROCYTES AND GLIOMA CELLS. V. Li, R.C. Chou and T.J. Langan*. Depts. of Neurology and Neurosurg, Sch. of Medicne, SUNY at Buffalo, Buffalo, NY 14222.

Mammalian astrocytes remain in a state of quiescence, but maintain the capacity to leave G_0/G_1 and enter S phase of the cell cycle in response to brain injuries/diseases or due to neoplastic transformation. The mechanisms involved in this activation process remain unknown, although the sterol synthetic pathway is believed to play a role. In the present study, the time courses of cell cycling in primary astrocytic and glioma cell cultures were compared. Primary astrocytes were harvested from Sprague Dawley rat pups of 0-48 hrs old, while C6 glioma cells were purchased from ATCC. These cell cultures were allowed to grow to 30-50% confluence in DMEM + 10% bovine calf serum (BCS), followed by serum deprivation in DMEM + 0.1% BCS for 48 hrs. The cell cultures were then incubated in DMEM + 10% BCS in the presence of two cell cycle phase-specific inhibitors, hydroxyurea and the HMG CoA reductase inhibitor mevinolin, which were added to the cell cultures at different time points of incubation. Rates of DNA synthesis were evaluated by measuring ^{3}H-thymidine incorporation and total cellular protein content. Results reveal that C6 glioma cells entered S phase 3-4 h earlier than primary astrocytes after synchronization. Maximum inhibition of DNA synthesis in primary astrocytes by mevinolin occurred with exposure at 10-14 h post-synchronization. Similar inhibition by mevinolin occurred 4 h early in C6 glioma cells. The inhibitory effect of hydroxyurea on both primary astrocytes and C6 glioma cells increased with incubation time. Early withdrawal of hydroxyurea resulted in more complete recovery of cell cycling in the C6 glioma cells.
(Supported by N.I.H. grant NS-30718 to T.J.L.)

407.4

TUMOR NECROSIS FACTOR AND CELL CYCLING IN PRIMARY ASTROCYTES. L. Pleban*, R.C. Chou and T.J. Langan. Depts of Physiology and Neurology, State University of New York at Buffalo, Buffalo, NY 14214.

Astrocytes, normally in a state of quiescence in the cell cycle (arrested in phase G_0), are activated and re-enter the cell cycle during reactive gliosis that ensues in response to brain injury/disease. The proinflammatory mediator tumor necrosis factor (TNF) has also been implicated in several neurological disorders. Production of TNF by astrocytes, as well as possible feedback augmentation on astrocyte proliferation by TNF, was examined in the present study. Astrocytes were obtained from neonatal Sprague Dawley rat pups and cultured in DMEM + 10% bovine calf serum (BCS). Sychronization of astrocytes occurred through serum deprivation for 48 hours, followed by serum up-shift in DMEM + 10% BCS, allowing astrocytes to re-enter the G_1 phase of the cell cycle. Kinetic studies of astrocyte proliferation were performed by measuring ^{3}H-thymidine incorporation and total cellular protein content at different time points. TNF production by astrocytes, as well as cell proliferation upon stimulation by bacterial lipopolysaccharide (LPS) was also determined. Results of our studies demonstrate that both LPS and recombinant TNF enhance astrocyte proliferation in a concentration-dependent manner. Additionally, an increase in TNF concentration was found upon graded stimulation of astrocytes by LPS. TNF levels were undetectable in control culture supernatant. However, significant downregulation of astrocyte proliferation was found upon addition of a polyclonal anti-TNF antibody for different periods of time during the G_0/G_1 phase. These results suggest that TNF released from astrocytes has an autoregulatory effect upon their progression through the cell division cycle.
(Supported by N.I.H. grant NS-30718 to T.J.L.)

407.5

IN VITRO EFFECTS OF CELL CYCLE INHIBITORS ON PRIMARY ASTROCYTES AND GLIOMA CELLS. R.C. Chou*, V. Li, M. Wong, and T.J. Langan. Depts. of Neurology and Neurosurgery, State University of New York at Buffalo, Buffalo, NY 14222.

Astrocytes remain in a state of quiescence in the mammalian central nervous system under normal circumstances, but enter the cell cycle during many pathological conditions, such as neoplasia. The sterol synthetic pathway is believed to play a role in regulating cell cycling. In the present study, we examined the effects upon astrocytic cell cultures of two cell cycle phase-specific inhibitors, hydroxyurea and the HMG CoA reductase inhibitor mevinolin. Primary astrocytes were obtained from neonatal Sprague Dawley rats pups. These cell cultures, as well as C6 glioma cells, were grown in DMEM + 10% bovine calf serum (BCS). After synchronization in DMEM + 0.1% BCS for 48 hours, they were incubated for different periods of time in DMEM + 10% BCS in the presence of graded concentrations of hydroxyurea and mevinolin. ^{3}H-thymidine incorporation and protein content in cultures were measured. Rate of DNA synthesis at the S phase of the cell cycle is significantly higher in C6 glioma cells. Both hydroxyurea and mevinolin caused significant concentration-dependent inhibition of cell proliferation in primary astrocytes and C6 glioma cells. However, hydroxyurea and mevinolin appeared differentially synergistic in their inhibitory effects upon primary astrocytes and C6 glioma cells. Thus, not only do these results indicate the presence of different cell cycle regulatory mechanisms in the two types of cells, they also suggest possible interventions for selective targeting of neoplastic astroglia. (Supported by N.I.H. grant NS-30718 to T.J.L.)

407.6

ACTIVATED ASTROCYTES INDUCE NITRIC OXIDE SYNTHASE-2 EXPRESSION IN CEREBROVASCULAR ENDOTHELIAL CELLS. R. Shafer, S.A. Moore, I. Campbell and S. Murphy*. Depts. Pathology and Pharmacology, Univ. of Iowa, Iowa City, IA 52242, and Dept. Neuropharmacology, Scripps Research Inst., La Jolla, CA 92037.

Activation of astrocytes with cytokines promotes release of a transcriptionally-regulated factor which can induce nitric oxide synthase (NOS)-2 mRNA and protein expression in cerebrovascular endothelial cells. To define the nature of this factor, RNA from activated astrocytes was subjected to ribonuclease protection assay with a panel of probes for cytokines and, based on this screening, TNFα and IL-6 were selected. TNFα alone or in combination with IL-6 will induce NOS-2 expression in endothelium. TNFα signals through the NF-κB pathway and we found translocation of the p65 subunit into nuclei of endothelial cells exposed to activated astrocytes. In addition, astrocyte induction of NOS-2 in endothelium could be blocked by N-acetylcysteine, an inhibitor of NF-κB activation. These results suggest intercellular signalling mechanisms by which NOS-2 expression in the CNS may be amplified under pathological conditions.
Supported by NS24621.

407.7

ASTROCYTE NITRIC OXIDE SYNTHASE-2 EXPRESSION IS BLOCKED BY DIVERSE FACTORS THAT INHIBIT NF-κB BINDING TO DNA.
S.K. Park*, H.L. Lin and S. Murphy. Dept. of Pharmacology, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

We have shown that prior exposure of astrocytes to a diversity of factors blocks subsequent activation of the nitric oxide synthase (NOS)-2 gene by cytokines. The purpose of this study was to reveal whether a single or multiple mechanisms of action are involved. Exogenous NO did not block cytokine-evoked translocation of the p50 or p65 subunits of NF-κB into the nucleus. Gel shift assay revealed that the NF-κB complexes which bind to the promoter response element are composed mainly of p50 homodimers. This binding of p50 to DNA was inhibited by NO in a redox-sensitive and reversible manner. The formation of other transcription factor complexes with DNA (IRF-1, STAT 1) was little affected by NO. Gel shift assay also showed diminished p50/DNA binding in cytokine-activated astrocytes pretreated with ATP and glutamate. but cytokine activation of NF-κB (deduced from p65 translocation) was unaffected. These results suggest that diverse factors can block activation of the NOS-2 gene at a common site, namely at NF-κB response elements in the promoter. Such regulation may explain the spatial and temporal pattern of NOS-2 expression associated with particular neuropathologies such as stroke and trauma.
Supported by NS29226.

407.8

SOLUBLE TNF-RECEPTOR INHIBITS IL-12 PRODUCTION BY STIMULATED HUMAN ADULT MICROGLIAL CELLS.
B. Becher, V. Dodelet, V. Fedorowicz and J. P. Antel*, Department of Neurology and Neurosurgery, Neuroimmunology Unit, McGill University, Montreal, QC, H3A 2B4, Canada.

Il-12 is a cytokine which is detected in active lesions in multiple sclerosis (MS) and which promotes the acquisition of a Th1 cytokine profile by $CD4^+$ T cells. Autoreactive T cells recovered from the CNS of animals with experimental autoimmune encephalomyelitis (EAE), a disease model for MS, display this phenotype. We demonstrate that human CNS-derived microglia, but not astroglia can produce IL-12 *in vitro*. Under basal culture conditions, human adult microglia do not express detectable levels of IL-12, although these cells show some degree of activation as assessed by expression of immuno-regulatory surface molecules, such as HLA-DR and B7-1/2, and low levels of TNF-α mRNA. Following activation with LPS, IL-12 p40 mRNA and p70 Protein can be readily detected. IL-12 p70 production is preceded by TNF-α production and is inhibited by recombinant soluble human TNF receptor (II)-IgG1 fusion protein (shu-TNF-R). These data indicate regulation of IL-12 by an autocrine TNF-α dependent feedback loop, providing an additional mechanism whereby shu-TNF-R, now used in clinical trials in MS, may be exerting its effect. *(supported by the MS society of Canada)*

407.9

OPPOSITE EFFECTS OF ADENOSINE A3 RECEPTOR AGONISTS ON ASTROCYTES: CELL PROTECTION AT LOW AND INDUCTION OF CELL DEATH AT HIGH CONCENTRATIONS.
M.P. Abbracchio[1]*, S. Ceruti[1], R. Brambilla[1], D. Barbieri[2], C. Franceschi[2], A.M. Giammarioli[3], G. Rainaldi[3], W. Malorni[3], DKJE von Lubitz[4], K.A. Jacobson[4], F. Cattabeni[1]. [1]Inst. Pharmacol. Sci., Milan, Italy; [2]Inst Gen Pathol., 41100 Modena, Italy; [3]Ist. Sup. Sanita, 00100 Rome, Italy; [4]Mol. Recogn. Sect., Lab. Bioorg. Chem., NIDDK/NIH, Bethesda, MD 20892, USA

Acute or chronic treatment with the adenosine A3 selective agonist N^6-(3-iodobenzyl)adenosine-5'-N-methyluronamide (IB-MECA) prior to ischemia in gerbils has been shown, respectively, to worsen or ameliorate survival rate and hippocampal cell death (von Lubitz et al., Eur. J. Pharm. 263:59, 1994). To investigate the role of astroglial cells in these effects, we exposed both rat brain primary astrocytes and ADF cells (a human astroglioma cell line) to either IB-MECA or its 2-chloro-derivative (Cl-IB-MECA) (0.01 nM-60 μM). In both systems, morphological and flow cytometric analysis of propidium iodide-stained nuclei revealed apoptosis at 30-60 μM agonist concentrations. At 10-100 nM concentrations, neither compound induced apoptosis, but instead a marked reduction of the number of spontaneously detached apoptotic cells was observed. These effects were also accompanied by profound morphological changes such as increased cell protusions, increased staining of microfilament markers and modified cell adhesion patterns. A3 agonists therefore induce biphasic and concentration-dependent changes of astrocytes, resulting in cell protection at nM and in apoptosis at μM concentrations. These effects may be related to the opposing actions of IB-MECA on ischemia-associated cell death after in vivo administration.
Partially supported by Italian National Research Council Contract No. 95.00920.CT04; D.B. was a recipient of an AIRC (Associazione Italiana Ricerca Cancro) fellowship. M.P.A. and F.C. are involved in the concerted action ADEURO (European Union, BIOMED 1 Programme).

407.10

CHARACTERIZATION OF ASTROCYTES AND PROGENITOR CELLS PROLIFERATING IN RESPONSE TO EXCITOTOXIC INJURY
E.M. Welter, S.D. Collins, and D.M.D. Landis* Dept. Neurology, Case Western Reserve University, Cleveland, Ohio 44106.

We have distinguished two populations of cells that proliferate in response to a kainic acid-induced lesion in the adult rat caudatoputamen, using retrovirus-mediated transfer of the ß-galactosidase gene and BrdU labelling. *In vivo*, ß-gal-IR cells were clustered near the lesion site and adjacent to the needle tract. Very few ß-gal-IR cells were positive for GFAP or vimentin through 30 days after injury. These cells did not express microglial markers (Ox42, ED1), immature oligodendrocyte markers (O4, A2B5), or nestin, an intermediate filament protein found in neuroepithelial cells during development, or in astrocytes following injury.

In contrast, in cultures obtained from dissociated injury sites, many of the ß-gal-IR cells were positive for GFAP or A2B5/GalC, depending on the culture conditions. This pattern of differentiation is characteristic of O-2A progenitor cells.

When BrdU was injected systemically in addition to the retrovirus, we found that a large proportion of the BrdU-IR cells near the lesion were GFAP-IR or nestin-IR, but only rare ß-gal-IR cells were also BrdU-IR.

These results suggest that GFAP-IR cells and glial progenitor cells proliferate in response to injury. *In vivo*, the ß-gal-IR progenitor cells fail to express nestin and do not differentiate into GFAP-IR cells, but *in vitro* they behave as O-2A progenitor cells. These may be properties of the labelled cell population, or differentiation *in vivo* may be altered by the viral genome. The low frequency of co-labelling for BrdU and ß-galactosidase may reflect cumulative toxicity.

This study was supported by grant NS22614 from the National Institutes of Health to D.M.D. Landis.

407.11

SCHWANN CELL APOPTOSIS IN DEVELOPING PERIPHERAL NERVE AND ITS REGULATION BY NEUREGULIN
Judith B. Grinspan*, Mark A. Marchionni, Matthew Reeves, Markella Coulaloglou, and Steven S. Scherer. Children's Hospital of Philadelphia and University of Pennsylvania, Phila, PA 19104 and Cambridge Neuroscience, Cambridge, MA 02139

In the developing nervous system, the differentiation of neurons and oligodendroglia is accompanied by a large amount of programmed cell death resulting from competition for limited amounts of growth factors. We have now demonstrated that Schwann cells are also subject to programmed cell death via apoptosis modulated by Schwann cell:axon interactions. Apoptotic Schwann cells expressing the S100 antigen can be detected during the first two weeks after birth in the rat and constitute up to two percent of the Schwann cells. Axotomy increases the amount of apoptosis ten fold, but only during this post-natal period. Apoptotic Schwann cells express the low-affinity nerve growth factor receptor and *erb*B2/*neu*, but not myelin-related proteins such as periaxin, suggesting that only the pre-myelinating Schwann cells are susceptible to apoptosis. Schwann cell death following axotomy can be completely inhibited by administration of neuregulin, a growth factor which stimulates Schwann cells in vitro. Neuregulin administration also lowers the endogenous rate of apoptosis during early development. Developing Schwann cells express a neuregulin receptor composed of *erb*B2 and *erb*B3 which are phosphorylated in vivo in neonatal but not adult nerves. These data indicate that axon-Schwann cell interactions regulate the number of premyelinating Schwann cells in developing nerves, at least in part, through the actions of neuregulins.
[supported by NIH NS01793, NIH NS34528, NIH NS01565, NMSS2153-B3]

407.12

Ceramide-dependent cell death in cultured cortical, oligodendrocytes.
P.Casaccia-Bonnefil*, L. Aibel, M. V. Chao. Department of Cell Biology and Anatomy, Cornell University Medical College, NY, NY 10021.

Ceramide is a lipid second messenger implicated in the mechanism of apoptotic cell death. The effect of the cell permeable ceramide analogue C2 has been tested on primary cortical cultures of oligodendrocytes, neurons, and astrocytes. C2-ceramide, but not the inactive analogue dihydro-C2-ceramide, induced a dose-dependent cell death in primary oligodendrocytes that correlated with the activation of jun kinase. Committment of oligodendrocytes to cell death occurred within the first 6 hours of treatment. In contrast, ceramide treatment of primary astrocytic cortical cultures had no effect on cell survival and did not activate jun kinase. Furthermore, bipotential progenitors that were differentiated towards astrocytes, also became resistant to ceramide treatment as they acquired a mature astrocytic phenotype.
These experiments suggest that cell type specific factors are required for ceramide-mediated cell death in the nervous system.
Support by grants from NIH/NRSA and Shearson Lehman.

407.13

REDOX REGULATION OF C-JUN N-TERMINAL KINASE / STRESS-ACTIVATED PROTEIN KINASE IN GLIA: IMPLICATIONS FOR A CYTO-PROTECTIVE RESPONSE AGAINST OXIDATIVE STRESS, N.R. Bhat*, Neurology Dept, Med Univ SC, Charleston, SC 29425

The c-Jun N-terminal kinases(JNKs)/stress-activated protein kinases (SAPKs) are activated by pro-inflammatory cytokines and a variety of other stress inducers. This study examines the relationship between oxidative stress and JNK/SAPK activation in primary cultures of rat brain glial cells. Treatment of cultured astrocytes and oligodendrocytes with the cytokine TNFα resulted in a rapid activation of JNK/SAPK (determined by a solid-phase "kinase capture" assay) which could be blocked by oxidants, such as H_2O_2, diamide and diethyl maleate, but enhanced by the antioxidant, N-acetylcysteine (NAC). In contrast, oxidative stress induced by homocysteate (HCA), an inhibitor of cystine transport, and buthionine sulfoximine (BSO), an inhibitor of glutathione biosynthesis, resulted in a delayed but sustained activation of JNK/SAPK. These conditions also induced cell death as determined by a cell viability assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT, a chromogenic compound cleaved by active mitochondria). Co-treatment with NAC inhibited oxidative stress-induced cytotoxicity at the same time potentiating JNK/SAPK activation. Gel mobility shift assay using AP-1 consensus sequences demonstrated changes in AP-1 DNA binding activity parallel to JNK activation/inhibition. These results suggest a complex regulation of JNK/SAPK by cellular redox state and implicate JNK/SAPK activation (and possibly AP-1 mediated gene regulation) in a protective response of glial cells to oxidative damage. Supported in part by NMSS grants RG2481 and RG2474.

407.14

A novel mechanism of antibody mediated oligodendrocyte cell death. Lixin Zhou* and Robert H. Miller, Dept. of Neurosci., Case Western Reserve Univ. School of Medicine, Cleveland, OH 44106.

We have described a mouse monoclonal antibody (Mab) termed 2B10 which identifies a novel protein expressed on cell surface of oligodendrocytes and mediates myelin disruption in adult rat CNS. To investigate the effect of Mab 2B10 on oligodendrocyte development and survival, affinity purified Mab 2B10 was added to dissociated neonatal rat whole spinal cord cultures. Two days later significant cell death was observed. Immunofluorescent studies indicated that the number of O1$^+$ oligodendrocytes was reduced by more than 80%. By contrast, the number of astrocytes in these cultures were not affected. Mab 2B10 did not affect proliferation and differentiation of A2B5$^+$/O4$^+$ oligodendrocyte precursors. The specific removal of O1$^+$ oligodendrocytes was independent of complement, but required environmental signals. In immunopanned purified oligodendrocyte cultures, binding of the antibody to the oligodendrocyte cell surface did not result in cell death. Transwell co-culture and conditioned medium experiments indicated that soluble signals alone were not adequate for the antibody killing. However, Mab 2B10 induced cell death was reconstituted in cultures where purified oligodendrocytes were grown on top of astrocyte monolayer, suggesting that a contact dependent signal from astrocytes is required. Such antibody mediated oligodendrocyte cell death may in part explain the loss of oligodendrocytes and myelin in such demyelination diseases as Multiple Sclerosis. (supported by NIH grant 30800)

407.15

THE EFFECT OF A FREE RADICAL GENERATOR ON MYELINATED RAT SPINAL CORD AGGREGATE CULTURES B.L. Bartnik and R.M. Devon,* Department of Anatomy and Cell Biology, University of Saskatchewan, Saskatoon, SK. S7N-5E5 Canada

Recent in vitro experiments suggest that, compared to astrocytes, oligodendrocytes and their precursors have reduced abilities to scavenge free radicals due to lower levels of glutathione and increased iron content. The following study was performed to determine the effect of free radicals on both myelin and oligodendrocyte mophology. Myelinating E15 rat spinal cord aggregate cultures were established and used as an in vitro model of CNS myelination. Aggregate cultures were maintained for 4 weeks in DMEM/F12 supplemented with 10% FBS and 1% glucose until adequately myelinated. Aggregates were then treatred with 3-morpholinosyndnonamine (SIN-1), an $O_2^{\cdot-}$ and NO$^{\cdot}$ generator, in concentrations ranging from 1mM through to 0.01μM and sampled at 24, 48 and 96 hours. Treating the cultures with 1mM, 100μM and 10 μM for 24 hours resulted in a dramatic loss of neuropil in the aggregate accompanied by widespread non-specific cell death. By 48 hours the sampled aggregates were almost completely devoid of cellular detail and by 96 hours all cells were killed. Subsequent experiments using concentrations of 1μM, 0.1μM and 0.01μM demonstrated that the SIN-1 had little effect on aggregate structure at 24 hours. However when sampled at 48 hours the aggregates showed a significant increase in damage to both the cells and myelin. This damage was both time and concentration dependent as determined by cell viabiltiy assays using Mitotracker as a viability probe. Alterations to the myelin structure in the form of delamination and demyelination of axons were determined by electron microscopy. Stereological analysis of cells in the aggregates indicate that at 1 μM SIN-1, there was a significant decrease in the cell number and nuclear volume fraction of oligodendrocytes by 48 hours and a concomitant increase in the nuclear volume fraction of astrocytes compared to the control aggregates. Taken together, these results suggest that oligodendrocytes appear to sustain free radical damage and that astrocytes may become reactive in response to the SIN-1 treatment.

This work was supported by a Multiple Sclerosis of Canada Grant to RMD.

407.16

CHARACTERIZATION OF PHOSPHODIESTERASE I EXPRESSION IN THE CNS OF NORMAL, MUTANT AND EAE MICE. B. Fuss[1]*, H. Baba[2], T. Phan[1], P. A. Dore-Duffy[3], V.K.Tuohy[1] and W. B. Macklin[1] [1]Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA. [2]Nat. Inst. for Physiol. Sci., Okazaki, Aichi 444, Japan. [3]Wayne State Univ Sch Med, Detroit, MI 48201, USA

The cDNA clone p421.HB/phosphodiesterase I was isolated by differential and subtractive screening techniques in order to identify new proteins expressed in oligodendrocytes. Sequence analysis revealed that it represents an alternatively spliced isoform of rat brain-specific phosphodiesterase I/pyrophosphatase and a species homolog of the human cytokine autotaxin. *In situ* hybridization of rat brain sections revealed positive staining in cells of the choroid plexus and white matter tracts. During development, expression in white matter tracts peaks around postnatal day 20, whereas it continues to increase with age in the choroid plexus. Upon further analysis, we found that the longer alternatively spliced form of p421.HB/phosphodiesterase I is brain-specific, while the shorter isoform is expressed ubiquitously. In order to study possible functional roles of p421.HB/phosphodiesterase I we characterized its expression in the mouse dysmyelinating mutant shiverer and in murine EAE (experimental autoimmune encephalomyelitis). The time course of its expression in wild type oligodendrocytes and lower levels of expression at p26 in shiverer mutant brains suggest an association of p421.HB/phosphodiesterase I with molecular events involved in myelin sheath formation. In the adult, p421.HB/phosphodiesterase I expression seems restricted to cells of the choroid plexus and possibly microvessels (shown by RT-PCR of isolated microvessels). Therefore, initial experiments, in which p421.HB/phosphodiesterase I expression appears to be downregulated in EAE brains, may suggest a possible functional role in the maintenance of body fluid - brain barriers. Supported by the National Multiple Sclerosis Society.

407.17

INTERFERONβ-1b CAN COUNTERACT THE INCREASED MIGRATION RATE OF HUMAN T LYMPHOCYTES INDUCED BY CHEMOKINES. S.S. Jung*, O. Stüve, V.W. Yong. Montreal Neurological Institute, Department of Neuroimmunology, McGill University, Montreal, Canada, H3A 2B4.

Interferonβ-1b (IFNβ-1b) has been shown to be effective in decreasing the number of relapses in relapsing-remitting multiple sclerosis (MS); magnetic resonance imaging studies indicate that the number of CNS lesions is also reduced by IFNβ-1b. The mechanism of action of IFNβ-1b remains unclear. Evidence suggests that MS is a predominantly T lymphocyte-mediated disorder, and that the migration of systemic T cells into the cerebral parenchyma, towards a gradient of chemokines, constitutes a key event in its pathogenesis. Using a Boyden chamber assay, we investigated whether T lymphocyte migration towards chemokines could be influenced by IFNβ–1b. We observed an anticipated increase in the migration rate of human T cells after exposure to the β chemokines MIP-1α, MCP-1 and RANTES. Pretreatment of these cells with IFNβ-1b reduced the migration rate to control levels. The mechanism of action of IFNβ-1b is currently being investigated. Our findings suggest that a potential mechanism of IFNβ-1b in MS is the reduction in migration of T lymphocytes into the central nervous system.

Supported by Berlex Laboratories, USA.

408.1

TWO CALCIUM-DEPENDENT CHANNELS IN A PANCREATIC BETA CELL LINE. A. Kozak* and D. E. Logothetis. Dept. of Physiology and Biophysics, Mt. Sinai Sch. of Med., C.U.N.Y., New York, NY 10029

Pancreatic β cells respond to stimulation by secretagogues with electrical burst activity and an increase in cytosolic calcium followed by insulin secretion. We used a mouse β cell line (βTC-3) to study the ionic currents activated by influx of calcium under physiological conditions. We applied the perforated-patch recording technique which leaves the calcium buffering system intact. When Cs^+ was used as the internal cation, depolarizing voltage steps (holding potential=-70 mV) activated an outward current followed by a slow inward tail. Removal of external Ca^{2+} or addition of Cd^{2+} abolished this current reversibly. The current reversed at -21 mV and shifted to more positive values when $[Cl]_O$ was reduced (consistent with the predictions of the Nernst equation for a Cl^- selective channel). The current was blocked by chloride channel blockers DIDS and niflumic acid. This $I_{Cl(Ca)}$ showed anion selectivity corresponding to Eisenman I series. When the internal cation used was K^+, depolarizing voltage steps could also activate an additional outward current which was followed by an outward tail current at the holding potential. This current was also abolished by removal of external calcium or Cd^{2+} addition and reversed at -80 mV, close to K^+ equilibrium potential. The current reversal potential value was sensitive to changes in extracellular K^+ concentration and was blocked by charybdotoxin and quinine but not TEA or apamin. We concluded that this represented a calcium-dependent potassium current ($I_{K(Ca)}$). These novel calcium-dependent currents may participate in setting the plateau potentials and the interburst intervals of electrically active β cells. [Supported by NIH (HL46383) and Life and Health Insurance].

408.2

IDENTIFICATION OF CA^{2+}-DEPENDENT Cl^--CONDUCTANCE IN NEONATAL RAT PHRENIC MOTONEURONS. C.K. Su* & J.L. Feldman. Depts. of Physiol. Sci. and Neurobiol., UCLA, LA, CA 90095-1527

To understand the postinspiratory changes of membrane excitability induced by inspiratory drive, ionic conductances were measured in neonatal rat phrenic motoneurons (PMNs) following long depolarizing pulses. We focused on Ca^{2+}-dependent conductances, especially the Ca^{2+}-dependent Cl^- conductance. Experiments were performed after blockade of synaptic transmission by TTX. To eliminate the confounding effects of K^+ currents, PMNs were loaded with high Cs^+ under whole-cell patch-clamp conditions. The Cl^- equilibrium potential (E_{Cl}) was raised to about -35 mV by increasing intracellular Cl^- concentration. A two-pulse paradigm was used: a depolarizing pulse (-60 to -10 mV, 100-400 ms) was followed by a step pulse to different levels (-120 to -50 mV, 10 mV/step, 900-600 ms). With a low Ca^{2+} buffer pipette solution (0.1 mM BAPTA), a long tail inward current was elicited by the depolarizing pulse. Extrapolation of the I-V curve indicated the tail current reversed close to E_{Cl}. Such tail currents were abolished by 50 μM Cd^{2+} or high Ca^{2+} buffer pipette solution (10 mM BAPTA). Activation (at -10 mV) and inactivation (at -80 mV) of this conductance were also time-dependent, with a 50% ratio at about 90 and 140 ms, respectively. Our data suggest that a Ca^{2+}-dependent Cl^- conductance underlies the tail responses induced by inspiratory drive. This conductance may facilitate membrane repolarization toward resting levels under normal physiological conditions and may stabilize PMN membrane potential during strong inspiratory drives. Supported by NIH Grant NS24742

408.3

CHARACTERIZATION OF EXPRESSION PATTERNS OF MEMBERS OF THE ClC CHLORIDE CHANNEL FAMILY. J.L. Kugler*†, C.E. Sedwick†, and D.J. Nelson†§ Depts. of §Neurology and †Pharmacology and Physiology, University of Chicago, Chicago, IL 60637.

Chloride currents are known to play important roles in such diverse activities as synaptic transmission, volume regulation, and morphological changes in microglia. Some have been well characterized at the molecular level, particularly CFTR and the $GABA_A$ and glycine receptor-ionophores. A new family of Cl^- channels has recently been identified by sequence homology to the voltage-gated Torpedo Cl^- channel, ClC-0. One obstacle to characterization of ClC family members has been an inability to obtain reliable functional electrophysiological expression of many of these channels in either *Xenopus* oocyte or mammalian expression systems. We have used heteroplex PCR techniques in an attempt to identify the molecular basis for a nearly ubiquitously expressed calcium-activated Cl^- conductance which we believe to be a member of the ClC family. By this assay, human monocytes and macrophages express ClC-3 but not ClC-4, while J774.1 cells (a murine monocyte-derived cell line) express ClC-4, ClC-5 and ClC-7, but not ClC-3 or ClC-6. Neither human macrophages nor J774.1 cells appear to express ClC-1 or ClC-2. Partial sequencing of the human isoform of rat ClC-3 has demonstrated strong homology with the rat isoform at the amino acid level with considerable divergence at the nucleotide level. Electrophysiological studies of rat ClC-3 transiently expressed in human macrophages and murine J774.1 cells has identified a Ca^{2+}-calmodulin dependent kinase II- (CaMK-II)-activated, nonselective ion conductance which, once activated, leads to cell death by osmotic swelling. Based on these observations, we conclude that neither ClC-1, ClC-2, ClC-3 nor ClC-4 is responsible for the ubiquitous calcium-activated Cl^- conductance. This current may be carried by another previously identified ClC family member, or perhaps a novel channel.

This work supported by GM RO1 36823 and GMT3207151.

408.4

A MULTI-LEVEL HIGH CONDUCTANCE CHLORIDE CHANNEL IN PC12 CELLS. R.J. Pearce* and Z. Galdzicki. LNS, NIA, NIH, Bethesda, MD 20892.

A chloride channel which reaches the upper limits of single channel conductance values has been observed in a number of cell types, including skeletal muscle, neurons and epithelia. We have now identified a similar channel in undifferentiated PC12 cells. Using the single channel patch-clamp technique we have recorded from PC12 cells (2-3 days in culture) in both the cell-attached and the excised-patch mode. Single channel activity was observed in 40% (n=27) of cell-attached patches. For excised patches, 70-90% showed activity depending on the recording solution (n=118). In general, activity was not seen immediately on patch excision, but started within a couple of minutes. In excised patches, under conditions of symmetrical 120 mM choline chloride, 10 mM $CaCl_2$, 10 mM TEA and 10 mM 4-AP, two correlated single channel levels were most frequently elicited: the first one corresponded to a maximum conductance level of 360 ± 55 pS (n=4) and the second (a substate) corresponded to a lower conductance of 142 ± 13 pS (n=4). We also observed other levels with lower probabilities of opening indicating the existence of numerous sub-levels. Replacing the major cation with either Na (120 mM) or Cs (120 mM) had no significant effect on the reversal potential for either conductance level (n=3-5). However, replacing 120 mM of the total 154 mM chloride with acetate resulted in a significant shift in the reversal potential at both conductance levels (n=3), confirming chloride as the major charge carrier for this channel. In no instance did the reversal potential of the two main conductance level differ significantly. Single channel activity was observed in the presence of $CaCl_2$ at 10 mM, 2 mM and nominal (μM) levels. Removing calcium with EGTA did not significantly alter the reversal potential or slope conductance of either the high or low conductance. In 120 mM symmetrical choline chloride and 10 mM EGTA only 42% of patches (n=12) showed activity, comparable with the activity of cell-attached patches, but lower than the activity observed in excised patches in Ca-containing solutions. This raises the possibility that while the channel is not calcium-dependent it may be calcium modulated.

408.5

THE Cl^- CURRENT ACTIVATED BY ALPHAXALONE IN EMBRYONIC HUMAN DRG CELLS A.Y.Valeyev*[‡], J.C.Hackman[#], P.M.Wood[+] and R.A.Davidoff[#] Neurophysiology Laboratory, Veteran's Administration Medical Center[#], Department of Neurology[‡] and Miami Project[+], University of Miami, School of Medicine, Miami, Florida 33101

Whole-cell voltage clamp recordings with Cl^--filled pipettes were performed at room temperature on cells cultured from embryonic (8-10 week) human dorsal root ganglia (DRG). Neurons were grown for 5 or more days in serum-supplemented medium. In the majority of cells recorded in 1 μM TTX and 10 mM $MgCl_2$ the baseline current signal fluctuated at all potentials except 0 mV. All cells responded to micromolar concentrations of GABA, pentobarbital, or alphaxalone (3α-hydroxy-5α-pregnane-11,20-dion) but not muscimol, baclofen or CACA (cis-4-aminocrotonic acid). With equal intra- and extracellular Cl^- concentrations, the reversal potential for alphaxalone-activated current was set near 0 mV which corresponds to E_{Cl}- calculated from the Nernst equation and suggests a dominant role for Cl^- ions in the response. GABA receptor agonists and modulators were applied by pressure pulses from closely positioned pipettes. The current activated by alphaxalone was linear at negative holding potentials, but displayed strong rectification at positive holding membrane potentials. The Cl^- current activated by alphaxalone was not blocked by bicuculline, picrotoxin or TBPS. Single-channel recording show unique properties of the alphaxalone-activated current. (Supported by VAMC MRIS #1769 and 3369 and USPHS #NS 17577).

408.6

SINGLE CHANNEL RECORDINGS OF THE INWARD RECTIFIER CURRENT I_H FROM HUMAN SURAL NERVE AXONS S. Quasthoff, J. Großkreutz, M. Kühn, Ch. Alzheimer and P. Grafe*. Dept. of Neurology (S.Q., J.G., M.K.), Technical Univ. and Dept. of Physiology, Univ. of Munich, Munich, Germany.

A time-dependent inward rectification has been demonstrated in myelinated axons of mammalian peripheral nervous system (Baker, Bostock, Grafe & Martius 1987 J.Physiol. 383:45-67). The functional role of the hyperpolarization-induced current (I_H) may be to maintain membrane potential at appropriate levels during high-frequency firing. After the patch-clamp technique was applied to the myelinated axon many different ion channels have been described. However, up to now single channel recordings of the ion channel responsible for this delayed inward rectification current (I_H) are absent. We applied the patch-clamp technique to enzymatically dissociated and para- and/or internodally demyelinated axons from human sural nerve biopsies. The following solutions were used to registrate the unspecific cation current: (mM) NaCl, 70; KCl, 70; CaCl2, 1.8; MgCl2, 1; BaCl2, 1; MnCl2, 2; and Hepes-NaOH, 5; (for electrodes) and potassium aspartate, 130; NaCl, 10; CaCl2, 2; EGTA, 5; and Hepes-KOH, 10 (for bath). A hyperpolarizing current pulse protocol (0.5-1 sec. duration) from a holding potential of 0 mV was applied to the inside-out membrane patches. In the presence of 10-100 μM c-AMP in the bath solution hyperpolarization in a range of -60 to -140 mV resulted in delayed activation of up to three single channel levels. Latency from the pulse start to the first opening of the channels ranged between 50 to 100 ms. No channel inactivation was observed during long lasting current pulses. The calculated slope conductance of the I/V curve was about 0.96 pS. The channel share many common characteristics with the (I_f) channel in cardiac pacemaker sino-atrial node cells described by DiFrancessco and Mangoni (J. Physiol. 1994 474: 473-482). In summary, we describe single channel recordings of the delayed inward rectifying channel (I_H) from human sural nerve axons. Channel activity seems to be directly dependent on long lasting hyperpolarization and on the presence of c-AMP. *Supported by the W. Sander Stiftung (S.Q.,M.K.) and the DFG.*

408.7

MOLECULAR AND PHARMACOLOGICAL CHARACTERISATION OF THE HYPERPOLARISATION ACTIVATED CHLORIDE CURRENT IN DISSOCIATED RAT SCG NEURONS. Samantha Clark, Maureen Docherty and Alistair Mathie*. Department of Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill St., London NW3 2PF, UK.

We have shown that neurons isolated from the superior cervical ganglion (SCG) of 7-8 day old rats possess a hyperpolarisation-activated inwardly-rectifying chloride current $I_{Cl(SCG)}$ (Clark & Mathie, *J. Physiol.* 485 48-49P, 1995). Of the chloride channels cloned to date, the functional properties of this current have most in common with ClC-2, however, a complete functional characterisation of both $I_{Cl(SCG)}$ and ClC-2 is lacking and it is not known whether ClC-2 is expressed in SCGs.

In this study, we have used RT-PCR and Southern blotting of PCR products to show that mRNA for ClC-2 is present in dissociated rat SCGs. In contrast, rat cerebellar granule neurons, which do not possess a hyperpolarisation activated chloride current, do not appear to have mRNA for ClC-2. We have further characterised the properties of $I_{Cl(SCG)}$ by examining the effects of five organic chloride channel blockers on the current using whole-cell patch-clamp recording. At -90 mV, DIDS (1 mM) blocked $I_{Cl(SCG)}$ by 35 ± 6% (n=3), 9AC (1 mM) inhibited the current by 34 ± 7% (n=5) and NPPB (200 μM) inhibited it by 59 ± 2% (n=7). In contrast SITS (1 mM, n=7) and niflumic acid (100 μM, n=3) had little effect on $I_{Cl(SCG)}$. Interestingly, the block produced by each of the three effective inhibitors was voltage-dependent with DIDS and NPPB producing a greater inhibition at depolarised potentials while 9AC was more effective at hyperpolarised potentials.

Thus we have shown that ClC-2 is expressed in SCG neurons and we have provided a description of the action of a number of chloride channel blockers on $I_{Cl(SCG)}$ which will aid future characterisation of this current.

Supported by the MRC.

408.8

A HYPERPOLARIZATION-ACTIVATED CURRENT CARRIED BY SODIUM AND POTASSIUM (Ih) IN PITUITARY CELLS. S.M. Simasko* and S. Sankaranarayanan. Dept. of VCAPP, College of Veterinary Medicine, Washington State Univ., Pullman, WA 99164.

The ionic mechanisms that underlie spontaneous electrical activity in lactotrophs and somatotrophs have yet to be fully elucidated. In this study we have investigated the possibility that an Ih- or If-like current could participate in maintaining spontaneous electrical activity as it does in cells of the SA-node in the heart. In the clonal pituitary cell line GH_3, which secrete both prolactin and growth hormone, a slow activating, sustained inward current was observed when cells were held at -60 mV and pulsed to -120 mV (1 sec to half maximal activation; -16.4 ± 1.5 pA in physiological solutions, n=71). The current was 50% activated at -99 mV, unaffected by 10 mM tetraethylammonium, 10 mM 4-aminopyridine, or 1 mM Ba^{2+}, but completely blocked by 3 mM Cs^+. Reversal potential measurements demonstrated a 3-fold selectivity for potassium over sodium. A similar but smaller Cs^+-blocked current was found in somatotrophs identified by reverse-hemolytic plaque assay (-2.4 ± 0.4 pA in physiological solutions, n=15) but not in plaque-identified lactotrophs. Exposing spontaneously spiking GH_3 cells to 3 mM Cs^+ either had no effect or increased spike frequency. This latter result suggests that spontaneously spiking pituitary cells probably rest too far depolarized for this current to have any role in mediating spontaneous depolarizations. However, the possibility remains that this current, at least in somatotrophs, could be important in depolarizing the cell when the cell recovers from somatostatin-induced hyperpolarization.

This work supported by NSF grant No. IBN-9319536.

408.9

HYPERPOLARIZATION-ACTIVATED CURRENT (I_h) IN LAYER II CELLS OF THE MEDIAL ENTORHINAL CORTEX (MEC). C.T. Dickson* and A. Alonso. Montreal Neurological Inst. and McGill University, Montreal, Que. Canada.

Stellate cells in MEC layer II display prominent time dependent inward rectification in current clamp recordings with sharp electrodes. Using the infrared guidance technique we have now performed whole cell patch-clamp recordings of MEC layer II neurons from adult rat brain slices and characterized under voltage clamp conditions the hyperpolarization activated current (I_h) underlying inward rectification. In response to hyperpolarizing voltage-clamp steps I_h activated slowly and showed no inactivation. The activation time course of I_h was well fitted by a double exponential and the time constant of both the fast and slow current components (τ_f and τ_s) decreased with hyperpolarization. The mean values of τ_f and τ_s at -80mV were 175ms and 500ms, respectively. I_h activated over the range -55 to -120mV with half activation at -85mV. The reversal potential of I_h was estimated by extrapolation of the instantaneous (chord) conductance computed from I/V plots at different holding potentials and was, on average, -30 mV. Over the entire range of voltage potential steps, raising $[K^+]_o$ was found to increase the amplitude of the current while lowering $[K^+]_o$ or $[Na^+]_o$ was found to decrease the amplitude of I_h. Similarly to inward rectification in current clamp, I_h was blocked by perfusion with 2mM Cs^+ but not 2mM Ba^{2+}. Blockade of I_h with Cs^+ decreased the rhythmicity and lowered the frequency of the subthreshold membrane potential oscillations typical of the stellate cells. Thus, it appears that this current plays a significant but not determinant role in pacing the active subthreshold membrane voltage behavior of the stellate cells. This work was supported by the MRC, the FRSQ and an NSERC fellowship to C.T.D.

408.10

A SLOW NONSELECTIVE CATION CONDUCTANCE ACTIVATED BY NEUROTENSIN IN MIDBRAIN DOPAMINERGIC NEURONS. P.-Y. Chien*[1], R. H. Farkas[1], S. Nakajima[2] and Y. Nakajima[1]. Dept. of Anat. and Cell Biol. [1], and Dept. of Pharmacol. [2], Univ. of Illinois, College of Med., Chicago, IL 60612.

We previosly showed that in cultured dopaminergic neurons from the ventral tegmental area, neurotensin (NT) induces a conductance permeable to Na^+ and Cs^+, but not to Cl^-. We used whole-cell recording to further characterize this NT-induced nonselective cation conductance. The relative permeability of K^+ to Na^+ was calculated from the shift of reversal potential of the NT response in individual cells upon switching from Na^+- to K^+-containing external solution. The average shift of reversal potential was only -0.1±1.9 mV (n = 9). Thus, the permeability of K^+ to Na^+ (P_K / P_{Na}) was about 1. These results indicated that the current induced by NT was indeed a nonselective cation current. The latency of the NT response was long (≥ 185 msec, average 406 msec at 30 °C), suggesting that NT did not induce the conductance through a ligand-gated channel. The NT response was unaffected by loading the neurons with 1 mM cyclic GMP or cyclic AMP (each with the phosphodiesterase inhibitor IBMX, 100 μM). Loading the neurons with either the Ca^{2+} chelator BAPTA (20 mM) or with 1 mM Ca^{2+} did not affect the size of the NT response. Therefore, the NT-induced nonselective cation conductance was neither activated by cyclic nucleotides nor by internal Ca^{2+}. Supported by NSF grant IBN 9319456 and F30MH10167.

408.11

PACAP ACTIVATES A NOVEL NON-SELECTIVE CATION CHANNEL IN RAT PINEAL CELLS N. Darvish, B. H. Nguyen, E. Neale* and J. T. Russell. LCMN, NICHD, NIH.

Analysis of membrane currents using patch clamp techniques in cultured rat pineal cells revealed a novel class of non selective cation channels activated by pituitary adenylate cyclase activating polypeptide (PACAP) and its second messenger cAMP. Channel activity was observed in 30% of the patches having a unitary conductance of 150± 22pS (n=25). The open probability (P_o) measured in the on-cell and in the inside-out modes increased at depolarizing voltage commands. Bath application of PACAP (1μM) in the cell attached mode increased P_o from 0.0027±0.001 to 0.26± 0.1 (n=12) suggesting a second messenger mediated mechanism. In the resting cell, the channel was found to carry only outward current (n=25). Upon PACAP application, however, inward current was detectable through the same channel (n=10) in the patch, suggesting a PACAP induced change in channel rectification properties. In the cell attached mode, 8-Br-cAMP (2mM) activated the channel within 3-8 min (n=5) while, the Rp isomer of cAMP did not, raising the possibility of a cAMP mediated phosphorylation step for channel activation (n=4). The permeability ratio (PK^+/PNa^+) of the channel calculated from inside-out data was 3:1. Reducing calcium concentration to $<10^{-8}$ M had no effect on channel P_o (n=6) and CsCl (10mM) applied to the inner mouth of the channel blocked all channel activity. Under the current clamp condition, PACAP application resulted in depolarization of resting membrane potential by 12±2mV which is in agreement with activation of the non selective cation channels.

408.12

CONCENTRATION DEPENDENCE OF CATION PERMEATION THROUGH CYCLIC AMP-GATED CHANNELS IN MAMMALIAN OLFACTORY RECEPTOR NEURONS

P. H. Barry, S. Balasubramanian, J. W. Lynch[†] and A. M. Cunningham[†]*, School of Physiology and Pharmacology, University of New South Wales, Sydney 2052 and [†]Neurobiology Division, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia.

We have already investigated the permeation and selectivity properties of the adenosine 3', 5'-cyclic monophosphate (cAMP)-gated channel in excised inside-out patches from the dendritic knobs of dissociated adult rat olfactory neurons and shown (1) that these cyclic nucleotide-gated (CNG) channels are permeable to large organic cations, having minimum pore dimensions of about 6.5 x 6.5 Å, and (2) that they do not exhibit anomalous mole fraction effects. We have now investigated the concentration dependence of the current-voltage relations of these channels. In symmetrical NaCl solutions without added divalent ions, the current-voltage curve was linear, reversing close to 0 mV. Varying internal [NaCl], from 25 to 300 mmol/l NaCl (osmotically balanced with sucrose) with external [NaCl] kept constant at 150 mmol/l, the reversal potential closely followed the Na^+ equilibrium potential, implying that $P_{Cl}/P_{Na} \cong 0$. Na currents through the channels did not obey the independence principle, with inward currents being much more strongly dependent on internal [Na] at negative potentials than would be predicted by equations such as the Goldman-Hodgkin-Katz equation. The patch currents exhibited saturation kinetics as internal [Na] increased, with an apparent dissociation constant of about 125 ± 1 mmol/l, at both positive and negative potentials. These results suggest multiple binding sites within the channel, presumably with sites located close to each end of the channel.

Supported by the Australian Research Council.

408.13

SODIUM-ACTIVATED CATION CHANNEL FROM LOBSTER OLFACTORY RECEPTOR CELLS: GATING AND CONDUCTION PROPERTIES. A. B. Zhainazarov and B. W. Ache*. Whitney Lab. and Depts. Zoology & Neurosci., Univ. of Florida, St. Augustine, FL 32086.

We further characterized a novel Na^+-activated channel in excised patches from lobster olfactory receptor cells (J. Neurophys. 73: 1774, 1995). The open probability vs [Na^+] relationship could be fit by the Hill equation with a coefficient of 3.1. At 210 mM (saturating) Na^+, the open probability was 0.5±0.1(S.D.) (n=5; -60 mV). At 30 mM Na^+, channel openings were infrequent and bursty. Increasing [Na^+] increased the frequency of transitions until at 180 mM Na^+ one observed long, continuous flickering transitions. At 30 mM Na^+, the closed time distribution could be fit by the sum of four exponential components with mean time constants of 0.2±0.1, 7.8±2.0, 80±10, and 896±23 ms. At 210 mM Na^+, the closed time distribution could still be fit by a four exponential function, but the longer tree components shortened considerably. The open time distributions could be fit by a single-exponential function [4.6±0.1 (S.E.M.) ms, 30 mM Na^+] that was not affected by [Na^+]. Permeability ratios calculated from the reversal potentials for monovalent and divalent cations in biionic conditions were Li(1.1)>Na(1.0)>K(0.5)>Rb (0.4)>Cs(0.2) and Ca(35)>Mg(30)>Mn(13)>Ba(11)>Na(1). The channel could be localized this channel to the outer dendrites, the site of odor transduction in these cells, but its role in transduction, if any, remains to be determined. Supported by the NIDCD (DC 01655).

408.14

EXPRESSION OF MTrpb-1 A MAMMALIAN *Trp* HOMOLOGUE IN NEURONAL AND NON-NEURONAL CELLS. S. DasGupta*, F. Qian, R.J. Miller and L.H. Philipson†. Depts. of Pharmacol. and Physiol. Sci. and Medicine†, University of Chicago, Chicago, IL 60637.

In many cells, production of IP_3 releases intracellular calcium, resulting in a biphasic calcium signal; the first from a transient rise in the cytosolic calcium concentration, followed by calcium influx across the plasma membrane. Proteins homologous to the *Drosophila* photoreceptor transient receptor potential (*trp*), have been suggested to be involved in voltage independent calcium entry while *trp1*-related proteins may encode an IP_3-activated nonselective cation current. We have recently cloned mtrpb-1, a mammalian *trp* and *trp1* homologue from mouse BTC3 cells and have now employed a reverse-transcription polymerase chain reaction method to determine the presence of mtrpb-1 mRNA in a variety of tissues, cells and cells lines. Using oligonucleotide primers flanking regions encoding the sixth and eighth transmembrane domains in the mtrpb-1 cDNA, we found mtrpb-1 to be expressed in the mouse and rat brain, mouse pituitary tumor AtT20 cells, insulin secreting BTC3 and NIT cells. While present in astrocytes and CG-4 glial precursor cells but absent in a oligodendrocyte derived cell line, mtrpb-1 message was not detected in PC12, F11 and IMR32 cell lines. Primary cultures of rat cerebellar neurons showed barely detectable levels of mtrpb-1. In conclusion, we find mtrpb-1 mRNA to be present in excitable as well as non-excitable cells. Detailed knowledge of expression of the family of mammalian *trp* and *trp1* related genes will aid in understanding the role of these gene products in regulating intracellular calcium-associated signalling pathways.

Supported in part by NIH DA-02575, DA-02121, MH-40165, NS 33502 (RJM), DK-48494, DK44840 and the Marilyn M. Simpson Charitable Trust (LHP).

408.15

FUNCTIONAL COUPLING OF CAPACITATIVE CALCIUM ENTRY AND SECRETION IN PC12 CELLS. S. Koizumi* and K. Inoue. Div. Pharmacol., Natl. Inst. Health Sci., Kamiyoga, Setagaya, Tokyo 158, Japan

The caffeine-evoked rise in intracellular Ca^{2+} concentration ([Ca]i) and release of dopamine in rat pheochromocytoma PC12 cells were investigated. Caffeine produced a transient rise in [Ca]i followed by a sustained elevation in [Ca]i in the cells. When extracellular Ca^{2+} was removed, the sustained [Ca]i rise was disappeared whereas the transient one was unaffected, showing that the sustained [Ca]i rise results from Ca^{2+} influx from extracellular spaces. Pretreatment of cells with cyclopiazonic acid, an inhibitor of Ca^{2+}-ATPase of endoplasmic reticulum, abolished the Ca^{2+} responses to caffeine, which suggests that both of the transient and the sustained [Ca]i rises depend on intracellular Ca^{2+} stores. Caffeine evoked the release of dopamine in the cells, which was totally dependent on extracellular Ca^{2+}. The effects of Zn^{2+}, Cd^{2+}, La^{3+} and nicardipine on the caffeine-evoked responses were examined. Both the caffeine-evoked dopamine release and the sustained rise in [Ca]i were inhibited by Zn^{2+} (30 μM) but not by Cd^{2+} (100 μM), La^{3+} (100 μM) or nicardipine (30 μM). The transient rise in [Ca]i induced by caffeine was not affected by Zn^{2+}. Taken together, we demonstrated that caffeine stimulates not only Ca^{2+} mobilization but also Ca^{2+} influx via a pathway which is highly sensitive to Zn^{2+} and that the sustained Ca^{2+} influx is essential for the release of dopamine from PC12 cells. It is strongly suggested that the sustained Ca^{2+} influx evoked by caffeine appears to be so-called "capacitative Ca^{2+} entry" and that one of the physiological significance of the Ca^{2+} entry is secretion in the cells.

408.16

THE LARGEST MAMMALIAN GAP JUNCTION CHANNEL (FORMED OF RAT CX37 IN TRANSFECTED N2A CELLS) EXHIBITS SATURATION AND CATIONIC SELECTIVITY. M. Tao, M. Waltzmann and D.C. Spray*, Dept. Neuroscience, Albert Einstein Coll. Med., Bronx, NY.

We stably transfected N2A neuroblastoma cells with a vector containing cDNA encoding rat Cx37, the gap junction channels formed displayed the highest unitary conductances of the gap junction channels that have been characterized (as high as 300 pS). Sensitivity of steady state macroscopic conductances to transjunctional voltage (V_j) was strong (V_0 ± 29 mV, g_{min}/g_{max} ~ 0.18 and n, equivalent gating charge about 2.25). In addition, instantaneous junctional conductance declined as command pulses exceeded ± 20 mV. Recordings of unitary junctional currents showed a mainstate and substate conductance. Mainstate conductance declined with voltage, apparently accounting for the changes in instantaneous macroscopic conductance; mainstate open time decreased with increasing V_j, apparently accounting for the behavior of steady state macroscopic conductance. When ionic strength was reduced by isosmotic substitution of CsCl with mannitol, channels saturated at about 50 mM salt. To quantitate selectivity, unitary conductances and macroscopic voltage sensitivity were assessed in symmetric 150 mM solutions of CsCl, Choline (Ch)Cl, tetramethylammonium (TMA)Cl and tetraethylammonium (TEA) Cl and under asymmetric conditions, where one electrode contained CsCl and the other ChCl, TMACl or TEACl. Under symmetric conditions, relative permeabilities for the cations were Cs:Ch:TMA:TEA:1:0.4:0.2:0.1 and permeabilities of Cl^- was estimated to be no more than 10% that of Cs. In asymmetric solutions, reversal potentials revealed relative permeability ratios of 0.5:0.5: 0.3 for Ch:TMA:TEA vs Cs. We conclude that Cx37 gap junction channels saturate at modest V_j and at physiological salt concentrations, and that Cx37 is a remarkably cation-selective gap junction channel that passes even large cations rather well.

OTHER ION CHANNELS II

409.1

THE CAPSAICIN RECEPTOR - TARGET FOR A NOVEL ACTION OF ASPIRIN-LIKE DRUGS? Kress M.*, Vyklicky L.§ and Reeh P.W., Dept. Physiol. & Exp. Pathophysiol., Universitätsstr. 17, D-91054 Erlangen, Germany; § Academy of Science, Dept. Physiol., Prague, Czech Rep.

Capsaicin (CAPS) has long been known as an algogenic substance selectively exciting and sensitizing nociceptors. As a possible mechanism of action, an excitatory non-selective cation current has been identified in a subpopulation of adult dorsal root ganglion (DRG) neurons in culture. Aspirin, on the other hand is an analgesic acting peripherally by inhibition of prostaglandin synthesis, as generally assumed.

In the present study we tested if aspirin-like drugs could counteract the excitatory effect of CAPS by performing whole cell patch-clamp recordings from acutely dissociated rat DRG neurons using a 12 channel computer controlled system for fast drug application. At -80 mV holding potential, CAPS (3 μM) induced an inward current in 80 % of small DRG neurones that showed little inactivation within 40 s. The current was inhibited by acetylsalicylic acid, salicylic acid (SA) and diclofenac in concentrations below those needed for inhibition of prostaglandin formation. Half-maximal inhibition of the current by SA could be overcome by increasing the CAPS concentration to 6 μM which may suggest competition of the drug at the binding site of the putative CAPS receptor-channel complex. The inhibition was also present at +40 mV holding potential though higher drug concentrations were needed.

The present results demonstrate an inhibition of the capsaicin-induced current by aspirin-like drugs in adult rat DRG neurons. Since the sensitivity to CAPS is specific for nociceptors this inhibitory action may represent a new and potentially important mechanism of the analgesic action of aspirin-like drugs though an endogenous ligand of the CAPS receptor has not been identified so far.

(Supported by the DFG, SFB 353/B12 and the European Union)

409.2

CHARACTERIZATION OF ION CHANNELS PRODUCED BY BOTULINUM TOXINS IN PC12 CELLS. R. E. Sheridan.* Neurotoxicology Branch, USAMRICD, Aberdeen Proving Ground, MD 21010-5425.

Botulinum neurotoxin (BoNT) is known to produce large conductance cationic channels in artificial bilayers. The purpose of this study was to examine these ion channels in membrane patches excised from transformed PC12 cells and to compare the properties of these ion channels produced by different serotypes of botulinum toxin. PC12 cells were grown in RPMI-1640 media supplemented with 10% horse serum, 5% fetal bovine serum and 50–100 ng/ml of nerve growth factor (NGF, 2.5S) on a polylysine coated glass substrate. After a minimum of 4 days, the cells were transferred to a bath containing either 200 mM CsCl or 200 mM RbCl, 5 mM Na•HEPES (pH 7.0) and 1 mM dithiothreitol (DTT) at 21°C. Patch pipettes were prepared with a matching salt solution but buffered to pH 5.0 with 5 mM Na•acetate and containing 2–10 μg/ml BoNT serotypes A or E. Patches of membrane were excised in the inside–out configuration and membrane currents were monitored over time. After a delay of 5–20 minutes, large conductance ion channel openings were observed. Unlike single channel currents endogenous to the membrane, which were predominantly K^+ channels, the toxin–induced channels were most active with a positive potential applied to the same side as the toxin (cis) and showed an increased mean open time and a decreased closed time. These ion channels started as a single conductance level but typically increased in equal steps up to 5 times the original channel conductance, with full openings and closings of even the largest channels observed as common events. There were no significant differences between BoNT/A and BoNT/E in ionic conductance or the voltage-dependence of channel gating. *Supported by the DoD.*

409.3

PROPERTIES OF A NOVEL SYNAPTIC CAN CURRENT ACTIVATED BY mGluRs IN CA1 HIPPOCAMPAL NEURONS

P. Congar, V. Crépel, A. Böhme * and Y. Ben-Ari - INSERM U29, 123 Bld de Port-Royal, 75674 Paris cedex 14 (France).

The currents evoked in CA1 pyramidal neurons by the selective metabotropic glutamate receptor agonist 1S,3R-ACPD were studied in whole-cell patch-clamp configuration (Cs-gluconate 125 mM) in the presence of TTX (1μM), kynurenate (1 mM) and bicuculline (10 μM) and K^+ channel blockers (6 mM Cs^+, 5 mM 4-AP and 10 mM TEA). As previously shown, bath application of 1S,3R-ACPD (100-200 μM, 2-3 min) induced a Ca^{2+}-activated nonspecific cationic current (CAN) sensible to external divalent cations (Crépel et al., *J. Neurophysiol.* 72: 1561-69, 1994; Congar et al. Soc. Neurosci. Abstr., 32: 10, 1995). In the present work, we have investigated the role of G protèins in the induction of CAN current, its pharmacological feafures and its synaptic expression.

We now report that: (i) CAN current is mediated exclusively through a G protein-dependent process; it becomes irreversible in the presence of intracellular GTPγS and it is blocked by GDPβS. (ii) It is activated by selective agonists of at least two types of metabotropic receptors linked to IP3 pathway: the group I mGluRs and the muscarinic receptors. (iii) It is not activated through cAMP pathway, since it is neither antagonized by forskoline (50 μM), nor evoked by selective agonists of group II and group III mGluRs (linked to cAMP pathway). (iv) CAN current can be synaptically evoked by high-frequency stimulation in the lacunosum-moleculare. Furthermore, this synaptic CAN current is considerably increased in duration and amplitude in presence of intracellular GTPγS. The mGluR CAN current may play an important role in the long lasting synaptic modulations of cell excitability.

409.4

"CHANNEL NOISE" MAY INFLUENCE THE FIRING PROPERTIES OF NEURONS IN THE MEDIAL ENTORHINAL CORTEX

J.A. White[*1], R. Klink[2], A. Alonso[2], and A.R. Kay[3], [1]Dept. of Biomed. Engr., Boston Univ., Boston, MA 02215; [2]Montreal Neurol. Inst., Montreal, PQ H3A 2B4 CANADA; [3]Dept. of Biol. Sci., Univ. of Iowa, Iowa City, IA 52242.

The theta rhythm, generated by partially synchronous neuronal activity at frequencies of 4-12 Hz in the hippocampus and associated areas, plays an important role in spatial learning. Neurons of layer 2 of medial entorhinal cortex, which deliver neocortical input to the hippocampus, exhibit slow subthreshold oscillations under current clamp. These intrinsic oscillations, driven by a persistent Na^+ current and a slow outward current, may help generate the theta rhythm.

The number of persistent Na^+ channels underlying this phenomenon is relatively small ($< 10^4$). Here, we examined the effects of this finite number of channels using stochastic simulation techniques. The probabilistic nature of the Na^+ channels can account for the noise in current-clamp records; this added noise makes oscillatory behavior less sensitive to changes in parameter values than in deterministic models. Preliminary results indicate that stochastic simulations may replicate higher order interspike interval statistics seen *in vitro*. Channel noise may shape cellular responses *in vivo* as well: the stochastic system has enhanced sensitivity to small periodic stimuli, in a form of stochastic resonance, and diminished sensitivity to large periodic stimuli.

Supported by the Whitaker Foundation (JAW), the MRC (AA), and NIH (ARK)

409.5

FAST AND SLOW ADPs IN CORTICAL NEURONS AND THEIR REGULATION BY MUSCARINIC RECEPTORS. S. Haj-Dahmane* and R. Andrade Dept. of Psychiatry and Behavioral Neurosciences, Wayne State Univ. School of Med. Detroit, MI 48201.

Aftercurrents play an important role in controlling neuronal firing pattern. We have previously shown that muscarinic stimulation in rat prefrontal cortex elicits a slow afterdepolarization (sADP) mediated by a calcium activated cation nonselective current. In the present study, we describe the presence of an additional fast afterdepolarising potential (fADP) which is also modulated by muscarinic stimulation.

Whole cell recordings are obtained from rat prefrontal cortex layer V pyramidal neurons *in vitro*. In cesium loaded cells a calcium spike in the presence of TTX (1 μM) induces a fast inward aftercurrent which decays completely in about 200 ms in neurons clamped at their resting membrane potential (~ -70 mV). Blocking the calcium influx using cadmium or buffering the intracellular calcium with 10 mM BAPTA strongly reduces the amplitude of the fast inward aftercurrent. Ion substitution experiments reveal that the fast inward aftercurrent is dependent on the extracellular concentration of sodium.This indicates that fADP is also mediated by a calcium activated cation nonselective current. Bath administration of carbachol (30 μM) enhances the fast component and activates a much slower inward aftercurrent which decays incompletely over a period of several seconds.

These results indicate that pyramidal neurons of rat prefrontal cortex have a constitutively fast depolarizing afterpotential. Stimulation of muscarinic receptors enhance this fADP and results in the appearance of a sADP.

This work was sponsored by MH 49355

409.6

INTERACTIVE MODELLING OF HIPPOCAMPAL NEURONS

R.C. Cannon, R.J. Holland, C.Bernard[1] and H.V. Wheal.*
Neuroscience Research Group, University of Southampton, SO16 7PX, UK.
[1] *INSERM U29, Hôpital de Port Royal, Paris, France.*

Interactions between excitatory and inhibitory synaptic circuitry in the CA1 region of the hippocampus play a crucial role in the control of cell excitability and consequently in the generation of epileptiform activity.

In order to explore this behaviour we have constructed multicompartmental models of CA1 pyramidal cells and inhibitory interneurons. These models follow a modified Hodgkin-Huxley formalism and have been derived from our own experimental data by an incremental fitting algorithm, constrained by published channel kinetics and synaptic properties.

Our connectivity database (Bernard and Wheal, 1994, Hippocampus 4, 497-529) is being extended to include detailed cellular morphology and physiology in order to build realistic local networks.

We have developed a graphical interface in java for interactive examination of the biophysical behaviour of the single cell models. It may be used across the web at *http://www.neuro.soton.ac.uk/ca1_neurons.html.*

Funded by the EC, the Wellcome Trust, the British Council and Southampton University.

ACETYLCHOLINE: STRUCTURE/FUNCTION I

410.1

HUMAN CHOLINE ACETYLTRANSFERASE mRNAS WITH DIFFERENT 5'-REGION PRODUCE A 69-kDa MAJOR TRANSLATION PRODUCT. H. Misawa*, J. Matsuura, Y. Oda, R. Takahashi and T. Deguchi. Dept. of Neurol., Tokyo Metropolitan Inst. for Neurosci., Tokyo 183, Japan. *Dept of Pathol., Kanazawa Univ., Sch. of Med., Ishikawa 920, Japan.

Previously unknown types of human choline acetyltransferase (ChAT) cDNAs (N1- and N2-types) were cloned and their translation products were analyzed. In four species of ChAT mRNAs (R-, N1-, N2- and M-types), the ATG initiation codon in the rat, mouse and pig was replaced by ACG, which does not serve as an initiation codon for translation. *In vitro* translation and mammalian expression analyses revealed that N1-, N2- and R-type mRNAs give rise to a single 69 kDa enzyme, while M-type mRNA produces both 82 and 69 kDa enzymes. The translation efficiency of M-type mRNA was lower than that of the other mRNA species. Moreover the translation efficiency of human ChAT mRNAs was considerably lower than that of rat ChAT mRNA, suggesting that the ATG codons for human ChAT mRNAs are unfavorable for translation initiation. These results provide rational explanations for the previous reports that human ChAT protein purified from the brain and placenta had 66-70 kDa molecular mass, and that ChAT activity in a single motor neuron of human was far lower than that of other vertebrates. Furthermore sequencing of monkey ChAT gene showed that the initiation ATG in rodent ChAT was also replaced by ACA in the monkey. (Supported by a research grant from Foundation Advanced Technology Institute)

410.2

ANTISENSE OLIGODEOXYNUCLEOTIDE TO THE MUSCARINIC M_2 RECEPTOR SUPPORTS ITS ROLE AS A NEGATIVE AUTORECEPTOR REGULATING ACETYLCHOLINE RELEASE. K. Kitaichi, A. I. Hersi, J. W. Richard, T. West*, L. K. Srivastava, R. Quirion. Douglas Hospital Research Centre, Dept. of Psychiatry, Faculty of Medicine, McGill University, Verdun, Quebec, Canada H4H 1R3.

At least five (m_1 to m_5) distinct muscarinic receptors have been cloned thus far. However, it is difficult to ascertain their respective physiological roles due to the lack of highly selective agonists and antagonists for a given subtype. For example, the pharmacologically defined muscarinic M_2 class consists of two subtypes (m_2 and m_4 receptors), both having high affinity for the AF-DX series of antagonists. Recently, much interest has focused on the M_2-like receptor family on the basis of its possible role as negative autoreceptor modulating acetylcholine (ACh) release in the brain. However, it is unclear if these autoreceptors belong to cloned m_2 or m_4 (or both) subtype(s). To address this issue, we utilized a combined antisense oligodeoxynucleotide-*in vivo* dialysis approach. Phosphorothioate-modified antisense oligodeoxynucleotides targeted to subtype specific sequences at the 5' region were continuously infused (1 μg/μL/hr for 3 days) into the third ventricle of adult male Sprague-Dawley rats. These animals were then used in *in vivo* dialysis to monitor ACh release as recently described (Hersi et al., J. Neurosci. 15: 7150, 1995). The m_2 antisense treatment reduced AF-DX 384 (0.1 μM)-induced hippocampal ACh release whereas a random sequence oligodeoxynucleotide did not. It thus appears that the molecularly defined m_2 receptors are involved in the regulation of ACh release in the rat hippocampus. This work was supported by the MRCC.

410.3

A ROLE FOR DOPAMINE D5 RECEPTORS IN THE MODULATION OF HIPPOCAMPAL ACETYLCHOLINE RELEASE REVEALED BY ANTISENSE OLIGONUCLEOTIDES A. I. Hersi, L. K. Srivastava, P. Gaudreau[2] and R. Quirion*. Depts. of Neurology/Neurosurgery and Psychiatry McGill University, Montreal, Quebec, Canada and Douglas Hospital Research Center, Verdun, Quebec, Canada H4H 1R3. [2]Neuroendocrinology Lab., Hopital Notre Dame, Université de Montreal, Montreal, Quebec, Canada.

Lately, D1-like (D1 and D5) receptors and their possible involvement in cognition have generated considerable interest. However, due to the lack of selective ligands, it has not been possible to ascertain which of these receptors is(are) involved in these processes. We and others have recently shown that D1-like receptors modulate hippocampal acetylcholine (ACh) release (Day and Fibiger, 1994; Hersi et al., 1995; Imperato et al., 1993). In the present study, the receptor subtype(s) responsible for this effect was investigated using a combined antisense in vivo dialysis approach. Phosphorothioate modified antisense oligodeoxynucleotides, targeted against specific regions of the D1 or D5 receptors were continuously infused into the third ventricle of Sprague Dawley rats (250-350g) for three days at a dose of 1ug/hr. Both antisense oligos reduced hippocampal-formation D1-like/[3H] SCH 23390 binding sites to similar extents. However, only the antisense directed against the D5 receptor was able to block the increase in hippocampal ACh induced by the D1-like receptor agonist, SKF 38393 (100μM). Administration of a random-sequence anti-D5 oligonucleotide failed to either decrease D1-like receptors or block the SKF 38393 induced increase in ACh release. Taken together, these findings suggest that the receptor subtype involved in the dopaminergic regulation of hippocampal ACh release is of the D5 subtype. To our knowledge, this is the first evidence of a physiological function for the D5 receptor in the mammalian brain. Supported by the MRCC and FRSQ.

410.4

HISTOCHEMICAL CHARACTERIZATION OF MATURE AND AGED RATS FOLLOWING INTRASEPTAL INJECTION OF 192-IgG-SAPORIN. G.R. Stevens[1]*, W.L. Klein[1], P. Curzon[2], M.W. Decker[2] and A.W. Bannon[2]. [1]Northwestern Univ., Dept. Neurobiol. and Physiol., Evanston, IL 60208; [2]Abbott Laboratories, Neuroscience Research, D-47W, AP-9A, Abbott Park, IL 60064-3500

192-IgG-saporin is an immunotoxin that selectively targets basal forebrain cholinergic neurons that express nerve growth factor receptors (NGFr). Previously, we found subtle behavioral effects in aged, but not mature Long-Evans rats following intraseptal injection of 192-IgG-saporin into the medial septum (Brain Res., in press). However, depletion of choline acetyltransferase (ChAT) activity in hippocampus and cingulate cortex was the same in both mature and aged rats. In this study, parvalbumin, ChAT, NGFr and alpha bungarotoxin (αBgT) staining were examined in mature and aged rats following intraseptal injection of 192-IgG-saporin. Consistent with other reports, 192-IgG-saporin treatment appears reduce NGFr and ChAT staining while sparing parvalbumin staining. Interestingly, loss of αBgT staining coincided with loss of NGFr and ChAT staining in the medial septum. These early data support the specificity of 192-IgG-saporin as a selective cholinergic toxin, and suggest the existance of αBgT binding sites on basal forebrain cholinergic neurons in the medial septum. (supported by Abbott Laboratories)

410.5

EFFECTS OF REPEATED IONTOPHORETIC ADMINISTRATION OF ACETYLCHOLINE ON THE RESPONSIVENESS OF RAT SOMATOSENSORY CORTICAL NEURONS BEFORE AND AFTER SYSTEMIC DFP TREATMENT. **A.A. Myasnikov*, G. Testylier, M. Maalouf, A.E. Butt & R.W. Dykes. Département de Physiologie, Université de Montréal, Québec, Canada H3C 3J7.**

We describe the evolution of the responsiveness of single units in the awake (24 cells) or urethane-anesthetized (37 cells) rat somatosensory cortex subjected to repeated (20-200 pulses) iontophoretic administration of acetylcholine (ACh; 1.0 s duration, 80 nA pulses), before and after systemic treatment with the irreversible acetylcholinesterase (AChE) inhibitor diisopropylfluorophosphate (DFP; 0.3-0.5 LD_{50}). The responses to ACh varied among cortical neurons and fluctuated across time. There were monophasic excitatory (E) or inhibitory (I) responses to ACh in some cells (n = 20), and biphasic (E-I, I-E, E-E, and I-I) or triphasic (E-E-I, I-I-E, and I-E-I) responses in others (n = 41). Although the direction and complexity of these responses remained consistent, the magnitude of the response to ACh fluctuated across time; most cells exhibited either an initial increase or decrease in responsiveness followed by fluctuations that diminished across time, such that the response magnitude eventually returned to initial levels. DFP treatment given 1 hr after beginning ACh administration resulted in a re-emergence of the increase or decrease in responsiveness followed by fluctuations in the response similar to those seen during the first part of the experiment. Results suggest the existence of dynamic, homeostatic mechanisms controlling the balance between excitatory and inhibitory influences within the cortical circuitry; these mechanisms are engaged by the prolonged increase in extracellular ACh levels caused either by repeated iontophoretic ACh administration or by AChE inhibition by DFP. The ability of the cholinergic system to rapidly adjust to changes in tonic levels of extracellular ACh raises questions about the potential efficacy of therapeutic treatments in Alzheimer's disease designed to increase ACh activity. (Supported by the Medical Research Council of Canada).

410.6

NEURONAL AND GLIAL S-100 IMMUNOREACTIVE CELLS IN THE AVIAN BRAIN.

E.Garcia-Ojeda, A. Garzino, G.C.Panzica*. °Dept. Anatomy, Pharmacology, & Forensic Medicine, Univ. Torino, I- 10126 Torino, Italy.

S-100 is a calcium-binding dimeric protein that has been classically regarded as a specific marker of central and peripheral glial cells. Recent studies had, however, demonstrated a wide population of S-100 positive neurons in the rat brain, as well as in some nuclei of other vertebrate species. In the present study we analyzed by immunocytochemistry the distribution of S-100 immunoreactivity (IR) in a well-studied avian species, the Japanese quail. Two different antisera raised against the whole molecule (polyclonal) or against its ß-subunit (ßS-100, monoclonal) were used. Both glial and neuronal cells were recognized by both antibodies, and the staining was more pronounced in the brainstem than in the forebrain. Immunostained glial elements were radial glia and tanycytes (stained with the ßS-100 only), or astrocytes (stained with both sera). A wide population of neurons, identified by morphological criteria, was stained with both antibodies. Their degree of staining varied from weak to strong according to their location. In the prosencephalon, scattered S-100 positive neurons were observed in the striatal complex, in the nucleus paraventricularis thalami, nucleus rotundus, and nucleus geniculatus lateralis. In the brainstem a rich S-100 neuronal population was detected in several discrete structures, including motor and sensitive nuclei of cranial nerves (3rd, 4th,Vth,VIth, VIIIth). The pattern of distribution of neuronal S-100 IR in quail is highly comparable to that previously reported for the rat brain. Some of these locations (motor nuclei of cranial nerves, striatal complex) have also high levels of IR for cholineacetyltransferase, suggesting hence a possible co-existence of S-100 IR in a portion of the cholinergic system. The present results confirm previous scattered reports for different vertebrates and represent the first detailed map of distribution of neuronal S-100 IR in a non mammalian vertebrate. Moreover they demonstrate that the neuronal distribution pattern of this calcium binding protein is highly conserved throughout phylogeny. *Work supported by grants from CNR, MURST and EU.*

410.7

PONTINE MICROINJECTION OF 4-AMINOBENZOVESAMICOL (ABV) BLOCKS CHOLINERGIC RAPID EYE MOVEMENT (REM) SLEEP ENHANCEMENT. R. Lydic* M.L. Capece, and S.M.N. Efange. Department of Anesthesia, Pennsylvania State University, College of Medicine, Hershey, PA 17033.

Pontine microinjection of the acetylcholinesterase inhibitor neostigmine produces a REM sleep-like state (REM-Neo), presumably by increasing the amount of acetylcholine (ACh) available to activate postsynaptic receptors (*J. Pharmacol. Exp. Ther. 231:173, 1984*). Recent studies revealed inhibition of REM sleep generation when vesicle uptake of ACh was blocked by intracerebroventricular administration of vesamicol (*Psychopharm 121:485, 1995*). ABV is a selective blocker of presynaptic ACh vesicular storage and an inhibitor of ACh release (*J. Med. Chem. 32:1217, 1989*). The present study is testing the hypothesis that ABV can decrease neostigmine-induced REM sleep when microinjected into the medial pontine reticular formation (mPRF) of intact, unanesthetized cat. To date, 3 cats have received unilateral mPRF microinjections (n) of saline (n=7), neostigmine (n=9), ABV (n=6), and ABV as a 15 min pretreatment to neostigmine (n=7). Polygraphic recordings were made for 2 hr after each injection to quantify percent time in sleep and wakefulness. Mean REM sleep time (16.3%, saline control) was decreased (-47.8%) by ABV (8.5% REM sleep). The REM sleep-like state elicited by neostigmine (61.2%) was significantly decreased (-36.1%) by pontine administration of ABV (39.1% REM-Neo). The finding that neostigmine-induced REM sleep enhancement can be blocked by decreasing presynaptic ACh release within the mPRF provides new support for the importance of pontine cholinergic neurotransmission in REM sleep generation. *Support: Grant HL-40881(RL); AG13621 (SMNE); Departments of Anesthesia and Neuroscience & Anatomy.*

410.8

CONTRIBUTION OF α_2-ADRENERGIC AUTO- AND HETERO-RECEPTORS IN MODULATING CORTICAL ACETYLCHOLINE (ACh) RELEASE *IN VIVO*. S. Tellez, F. Colpaert and M. Marien*, Pierre Fabre Res. Ctr., Castres, France.

Cortical ACh release *in vivo* can be modulated by α_2-adrenoceptor agonists and antagonists. The systemic administration of α_2-antagonists including (+)-efaroxan (EFX) increase, while α_2-adrenoceptor agonists such as UK-14304 (UK) decrease the release of ACh in the medial prefrontal cortex (mPFC) of conscious rats as measured by microdialysis (Tellez *et al*, 1995, *Soc. Neurosci. Abstr.* 21, 2138). To evaluate the contribution of α_2-adrenergic autoreceptors in mediating these drug effects, we re-examined the drug-induced changes in ACh release in rats which had previously undergone noradrenergic deafferentation procedures. Male SD rats were pretreated with the noradrenergic neurotoxin DSP-4 (40 mg/kg i.p.), or prepared with bilateral 6-OHDA-induced lesions of the locus coeruleus (LC). At 3 and 7 days following these respective pretreatments, microdialysis of the mPFC was performed. No significant difference in basal ACh outflow was observed in either the DSP-4 or LC-lesioned rats when compared to their control (vehicle or LC-sham) counterparts. In the control rats, ACh outflow was increased to 250% of baseline values by EFX (0.63 mg/kg i.p.), and decreased to 40% of baseline by UK (2.5 mg/kg i.p.), confirming our previous findings. In the DSP-4 pretreated rats, the inhibitory effect of UK on ACh outflow was preserved, while the ability of EFX to increase outflow was essentially eliminated. In LC-lesioned rats, EFX still significantly increased ACh outflow, but only to 170% of baseline values. Postmortem tissue concentrations of noradrenaline in the cortex were reduced by 81 ± 6% and 61 ± 6% in the DSP-4 and LC-lesion groups, respectively, as compared to controls. Results suggest that α_2-adrenoceptors both on noradrenergic neurons (autoreceptors) and on non-noradrenergic elements (heteroreceptors) can participate in modulating cortical ACh release *in vivo*. The ACh release-enhancing effect of EFX appears to be dependent on at least a partially intact noradrenergic innervation.

410.9

DIAGONAL BAND (NDB) STIMULATION INCREASES THE SPONTANEOUS ACTIVITY OF CELLS IN THE PIRIFORM CORTEX (PC) IN VIVO. L.A. Zimmer*, M. Ennis, and M.T. Shipley, Dept. Anatomy, Univ. Maryland School of Medicine, Baltimore, MD 21201

Electrical stimulation of NDB, the sole source of cholinergic input to PC, rapidly (<45 min) increases Fos protein expression in PC neurons. NDB-evoked Fos expression is prevented by muscarinic receptor antagonists. In vitro, high doses of cholinergic agonists in PC increases the excitability of pyramidal cells to afferent stimulation of the lateral olfactory tract (LOT). Taken together, these results suggest that activation of NDB cholinergic neurons should increase the activity of PC neurons. To test this hypothesis, extracellular single unit and field potential responses were recorded in PC before and during electrical activation of NDB neurons in anesthetized rats. Recordings were made from PC neurons (n=23) that exhibited stable spontaneous activity and a short-latency excitatory response to LOT stimulation (0.2 Hz; 100-500 μA). NDB stimulation (4 pulses, 100 Hz, 400 μA; n=7) increased the spontaneous discharge rate of PC cells from 4.9±1.6 to 7.3±1.4 spikes/s (49% increase, $p \leq 0.05$).

LOT-evoked field potentials in PC consisted of an initial (A1; afferent mediated) negative wave (onset=6.0±0.7ms; slope=-2.1±0.3mV/ms; mag=5.0±0.9mV) followed by a second (B1; associational mediated) negative wave (onset 12 ms), and a positive (P2) wave (onset=26.6±1.4ms; slope=0.6±0.06mV/ms; mag=3.8±0.4mV); P2 has been associated with LOT-evoked inhibition of PC pyramidal cells. NDB stimulation produced no field potentials in PC. Following paired electrical stimulation of NDB and LOT (n=8; LOT pulse occurring at the 4th NDB pulse), the A1 and B1 waves were unchanged. However, the magnitude of P2 decreased 37% to 2.4±0.4mV ($p \leq 0.05$); the slope was unchanged. Electrical activation of NDB increases the spontaneous activity of PC neurons. This may result from inhibition of LOT-evoked activation of GABAergic interneurons or inhibition of associational synapses onto GABAergic interneurons. *Support: US Army Contract DAMD-17-95-C-5031 and NIH DC-02588.*

410.10

EFFECT OF DNA α-POLYMERASE INHIBITION ON THE CHOLINERGIC PROPERTIES OF A NEUROBLASTOMA CELL LINE (SN-56). **M.A. Malik[1], S. Muntasser[1], J.K. Blusztajn[2] and C.E. Greenwood[1,*]. [1]Dept. Nutritional Sciences, Univ. of Toronto, Toronto, Ontario, Canada M5S 3E2 and [2]Dept. Pathology, Boston Univ. School of Medicine, Boston, MA.**

The use of agents which inhibit cell proliferation provide a useful tool to study the relationship between neuronal proliferation and differentiation. Aphidicolin, a DNA α-polymerase inhibitor, has been successfully used to study this relationship in the catecholaminergic neuroblastoma cell line, SH-SY5Y (LoPresti et al, 1992). Inhibition of proliferation was accompanied by morphologic differentiation including neurite extension. Our objectives were first to determine if comparable effects of aphidicolin on morphologic differentiation could be seen in the cholinergic neuroblastoma cell line SN56.B5.G4 and second to examine whether biochemical differentiation (acetylcholine (Ach) production) accompanied these changes. To assess the magnitude of response to aphidicolin, the changes in Ach production were compared to those previously reported for the neurotrophic agent, retinoic acid. Various aphidicolin concentrations (0.01 to 1.0 μM) were tested, cells were harvested and DNA and protein analyses were performed after 48 hrs. Treatment of SN-56 cells with 0.5 μM aphidicolin stopped cell proliferation with the least amount of cell death amongst the doses tested. Associated with this decrease in cell proliferation was an increase in neurite extension and intracellular Ach levels (measured by HPLC). The magnitude of increase in Ach levels was comparable to, or even greater than, the observed increase with saturating concentrations of all-trans retinoic acid (1 μM). No further increase in Ach levels were observed with a longer aphidicolin treatment period (4 days). These results indicate that treatment of cells with an optimal dose of aphidicolin results in a morphological change that is accompanied by an increased expression of its neurotransmitter, Ach. (NSERC).

410.11

ACTIVATION OF CHOLINE ACETYLTRANSFERASE BY CALPAIN D.H. Wu*, Y. Gu, W. Lian, L.B. Hersh, and J. Elce* Department of Biochemistry, University of Kentucky, Lexington, KY 40536-0084; *Department of Biochemistry, Queen's University, Kingston, Canada

Choline acetyltransferase (ChAT, E.C. 2.3.1.6), catalyzes the biosynthesis of the neurotransmitter acetylcholine (ACh). Regulation of ChAT activity could be an important determinant in the generation and maintenance of the terminal concentration of the neurotransmitter ACh. In this study, we examine the possible regulatory effects of Ca^{2+} on the activity of ChAT. In particular, we report that the Ca^{2+} dependent neutral proteinase (calpain) increases ChAT activity in a well defined in vitro system. Using recombinant calpain I and its inactive mutant (Cys105Ser), the effects of proteolysis of this Ca^{2+} dependent neutral proteinase on ChAT activity were measured. We found that incubation with calpain I but not its inactive mutant increases ChAT activity 2 to 3 fold. However, prolonged digestion resulted inactivation of ChAT. This observation could provide a potential mechanism for the regulation of neurotransmitter synthesis in response to synaptic activity and possibly a mechanism of ChAT turnover.

This work was supported in part by a research fund from UKMC and by AG05893from the National Institute on Aging.

410.12

PHARMACOLOGICAL REGULATION OF SENSORY STIMULATION-EVOKED INCREASES IN CORTICAL ACETYLCHOLINE RELEASE E. ACQUAS*, H.C. FIBIGER Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, B.C., V6T 1Z3 Canada

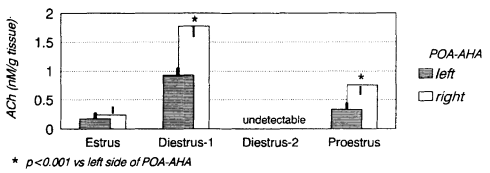
Recent reports have indicated that sensory stimuli increase the release of acetylcholine (ACh) in rat frontal cortex. The aim of the present study was to elucidate the neurochemical regulation of basal forebrain cholinergic neurons. To this end the effects of systemic administration of agonists and antagonists of various neurotransmitter receptors were studied on tactile stimulation (TS) evoked increases in frontal cortical ACh release. TS, produced by gently stroking the rat's neck with a nylon brush for 20 min., significantly increased frontal cortical ACh release by 116% above baseline. Administration of the benzodiazepine agonist diazepam (5 mg/kg) significantly reduced basal and TS-evoked cortical ACh release. Similarly, administration of the noradrenergic α_1 antagonist prazosin (1 mg/kg) or the α_2 agonist clonidine (0.1 or 0.2 mg/kg) significantly reduced the increases in ACh release produced by TS. The dopaminergic D_1 and D_2 receptor antagonists, SCH 23390 (0.15 mg/kg) and raclopride (1 mg/kg) failed to significantly reduce TS-induced increases in cortical ACh release. However, co-administration of SCH 23390 and raclopride significantly reduced the increases in cortical ACh release evoked by TS. These results, confirm that cortically projecting cholinergic neurons are activated by unconditioned sensory stimuli, and indicate that the increases in cortical ACh release produced by TS are inhibited by enhanced GABAergic transmission, by stimulation of α_2, and by blockade of α_1 noradrenergic receptors. In addition, these findings indicate that concurrent blockade of D_1 and D_2 receptors is necessary to achieve a significant reduction of sensory stimulation-evoked ACh release in the frontal cortex. These results are consistent with a role for basal forebrain cholinergic neurons in the regulation of arousal/attention. *Supported by the Medical Research Council of Canada and by the Human Frontiers Science Program.

410.13

THE ACETYLCHOLINE CONCENTRATION IN POA-AHA PRESENTS ASYMMETRIC VARIATIONS DURING THE ESTROUS CYCLE OF THE RAT. P.R. Arteaga and M.E. Cruz*. UIBR FES- Zaragoza, UNAM. México.

There is evidence that the cholinergic system of POA-AHA plays a stimulatory role on the regulation of gonadotropine secretion and ovulation. By the analysis of the effects of unilateral implants of atropine and the cholinoacetiltransferase (ChAT) activity, we have shown asymmetry in the activity of the cholinergic system in right and left side of POA-AHA during the estrous cycle. In present study, the concentration of acetylcholine (Ach) in the left and right side of POA-AHA at 13:00 hours of each day of the estrous cycle was cuantificated.

The figure shows the changes in the concentration of the Ach in the left anf right side of POA-AHA during the estrous cycle.



The differences in the Ach concentration in POA-AHA during the cycle could reflect the differences and asymmetry observed in the ChAT activity during the estrous cycle. (NeuroReport 5:433; 1994). · Supported by DGAPA:IN204693, PUIS.

410.14

REGIONAL DIFFERENCES IN THE EFFECTS OF PERINATAL LEAD EXPOSURE ON ChAT ACTIVITY IN THE CNS OF THE POSTNATAL RAT. X. Tian*, and J. B. Suszkiw. Dept. of Molecular & Cellular Physiology, University of Cincinnati, Cincinnati, OH 45267-0576.

We have previously reported that lead (Pb) exerts cholinotoxic effects in the septum of developing rat. In order to determine if developmental cholinotoxicity of Pb is region-specific, we compared the effects of low-level Pb exposure on the cholinergic marker enzyme, cholineacetyltransferase (ChAT) activity in the septum, striatum, brainstem and spinal cord. Rat pups were exposed to lead from gestational day 16 via administration of 0.2% lead acetate in drinking water to the dams. ChAT activity was assayed on postnatal (PN) days 7 and 14. The ChAT activities (in nmol/mg P/min) in control PN7 and PN14 animals were respectively, 0.32 and 0.92 in the septum, 0.65 and 1.12 in the striatum, 0.66 and 1.04 in the brainstem, and 0.94 and 1.44 in the spinal cord. Lead exposure resulted in a 63% and 37% reduction of septal ChAT, and a 38% and 34% reduction of striatal ChAT activity levels at PN7 and PN14, respectively. In contrast, Pb exposure had no significant effect on ChAT activities in the brainstem or spinal cord. These results suggest that developmental Pb exposure causes a reduction of ChAT activity in the forebrain cholinergic neurons without affecting the expression of ChAT activity in the cholinergic cells in the brainstem and spinal cord. *This work was supported by NIEHS grant ES06365.*

410.15

REGULATION OF CHOLINERGIC GENE EXPRESSION IN *C. ELEGANS*. D. Frisby, J. McManus, J. Duerr, and J. Rand*. Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

The genomic organization of the *cha-1* and *unc-17* genes in *Caenorhabditis elegans*, encoding choline acetyltransferase (ChAT) and a vesicular acetylcholine transporter (VAChT) respectively, resembles that of an "operon." The *unc-17* coding sequence is completely nested within the first intron of *cha-1*, and both genes appear to be expressed in a common transcription unit that is alternatively spliced to give rise to each mRNA. A similar genomic arrangement of these genes is conserved from nematodes to vertebrates, suggesting that coordinated regulation is important. Our data indicate that the all of the important upstream regulatory loci reside within 3.1 kb of exon-1. Comparison of the nucleotide sequence of the DNA upstream of *cha-1/unc-17* with the *C. briggsae* homologue revealed several highly-conserved regions which may be important for regulation. Deletion analysis has confirmed the existence of at least two important upstream regulatory loci. The first is located within a 747 bp region about 2.4 kb upstream, and is necessary for expression in most of the body cholinergic neurons. A previously identified point mutation, called β, located within this 747 bp region has a similar phenotype. The second is located within an 824 bp region located about 1.3 kb upstream of exon-1. Deletion of this region, which contains three conserved sequences, results in reduced expression in the dorsal and ventral sublateral nerve cords.

Supported by grants from NIH and OCAST

410.16

STRUCTURE AND REGULATION OF CHOLINE ACETYLTRANSFERASE AND VESICULAR ACETYLCHOLINE TRANSPORTER GENES IN *DROSOPHILA*. T. Kitamoto*, W. Wang, B. Sun and P. M. Salvaterra. Div. Neuroscience, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Functional cholinergic neurons require coordinated expression of two cholinergic macromolecules: choline acetyltransferase (ChAT; the biosynthetic enzyme for acetylcholine) and vesicular acetylcholine transporter (VAChT; the proton exchanger that packages acetylcholine into synaptic vesicles). We are using *Drosophila* as a model system to understand the mechanism and physiological significance of regulation of these two genes. In the present study we have isolated cDNA and genomic DNA clones for *Drosophila* VAChT. The *Drosophila* VAChT gene shares the 1st exon with ChAT and the rest is nested within an intron of the ChAT gene. RNase protection analyses reveal that all VAChT mRNA contains the shared 1st exon, indicating that cholinergic neuron-specific transcription of the ChAT and VAChT genes is mediated by the common regulatory region. The ratios of ChAT and VAChT mRNAs, however, differ in different tissues and in *Cha* temperature-sensitive mutants. This suggests the levels of ChAT and VAChT mRNAs can be independently regulated by post-transcriptional mechanisms. The *Drosophila* VAChT cDNA is 7.1 kb long and has unusually long 5' and 3' untranslated regions (UTR). The VAChT 5' UTR contains the GTG codon for ChAT translational initiation and three ATG codons, which are followed by a number of termination codons in all reading frames. These features of the VAChT 5'-UTR suggest that VAChT translational initiation is mediated by internal ribosome binding. (Supported by NIH-NINDS and the French foundation.)

410.17

EXPRESSION OF PRESENILIN-1 AND -2 mRNAs IN THE HIPPOCAMPUS AND CEREBELLUM IN ALZHEIMER DISEASE

K. Terai, K. Takami, D. G. Walker, A. Matsuo, A. Jakubovic* and P. L. McGeer, Kinsmen Laboratory of Neurological Research, University of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C., V6T 1Z3, Canada

Recently, new genetic linkages have been identified for early onset familial Alzheimer disease (AD) in the presenilin (PS)-1 (S182) gene on chromosome 14 and the PS-2 (STM2/E5-a) gene on chromosome 1. The genes are 67% homologous. In order to understand their role in AD, it is important to identify their expression in brain. We investigated the localization of the mRNAs for PS-1 and PS-2 in the hippocampus and cerebellum from 4 AD cases and 4 controls by in situ hybridization. Oligonucleotide probes from the 5'-region, which has the lowest homology between the two PSs, were synthesized and labeled at the 3'-end with 35S-dATP. The sequences corresponded to nucleotide bp 200-244 and 353-397 of mouse PS-1, bp 261-305 and 414-458 of human PS-1 and bp 380-424 and 503-547 of human PS-2. PS-1 mRNA expression was seen in hippocampal pyramidal and dentate granule neurons, as well as cerebellar Purkinje and granule neurons of both AD and control cases. The distribution of PS-2 mRNA expressing cells was similar. The expression of both mRNAs in hippocampal neurons was decreased in comparison to cerebellar neurons in the AD but not the control cases. However, PS-2 but not PS-1 expressing astrocytic-like cells were seen in AD hippocampus. The present data indicate that the PSs may play important roles in specific neurons in normal brain, and that a decrease of PS-1 and PS-2 expressing neurons may occur in affected areas of AD brain.

Supported by grants from the Alzheimer Society of B.C. and the Jack Brown and Family A.D. Research Fund

ACETYLCHOLINE RECEPTORS: NICOTINIC—REGULATION OF GENE EXPRESSION

411.1

DIFFERENTIAL REGULATION OF NEURONAL NICOTINIC RECEPTOR SUBTYPES FOLLOWING CHRONIC NICOTINE TREATMENT IN RATS. C.M. Flores[1]*, M.I. Dávila-García[2], Y.M. Ulrich[1] and K.J. Kellar[2]. [1]Department of Restorative Sciences, University of Minnesota, Minneapolis, MN 55455 and [2]Department of Pharmacology, Georgetown University, Washington, D.C. 20007.

Chronic nicotine administration to rats leads to an increase in neuronal nicotinic receptor binding sites throughout the central nervous system. Moreover, the increased sites that are labeled by ^{3}H-cytisine in cerebral cortex are composed exclusively of α4 and β2 subunits. Although it is unknown whether other potential subtypes that do not bind ^{3}H-cytisine with high affinity (e.g. α3β4) are also increased following chronic nicotine treatment, the recent availability of ^{3}H-epibatidine has made possible the detection of several different receptor subtypes in nervous tissue and in transfected cell lines. In the present studies, we tested the hypothesis that chronic nicotine administration differentially regulates neuronal nicotinic receptor subtypes in rat nervous tissues. Thus, in rats receiving twice daily injections of nicotine (2mg/kg s.c.) or saline vehicle, we compared the binding of ^{3}H-cytisine and ^{3}H-epibatidine to neuronal nicotinic receptors in brain, spinal cord and trigeminal ganglion. In each of these, chronic nicotine treatment led to an increase in binding sites labeled with ^{3}H-cytisine as well as with ^{3}H-epibatidine; however, the magnitude of the increase in the ^{3}H-cytisine binding was always greater than that for ^{3}H-epibatidine binding. In fact, the entire extent of nicotine-induced increases in binding sites could be accounted for by an increase in the fraction of receptors bound by ^{3}H-cytisine. In contrast, no increase in ^{3}H-epibatidine binding was seen in the adrenal gland, which is thought to express a receptor subtype that is composed of α3 but not α4 subunits and does not bind ^{3}H-cytisine with high affinity. These data indicate that, under these conditions, chronic nicotine administration leads to a preferential increase in the α4β2 but not non-α4β2 subtypes of neuronal nicotinic receptor. This differential effect may be related to the differing affinities of these subtypes for nicotine and suggests that the expression of receptor subtypes is subject to distinct regulatory mechanisms.

Supported by Smokeless Tobacco Research Council 0490 and NIH DA06486.

411.2

VALIDATION OF A CHRONIC INFUSION MODEL FOR PRODUCING NICOTINE-INDUCED INCREASES IN NEURONAL NICOTINIC RECEPTORS IN RATS. Y.M. Ulrich*, K.M. Hargreaves and C.M. Flores. Department of Restorative Sciences, University of Minnesota, Mpls, MN 55455.

Chronic nicotine exposure in rats produces a characteristic increase in neuronal nicotinic binding sites in many brain regions and spinal cord. The classical paradigm for inducing such increases consists of a twice daily injection regimen. This schedule was initially developed for its convenience and typically utilizes a near maximal, sub-convulsive dose of nicotine (e.g. 2 mg/kg of the bitartrate dihydrate salt). An alternative to this paradigm involves the chronic infusion of drug using an osmotic mini-pump. Although well characterized in mice, to our knowledge the use of osmotic mini-pumps in rats to produce chronic nicotine-mediated receptor increases has yet to be validated. To determine whether the use of osmotic mini-pumps could serve as a viable alternative to twice daily injections, we chronically (i.e. 10 days) exposed separate groups (n=8) of adult, male Sprague-Dawley rats to saline vehicle or nicotine bitartrate via either injections (2 mg/kg s.c., twice daily) or osmotic pumps (1 mg/kg/hr). This infusion dose, which represents a 6-fold greater overall daily dose compared to injections, was chosen to maximize the probability that steady state nicotine levels would be of sufficient magnitude to produce receptor changes. Radioreceptor binding analyses were performed in cortex, thalamus and dorsal and ventral lumbar spinal cord from each group of animals using [^{3}H]-epibatidine under saturating concentrations and 300 μM nicotine to define non-specific binding. In all regions examined, compared with saline treated controls, neuronal nicotinic receptor levels were significantly increased ($p < 0.05$) in the nicotine treated animals in both the injection and infusion paradigms. Interestingly, the magnitude of the increase in each region was approximately four times greater in the pump animals vs the injection animals. These data demonstrate the efficacy of osmotic mini-pumps for producing nicotine-induced receptor changes in rats and indicate that, under these conditions, the magnitude of the increases obtained by chronic infusion can exceed those achieved using the classical injection paradigm.

Supported by Smokeless Tobacco Research Council 0490 and NIH DE09860.

411.3

TRANSCRIPTION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR α-3, β-4 AND α-5 SUBUNIT GENES BY THYMOCYTES AND THYMIC EPITHELIAL CELLS. DOWN REGULATION DURING THE PROCESS OF IMMUNOCYTE MATURATION. Mirta Mihovilovic*[1], Stephen Denning[2], Yun Mai[1], Leona Whichward[3], Dhaval Patel[3], and Allen D. Roses[1,4]. [1]Department of Medicine, Division of Neurology, [2]Division of Cardiology, [3]Division of Rheumatology and Immunology, [4]Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Thymic tissues express transcripts encoding for the α-3, α-5 and β-4 subunits of nicotinic neuronal acetylcholine receptors (AcChRs). This pattern of transcriptional expression suggests that neuronal AcChRs similar to those expressed in ganglia are expressed in the thymus and that these receptors may be of importance in transducing signals delivered to the thymus through the autonomic nervous system or be responsive to local production of AcCh.

To determine the cell type responsible for thymic transcription we used freshly purified thymocyte preparations and cultured thymic epithelial cells as a source of RNA. Our results indicate that thymocytes express transcripts at levels comparable to those of the thymic tissue as a whole. When compared to thymocytes, epithelial cells express 100 fold less of the α-3, and similar levels of the α-5 and β-4 AcChR subunit transcripts. Transcriptional analyses of thymocyte subsets characterized as CD4+8-, CD4-8+ and CD4+8+ show higher levels of the α-3 and β-4 transcripts in immature CD4+8+ thymocytes, lower levels in CD4+8- thymocytes and very poor transcription, if any, in CD4-8+ thymocytes. α-5 AcChR subunit gene transcription, however, appears to be constant in all thymocytes subsets tested.

Overall our results indicate that neuronal nicotinic AcChR transcripts are expressed in both lymphoid and non-lymphoid thymic cells. In addition, the observation that peripheral lymphocytes do not express neuronal α-3 and β-4 AcChR transcripts and low levels of α-5 transcripts suggest that during the process of thymocyte maturation there is transcriptional regulation of neuronal AcChR subunit genes.

411.4

THE TRANSCRIPTIONAL ACTIVATOR SP1 BINDS TO A PROMOTER ELEMENT WITHIN THE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR β4 GENE. C.B. Bigger and P.D. Gardner*. Center for Molecular Medicine, Institute of Biotechnology, University of Texas Health Science Center, San Antonio, TX 78245.

To date, eleven members (α2-α9 and β2-β4) of the neuronal, nicotinic acetylcholine receptor (nAChR) gene family have been identified. These subunits have been demonstrated to form a variety of distinct receptors with different pharmacological and physiological profiles and are expressed in cell-restricted patterns within the CNS and PNS. Distinct transcriptional mechanisms probably direct this segregation in nAChR gene expression, although evidence identifying transcriptional control elements and their cognate DNA-binding proteins is minimal. Efforts to characterize β4 subunit gene expression have revealed the presence of several sequence motifs within the proximal promoter that are required for gene expression. Among these include a previously identified C/T-rich, 19bp sequence shown to be required for expression of β4 promoter-reporter gene constructs. Here we report the identification (by site-directed mutagenesis) of a CACCC motif that is required for β4 gene expression. This element was shown to interact with Sp1-like proteins based upon mobility shift competition assays and DNaseI footprinting experiments. Mobility supershift assays using specific antiserum to Sp1 confirmed the identification of the CACCC binding activity as Sp1. Investigations into the possible interaction between Sp1 and proteins binding to the adjacent 19bp sequence are in progress. Supported by The National Institutes of Health and The Council for Tobacco Research.

411.5

PURIFICATION OF A DNA-BINDING ACTIVITY THAT SPECIFICALLY INTERACTS WITH A NICOTINIC ACETYLCHOLINE RECEPTOR GENE PROMOTER. Q. Du, S.G. Britt* and P.D. Gardner. Center for Molecular Medicine, Institute of Biotechnology, University of Texas Health Science Center, San Antonio, TX 78245.

Nicotinic acetylcholine receptors constitute a multigene family (α2 - α9, β2 - β4) expressed in discrete temporal and spatial patterns within the nervous system. The receptors are critical for proper signal transmission between neurons and their targets. The molecular mechanisms underlying receptor gene expression have not been completely elucidated but clearly involve regulation at the level of transcription. We previously identified a novel 19-bp transcriptional regulatory element in the promoter region of the rat β4 subunit gene. The 19-bp element specifically interacts with DNA-binding proteins enriched in nuclear extracts prepared from adult rat brain. We have begun the biochemical purification of these nuclear proteins. *In vitro* binding assays indicated that the proteins are conserved between rat and bovine and therefore, bovine brain was used as a source of starting material. Using a combination of cation exchange (cellulose phosphate) chromatography and sequence-specific affinity chromatography, we purified the 19-bp element-binding activity approximately 20,000-fold. SDS-PAGE revealed the presence of four bands in the final product, ranging in molecular weight between 43 kDa and 100 kDa. Denaturation/renaturation studies are underway. Supported by The National Institutes of Health, The Council for Tobacco Research and The Smokeless Tobacco Research Council.

411.6

EFFECTS OF CHRONIC NICOTINE ON THE PHARMACOLOGY AND FUNCTION OF NEURONAL NICOTINIC RECEPTOR SUBTYPES. **E. L. Meyer*, Y. Xiao, M. I. Dávila-García, and K. J. Kellar. Dept. of Pharmacology, Georgetown Univ. Sch. of Med., Washington D. C. 20007.**

Neuronal nicotinic acetylcholine receptors comprise a diverse group of ligand gated ion channels differing in subunit composition and biophysical and pharmacological properties. These receptors are thought to be pentamers, assembled from two kinds of subunits, termed α and β. At least eight different genes encoding α subunits (α2-α9) and three different genes encoding β subunits (β2-β4) have been identified in vertebrate brain. It is believed that two α subunits and three β subunits combine to form neuronal nicotinic receptors which function as ion channels permeable to Na^+, K^+ and in some cases Ca^{++}. Chronic administration of nicotine to rats or mice, and smoking in humans increases the density of nicotinic receptor binding sites in brain. Brain may contain several different subtypes of nicotinic receptors; as a result, it is difficult to study the effects of chronic nicotine on any one particular subtype. Therefore, we have generated a library of transfected HEK 293 (human embryonal kidney) cell lines, each stably expressing one subtype of neuronal nicotinic receptor. These cell lines, in conjunction with tumor cell lines, provide models for studying nicotinic receptor subtypes. In this study, we compared the effects of chronic nicotine treatment on agonist stimulated $^{86}Rb^+$ efflux and on [^{3}H]epibatidine ([^{3}H]EB) binding sites in cells transfected with α2 and β4 nicotinic receptor subunits (KXα2β4R1 cells) and in IMR-32 cells, a human neuroblastoma cell line which expresses α3, α5, α7, β2, and β4 subunits. In IMR-32 cells treated with 100 μM nicotine for eight days, nicotinic receptors labeled by [^{3}H]EB are increased by ≈ 100%. However, these cells show an ≈ 50 % decrease in function measured by $^{86}Rb^+$ efflux. A six day treatment of KXα2β4R1 cells with 100 μM nicotine resulted in a four fold increase in [^{3}H]EB binding sites with little or no change in nicotinic receptor function measured by $^{86}Rb^+$ efflux. (NIH grant DA06486)

411.7

IDENTIFICATION OF A NEURAL-SPECIFIC ENHANCER IN THE 3'-UNTRANSLATED EXON OF THE β4 NICOTINIC RECEPTOR GENE. J. A. McDonough* and E. Deneris. Department of Neuroscience, School of Medicine, Case Western Reserve University, Cleveland, OH 44106.

The transcriptional mechanisms that generate complex patterns of neuronal nAChR subunit gene expression are not understood. We have identified an enhancer which activates transcription of both α3 and β4 nAChR promoters in PC12 cells. This enhancer, designated β43', is composed of two 37bp direct repeats separated by 6bp and is located within the 3' untranslated region of the β4 gene, about 3kb upstream of the α3 transcription start site region. The enhancer activates transcription from reporters in the PC12 neuroendocrine cell line, but not in cell lines derived from rat fibroblasts, liver, pancreas, or from a human carcinoma cell line. It is weakly active in the C6 glial and C1300 neuroblastoma cell lines. Thus β43' activity is restricted among neural lines. To characterize nuclear proteins that interact with β43', an oligonucleotide bearing the first repeat of the enhancer was used as a probe in electrophoretic mobility shifts. Two protein/DNA complexes, RBP1 and RBP2, were formed with nuclear extracts from PC12 cells. In nuclear extracts from non-neural lines and the C6 line, a complex that co-migrated with RBP2 but not RBP1 was detected. An RBP2 co-migrating complex and a trace of complex co-migrating with RBP1 were detected in the C1300 line. β43' is the first example of a neural cell-type specific enhancer controlling neuronal nAchR genes. Its cell-type restricted activity makes it an attractive candidate for a component of the cis-regulatory information that restricts expression of clustered neuronal nAChR genes to specific populations of neurons. We are currently making mutations through the repeat region to identify cis elements necessary for enhancer activity.

This work was supported by National Institutes of Health Grant NS29123.

411.8

NEURONAL NICOTINIC RECEPTOR mRNA EXPRESSION PATTERNS IN DEVELOPING POSTNATAL RAT BRAIN. J. J. Shacka* and S. E. Robinson. Department of Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.

The purpose of this study was to determine postnatal development patterns of neuronal nicotinic receptor (nNR) subunit mRNA in Sprague Dawley rat brain. Total RNA from male and female postnatal days (P)1, 7, 14 and 28 rat hippocampus (hippocampus +septum) and cortex was size-fractionated and transferred to nylon. Northern hybridization was performed using α-^{32}P dCTP-labelled 216 and 339 bp cDNA probes targeting the respective α7 and β2 nNRs' putative non-conserved 3' intracellular loops. γ-^{32}P-labelled 28S rRNA served to normalize for gel loading. All data were quantified using a Molecular Dynamics® PhosphorImager and expressed as the ratio of transcript mRNA to 28S rRNA. These studies identified four transcripts (3.7, 5.0, 7.5 and 10.0 kb) homologous to β2 cDNA, and a single 5.7 kb transcript homologous to α7 cDNA. Among the four β2 transcripts, the 5.0 kb transcript was consistently the most intense. All transcripts were present at each time point tested. ANOVA indicated no sex differences for each transcript at any time point, and that cortical α7 transcript levels peaked at P7 ($p<.005$, as compared to P1 and P28). Hippocampal/septal α7 transcript levels appeared higher at P7 as compared to P1. β2 transcript levels appeared greater in P14 hippocampus/septum and cortex as compared to P1 and P7. Tissue distribution patterns correlated well with previously published reports in adult Sprague Dawley rats. At all time points, α7 transcript levels appeared higher in the hippocampus/septum than in the cortex. However, hippocampal/septal versus cortical levels of all four β2 transcripts were similar; this may be due in part to β2's putative association with numerous alpha and beta subunits. The existence of multiple β2 transcripts may be the result of post transcriptional modification events, or evidence of still uncloned nNR subunits. [Supported in part by DA 07027]

411.9

COEXPRESSION OF RAPSYN SLOWS THE TURNOVER OF THE NICOTINIC ACETYLCHOLINE RECEPTOR (AChR) IN COS CELLS. Z.-Z. Wang* and Z. W. Hall. Laboratory of Cell Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, MD20892.

Development of the neuromuscular junction is characterized by clustering of AChRs in the postsynaptic membrane as well as a marked increase in their metabolic stability. Rapsyn, a 43 kDa protein which is closely associated with the AChR, is critical for the redistribution of the receptor in the muscle membrane during synaptogenesis. We examine here whether association of rapsyn with AChRs also affects the turnover rate of the receptor protein. Coexpression of rapsyn with AChR in COS cells significantly reduced the degradation rate of surface AChRs labeled by ^{125}I-α-bungarotoxin (10 hours in control vs. >24 hours with rapsyn). The effect was more prominent with increased amounts of rapsyn cDNA cotransfected, and was seen for both embryonic ($\alpha_2\beta\delta\gamma$) and adult ($\alpha_2\beta\delta\varepsilon$) AChRs, as well as for an AChR whose β subunit lacks the putative tyrosine phosphorylation site in the cytoplasmic loop. Rapsyn mutants lacking the myristylation signal, those with *N*-terminal residues 16-254 deleted, and those with deletion of C-terminal region containing the zinc-finger domain and PKA phosphorylation site, all failed to cluster the AChR and to slow down its turnover. These data suggest that rapsyn may contribute to the metabolic stabilization of AChR in mature neuromuscular junctions. (Supported by extramural and intramural grants from National Institutes of Health and by funds from Muscular Dystrophy Association).

411.10

THE EFFECTS OF ENUCLEATION ON THE DEVELOPMENTAL EXPRESSION OF NEURONAL NICOTINIC RECEPTOR SUBUNIT mRNA IN PRIMARY VISUAL CORTEX. B.S. Stoveken,* R. T. Robertson, and F.M. Leslie. Depts of Pharmacology and Anatomy and Neurobiology, University of California, Irvine Irvine, CA 92717

Studies have shown that during the second week of postnatal rat development acetylcholinesterase (AChE) (R.T. Robertson et al 1985 Dev. Brain Res 21: 203-214, R.T. Robertson et al 1987 Dev. Brain Res 33: 185-197), and [^{3}H]nicotine binding (Prusky et al 1988 Brain Res, 467:154-159) are transiently upregulated in layer IV of the primary visual cortex (V1). With binocular enucleation at birth, this transient [^{3}H]nicotine binding and AChE staining are lost (Prusky et al 1988 Brain Res, 467:154-159). We have previously shown the mRNA expression of the neuronal nicotinic acetylcholine receptor (nAChR) subunits α3 and β4 exhibit a transient expression in V1 with a spatial and temporal pattern similar to AChE staining and [^{3}H]nicotine binding. The present study was therefore undertaken to determine whether removal of peripheral sensory input influences the expression of α3 and β4 mRNA transcripts in developing V1. Unilateral and bilateral enucleations were done at P0 and litter mates served as controls. Coronal sections from pups sacrificed on P7, P13, and P17 were processed for in situ hybridization using [^{35}S]-labelled riboprobes. Data were analyzed using computer assisted quantitation of the resulting autoradiograms. β4 mRNA expression in V1 layer IV was found to be high at P7 and to have declined to the adult level by P17. This decline was not correlated with eye-opening since decreased expression of β4 mRNA was already evident at P13 prior to eye-opening. Enucleation did not significantly affect either β4 or α3 mRNA expression at any time point examined. This finding suggest that while both α3 and β4 nAChR mRNA show increased expression during early postnatal development this expression is not regulated by sensory input form the periphery. Consequently if either or both of these mRNA's contribute to transient expression of [^{3}H]nicotine binding in layer IV of V1 during the second postnatal week, then regulation is at the translational, not the transcriptional level. Supported by PHS grant #30109

411.11

MANIPULATING ALPHA-3 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT EXPRESSION IN CULTURED NEONATAL RAT SYMPATHETIC NEURONS USING ADENOVIRUS-MEDIATED GENE TRANSFER. Madelaine Rosenberg* and Ellis Cooper. Dept. of Physiology, McGill University, Montréal, Québec, Canada, H3G 1Y6.

We have previously shown that neonatal rat sympathetic neurons express five nicotinic acetylcholine receptor (nAChR) subunit genes: α3, α5, α7, β2 and β4. During early postnatal development, and in culture, the mRNA levels of α5, β2 and β4 remain constant, whereas α3 mRNA levels increase and correlate closely with increases in ACh-evoked current densities on these neurons. These results indicate that changes in α3 expression influence the number of functional nAChRs on neonatal sympathetic neurons.

To explore further the relationship between α3 mRNA levels and functional nAChRs, we have constructed a replication-deficient (E1 deleted) adenovirus vector containing the rat α3 cDNA under the control of the HCMV promoter. This vector was produced in HEK 293 cells by homologous recombination of a shuttle plasmid containing the full length α3 cDNA and a portion of the left end of the Ad 5 genome, and a second plasmid (pJM17) containing overlapping left end Ad 5 regions and the remainder of the Ad 5 genome. In addition, we have inserted the sequence coding for the epitope FLAG at the 3' end of the α3 cDNA to localize the expressed proteins in infected cells. We have co-expressed the α3-shuttle plasmid with β4 cDNA in *Xenopus* oocytes to ensure that the modified α3-FLAG subunit can co-assemble with β subunits into functional receptors. Our preliminary data indicate that neurons infected with α3-FLAG-containing adenovirus express mRNA for α3-FLAG and immunostain for α3-FLAG proteins. We are now examining infected neurons electrophysiologically to quantify changes in ACh-evoked current densities. (Supported by CHSF and MRC of Canada.)

411.12

STABLE EXPRESSION OF RAT NICOTINIC ACETYLCHOLINE RECEPTOR SUBTYPES IN MAMMALIAN CELLS. Y. Xiao*, E. L. Meyer, R. A. Houghtling, J. M. Thompson and K. J. Kellar. Dept. of Pharmacology, Georgetown Univ. Sch. of Med., Washington, D.C. 20007

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels composed of α and β subunits. At least eight α and three β subunits of neuronal nAChRs have been identified. We have transfected human embryonal kidney cells (HEK 293 cell line) with six rat nAChR subunit genes. Cell lines stably expressing the putative nAChR subtypes α2/β2 (KXα2β2R1), α2/β4 (KXα2β4R1), α3/β4 (KXα3β4R1) and α4/β2 (KXα4β2R1), as well as cell lines stably expressing a single α or β subunit, have been established. [^{3}H]Epibatidine ([^{3}H]EB) binds with high affinity in membranes of cells that express each of these nAChR subtypes but not in membranes of cells that express only a single subunit gene. The K_d values of [^{3}H]EB binding in these cell lines range from 15 pM (KXα2β2R1) to 130 pM (KXα2β4R1). Interestingly, the K_d value of [^{3}H]EB binding in KXα4β2R1 cells (20 pM) appears to be slightly lower than in rat forebrain (45 pM). Cytisine, nicotine, carbachol, DHβE, curare and ACh compete for all of the binding sites labeled by [^{3}H]EB in each cell line, but with different rank orders of potency. The cell line KXα2β4R1 has a very high density of [^{3}H]EB binding sites ($B_{max} \approx 800$ fmol/mg protein) and preliminary studies indicate most of these sites are on the cell surface. Nicotine stimulates $^{86}Rb^+$ efflux in KXα2β4R1 and KXα3β4R1 cells, but not in cells expressing a single subunit gene. Chronic exposure of cells expressing nAChR subtypes to 100 μM nicotine results in a marked increase in the B_{max} values of [^{3}H]EB binding. These results show that functional rat nAChR subtypes can be stably expressed in HEK 293 cells. A library of such cell lines expressing different subunit combinations should be very useful for studying the pharmacological properties and regulation of nAChR subtypes and the contributions to these characteristics imparted by each α and β subunit. This study was supported by NIH grant DA06486.

411.13

CNS SITES AFFECTING NICOTINE-INDUCED NOREPINEPHRINE SECRETION IN THE PARAVENTRICULAR NUCLEUS (PVN). Y Fu*, S Matta, J Valentine and B Sharp. Endocrine Neuroscience Laboratory, Minneapolis Med Res Fndn and Depts of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis MN 55404.

Brainstem noradrenergic projections to the hypothalamic PVN are involved in nicotine-induced ACTH secretion from the pituitary. To determine whether iv nicotine affects norepinephrine (NE) terminals within the PVN in addition to NE neurons within the brainstem, in vivo PVN microdialysis studies were conducted and mecamylamine (Mec), a nicotinic antagonist, was administered into the PVN or the fourth ventricle. A concentric microdialysis probe was inserted via chronic guide cannula into the PVN of freely moving rats (male Holtzman) during their active (dark) phase and perfused at a rate of 1μl/min. Studies showed that both baseline and peak NE responses were similar when dialysis was performed on 3 alternate days (d 1, d 3, d 5) in each rat. Nicotine (0.045 mg/kg per 30 s for 30, 60 or 90 s.) dose-dependently increased NE secretion with an ED_{50} of 0.09 mg/kg. In subsequent experiments, Mec was infused via the PVN microdialysis probe (0.4 - 2.0 μg/μl/min for 40 min prior to nicotine 0.09 mg/kg iv) or microinjected into the fourth ventricle (0.4 - 4.8 μg/0.5 μl, 5 min prior to iv nicotine). Infusion of Mec into the PVN blocked nicotine-stimulated NE secretion by 40%. In contrast, Mec delivered into the 4th ventricle (ED_{50} of 0.4 μg) inhibited at least 85% of the PVN NE response to nicotine. These results indicate that nicotine dose-dependently stimulated NE secretion in the PVN through nicotinic receptors located adjacent to the 4th ventricle and, to a lesser extent, through receptors located in the PVN. (Supported by NIDA DA03977)

411.14

COMPARATIVE EXPRESSION OF IMMEDIATE EARLY GENES IN BRAINSTEM AND PARAVENTRICULAR NUCLEUS (PVN) IN RESPONSE TO NICOTINE. SG Matta*, JD Valentine, JT Hokanson and BM Sharp. Minneapolis Medical Research Foundation and Departments of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55404

cFos has been used in this laboratory as an anatomical marker to correlate activation of PVN neurons with phenotypically identified (and unidentified) neurons in catecholaminergic regions of the brainstem. cFos also is important for transcriptional regulation of other genes in nicotine-responsive neurons. The present studies were conducted to determine whether immediate early genes related to cFos, involved in formation of AP-1 complexes or otherwise involved in transcriptional regulation were responsive to nicotine. Rats were cardiac perfused 1, 6 or 24 h after receiving saline or nicotine (0.09 mg/kg over 60 sec) iv and brains were processed for immunocytochemistry for cFos, FosB, FRA1, FRA2, junB, junD or KROX24. As expected, cFos was activated in parvocellular PVN (pcPVN) and NTS-A2 and -C2 catecholaminergic regions; only A2 was still elevated at 6 h. FosB expression was increased at 1 h and peaked at 6 h in pcPVN. FRA1 and FRA2 were unaffected by nicotine. JunB was induced within 1 h in pcPVN and A2, remaining elevated at 6 h. In contrast, junD was expressed in magnocellular PVN at 1h, but not pcPVN. Expression of KROX24 was seen at 1 h in pcPVN and A2. These studies demonstrate that nicotine selectively increased the number of cells positive for KROX24 and for transcription factors involved in the formation of AP-1 complexes. (Supported by NIDA DA03977)

411.15

DYSTROPHIN AFFECTS ACETYLCHOLINE RECEPTOR (AChR) DEGRADATION Rufeng Xu* and Miriam M. Salpeter. Neurobiology and Behavior, Cornell University, Ithaca NY 14853

Neural regulation of acetylcholine receptor (AChR) turnover is well established but not well understood. In addition the possible role played by muscle cytoskeletal elements or other potential anchoring molecules remains elusive. The present study reports that the absence of dystrophin, an integral cytoskeletal element, affects the degradation rate of the Rs but not that of the Rr AChRs. Rs and Rr AChRs are two populations of receptors expressed at the neuromuscular junction (nmj) dependent on the state of muscle innervation. The Rs is synthesized in innervated muscles and has a degradation t1/2 of about 8-10 days. When the muscle is denervated the Rs degradation accelerates to a t1/2 of about 3 days but can be restabilized to adult values by reinnervation or elevation of intracellular cAMP. The Rr, on the other hand, is synthesized in denervated muscles, has a degradation t1/2 of ~ 1-2 d, like that of embryonic AChRs, and is not stabilized to adult values by reinnervation. In early post denervation periods before all the Rs has degraded and been replaced by Rr, both AChR populations are interspersed at the top of the junctional folds at a total site density as seen in adult muscle.

To ask whether cytoskeletal integrity plays a role in the regulation of AChR turnover, we examined AChR degradation in the mutant *mdx* mouse known to be devoid of dystrophin. We found no difference in the degradation rates of Rr AChRs between mdx and control muscles from same strain of mice. This was true for the degradation of Rr both at long term denervated nmjs, where Rr had fully replaced the Rs, and on myotubes in tissue culture. A significant difference was however seen in the degradation of the Rs AChRs. At several muscles (sternomastoid, soleus, and diaphragm) *in vivo*, the degradation rate of Rs AChRs in the innervated mdx mice resembled that of the post denervation accelerated Rs (t1/2 ~3-5 days) seen in control mice. Denervating these mdx muscles did not further accelerate their Rs degradation rate. It appears that the absence of dystrophin, with possible resultant disruption of overall cytoskeletal organization, prevents the neural stabilization of Rs AChRs normally seen in innervated muscles. Supported by NIH NS09315 and 2 T32 GM 07469.

EXCITATORY AMINO ACIDS: PHARMACOLOGY—METABOTROPIC RECEPTORS

412.1

SUPERSENSITIVITY OF METABOTROPIC GLUTAMATE RECEPTORS IN 6-HYDROXYDOPAMINE (6-OHDA) LESIONED HEMIPARKINSON RATS. K.A. Serpa* and L.T. Meltzer, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105

Recent findings have indicated that interactions between the dopaminergic (DA) and glutamatergic (GLU) systems in the basal ganglia may play a role in the symptomology of Parkinson's disease (PD). We examined the behavioral effects of stimulation of the metabotropic subtype of the GLU receptor (mGluR) in the caudate of both normal rats and rats made hemiparkinson by injection of 6-OHDA into the right medial forebrain bundle.

Normal or lesioned rats were implanted with a chronic guide cannula in the right caudate nucleus. At least 8 days post-surgery, rats were injected with the mGluR agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) into the caudate and rotational activity monitored for 6 h. In normal rats, ACPD 1 μmol produced a low level of predominantly contraversive turning (mean 43 $\pm$ 21 turns/6h, n=11). In lesioned animals, injection of ACPD 1μmol into the denervated caudate produced more robust contraversive turning than that seen in normal rats (468 $\pm$ 70 turns/6h, n=19). Most (>90%) of the turning was observed in the first 2 hours post injection. Injection of ACPD 0.1 μmol resulted in a similar total number of turns/6h (454 $\pm$ 186, n=6), but the turning occurred at a lower rate and occurred over more than 4h. The turning induced by ACPD 1μmol in lesioned rats was not antagonized by pretreatment with SCH 23390 30 μg/kg SC + haloperidol 0.3 mg/kg IP, indicating that ACPD is acting independent of DA mechanisms. The mGluR1/2 receptor antagonist, α-methyl-4-carboxyphenylglycine 1μmol, co-injected with ACPD 1μmol, did not reduce the number of turns/6h (471 $\pm$ 82, n=4), but reduced the rate and increased the duration of the response to that seen following ACPD 0.1 μmol. Thus, 6-OHDA lesioned rats appear to have functionally supersensitive mGluRs. (Supported by Warner-Lambert.)

412.2

1S,3R-ACPD-INDUCED INCREASES OF cAMP IN THE NEONATAL RAT HIPPOCAMPUS ARE MEDIATED BY A SYNERGISTIC INTERACTION BETWEEN PHOSPHOINOSITIDE HYDROLYSIS- AND INHIBITORY cAMP-COUPLED mGluRs B. G. Johnson*, D. D. Schoepp, and J. A. Monn Central Nervous System Research Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, Indiana 46285.

A pharmacological approach was used to investigate the cellular mechanism and subtypes of metabotropic glutamate receptors (mGluRs) that mediate stimulation of basal cAMP formation in slices of the neonatal rat hippocampus. 1*S*,3*R*-ACPD, an agonist for phosphoinositide-coupled and inhibitory-coupled cAMP-linked mGluRs in cloned and in situ preparations, produced prominant stimulations of basal cAMP. However, the agonists DHPG and 2*R*,4*R*-APDC, that selectively act on phosphoinositide-coupled and inhibitory cAMP-coupled mGluRs, respectively, only weakly increased cAMP. When these two mGluR subtype selective agonists were added in combination, increases in cAMP, similar to those observed for 1*S*,3*R*-ACPD, were found. Stimulations of cAMP evoked by 1*S*,3*R*-ACPD and combined additions of DHPG + 2*R*,4*R*-APDC occured at concentrations of these agents which directly couple to other mGluR second messenger responses. However, these stimulatory cAMP responses were prevented by the presence of adenosine deaminase, and 8-para-sulfophenyltheophylline (an adenosine receptor antagonist), as well as (+)-MCPG (an mGluR receptor antagonist). Thus, 1*S*,3*R*-ACPD-induced increases in cAMP formation in the neonatal rat hippocampus are mediated by a synergistic interaction between phosphoinositide- (group 1) and inhibitory cAMP-coupled (group 2) mGluRs, which are indirectly expressed by potentiation of cAMP responses to other agonists (in this case endogenous adenosine).

412.3

PHARMACOLOGICAL ACTIVITIES OF FLUORID CCG DERIVATIVES: DISCOVERY OF A NOVEL AGONIST FOR METABOTROPIC GLUTAMATE RECEPTORS. H. Shinozaki[1]*, M. Ishida[1], T. Saitoh[1], Y. Uyama[1] and T. Taguchi[2]. [1]The Tokyo Met. Inst. Med. Sci., Tokyo 113 and [2]Tokyo College Pharmacy, Tokyo 192-03, Japan

Derivatives of 2-(carboxycyclopropyl)glycine (CCG), a conformationally restricted glutamate analogue with an extended and folded form, are proving useful in determining the active conformation of glutamate at various receptors. We have already found that the extended CCG derivatives, L-CCG-I, DCG-IV and *cis*- and *trans*-MCG-I, are potent agonists for metabotropic glutamate receptors (mGluR), but are weak agonists of ionotropic glutamate receptors (iGluRs), suggesting that mGluRs are activated by extended conformation of L-glutamate, while iGluRs are activated by a folded form. Fluoridation of the 3'-carbon of CCG compounds would be expected to further restrict their conformational freedom. In the present paper, we compared the pharmacological actions of eight isomers of 3',3'-difluoro-CCG (3'-F_2CCG) with those of CCG in the isolated spinal cord of newborn rats. L-3'-F_2CCG-I (extended form, corresponding to L-CCG-I) was respectively about 3 and 1.5 times a more potent agonist of Group I and Group II mGluRs than L-CCG-I. L-CCG-III is a potent uptake inhibitor for L-glutamate, but L-3'-F_2CCG-III (folded form, corresponding to L-CCG-III) did not enhance glutamate-induced depolarization, suggesting no inhibitory action on the L-glutamate uptake system. Other fluoro-CCG compounds did not show large differences from CCG, although were generally less active than the corresponding CCGs. In addition to potent activation of mGluRs, L-3'-F_2CCG-I demonstrated novel pharmacological features, suggesting that L-3'-F_2CCG-I may be a useful probe for elucidating the physiological roles of mGluRs.

412.4

GTPγS BINDING AS A TOOL TO CHARACTERIZE METABOTROPIC GLUTAMATE RECEPTOR (mGluR) AGONISTS IN CHO CELLS EXPRESSING SUBTYPES 2 AND 6. C. J. Spencer*, C. B. Nelson and R. D. Schwarz. Parke-Davis Pharmaceutical Res., Div. of Warner Lambert Co., Ann Arbor, MI 48105

mGluRS belong to the superfamily of G-protein coupled receptors. In the resting state, GDP is bound to the associated heterotrimeric G proteins; upon agonist activation, GTP exchanges with the GDP. By using [^{35}S]GTPγS, agonist stimulated GDP-GTP exchange can be assessed by standard binding techniques. This method was applied to subtypes 2 and 6 expressed in CHO cells. Membrane preparations were incubated at 30° C in a pH 7.4 buffer containing NaCl, $MgCl_2$, and GDP at optimal concentrations for each subtype. Agonists and radioligand were added for 30 min. Bound ligand was separated from free by rapid filtration. Agonists examined were (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (CCG), (S)-4-carboxy-3-hydroxyphenyl-glycine (4C3HPG), O-phospho-L-serine (SOP) and the acids L-glutamic (glu), L-quisqualic (quis), ibotenic (ibo), (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic (ACPD) and L(+)-2-amino-4-phosphonobutyric (AP4). EC_{50} values were determined and compared to EC_{50} values for the same compounds' ability to reverse forskolin stimulated cAMP accumulation. In mGluR2 membranes, the rank order of potency for [^{35}S]GTPγS binding was CCG>glu>ACPD=4C3HPG>ibo>>quis=AP4=SOP; the rank order for cAMP was CCG>glu>ACPD>4C3HPG>>ibo=quis=AP4. For mGluR6, the [^{35}S]GTPγS order was AP4≈SOP>glu>ACPD; others were inactive. The cAMP rank was similar. These results show good correlation between the two methods. [^{35}S]GTPγS binding has major advantages over other second messenger assays since GDP-GTP exchange is common to all the subtypes regardless of the subsequent pathways and thus can be used to directly compare agonist activity between subtypes. (Supported by Warner-Lambert.)

412.5

GLUTAMATE METABOTROPIC RECEPTORS : ANTICONVULSANT AND CONVULSANT EFFECTS OF GROUP II AGONISTS AND ANTAGONISTS
B.S. Meldrum[1], M. Ghauri[1], E. Tang[1], P.K. Yip[1], D.E. Jane[2], J.C. Watkins[2] and A.G. Chapman[1]*. [1]Dept. of Neurol, Inst. of Psychiatry, London, SE5 8AF, U.K., [2]Dept. of Pharmacol., School of Med. Sci., Bristol BS8 1TD, UK.

Metabotropic glutamate receptors are classified into three groups on the basis of their sequence homology, pharmacology and signal transduction mechanism. Groups II and III are negatively coupled to adenylate cyclase and act presynaptically to depress the release of glutamate and GABA. We have examined proconvulsant action of group II selective compounds in non-epileptic Swiss-Webster (SW) and epileptic DBA/2 strains of mice, and anticonvulsant action against sound-induced (95-110dB; 12-16 KHz) seizures in DBA/2 mice. Groups of mice were observed for 30 min for clonic and tonic seizures and/or response to sound-exposure at 5, 15 or 30 min following intracerebroventricular (icv) 10µl injections. The non-selective metabotropic agonists (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (1S,3R-ACPD; 500-700 nmol) and (2S,1'S,2'S)-2-(2-Carboxycyclopropyl) glycine (L-CCG-I) at low doses (20-50 nmol) suppressed sound-induced clonic seizures in DBA/2 mice at 5 and 15 min. The selective group II agonist (1S,3S)-1-amino-cyclopentane-1,3-dicarboxylate (1S,3S-ACPD; 50-500 nmol), in contrast, was convulsant in both SW and DBA/2 mice. The potent, selective group II antagonist α-ethylglutamate (EGLU; 3-7 µmol) was also convulsant in both strains of mice. The anticonvulsant effects of the group II agonists are consistent with those reported by Dalby and Thomsen (JPET, 276; 516-522; 1996); the proconvulsant effects of both agonists and antagonists of group II match those previously reported by us for group III agonists and antagonists (Ghauri et al., Neuroreport, in press).

412.6

EFFECT OF THE NOVEL ANTAGONIST MPPG ON ADENYLATE CYCLASE-LINKED GLUTAMATE RECEPTORS IN MESENCEPHALIC AND STRIATAL NEURONS *IN VITRO*.
L. Bresciani, F. Fidone, N. Brunello^* G. Racagni and A. Ambrosini. Center of Neuropharmacology, Univ. of Milan, and ^Dept. Pharmaceutical Sciences, Univ. of Modena -(I).

New selective agonists enabled us to characterise the activity of metabotropic glutamate receptors (mGluRs) of class I, that trigger the phosphoinositide (PI) hydrolysis, and classes II or III, that modulate cAMP levels, in mesencephalic (Mol. Pharmacol. 47, 1057, 1995) and in striatal neurons. In both neuronal systems, the agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) was more potent on PI hydrolysis stimulation than on the modulation of cAMP levels, a difference that was more evident in striatal neurons, in which the EC_{50} values on the two pathways were 3 µM and 25 µM, respectively. On the contrary, (2S,3S,4S)-alpha-(carboxycyclopropyl)glycine (CCGI) inhibited cAMP formation at lower concentrations than those required to activate PI turnover. In striatal neurons, its EC_{50} values were 50 µM on the PI system and 0.8 µM on cAMP modulation. Aminophosphonobutyrate (AP4) and serine-O-phosphate (SOP) also inhibited adenylate cyclase activity both in mesencephalic and in striatal neurons. The effect of the novel antagonist (RS)-alpha-methyl-4-phosphonophenylglycine (MPPG), proposed as selective for class III mGluRs, was then tested. MPPG (10 µM) did not affect basal cAMP values, but effectively blocked the response to AP4, SOP and CCGI, confirming its pure antagonist action at mGluRs that inhibit adenylate cyclase, although a selectivity between class II and III was not evident. The pharmacological distinction of the mGluR effects may help to clarify their role in the basal ganglia. *Study partially supported by Pharmacia-Upjohn*

412.7

4C3HPG ACTIVATES PHOSPHATIDYL INOSITOL (PI)-LINKED METABOTROPIC GLUTAMATE RECEPTORS (mGLURs): DIFFERENTIAL EFFECTS IN NEONATAL RAT BRAIN REGIONS.
E. M. Santori*, A. I. Sacaan, T. S. Rao, M. A. Varney, E. C. Johnson, G. Velicelebi, and G. K. Lloyd. SIBIA Neurosciences Inc., La Jolla, CA 92037.

To characterize the native mGluRs expressed in different brain regions, the efficacy and potency of several mGluR agonists and antagonists were compared using PI hydrolysis in neonatal rat striatal, hippocampal, cortical and cerebellar slices. In the striatum, hippocampus and cortex, all agonists tested were equiefficacious (normalized to 10 µM Quis) with rank order of potency of Quis>(RS)-3,5,DHPG>1S,3R-ACPD>>Glu. Interestingly, 4C3HPG, a putative class I mGluR antagonist, also displayed full agonist activity with a potency similar to that of Glu in the striatum, hippocampus and cortex, and this effect was significantly blocked by (S)-4CPG. In the cerebellum however, 4C3HPG was a very weak agonist (37% of that of 10 µM Quis). The stimulatory effects of 4C3HPG are in contrast with its antagonistic properties shown in studies on recombinant receptors (*Neuropharmacol.* 34: 887-94 and 895-903, 1995). 4C3HPG did not show any agonistic activity in cell lines stably expressing hmGluR1b or 5a., and selectively antagonized Quis response in hmGluR1b but not 5a cell lines. The effect of the mGluR antagonists (+)-MCPG, (S)-4CPG, and UPF523 on a class I selective mGluR agonist, 3,5-DHPG (10 µM)-stimulated PI hydrolysis was evaluated. Results are summarized below:

Antagonist	K_b (µM)			
	Cerebellum	Striatum	Hippocampus	Cortex
(+)-MCPG	59	78	80	87
(S)-4CPG	238	208	115	159
UPF523	648	484	493	not tested

The similar K_b values observed for these antagonists in the different brain regions contrasts with the differential distribution of mGluR subtypes in these regions as demonstrated by in-situ hybridization and immunohistochemistry studies. These results suggest that the pharmacology of these compounds on native mGluR receptors substantially differ from their pharmacology on recombinant class I mGluRs. Furthermore, stimulatory effects of 4C3HPG observed in this study may reflect activity at a novel PI-linked mGluR subtype.

412.8

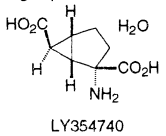
THE METABOTROPIC GLUTAMATE RECEPTOR AGONIST LY354740 ATTENUATES INCREASES IN SENSORIMOTOR REACTIVITY RESULTING FROM WITHDRAWAL OF CHRONIC NICOTINE
D.R. Helton*, J.P. Tizzano, J.A. Monn, D.D. Schoepp and M.J. Kallman Lilly Research Laboratories, Greenfield Laboratories, Eli Lilly and Company, Greenfield, IN 46140, USA

LY354740 monohydrate is a highly selective metabotropic glutamate receptor agonist (Group 2: $mGluR_2/mGluR_3$) which has been shown to be efficacious in animal models of anxiety and benzodiazepine withdrawal (see Monn et al. and Kallman et al., this meeting). To explore the potential clinical efficacy of LY354740 for the alleviation of nicotine withdrawal symptoms or as an aid in smoking cessation, the auditory startle reflex was examined in rats undergoing withdrawal from chronic administration of nicotine. We have previously described a model for evaluation of nicotine withdrawal using the acoustic startle reflex in rats (Helton et al., Psychopharmacology, 113:205-212;1993). Animals were exposed to nicotine continuously for 12 days (6 mg/kg/day, subcutaneous) via osmotic minipumps. After 12 days the pumps were removed and the animals were allowed to go through spontaneous withdrawal. The acoustic startle reflex was significantly increased on days 1, 2, 3, and 4 following nicotine withdrawal. Withdrawal-evoked increases in startle responding were suppressed by readministration of nicotine or treatment with the anxiolytic, diazepam. Treatment with LY354740 (0.03 to 3 mg/kg, oral) produced a dose-dependent suppression of the enhanced auditory startle responding following nicotine withdrawal (ED_{50} = 0.7 mg/kg). LY354740 did not alter baseline startle responding at doses which suppressed nicotine withdrawal. The (-)-enantiomer of LY354740, LY366563, which is inactive as a Group 2 mGluR agonist, had no effect on nicotine withdrawal at an oral dose of 10 mg/kg. Thus, the Group 2 mGluR agonist LY354740 may be an effective treatment for nicotine withdrawal and/or smoking cessation in man.

412.9

IN VITRO CHARACTERIZATION OF LY354740 MONOHYDRATE: A HIGHLY POTENT AND SELECTIVE AGONIST FOR GROUP 2 METABOTROPIC GLUTAMATE RECEPTORS.
J. A. Monn, M. J. Valli, R. A. Wright*, B. G. Johnson, C. R. Salhoff, J. P. Burnett, N. G. Mayne, R. Belagaje, S. Wu and D. D. Schoepp Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, 46285, and Greenfield, IN, 46140, USA

Metabotropic glutamate receptors (mGluRs) are a heterogenous family of G-protein coupled receptors that are linked to multiple second messenger systems. We report here that the conformationally constrained analog of glutamic acid, 1*S*,2*S*,5*R*,6*S*-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate (LY354740 monohydrate) binds with high affinity to mGluRs in rat forebrain homogenates (ACPD-sensitive ^{3}H-glutamate binding IC_{50} = 180 ± 52 nM) and is a highly potent agonist at group 2 mGluRs, both in the adult rat hippocampus (EC_{50} = 22 ± 3 nM) and in cells expressing recombinant human group 2 mGluRs (mGluR2 EC_{50} = 5.1 ± 0.3 nM; mGluR3 EC_{50} = 24.3 ± 0.5 nM). Unlike other selective group 2 mGluR agonists, LY354740 does not produce group 1 mGluR agonist or antagonist effects (up to 100,000 nM) either in the rat hippocampus or in cells expressing human group 1 mGluRs (mGluR1 or mGluR5). In addition, this compound is not active as an agonist or antagonist (up to 10,000 nM) at recombinant human group 3 mGluRs (mGluR4 or mGluR7) and shows no measurable ionotropic glutamate receptor affinity up to 100,000 nM. Thus, LY354740 monohydrate is a useful pharmacological agent for exploring the functional consequences of group 2 mGluR activation in vivo (see Helton et al., Kallman et al. and Tizzano et al., this meeting).



LY354740
Monohydrate

412.10

LY354740: IN VIVO PROPERTIES OF A NOVEL METABOTROPIC GLUTAMATE RECEPTOR GROUP 2 AGONIST FOR THE POTENTIAL TREATMENT OF ANXIETY
M.J. Kallman*, J.P. Tizzano, J.A. Monn, D.D. Schoepp and D.R. Helton Lilly Research Laboratories, Greenfield Laboratories, Eli Lilly and Company, Greenfield, IN 46140, USA

LY354740 monohydrate is a conformationally constrained analog of glutamate with highly selective nanomolar agonist affinity for Group 2 cAMP coupled metabotropic glutamate receptors ($mGluR_2$ and $mGluR_3$). The discovery of this novel pharmacological agent has allowed the exploration of the functional consequences of activating Group 2 mGluRs *in vivo* (see Helton et al., and Tizzano et al., this meeting). In an effort to evaluate the clinical utility of LY354740 as an anxiolytic, we examined its effects in the fear potentiated startle and elevated plus maze models of anxiety (0.1 to 10 mg/kg, oral) and compared the results with the clinically prescribed anxiolytics diazepam (ip) and buspirone (ip). In the fear potentiated startle and elevated plus maze models, LY354740, diazepam and buspirone produced significant anxiolytic activity (ED_{50} values of 0.3, 0.4, and 0.6 mg/kg for fear potentiated startle and 0.2, 0.5, and 0.2 mg/kg for the elevated plus maze, respectively). The duration of pharmacological effect for LY354740 in the efficacy models was 4 to 6 hours following administration. The effects observed were stereoselective since the (-)-enantiomer of LY354740, LY366563, was without effect in these models. In contrast to diazepam, acute administration of LY354740 did not produce sedation, cause deficits in neuromuscular coordination, interact with CNS depressants, produce memory impairment, or change convulsive thresholds at doses 100 to 1000 fold the efficacious doses in animals. Thus, LY354740 has atypical anxiolytic activity in animals comparable to diazepam. More importantly, at maximally effective doses in these models, LY354740 produced none of the unwanted secondary pharmacology associated with other currently prescribed benzodiazepines.

412.11

THE NOVEL METABOTROPIC GLUTAMATE RECEPTOR AGONIST LY354740 BLOCKS THE EFFECTS OF DIAZEPAM WITHDRAWAL ON AUDITORY STARTLE RESPONDING J.P. Tizzano*, M.J. Kallman, J.A. Monn, D.D. Schoepp and D.R. Helton Lilly Research Laboratories, Greenfield Laboratories, Eli Lilly and Company, Greenfield, IN 46140, USA

In humans, withdrawal from the chronic administration of benzodiazepines can result in a variety of undesirable side-effects, including anxiety. Previously, we have shown that benzodiazepine withdrawal produces anxiogenic effects in rats as indicated by enhanced auditory startle response (NeuroReport, 5:154-156;1993). To investigate the potential clinical utility of mGluR agonists for the treatment of benzodiazepine withdrawal symptoms, we examined the effects of the selective mGluR Group 2 ($mGluR_2/mGluR_3$) agonist, LY354740 monohydrate on the increased auditory startle response seen in rats undergoing withdrawal from the chronic administration of diazepam. Furthermore, the potential for LY354740 to produce benzodiazepine-like signs of withdrawal was evaluated under the similar experimental conditions as diazepam. Rats were exposed to diazepam (10 mg/kg/day) or LY354740 (1, 3, 10 mg/kg/day) continuously for 12 days via osmotic minipumps (sc). After 12 days the pumps were removed and the animals were allowed to go through spontaneous withdrawal. Significant increases in sensorimotor reactivity were observed on days 1, 2, 3, and 4 following cessation of diazepam. Unlike diazepam, removal of LY354740 following chronic administration did not produce signs of withdrawal. Acute treatment with either diazepam (3 to 30 mg/kg, ip) or LY354740 (0.01 to 0.3 mg/kg, po) dose-dependently blocked withdrawal-induced increases in the auditory startle response following cessation of diazepam. Thus, in addition to the anxiolytic potential of LY354740, these data suggest that LY354740 may be beneficial in the treatment of benzodiazepine withdrawal or as an intermediate drug treatment when shifting individuals from typical to atypical anxiolytics.

EXCITATORY AMINO ACID RECEPTORS: STRUCTURE, FUNCTION, AND EXPRESSION—METABOTROPIC GLUTAMATE RECEPTORS

413.1

PROTEIN KINASE C-MEDIATED DESENSITIZATION OF mGluR5 IN *XENOPUS* OOCYTES. R.W. Gereau IV* and S.F. Heinemann. Molecular Neurobiology Laboratory. The Salk Institute for Biological Studies, La Jolla, CA 92037.

Much evidence from studies using cultured neurons and brain slices indicates that metabotropic glutamate receptors (mGluRs) coupled to phosphoinositide hydrolysis desensitize in response to prolonged or repeated agonist exposure, and that this desensitization is mediated by a mechanism involving activation of protein kinase C (PKC). The present studies were undertaken to determine if this desensitization could be mediated by direct PKC-mediated phosphorylation of mGluR5.

When expressed in *Xenopus* oocytes, both mGluR5a and mGluR5b showed pronounced desensitization which recovered slowly over 30min following a 1min exposure to 100μM Glu. The extent and timecourse of desensitization were very similar for mGluR5a and mGluR5b. This desensitization was mimicked by direct activation of PKC and was blocked by selective PKC inhibition. Furthermore, the recovery from the desensitized state was prevented by phosphatase inhibitors. These data suggest that PKC-mediated phosphorylation is involved in the desensitization of mGluR5a-mediated responses observed in this system.

By constructing point mutants to knock out various PKC consensus phosphorylation sites, we provide evidence that this desensitization is likely mediated by direct PKC-mediated phosphorylation of mGluR5.

Supported by grants from the NIH and the McKnight Foundation to SFH and an NIH postdoctoral NRSA to RWG.

413.2

SYNAPTIC TRANSMISSION AND PLASTICITY IN MICE LACKING mGluR5. Y.M. Lu*[1], Z. Jia[2], J. Roder[2], J.M. Wojtowicz[1]. Dept. of Physiol.[1], Samuel Lunenfeld Res. Inst.[2], Univ. of Toronto, Toronto, Ont, Canada M5S 1A8.

mGluR5 is highly expressed in the hippocampus but its function remains unknown due to lack of selective pharmacological blockers. We used gene targeting techniques to delete mGluR5 in mice and measured synaptic transmission in hippocampal slices prepared from knockout (KO) animals and control littermates. Gross anatomical features and organization of synaptic pathways appeared normal in KO animals. Field responses in stratum radiatum or whole-cell recordings were used to measure synaptic functions. The basal level of synaptic transmission and the magnitude of the paired-pulse facilitation did not differ in the two groups, suggesting normal level of transmitter release in the KO animals. However, KO mice showed learning deficits and reduced long-term potentiation in the hippocampus (see accompanying abstract by Z. Jia *et al.*). To explore possible causes of these deficits we applied a metabotropic glutamate receptor agonist, 1S,3R ACPD (ACPD), which inhibited the evoked fEPSPs at 10-100 μM in control mice with $EC_{50} = 40\mu M$. In conrast, in KO mice EC_{50} for ACPD was increased to 300 μM indicating less effective inhibition. The effect was receptor specific since similar inhibition caused by a cholinergic agonist carbachol ($EC_{50} = 5\mu M$) was not affected. Whole-cell current clamp recordings from CA1 pyramidal neurons showed decrease of evoked EPSPs and membrane depolarization during applications of 50 μM ACPD in control but not in KO mice. Evoked population spike in stratum pyramidale was enhanced by 50 μM ACPD in the control but not in KO mice. We conclude that mGluR5 may be involved in modulation of neuronal excitability and long-term potentiation in the hippocampus. Supported by MRC and NCE of Canada.

413.3

REDUCED HIPPOCAMPAL LTP AND IMPAIRED SPATIAL AND CONTEXTUAL LEARNING IN MICE LACKING mGluR5. Z. Jia[1], Y.M. Lu[2], J.M. Wojtowicz[2], W. Abramow-Newerly[1], J. Henderson*[1], F. Taverna[1], R. Gerlai[1], C. Janus[1] and J. Roder[1]. Samuel Lunenfeld Research Inst., Mt. Sinai Hospital[1], and Dept. of Physiology[2], University of Toronto, Toronto, Ont. Canada M5S 1A8.

Metabotropic glutamate receptors (mGluR) have been implicated in a variety of physiological phenomena including synaptic plasticity, for example, long-term potentiation (LTP) and learning and memory. While it has been clearly shown that mGluR1, a member of group I mGluRs coupled with G-protein linked to PI pathway, is important in cerebellar long-term depression and motor coordination, the role of mGluR5, the other known member of group I mGluRs, remains obscure. Since there is no available agonist and antagonist specific to mGluR5, and since it is highly expressed in hippocampus suggesting its role in hippocampal dependent functions, we have used gene targeting techniques to make transgenic mice lacking mGluR5. This allows us to investigate the roles of mGluR5 specifically in synaptic physiology and behaviour. We show here that the mGluR5 mutant mice exhibit substantially reduced LTP in both CA1 region and dentate gyrus of hippocampal slices, whereas synaptic responses, the paired-pulse facilitation, and LTP in the CA3 region of the hippocampus are normal. mGluR5 mutant mice also showed impaired learning in both spatial and contextual performance, whereas other activities including motor coordination were normal. Our data suggests crucial roles of mGluR5 in regulating NMDA-dependent LTP and in learning and memory. Details of synaptic responses and behaviour of mGluR5 mutant mice will be discussed. Also, see accompanying abstract by Y. Lu *et al.* Supported by MRC and NCE of Canada.

413.4

CLONING AND FUNCTIONAL CHARACTERIZATION OF DROSOPHILA METABOTROPIC GLUTAMATE RECEPTORS. M. L. Parmentier, V. Brand, J. P. Pin, J. Bockaert* and Y. Grau. UPR 9023, C.C.I.P.E. Montpellier, FRANCE.

The excitatory neurotransmitter glutamate plays very important roles in the mammalian brain. To mediate its effects, glutamate activates two types of receptors, ligand-gated channels and metabotropic receptors coupled to G-proteins (mGluRs). Both families of glutamate receptors share no sequence homology and possess original structural features compared to other ligand-gated channels and G-protein coupled receptors respectively. Glutamate-gated receptor-channel subunits have already been characterized in invertebrates. Here, we report the cloning of two cDNAs coding for proteins homologous to metabotropic glutamate receptors in *Drosophila melanogaster*: DmGluRA and DmGluRB. DmGluRA proteic sequence is 43% and 45% identical to its mammalian homologues mGluR2 and mGluR3 respectively. When expressed in Human Embryonic Kidney 293 cells, it displays a very similar pharmacology and transduction mechanism than mGluR2 and 3. In situ hybridization data show that DmGluRA RNAs are expressed in the central nervous system of the late embryo. These results indicate that the original structural and functional features of all glutamate receptors types are conserved from insects to mammals and suggest that such receptors trigger fundamental functions highly conserved during evolution.

This work was supported by grants from the CNRS, CEE (BIO2-CT93-0243 and PL95-0228), the french ministry of Education, Research and Professional Insertion (ACC-SV5, n°9505077) and Bayer company (Wupertal; Germany).

413.5

MOLECULAR CLONING AND FUNCTIONAL EXPRESSION OF A NOVEL HUMAN METABOTROPIC GLUTAMATE RECEPTOR, mGluR8. R.T. Simin[1], L.G. Hammerland[1], D. Riccardi[2], K.J. Krapcho[1], F. Fuller[1], S.C. Hebert[2] and T.M. Stormann*[1]. [1]NPS Pharmaceuticals, Inc., Salt Lake City, UT 84108 and [2]Renal Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115.

The metabotropic glutamate receptors (mGluRs) and calcium receptors (CaRs) make up a family of G-protein-coupled receptors with unique structural features. In an effort to identify novel mGluR/CaR sequences, degenerate PCR primers were designed against regions that are highly conserved between mGluRs and CaRs. These primers were used to amplify from human genomic DNA, and PCR products within an expected size range were cloned. Sequence analysis of these clones resulted in the identification of a novel mGluR sequence. Northern blot analysis revealed that a hybridizing 3.8 Kb mRNA was expressed at low to moderate levels throughout the human brain. The highest levels of expression were detected in subthalamic nucleus and cerebral cortex. A full length, 3.8 Kb cDNA clone was isolated from a human cerebral cortex cDNA library and subsequently identified as the human homologue of mouse mGluR8 (Duvoisin et al., *J. Neuroscience* 15:3075, 1995). Both mouse and human mGluR8 cDNAs encode 908 amino acid proteins that share an amino acid identity of 97.5% up to residue 894, at which point the sequences diverge for the remaining 15 amino acids. PCR experiments indicate that this divergence is the result of alternative splicing and that two splice variants are expressed in human retina, subthalamic nucleus and cerebral cortex. Functional activity of the human mGluR8 cDNA was assessed in *Xenopus* oocytes. Application of L-glutamate or (S)-2-amino-4-phosphonobutyric acid (L-AP4) evoked inwardly-rectifying potassium currents in oocytes co-injected with cRNAs for human mGluR8 and the rat GIRK/CIR potassium channel subunits. Oocytes injected with the GIRK/CIR cRNAs alone did not respond to these agonists. Further pharmacological characterization of human mGluR8 is currently underway.

Supported by NPS Pharmaceuticals, Inc.

413.6

ANATOMICAL DISTRIBUTION OF METABOTROPIC GLUTAMATE RECEPTOR MGLUR8 EXPRESSION AND ITS PHYSIOLOGICAL REGULATION IN THE OLFACTORY BULB. N. Min*, H. Baker and R. M. Duvoisin, Margaret M. Dyson Vision Res. Inst. and Burke Med. Res. Inst., Cornell University Medical College, New York, NY.

Eight metabotropic glutamate receptors have been identified, including mGluR8 (Duvoisin et al., 1995), whose function remains unknown. First, an in-situ hydridization study was performed to determine the anatomical distribution of mGluR8 gene expression in the rodent brain. Strong expression was found in the olfactory bulb, and widespread expression was detected in many parts of the brain. For a more detailed mapping of mGluR8 expression, a polyclonal antibody was raised against a bacterial fusion protein, and comparisons between the distribution of mGluR8 message and protein were performed. Since there is a strong expression of mGluR8 in the olfactory bulb, its regulation was studied in a sensory deprivation paradigm. Unilateral naris closure causes odor deprivation, and as a result it induces several biochemical changes, for instance a profound down-regulation of tyrosine hydroxylase expression in the glomerular layer of the olfactory bulb. Since glutamate is the neurotransmitter used by the olfactory epithelium, we are investigating whether glutamate receptor levels are also affected. Preliminary results indicate an alteration of mGluR8 expression. The understanding of the functional roles of the mGluR8 will further our knowledge of glutamate neurotransmission in physiological and pathological conditions.

(Supported by EY09534, AG09686, RPB, Rudin Foundation and New York Academy of Medicine).

413.7

FUNCTIONAL COUPLING OF mGluR6 TO TRANSDUCIN.
K. Wenq*, C.-C. Lu, L.P. Daggett, G. Velióelebi, E.C. Johnson, and P.R. Robinson. SIBIA Neurosciences, Inc., La Jolla, CA 92037 and Dept. of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21228

Overlapping cDNA fragments isolated from human retinal cDNA libraries were used to construct a full-length hmGluR6 cDNA. The deduced amino acid sequences of the hmGluR6 receptor is 877 residues which is 6 amino acids longer than the rat mGluR6. Comparison of the amino acid sequences of human and rat mGluR6 revealed an overall divergence of 5.7%. In rat retina, mGluR6 has been shown to be exclusively expressed in ON-bipolar cells.

The cDNA encoding hmGluR6 and an 8-amino acid antibody epitope (1D4) was transiently transfected in COS-7 cells. Membranes prepared from transfected COS-7 cells were used in an in vitro transducin assay. Transducin is the rod photoreceptor specific G-protein. Activation of transducin was observed by transducin-dependent GTPgammaS binding assay. The activation by 1 mM Glutamate was comparable to light-activated wild-type rhodopsin at a similar concentration of receptor.

Our results demonstrate that hmGluR6 can couple to transducin. The rat homolog of mGluR6 has been shown to be expressed exclusively in retinal on-bipolar cells. Thus, these results support the hypothesis that the transduction machinery in on-bipolar cells is similar to that in photoreceptors. Furthermore, since mGluR6 is a G-protein coupled receptor that does not have substantial sequence homology to opsin, our results suggest a novel coupling of a non-rhodopsin G-protein coupled receptor to transducin.

Supported by NEI Grant EY10205-01 to P.R.R.

413.8

ENHANCED, BILATERAL EXPRESSION OF mGLUR3 IN THE RAT SPINAL CORD AFTER UNILATERAL UV-IRRADIATION OF THE HINDPAW. S.J. Boxall, [1]A. Berthele, [2]D. Laurie, [2]B. Sommer, [1]W. Zieglgänsberger, L. Urban*, [1]T.R. Tölle. Dept. of Pharmacology, Sandoz Inst. for Med. Res., London WC1E 6BN, UK, [1]Clin. Neuropharm., Max Planck Inst. of Psychiatry/Clin. Inst., Munich, Germany and [2]Dept. of Mol. Biology, Sandoz Pharma, Basel, Switzerland.

Metabotropic glutamate receptors (mGluR) play a role in the development of spinal hyperexcitability in inflammation. For further characterisation of the particular subtypes involved, we have monitored the distribution of mGluR1-5 and 7 in the rat spinal cord during the development of inflammatory hyperalgesia.

Lumbar segments of the spinal cord were removed from age matched control and UV treated Sprague-Dawley rat pups (postnatal day 11, 12 and 13). Bilateral mechanical hyperalgesia was induced on day 9 by exposure of the glabrous surface of the left hindpaw to a UVA light source for 90 secs in two sessions within an 18 hour interval. Pups were sacrificed one, two or three days later. *In situ* hybridisation with specific oligonuceotide probes was used to localise mGluR mRNA in the spinal cord. Silver grain density from emulsion-dipped sections was analysed using a computer-assisted image analyser. mGluR mRNA was seen in all areas of the spinal cord with distribution being specific for each subtype. No changes have been seen in the levels of expression of mGluR1, 2, 4, 5 and 7 in spinal cords taken from hyperalgesic animals. However, a significant increase in the expression of mGluR3 mRNA in large neurones in the superficial dorsal laminae and in interneurones in the deep dorsal horn was seen one day after the onset of inflammation on both sides. The increase was most pronounced in laminae III and IV but was transient and had disappeared by the third day after the onset of inflammation. These observations suggest that under conditions of inflammation, the expression of mGluR3 is enhanced and that these receptors may participate in the development or regulation of plastic changes within the spinal cord.

413.9

NEURECTOMY OF THE SCIATIC NERVE DIFFERENTIALLY AFFECTS EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE mRNA IN RAT MOTONEURONS. T.R. Tölle[a,b], J.M.H. Anneser[a], A. Berthele[a], D.J. Laurie[c], B. Sommer[c], J.M. Castro-Lopes[d], B. Conrad[b], W. Zieglgänsberger*[a]. [a]Max-Planck-Institute of Psychiatry/Clin. Inst., 80804 Munich, [b]Dept. of Neurology, Tech. University, 81675 Munich, Germany; [c]Sandoz Pharma AG, 4002 Basle, Switzerland; [d]Inst. of Histology and Embryology, 4200 Porto, Portugal.

Previous studies indicated that neurectomy (NCT) increases the susceptibility of motoneurons (MN) to glutamate induced neurotoxicity and downregulates NMDAR1 receptor mRNA. To characterize the involvement of metabotropic glutamate receptors we have monitored the distribution of mGluR (1-5,7) mRNA in MN following 1,2,3 and 4 weeks (w) of sciatic nerve transsection in adult rats. The spinal cord lumbar enlargements (N=4 each group) were processed with specific oligonucleotides for the detection of mGluR 1-5,7 mRNA with in situ hybridization techniques. From emulsion dipped slices (14 µm) silver grain densities over neuron profiles in the sciatic nerve alpha-motoneuron (MN) pool were counted, averaged (N=40 MN per animal and oligonucleotide) and compared to control animals and contralateral values of NCT animals. MN in control animals expressed mGluR 1,4 and 7 mRNA. NCT did not change the overall number of MN on the ipsi- or contralateral side. In ipsilateral MN, a transient swelling with a maximum after 1w and a return to normal cell size after 4w was observed. The mGluR1 mRNA signal was step-wise reduced to 55±8% of control levels after 1w NCT and 26±4% after 4w NCT. Following 1w of NCT the mGluR4 signal showed a initial decline to 22±3% with no apparent further decrease. During the whole observation period of NCT the mGluR7 mRNA was not significantly altered. An en passant observation concerned stable expression of all mGluR subunits in the dorsal horn of the spinal cord. The differential regulation of mGluR subtypes may be part of an adaptive cell program that helps to rescue adult motoneurons from excitotoxic cell death during the stress of peripheral deafferentation. (Supported by SFB 391).

413.10

EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE MESSENGER RNA IN THE HUMAN CEREBRAL AND CEREBELLAR CORTEX. A. Berthele*[a], S. Platzer[a], D.J. Laurie[c], B. Sommer[c], T. Arzberger[b], A. Weindl[b], B. Conrad[b], W. Zieglgänsberger[a], T.R. Tölle[a,b]. [a]Max-Planck-Institute of Psychiatry, Clinical Institute, 80804 Munich, Germany; [b]Dept. of Neurology, Technical University, 81675 Munich, Germany; [c]Sandoz Pharma AG, 4002 Basle, Switzerland.

Glutamate mediates excitatory neurotransmission by activating ionotropic receptor channels as well as G protein coupled metabotropic receptors. Currently, eight subtypes of the metabotropic glutamate receptor family (mGluR) have been characterized in rodents and corresponding sequences for the human mGluR1 to 5 subtypes (hmGluR1-5) have recently been described. Here we report the expression patterns of mRNAs encoding hmGluR1 to 5 subtypes in the human cerebral and cerebellar cortex as examined by *in situ* hybridization with specific oligonucleotide probes. Human brain tissues (N=5) were obtained at autopsy from patients with no history of neurological or psychiatric disease. In frontal cortex, considerable amounts of hmGluR1, 2, 3 and 5 mRNA were expressed, whereas hmGluR4 mRNA was not detectable. Cellular resolution revealed a differential distribution of these hmGluR mRNAs in different cortical laminae with overall levels of hmGluR1 and 2 transcripts being much lower than those of hmGluR3 and 5. In cerebellar cortex, hmGluR1 and 4 mRNA expression was most abundant with high amounts of mRNA detectable in Purkinje and granule cells; mRNA encoding the hmGluR4 subtype was additionally expressed in Bergmann glia cells. hmGluR2 transcripts were exclusively detected in Golgi cells, whereas hmGluR3 mRNA expression was prominent in Golgi cells as well as in Bergmann glia. The hmGluR5 signal was moderate in Bergmann glia and low in Purkinje cells.

The present data show that hmGluR mRNA is differentially distributed in human brain. However, expression patterns of mGluR mRNA are highly conserved between corresponding structures of human and rodent CNS. (Supported by SFB 391).

413.11

DEPLETION OF GLUTAMATERGIC PRIMARY INPUT TO RAT OLFACTORY BULB MODULATES THE EXPRESSION OF *c-fos* AND mGluR1a mRNAs. P.Bovolin*, N. Ferraris, S. De Marchis, I. Perroteau, A. Fasolo. Dept. Animal Biology, Univ. of Torino, 10123 Torino, Italy.

Glutamatergic primary olfactory neurons located in the nasal mucosa send their axons to the glomerular layer of the olfactory bulb (OB) where they make synapses with relay neurons, mitral and tufted (M/T) cells, and with periglomerular (PG) interneurons. We have previously demonstrated that deafferentation by nasal irrigation with 0.17 M $ZnSO_4$ produces a 50%-60% reduction in TH mRNA, expressed by PG neurons, accompanied by a 40% decrease of the $\gamma 2$ $GABA_A$ receptor subunit mRNA, expressed by M/T cells. On the opposite, the mRNA expression of the ionotropic glutamate receptor subunit GluR1 and NMDAR1 remains unaltered. In the present work we examined 1) the possible involvement of *c-fos* in the neuronal plasticity mechanisms occurring in response to glutamatergic input loss, and 2) the mRNA content of two different splicing forms of the metabotropic glutamate receptor 1 expressed by M/T cells, mGluR1a and mGluR1b. Control and $ZnSO_4$ bilaterally irrigated rats were sacrificed 2 and 16 days after the lesion. Both morphological analysis and quantitative RT-PCR have been performed on olfactory mucosae and OB. First we measured the expression of the Olfactory Marker Protein (OMP), which is exclusively expressed by mature olfactory neurons and whose mRNA is transported along the axonal processes to the OB. OMP mRNA quantitation showed a 60% decrease in the lesioned mucosae and 70-90% decrease in the correspondent OBs. *c-fos* mRNA in the OB was 30% lower at 2 days and 60 % higher at 16 days post-lesion. The mRNA for mGluR1a form was 40% reduced in lesioned rats, while the b form did not show any significant change. In conclusion, the long-term up-regulation of *c-fos* mRNA and the expression regulation of selected neurotransmitter receptors following glutamatergic deafferentation suggest an on-going circuitry adaptation of the OB directed by modulatory events at the transcriptional level. (Supported by grants from Italian MURST).

413.12

DIFFERENTIAL LOCALIZATION OF METABOTROPIC GLUTAMATE RECEPTORS IN CULTURED HIPPOCAMPAL NEURONS J.N. Stowell*, M.E. McGrath and A.M. Craig. Dept. of Cell and Structural Biology, University of Illinois, Urbana, IL 61801.

Metabotropic glutamate receptors (mGluRs) can be functionally and pharmacologically divided into three groups; group I is positively coupled to phospholipase C, while groups II and III are negatively coupled to adenylyl cyclase. Some function as autoreceptors pre-synaptically while others act at postsynaptic sites along with ionotropic receptors. To study the subcellular targeting of mGluRs, wild-type or epitope-tagged cDNAs of mGluR1α (group I), mGluR2 (group II), and mGluR7 (group III) were expressed from defective herpes simplex virus vectors in cultured embryonic rat hippocampal neurons. mGluR1α exhibited a marked colocalization with the endogenous AMPA-selective ionotropic receptor GluR1. Dendritic spines that were immunopositive for mGluR1α were also immunopositive for GluR1, and there was a correlation in the relative intensities of immunofluorescence at individual synapses(r=0.72,n=339). These results suggest that the colocalization of these unrelated glutamate receptors may be regulated by similar mechanisms, with the consequence that their coactivation may contribute to many forms of synaptic plasticity. mGluR1α was targeted to the somatodendritic domain but excluded from axons of mature neurons. Myc-tagged mGluR2 exhibited a similar distribution to mGR1α. In contrast, myc-tagged mGluR7 was present throughout the neuron, in the axon as well as the dendrites, suggesting a presynaptic function. Analysis of chimeric proteins may lead to the identification of subcellular localization signals.
Supported by NIH and the Markey Charitable Trust.

413.13

GROUP-I METABOTROPIC GLUTAMATE RECEPTORS (mGluRs) AND APOPTOSIS BY TROPHIC DEPRIVATION IN CULTURED CEREBELLAR GRANULE CELLS. F. Nicoletti[1,2*], A. Copani[3], G. Casabona[2,3], V. Bruno[2,3], A. Caruso[4], D.F. Condorelli[4], R. Kuhn[5] and T. Knöpfel[1]. Institutes of Pharmacology, [1]School of Pharmacy or [3]Medicine, and [4]Biochemistry, Catania University, Italy; [2]I.M.N. "Neuromed", Pozzilli, Italy; [5]CIBA-GEIGY, Basel, Switzerland.

Cultured cerebellar granule cells grown in "low-K^+" (10 mM) containing medium undergo apoptotis after 4 DIV and express high levels of mGluR5, which appears early in the development. Expression of mGluR1a is instead observed at later stages of maturation and predominates in cultures grown in 25 mM K^+. In cultures grown in 10 mM K^+ at 4 DIV, mGluR5 expression was restricted to the population of viable cells and was not observed in cells which had already entered the executive phase of apoptosis. In contrast, at this age, mGluR1a was exclusively present in apoptotic cells. Enzymatic depletion of extracellular glutamate or addition of the mGluR antagonist, MCPG, increased the percentage of apoptotic neurons in cultures grown in 10 mM K^+. NMDA receptor antagonists, as well as preferential antagonists of group II or -III mGluRs were ineffective. We speculate that activation of mGluR5 protects granule cells against apoptosis by trophic deprivation during the first 4 DIV in cultures. Transfection with antisense oligonucleotides or mGluR5 cDNA is currently carried out to verify this hypothesis.

413.14

EXPRESSION AND COUPLING TO PHOSPHOINOSITIDE (PI) HYDROLYSIS OF GROUP-I METABOTROPIC GLUTAMATE RECEPTORS (mGluRs) IN EARLY POSTNATAL AND ADULT RAT BRAIN. G. Casabona[1,2], R. Parenti[3], M.V. Catania[4*], R. Kuhn[5], T. Knöpfel[6], F. Gasparini[5], F. Cicirata[3], and F. Nicoletti[2,6]. Institutes of Pharmacology, [1]School of Medicine or [6]Pharmacy, and [3]Physiology, Catania University, Italy; [2]I.M.N. "Neuromed", Pozzilli, Italy; [4]I.B.F.S.N.C., CNR, Catania, Italy; [5]CIBA-GEIGY, Basel, Switzerland.

We have studied the expression of group-I mGluR subtypes by Western blot analysis in different brain regions of both adult and 9 day-old (PND9) rats. A high level of expression of mGluR5 protein was found in all the brain regions examined at PND9. mGluR5 expression was reduced in adult tissue, and this reduction was more prominent in the cerebellum and hypothalamus than in the cerebral cortex, corpus striatum, olfactory bulb and hippocampus. mGluR1a showed a different pattern of expression and, at least in the cerebellum, substantially increased with age. mGluR5 expression was well correlated with the extent of the PI response to mGluR agonists in brain slices, which was uniformely high at PND9 and was negligible in the adult hypothalamus and cerebellum. Cyclopropan [b]chromen-1a-carboxylic acid, at concentrations which antagonized mGluR1- but not mGluR5-mediated responses in recombinant cells, did not affect the stimulation of PI hydrolysis by quisqualate or $1_S,3_R$-ACPD in brain slices from PND9 or adult rats. We conclude that it is mGluR5 which mediates the PI response to mGluR agonists in brain tissue.

413.15

CHANGES IN mRNA FOR METABOTROPIC GLUTAMATE RECEPTORS AFTER IN UTERO HYPOXIA-ISCHEMIA. A. Simonyi, H.C. Scott* and G.Y. Sun Dept. Biochem. and Pathology, Univ. Missouri, Columbia, MO, 65212

The expression of the metabotropic glutamate receptor 1 (mGluR1) and 5 (mGluR5) mRNA was studied by in situ hybridization in the developing rat hippocampus after in utero hypoxic-ischemic insult. Both receptors are coupled to the phosphoinositide (PI) signaling pathway and appear to be particularly active during long term processing of neuronal plasticity and neurodegeneration. In utero hypoxia-ischemia was induced by clamping the uterine blood vessels of near-term fetuses for 10 min. Fetuses were delivered surgically, resuscitated and raised by foster mothers until 7 (P7) or 14 (P14) days. Results from in situ hybridization indicated a decrease in the level of mGluR1 mRNA in the dentate gyrus of the 7 day-old animals. From P7 until P14, the mGluR1 mRNA expression was increased and similar levels were attained by both the control and the ischemic groups by 14 days. During the same period of time, mGluR5 mRNA showed a constant high expression in the CA1 region of the control animals and a significant increase of mRNA levels in the ischemic group. Since long-term potentiation (LTP) activity is substantially increased between P7 and P15, our results suggest that in utero hypoxia-ischemia can alter the temporal expression pattern of the metabotropic glutamate receptors during postnatal hippocampal development (supported in part by the Children's Miracle Network Telethon Research Fund, University of Missouri).

413.16

APOPTOTIC DEATH IN CHO CELLS EXPRESSING METABOTROPIC GLUTAMATE RECEPTORS COUPLED WITH PHOSPHOLIPASE C. S. Pshenichkin*, A. Surin, E. Surina and J.T. Wroblewski. Department of Pharmacology, Georgetown Univ. Sch. of Med., Washington, D.C. 20007

Metabotropic glutamate receptors (mGluRs) coupled to the activation of phospholipase C and to subsequent release of calcium from intracellular stores (group I) were reported to contribute to glutamate neurotoxicity. We examined the viability of Chinese Hamster Ovary (CHO) cell lines stably transfected with DNA encoding for mGluR1a and mGluR5a receptors. The expression of the respective receptors in CHO cells was established by PCR, immunoblotting and functional measurements of phosphoinositide hydrolysis and intracellular calcium release. The agonist selectivity of the transfected receptors was similar to that reported for mGluR1 and mGluR5 in other studies. Cell viability was assessed by MTT reduction and by fluorescent staining. Cells transfected with mGluR1a showed a decreased life span after reaching confluency in contrast to cells expressing mGluR5a and to untransfected cells. Cells transfected with mGluR1a showed a decreased rate of protein biosynthesis as assessed by ^{3}H-arginine incorporation, as compared to untransfected and mGluR5a-transfected cells. DNA analysis performed by agarose gel electrophoresis and ethidium bromide staining revealed a pattern of fragmentation typical of apoptosis in mGluR1a-transfected cells but not in cells expressing mGluR5a. Cell cultures expressing mGluR1a, but not those expressing mGluR5a, also showed increased accumulation of lactate in the medium accompanied by decreased pH, suggesting a profound alteration of oxidative metabolism. The addition of glutamate (1 mM) to the medium was strongly toxic to cells expressing mGluR1a but not to those expressing mGluR5a. However, cells expressing mGluR1a were not protected by mGluR antagonists 4-carboxy-3-hydroxyphenylglycine or α-methyl-4-carboxy-phenylglycine (up to 1 mM). Moreover, 4-carboxyphenylglycine, the mGluR1-selective antagonist not only failed to protect from toxicity but, when used alone, was toxic in cells expressing mGluR1a receptors. These results suggest functional differences between the two phospholipase C-coupled mGluRs and point to mGluR1, rather than mGluR5, as the receptor contributing to apoptotic cell death. *(Supported by NIH grants NS28130 and NS01720.)*

413.17

MODULATION OF INTRACELLULAR CALCIUM SIGNALS EVOKED BY PHOSPHOLIPASE C-COUPLED AND CHIMERIC METABOTROPIC GLUTAMATE RECEPTORS EXPRESSED IN HEK-293 CELLS.

A. Surin*, S. Pshenichkin, E. Surina and J.T. Wroblewski. Department of Pharmacology, Georgetown Univ. Sch. of Med., Washington, D.C. 20007

Metabotropic glutamate receptors mGluR1 and mGluR5 stimulate phospholipase C leading to an increase of IP_3 level and to Ca^{2+} release from intracellular stores. The specific roles of mGluR1 and mGluR5 in intracellular Ca^{2+} homeostasis are poorly understood. Using Fura-2 fluorescence imaging, we examined the increases of intracellular free Ca^{2+} concentrations ($[Ca^{2+}]_i$) induced by mGluR agonists in HEK-293 cells transiently transfected with rat mGluR1a, and mGluR5a receptors. We also constructed two chimeric receptors in which the N-terminal extracellular domain of mGluR1a was substituted with the N-terminal domains of either mGluR2 (mGluR2/1a chimera) or mGluR3 (mGluR3/1a chimera). These receptors, expressed in HEK293 cells conserved the coupling characteristic for mGluR1 and induced $[Ca^{2+}]_i$ increases while their pharmacology was similar to that reported for unmodified mGluR2 and mGluR3 receptors. The time patterns of Ca^{2+} mobilization induced by glutamate, quisqualate and *trans*ACPD were different in cells transfected with mGluR1a and mGluR5a. Activation of mGluR5a evoked $[Ca^{2+}]_i$ oscillations, while mGluR1a induced only transient $[Ca^{2+}]_i$ increases. Activation of mGluR2/1a and mGluR3/1a chimeras caused transient $[Ca^{2+}]_i$ increases similar to those evoked by mGluR1a. Cyclothiazide (CTZ), known as a blocker of AMPA receptor desensitization, inhibited *trans*ACPD-induced elevation of $[Ca^{2+}]_i$ in cells transfected with mGluR1a, while only slightly increased the period of $[Ca^{2+}]_i$ oscillations with no effect on their amplitudes in mGluR5a transfected cells. CTZ also inhibited phosphoinositide hydrolysis in cells transfected with mGluR1a but not with mGluR5a, and was without effect on cAMP-coupled mGluR2 receptor. In contrast, CTZ inhibited $[Ca^{2+}]_i$ increases induced by mGluR2/1a and mGluR3/1a chimeras. These results suggest that the modulatory effect of CTZ is not determined by interactions with the N-terminal extracellular domain of mGluR1a but possibly with one of the extracellular loops which are preserved in the chimeric receptors. *(Supported by NIH grants NS28130 and NS01720.)*

413.18

DEVELOPMENT OF MONOCLONAL ANTIBODIES TO THE mGLUR1 ALPHA RECEPTOR.

S. Singh*[1], C.Wu[1], L. Shi[1], J. Wei[1], P. Dias[1], R.S. Petralia[2], Y.-X. Wang[2], and R.J. Wenthold[2]. [1]PharMingen, 10975 Torreyana Road, San Diego, CA 92121; [2]NIDCD/NIH, 36 Convent Drive, Bethesda, MD 20892-4162.

Molecular cloning and pharmacological studies have identified eight metabotropic glutamate receptor (mGluR) subtypes, mGluR1-8. mGluR1, consists of three splice variants, mGluR1a, mGluR1b, and mGluR1c, differing at the carboxy terminus. Monoclonal antibodies were generated against C-terminus domain (aa 842-1200) of human mGluR1a receptor protein. Several clones which specifically reacted in wetsern blot with mGluR1a protein in rat brain synaptic plasma membrane and transfected mammalian cells were tested for reactivity in immunocytochemistry using rat brain sections. Overall distribution of mGluR1 immunostaining using one of these clone, G209-1590, was similar to that described using polyclonal C-terminus antibodies (Martin et al., Neuron 9, 259, 1992; Baude et al., Neuron 11, 771, 1993); e.g., abundant staining was found in the olfactory bulb, globus pallidus, thalamus, and Purkinje and Golgi cells of the cerebellum. Ultrastructural studies of the hippocampus showed that immunostaining was prevalent in the postsynaptic portion of synapses, but was absent from the synaptic cleft and presynaptic terminal. Stained synapses were found on dendrite shafts and occasionally on dendrite spines. Staining was found in patches throughout the cytoplasm of neuron somas, as described with other glutamate receptor antibodies, and was particularly prevalent in portions of the endoplasmic reticulum.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS II

414.1

DISTRIBUTION OF ρ_1 and ρ_2 GABA RECEPTOR SUBUNIT TRANSCRIPTS IN THE RAT BRAIN

K. Wegelius[1], M. Reeben[2], M. Saarma[2], K. Kaila[1]* and M.Pasternack[1]
[1]Dept. of Biosciences, Div. of Animal Physiology, P.O.Box 17 and [2]Institute of Biotechnology P.O.Box 56, FIN-00014 University of Helsinki, Finland.

The ρ_1 and ρ_2 GABA receptor subunits form pharmacologically distinct GABA receptors with properties similar the so-called $GABA_C$ receptors observed in vertebrate retinal interneurons. So far the physiological significance of these receptors is poorly understood.

We have cloned the full cDNA of the rat ρ_1 subunit and a 750 bp segment of the rat ρ_2 subunit, and studied the expression of the transcripts of these subunits in different areas of the rat CNS using *in situ* hybridization, Northern blotting, and RT-PCR techniques.

ρ_1 mRNA was detected with Northern blotting exclusively in the retina, whereas a 2.0 kb and a 2.3 kb ρ_2 mRNA was found in retina, hippocampus and superior colliculus (SC). In cortex only a 2.0 kB ρ_2 mRNA could be detected. *In situ* hybridization showed a high level of ρ_2 expression in the retinal inner nuclear and external plexiform layers. Interestingly a relatively high level of ρ_2 expression was seen in a limited subset of cells in the dorsal lateral geniculate nucleus (DLG) and in the superficial gray layer of the SC. ρ_2 mRNA was also detected in area CA1 of the hippocampus and at a low level in the layer 6 of the visual cortex. RT-PCR combined with Southern blotting revealed ρ_1 mRNA expression in hippocampus, cortex, brain stem, thalamus, SC and dorsal root ganglia. Our preliminary findings also indicate higher expression of ρ_1 mRNA in the 8-day old rat hippocampus as compared to the adult. The RT-PCR results were normalized with light neurofilament PCR results obtained from the same specimen.

Our results show that ρ_1 and ρ_2 subunits are expressed in the retina, hippocampus and in formations pertaining to the visual pathway. These findings may be of importance in elucidating the functional role of the GABA receptors formed from ρ_1 and ρ_2 subunits.

Supported by the Academy of Finland.

414.2

GABA, GAD, AND $GABA_A$-RECEPTORS IN THE RAT PARABRACHIAL COMPLEX.

A. Guthmann[1], J.-M. Fritschy[2], O.P. Ottersen[3], R. Torp[3], K. Feil[1]* & H. Herbert[1], [1]Dept. Animal Physiol., Univ. of Tübingen, Germany; [2]Dept. Pharmacol., Univ. of Zürich, Switzerland; [3]Anatom. Inst., Univ. of Oslo, Norway.

While the afferent and efferent connections of the Parabrachial (PB) and Kölliker-Fuse nuclei (KF) are fairly well known, there is still a lack of knowledge as to the neurotransmitters and their receptors in these anatomically distinct nuclei. In the present study we sought to investigate whether the inhibitory amino acid GABA might participate in signal transduction within the PB/KF. ***GABA-immunoreactivity (-ir)*** was demonstrated on semithin plastic sections using polyclonal rabbit anti-GABA antisera and the PAP-technique. ***GAD65-mRNA*** was demonstrated on cryostat sections by using riboprobes (cDNA GAD clones, courtesy of A.J. Tobin, Brain Res. & Mol. Biol. Inst. Los Angeles) and the DIG-technique. ***$GABA_A$-receptor subunits*** were demonstrated on frozen sections by using polyclonal antisera raised against various $GABA_A$-subunits and the Avidin-Biotin-technique.

Our data show that all nuclei of the PB/KF-complex are densely innervated by GABA-ir axons. Only slight differences in staining intensity were observed between medial vs. lateral PB nuclei and the KF. GAD65 positive somata, indicative for GABAergic neurons, were found only in small numbers caudally in the PB/KF and embedded in the ventral aspect of the superior cerebellar peduncle. The remaining nuclei of the PB were largely devoid of GAD65 positive cells. Immunocytochemical staining for the $\beta_{2/3}$-subunit of the $GABA_A$-receptor revealed a discrete pattern of expression. Most prominent staining was observed in the dorsal lateral PB and in the waist area while the remaining PB nuclei and the KF showed a moderate staining intensity. In contrast, the external lateral PB was totally devoid of $\beta_{2/3}$-ir. Stainings for other $GABA_A$-subunits are in progress. Our data strongly suggest that the inhibitory amino acid GABA plays an important role in neurotransmission in the PB/KF. *Supported by Graduiertenkolleg Neurobiologie Tübingen and DFG He 1842/6-1*

414.3

NEUROCHEMICAL DIFFERENTIATION OF GABAERGIC AMACRINE CELLS IN THE FISH RETINA.

H.-J. Wagner, B. Nordmann and U. D. Behrens*.
Anatomisches Institut, Universität Tübingen, D-72074 Tübingen, Germany

GABA is the dominant neurotransmitter of the inner plexiform layer, used by 30-50% of the amacrine cells (ACs). In view of the morphological diversity of this class of retinal cells, we used the pattern of colocalisation with various neuropeptides to study the heterogeneity of GABAergic ACs.

Immunohistochemical localisation of GABA was performed on cryostat sections of cyprinid retinae using a polyclonal antiserum (guinea pig) which was visualised with DTAF. The same sections were then incubated with one of the following polyclonal antisera (rabbit): SP, NPY, CGRP, met-ENK, VIP, and SST, and visualised with Texas Red. Colocalisation of a peptide and GABA was detected with a Zeiss LSM 410 and an image analysis feature detecting overlap of the two spectral channels. For a quantitative evaluation, a minimum of 1,000 GABA-ir perikarya (for each of the peptides) were counted in radial sections of various retinal locations and tested for colocalisation.

Between 3% (CGRP) and 1% (VIP, NPY, met-ENK, SST) of all GABAergic cells are colocalised with the respective neuropeptide. Conversely, about 80% of the peptidergic cells are immunoreactive for GABA. The ramification pattern and fine structure specific for each neuropeptide allowed a further differentiation of the GABAergic ACs. The double immunoreactive ACs ramify most strongly in sublayers 1, 3 and 5, with CGRP-and SP-ir cells being monostratified (sl3), and ENK-, SST-, VIP-, and NPY-ir cells trilaminar. In addition, the individual peptidergic cells are characterised by the calibre of their processes and the density and size of varicosities.

These investigations provide an approach to further analyse the "GABAergic system" of the retina. They clearly demonstrate that this "system" is morphologically highly differentiated and suggest that this is reflected on the functional level.

Supported by a grant from the Deutsche Forschungsgemeinschaft (Wa 348/14)

414.4

IMMUNOCYTOCHEMICAL LOCALIZATION OF TAURINE AND GLUTAMATE IN THE PERIPHERAL OLFACTORY SYSTEM.

A. Didier* (1), O.P. Ottersen (2), E. Moyse (1), F. Jourdan (1) and J. Storm-Mathisen (2). (1) Physiologie Neurosensorielle, CNRS-ERS 5643, Université Claude Bernard, F-69622, Villeurbanne, France and (2) Anatomical Institute, University of Oslo, PO Box 1105, Blindern, N-0317, Oslo, Norway.

We have previously shown that taurine and glutamate are co-localized in the terminals of primary olfactory neurons in the olfactory bulb (OB), with a subcellular distribution supporting a transmitter role for glutamate in these neurons (*Didier et al., Neuroreport 6,145-148, 1994*). Here, we further describe the cellular localization of these two amino acids in the other cell types of the OB and of the olfactory epithelium (OE). Immunocytochemistry of taurine and glutamate was performed by using fully characterized antibodies raised in rabbits against glutaraldehyde conjugates of taurine and glutamate. At the light microscopic level on semi-thin sections of the OB, glutamate is found in primary olfactory terminals, in mitral cells and in cell bodies of the external plexiform layer. Taurine is present in the primary olfactory terminals, in cell bodies of the periglomerular area and of the granular layer. In the OE, a semiquantitative study at the electron microscopic level using colloidal gold-coupled second antibody, reveals that the density of particles (particles/μm^2) reflecting the level of fixed taurine is higher in olfactory neurons (100.8±43.0, mean ± SD, n=9) and horizontal basal cells (78.2±15.5, n=5) than in globose basal cells (35.6±16.3, n=13). In contrast, glutamate distribution among the different cell types of the OE is homogeneous (25.26±11.54, n=7, 28.4±17.7 n=5, 18.4±9.1, n=8 for densities of particles in primary neurons, horizontal and globose basal cells respectively). Our data suggest neuromodulatory actions for both amino acids in different neuronal types of the OB. In the OE, the functional implication of the non homogeneous distribution of taurine requires further investigations.

Supported by a NATO fellowship to A.D. and the Norwegian Research Council.

414.5

LOCALIZATION OF GLUTAMATE RECEPTOR SUBUNITS OF THE -AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLEPROPIONATE (AMPA) TYPE IN THE PANCREAS OF NEWBORN GUINEA PIG. H.P. LIU, S.S.W. TAY* and S.K. LEONG. Department of Anatomy, National University of Singapore, Singapore 119260.

Glutamate and related molecules are the major excitatory neurotransmitters in the central nervous system, but little is known about them in the peripheral nervous system. The present study investigated the localization of glutamate receptor subunits of the AMPA type (GluR1, GluR2-3, and GluR4) in the pancreas of newborn guinea-pigs. With double labelling method of immunofluorescence and immuno-DAB-reaction, GluR1 and GluR4 were localized in the insulin-secreting islet cells, and GluR2-3 in the non-insulin secreting islet cells. With double labelling method employing immunofluorescence and NADPH-d histochemistry, GluR2-3 and GluR4 immunoreactivities were localized in most of the NADPH-d positive pancreatic ganglion cells. GluR1 immunoreactivity was undetected in the pancreatic ganglion cells. These results suggest that glutamate receptor of AMPA type may play an important role in the regulation of hormone secretion in the pancreas, and its neural effects may be mediated by the novel neurotransmitter, nitric oxide.

414.6

DIFFERENTIAL LOCALIZATION OF TWO METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES IN SOMATOSENSORY THALAMUS OF THE RAT. X.-B. Liu*, R.M. Mansour and E.G. Jones. Department of Anatomy and Neurobiology, University of California, Irvine, Irvine, CA92717.

Recent neuropharmacological studies have demonstrated that metabotropic glutamate receptors (mGluRs) play important roles in regulating excitatory and inhibitory neurotransmission in the thalamus. *In situ* hybridization data have confirmed that mGluR mRNAs are expressed in varying patterns in the thalamus. However, little is known about the localization of mGluR proteins in somatosensory thalamus (VP nucleus). Information regarding distribution patterns of different mGluR subtypes and their relationships with identified thalamic synapses is lacking. The aims of the present study are (1) To reveal the cellular distributions of mGluR1α and mGluR2/3 and correlate with the localization of α-calcium/calmodulin protein kinase (CAM II kinase-α). (2) To visualize the subcellular distribution patterns of mGluR1α and mGluR2/3 and their relationships with specific thalamic synapses. At the light microscopic level, large numbers of mGluR1α immunostained neuronal processes and punctate structures were found in VP. The thalamic reticular nucleus (RTN) was devoid of immunostaining which is identical to the CAM II kinase-α immunostaining pattern. In contrast, mGluR2/3 showed less dense but fairly even labelling in VB and RTN, but most of the neurons lacked mGluR2/3 immunostaining. At the subcellular level, mGluR1α immunoreactivity was primarily confined in postsynaptic densities associated with RS type (corticothalamic) terminals, distal dendritic profiles and small appendages presumably deriving from relay cells. mGluR2/3 immunoreactivity was mainly localized within astroglial processes which were frequently found to wrap the RS terminals and some of the F type or RL type terminals. Neither mGluR1α nor mGluR2/3 was found in presynaptic terminals. The findings indicate that the two mGluR subtypes have distinct cellular and subcellular localization patterns in VP.

Supported by NIH grant NS21377.

414.7

LOCALIZATION OF GLYCINE RECEPTORS ON THE MEMBRANE OF RAT ABDUCENS MOTONEURONES INTRACELLULARLY LABELLED WITH NEUROBIOTINE. A CONFOCAL AND ULTRASTRUCTURAL ANALYSIS. H. Bras* and A. Barbe Unité de Neurocytochimie cellulaire, CNRS UPR 9041, 280 Bd Ste Marguerite, 13009 Marseille, France

We have recently shown that GABA and glycine, which are the major inhibitory neurotransmitters in the central nervous system, are colocalized in 9% of the axon terminals apposed on the membrane of the soma and proximal dendrites of rat abducens motoneurones. The analysis of the organisation of the post synaptic membrane, ie the visualisation of glycine and/or $GABA_A$ receptors constitute the present aim of our study. Wistar rats were deeply anaesthetized with pentobarbitol. Abducens motoneurones were identified by their antidromic responses to the stimulation of the ipsilateral muscle rectus lateralis, and intracellularly injected with neurobiotine. After tanscardiac perfusion with 4 % paraformaldehyde, the brainstem was sectionned with a vibratome and the sections were processed for immunocytochemistry using a monoclonal antibody to glycine receptors (Boehringer). The neurobiotine was coupled to a rhodamin-avidin while the receptors were labelled with a fluorescein FITC-conjugated specific antibody. A confocal microscope (CM) analysis revealed the presence of glycine receptors on the membrane of the soma and the dendendrites of the aducens motoneurones. Special attention was paid to local geometrical features such as branching points and dendritic spines. The abducens motoneurones were reconstructed in 3 dimensions using a software program for reconstruction of single neurones, to obtain a quantitative description of the geometry of the arborization. This sofware allowed to calculate the path distance from the soma of any local dendritic parts observed in CM. The vibratome sections were serially ultrathin sectionned and an electron microscopic was performed. These observations were compared to the data of the confocal microscopic analysis.

414.8

GLYCINE, GLYCINE-RECEPTORS AND GLYCINE-TRANSPORTERS IN THE RAT PARABRACHIAL COMPLEX. H. Herbert[1]*, A. Guthmann[1], O.P. Ottersen[2], & F. Zafra[3], [1]Dept. Animal Physiol., Univ. Tübingen, Germany; [2]Anatom. Inst., Univ. Oslo, Norway; [3]Centro de Biol. Molec., Univ. Madrid, Spain.

In the present study we sought to investigate whether the inhibitory amino acid glycine might play a role in signal transduction within the Parabrachial (PB) and Kölliker-Fuse nuclei (KF). ***Glycine (Gly) immunoreactivity (ir)*** was demonstrated on semithin plastic sections using a polyclonal rabbit anti-Gly antiserum and the PAP-technique. ***Glycine-Receptor (GlyR)-ir*** was demonstrated on cryostat sections by using a monoclonal mouse anti-GlyR antibody (mAb 2b, courtesy of H. Betz, MPI Frankfurt) and the indirect immunofluorescence technique. ***Glycine-Transporter (GlyT)-ir*** was demonstrated on vibratome sections employing two different polyclonal rabbit anti-GlyT antisera (Ab#176: $GlyT_1$ and Ab#218: $GlyT_2$) and the PAP-technique. Sections were analyzed by light and fluorescence microscopy and by confocal laser-scanning microscopy.

Our results show that Gly-ir varicose fibers, GlyR-ir, and $GlyT_2$-ir are all co-distributed and most prominent in a specific set of PB nuclei, namely the internal lateral (il) PB and the lateral crescent of the PB, and in the KF. Gly-ir neurons were only occasionally seen in the PBil and the KF. In contrast, the external, central, and dorsal lateral PB were totally devoid of these immunoreactivities. Furthermore, we observed that the pattern of $GlyT_1$-ir in the PB/KF is different from the $GlyT_2$-ir distribution. This indicates that the $GlyT_1$ is not necessarily associated with the strychnine-sensitive inhibitory glycinergic neurotransmission. From previous tracing experiments we know that the PBil, which is rich in Gly-ir fibers, GlyR-ir, and $GlyT_2$-ir, receives a prominent and exclusive input from spinal neurons located in the lateral reticulated area of lamina V. Thus, it is conceivable that the inhibitory amino acid glycine is used in this presumed somatosensory spino-parabrachial pathway. Furthermore, our data indicate that the respiratory related neurons in the KF are also under inhibitory control. However, the origin of the glycinergic input in the KF is not yet known. *Supported by DFG He 1842/6-1*

414.9

TRKA IMMUNOREACTIVE NEURONS IN THE RAT BASAL FOREBRAIN: RELATIONSHIP WITH GALANIN AND NITRIC OXIDE SYNTHASE. T.Sobreviela*, S. Jaffar and E.J. Mufson. Dept. of Neurol. Sci.2, Rush Med. Ctr., Chicago, IL 60612.

Cholinergic basal forebrain (BF) neurons express the high affinity trkA receptor for NGF, the neuromodualator galanin (GAL) and nitric oxide synthase (NOS), a marker for the neurotransmitter nitric oxide. We defined the expression of these markers within the medial septal/vertical limb of the diagonal band (MS/VDB), horizontal limb of the diagonal band (HDB) and nucleus basalis (NB) using colchicine pretreated rat tissue. TrkA positive neurons were seen throughout all BF subfields. In contrast, NOS and GAL neurons were mainly distributed within the MS/DB complex. Colocalization experiments revealed that virtually all NOS neurons (visualized by NADPH-d histochemistry) also contained trkA (>90%), whereas, only 25% of trkA neurons contained NADPH-d within the MS/VDB. Within the HDB NADPH-d neurons expressed trkA (75-85%), whereas, only a few trk-A neurons expressed NADPH-d (~15%). Within the NB very few neurons were NADPH-d reactive, although most contained trk-A. Only an occasional trk-A neuron (2-5%) expressed NADPH-d. Colocalization revealed that 10-25% of GAL neurons expressed NADPH-d, whereas, 20-30% of NADPH-d cells displayed GAL within the MS/DB complex. No colocalization occurred within the NB. Adjacent sections stained for GAL and trkA revealed twice as many trkA as GAL neurons within MS/DB complex. Within the NB there were ten times as many trkA as GAL neurons. These findings suggest that NGF via trkA modulates select BF neurons containing NOS and GAL. Support: AG10668, AG10161, AG09466 and IDPH

414.10

NITRIC OXIDE SYNTHASE (NOS)-IMMUNOREACTIVE NEURONS IN THE RAT HIPPOCAMPUS AND DENTATE GYRUS ARE CHEMICALLY HETEROGENEOUS. C.E. Adams* and R. Freedman. Department of Psychiatry, University of Colorado Health Sciences Center, Denver, CO 80262.

Non-principle neurons within the rat hippocampus and dentate gyrus can be subdivided based upon whether they contain peptides, calcium binding proteins or nitric oxide synthase. The degree of overlap between these chemically-defined groups of neurons has not been completely characterized. The wide-spread distribution of neurons containing nitric oxide synthase within the rat hippocampus and dentate gyrus suggests that NOS-positive neurons might overlap to some degree with both the peptide and calcium binding protein subgroups. As an initial examination of this possibility, immunocytochemical staining of NOS was compared to that of somatostatin, cholecystokinin, parvalbumin, calbindin or calretinin in the same tissue sections. Our preliminary results indicate that subpopulations of NOS-immunoreactive neurons also contain somatostatin, parvalbumin, cholecystokinin and calretinin, but not calbindin. Thus, neurons containing nitric oxide synthase within the rat hippocampus and dentate gyrus overlap, in part, with both the peptide and calcium binding protein subgroups of non-principle cells. [This research was supported by USPHS Grant MH44212]

414.11

LATERAL PARIETAL AND TEMPORAL NEOCORTEX OF THE OPOSSUM HAVE A REMARKABLE CONCENTRATION OF DIAPHORASE-POSITIVE NEURONS. J.G. Franca, R.L.S. Oliveira and E. Volchan. Lab. of Neuroblology II, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, 21949-900, Brazil.

Many authors utilize NADPH diaphorase histochemistry as a means to label nitric oxide synthase-positive (NOS-+) neurons and study their morphology. There are though few studies devoted to quantify their distribution in neocortex. Four adult opossums (*Didelphis marsupialis aurita)* were used. Three hemispheres were flattened and tangential sectioned. The fourth was cut coronal. All 60µm-thick-sections were incubated in a solution for 'indirect' NADPH diaphorase histochemistry. Analysis of labeled cells was performed in medial x lateral portions in both coronal and tangential sections. In the latter, three other sectors were also analyzed: anterior x intermediate x posterior. In all hemispheres, posterior parts show very low labeled cell densities. The intermediate part is the most crowded region, followed closely by the anterior one. The opossum neocortex shows much higher labeled cell densities in lateral than in medial half. In one of the tangential cut hemispheres, medial x lateral distribution was compared in the 3 antero-posterior sectors. Densities in each lateral half were two-fold that of the corresponding medial one. Supragranular layers have very few cells. Laminar densities increase dorso-ventrally reaching their highest values in infragranular layers. The results suggest that visual areas in this species are rather deprived of NOS-+ neurons. On the other hand, the lateral parietal cortex has a high density of these cells. This region might correspond to the somatosensory representation of the snout. Auditory temporal cortex has also a high concentration of NOS-+ neurons. Functional significance of this heterogeneous distribution is still unknown.

Funding Sources: CNPq, FINEP, CEPG.

414.12

DISTRIBUTION OF NADPH-DIAPHORASE IN THE FOREBRAIN OF THE SNAKES *Thamnophis sirtalis* AND *Lamprophis fulginosis*. E. Lanuza and M. Halpern*. Dept. of Anatomy and Cell Biology, Health Science Center at Brooklyn, SUNY, Brooklyn, NY 11203

The distribution pattern of NADPH-diaphorase (NADPH-d) activity in the prosencephalon of the snakes *Thamnophis sirtalis* and *Lamprophis fulginosis* exhibit some important interspecific differences. In the telencephalon of both species NADPH-d reactive neurons were numerous in the retrobulbar formation, olfactory tubercle, striatum, amygdala and dorsal ventricular ridge. A moderate number of stained neurons were present in the septum, medial, dorsal and lateral cortices. Areas of staining (probably terminal) were observed in the glomeruli of the main and accessory olfactory bulbs, nucleus accumbens, striatum, dorsolateral septum, hilus of the nucleus sphericus and lateral amygdala. The glomeruli of the accessory olfactory bulb showed the strongest NADPH-d reactivity of the entire brain. In *L. fulginosis*, but not in *T. sirtalis*, labeled neurons were present in the ventral amygdala, and a NADPH-d reactive ventral amygdalofugal tract was observed.

In the thalamus, labeled neurons and fibers were observed in the lateral geniculate complex, ventrolateral thalamus, nucleus rotundus and nucleus lentiformis thalami. Labeled neurons, but not fibers, were present in the nucleus ovalis and the medial thalamic nucleus. The neuropil of the lateral habenula was NADPH-d positive. In the hypothalamus of both species labeled neurons were present in the anterior hypothalamus and periventricular organ. The ventromedial edge of the optic tract showed a bundle of positive fibers. In the hypothalamus of *L. fulginosis*, but not in *T. sirtalis*, a number of labeled neurons were seen in the lateral hypothalamic area, and the ventromedial hypothalamic neuropil was NADPH-d reactive.

In contrast with our results, the NADPH-diaphorase activity in the brain of the snake, *Trimesurus flavoviridis* (Jiang et al., 1996; Brain Res. 713:168-177), was almost absent in the forebrain.

This research was supported in part by a NIH grant (DC00104), and EL was supported by a grant of "la Caixa".

414.13

REGIONAL AND SUBCELLULAR DISTRIBUTION OF PDE4 SUBTYPES IN RAT BRAIN. Y. Ye, J. Chandler, J. M. O'Donnell*. LSU Medical Center, Shreveport, LA 71130.

Immunoblot analysis revealed that in rat cerebral cortex there are four specific proteins reacting to K116, an antibody generated against rolipram-sensitive, high affinity, cyclic AMP phosphodiesterase (PDE4). These four proteins have molecular masses of 109, 102, 98, and 91 KDa. Using subtype-specific PDE4 antibodies, we identified 109 KDa protein as PDE4A and 91 KDa as PDE4D; the other proteins remain to be identified. Comparison among the regions showed that pituitary and pineal gland have only 102 and 98 KDa bands, whereas the other regions have all four bands which vary in intensity. Brain stem is low in all four bands; cerebellum is high in 109 and 102 KDa PDE4, but low in the other two bands; cerebral cortex, hippocampus, and striatum have similar patterns for the four bands. Subcellular fractionation indicated that the 102 KDa PDE4 exists exclusively in the P2 fraction and the 98 and 91 KDa PDE4s are concentrated in the S2 fraction, except for the pineal gland. Studies using primary cultures showed that all four bands are present in neurons, while only 102 and 98 KDa PDE4s are seen in astroglia. The findings are consistent with the complex nature of PDE4 and support the notion that PDE4 subtypes may be differentially involved in signal transdution systems underlying different physiological processes.

414.14

GTPγ[^{35}S] AUTORADIOGRAPHY ALLOWS REGION-SPECIFIC DETECTION OF G PROTEIN ACTIVATION IN THE CHICK OPTIC TECTUM. **K. Kurkinen[1,2]*, M. Jokinen[1], J.M. Saavedra[3] and J.T. Laitinen[1].** **1)Department of Physiology and 2) A.I.Virtanen Institute, University of Kuopio, P.O.B. 1627, FIN-70211 Kuopio and 3) Section of Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD, USA.**

A recently introduced technique of GTPγ[^{35}S] autoradiography was used to visualize agonist-dependent activation of G proteins in tissue sections of chick optic tectum, a brain region with relatively high expression of G protein-coupled receptors (GPCRs) for the neurotransmitter acetylcholine and pineal hormone melatonin. Quantitative comparison of GTPγ[^{35}S] binding responses between tectal sections and membrane preparations revealed a close match between the two tissue preparations for the response elicited by the cholinergic agonist carbachol (CCh), its dose-dependent blockade with the muscarinic antagonist atropine, its 100-fold selectivity towards blockade with M1-type (pirenzepine) over M2-type (gallamine) muscarinic antagonist, as well as absolute requirement for micromolar concentrations of GDP (EC_{50} 10 µM) for the agonist-dependent GTPγ[^{35}S] response. By using spesific antisera against the C-terminus of G protein α subunits, we found that the pirenzepine-sensitive muscarinic receptors efficiently interacted with $\alpha_{i1/2}$ providing first functional data on chick tectal mAChRs. However, we failed to detect any melatonin receptor-mediated GTPγ[^{35}S] response in conditions where CCh, somatostatin and 5-HT all elicited region-spesific increases in GTPγ[^{35}S] binding. As already demonstrated for the rat brain, GTPγ[^{35}S] autoradiography represents a promising tool for functional neuroanatomical studies on G protein activation by several (but perhaps not all) GPCRs. GTPγ[^{35}S] autoradiography should greatly facilitate studies on endogenous G protein-coupling specificity of various receptor subtypes *in situ* at physiological expression levels of the signaling components.

414.15

IMMUNOHISTOCHEMICAL LOCALIZATION AND QUANTIFICATION OF GTP CYCLOHYDROLASE I (GTPCH), THE GENE DEFECTIVE IN HEREDITARY PROGRESSIVE DYSTONIA (HPD), WITHIN RAT BRAIN MONOAMINE (MA) NEURONS K. Hirayama*, R. Shen, M. Khalil and G. Kapatos. Cellular and Clinical Neurobiology Program, Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI 48201.

GTPCH is the first and rate-limiting enzyme in the biosynthesis of tetrahydrobiopterin (BH4), the obligatory cofactor for tyrosine and tryptophan hydroxylases. Intracellular levels of BH4 are believed to be subsaturating with respect to these enzymes. Using *In situ* hybridization, we have localized GTPCH mRNA to MA neurons in rat brain. There is significant heterogeneity in the abundance of this transcript in different MA cell populations, with the highest levels of expression in 5-HT and the lowest in DA neurons. In order to determine whether heterogeneity in GTPCH mRNA levels are reflected in GTPCH protein, we have raised an antibody directed against the N-terminal 12-36 amino acids of GTPCH. GTPCH immunoreactivity was detected within MA neurons throughout the rat brain and was eliminated by absorption with the peptide antigen. Through the use of ^{35}S-labeled secondary antibody and autoradiographic emulsion detection, GTPCH protein levels were quantitated within individual MA neurons known to express different levels of GTPCH mRNA. These studies indicate that heterogeneity in GTPCH protein levels is expressed to an even greater degree than previously observed for GTPCH mRNA. This heterogeneity presumably underlies cell-specific differences in the intracellular concentration of BH4 and, therefore, the capacity to synthesize MA. Very low levels of GTPCH protein were found within A9 DA neurons. This may help to explain why A9 DA neurons are more sensitive than other MA neurons to the mutations in GTPCH that affect individuals with HPD, a movement disorder characterized by a selective inability to synthesize DA within the basal ganglia. (Supported by NS26081)

415.1

AGONIST-INDUCED INTERNALIZATION OF THE HUMAN P_{2Y2} PURINERGIC RECEPTOR. S.M. Sromek* and T.K. Harden#. Curr. in Neurobiology and Dept. of Pharmacology#, Univ. of North Carolina, Chapel Hill NC 27599.

The human P2Y2 purinergic receptor (P2Y2-R) is a 377 amino acid G protein-coupled receptor that is activated by ATP and UTP and stimulates phospholipase C (PLC). P2Y2-R undergo agonist-induced desensitization as demonstrated by a reduced capacity of P2Y2-R agonists to activate PLC. This desensitization is reversible upon agonist removal (resensitization). Agonist-induced receptor internalization has been observed for many G protein-coupled receptors. Recycling of internalized receptors back to the plasma membrane has been suggested to be a mechanism underlying receptor resensitization. To study whether internalization plays a role in P2Y2-R agonist-induced desensitization and/or resensitization, an epitope-tagged P2Y2-R was constructed by addition of the hemagluttinin A (HA) tag to the amino terminus (P2Y2-HA-R). When expressed in 1321N1 human astrocytoma cells, P2Y2-HA-R exhibited a similar pharmacological selectivity and a similar desensitization profile to that of wild-type P2Y2-R. ELISA-based assays demonstrated that cell surface P2Y2-HA-R immunoreactivity was decreased within 15 min of agonist treatment. The methodology for viewing cell-surface distribution of P2Y2-HA-R by indirect immunofluorescence (IIF) has been established. IIF microscopy and ELISA-based assays are currently being utilized to study agonist-induced redistribution of P2Y2-R.

This project is supported by USPHS grant HL34322.

415.2

EXPRESSION AND PARASYMPATHETIC TRANS-SYNAPTIC REGULATION OF P_{2X} PURINOCEPTORS IN RAT PAROTID ACINAR CELLS Barbara Talamo and Lalitha Tenneti*. Department of Neuroscience, Tufts U. Medical School, Boston, MA

ATP activates P_{2X} and P_{2Z} (through ATP^{4-}) -type purinoceptors in rat parotid acinar cells to gate non-selective cation currents. Cytosolic calcium (Ca_i) is regulated through Ca^{2+} influx gated by these receptors as well as through intracellular Ca^{2+} release activated by muscarinic, α-adrenergic and substance P receptors coupled through G proteins to phospholipase C. Fluorimetric ratio imaging of Ca_i in single dissociated cells labeled with fura-2AM showed that most cells are responsive to ACh, Substance P and ATP^{4-}. However, MgATP-activated Ca^{2+} influx was observed in only 20% of the cells, with a pharmacology similar to that of the recently cloned P_{2X4} receptor. RT-PCR showed abundant mRNA for this receptor in parotid tissue. Following postganglionic parasympathetic denervation, all of these receptor-mediated Ca_i responses became more sensitive to activation. The fraction of cells responding to MgATP tripled and the fraction of cells "supersensitive" to ATP increased 7-fold. Analysis of Ca_i responses in single cells showed that sensitivity to muscarinic activation and to MgATP activation is distributed across different subsets of cells and appears to depend on different mechanisms and be regulated independently. Previous data suggested that muscarinic responses are regulated downstream from the receptor. We hypothesize that parasympathetic signals regulate P_{2X4} receptors trans-synaptically. Supported by NIH R01 NS28556 and in part by NIH P30 DK34928.

415.3

ROLE OF ADENOSINE IN PRODUCING CHANNEL ARREST IN THE ANOXIC TURTLE BRAIN. M.Pek, P.L.Lutz* Dept.of Biology, Florida Atlantic University, Boca Raton, FL 33431.

The turtle brain's remarkable ability to survive anoxia is mainly based upon the flexible regulation of energy demand and energy production. Earlier studies indicate that extracellular adenosine is involved in reducing the brain's energy demand and increasing the cerebral blood flow. We tested the involvement of adenosine in the reduction ofpotassium flux(channel arrest)that occurs in the anoxic turtle brain. Changes in extracellular K+ in the in situ brain of the turtle Trachemys scripta were monitored following inhibition of the Na-K ATP-ase with ouabain. The consequentincrease of extracellular K+ was substantially(3-fold)slower in the anoxic brain compared to normoxic controls. Superfusing the brain before and during anoxia with theophylline, or the specific adenosine A1 receptor blocker 8-cyclopenthyltheophylline(CPT), resulted in a significant increase in potassium efflux in the anoxic brain, and the lowering of the depolarization K+ inflexion point. This is the first demonstration of the involvement of the adenosine A1 receptors in the reduction of ion flux in the anoxic brain, and the inflexion point change under anoxia.

NSF grant, IBN:9507961

415.4

A1 ADENOSINE RECEPTOR REGULATION IN CEREBELLAR GRANULE CELLS . B.D. Hettinger-Smith*, M. Leid and T.F. Murray. College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Adenosine is a ubiquitous physiological regulator and neuromodulator acting at A1, A2a, A2b and A3 adenosine receptors all of which are coupled to G-proteins. A1 adenosine receptors (A1AR) are coupled to the inhibition of adenylyl cyclase and regulation of these receptors provides a means for cells to adapt to chronic agonist or antagonist exposure. Primary cultures of cerebellar granule cells from 8 day old Sprague Dawley rat pups were established and used to study A1AR regulation in response to chronic exposure to agonists, antagonists and forskolin. A 48 hour exposure to the A1AR agonist, cyclopentyladenosine (CPA) effected a decrease in receptor density, impaired A1AR coupling to G-proteins and a blunted adenylyl cyclase response to A1AR agonists. Agonist exposure also increases the fraction of sequestered A1AR. In order to assess the role of cAMP in receptor regulation, cells were treated with forskolin. Forskolin exposure did not affect A1AR density or affinity indicating that intracellular cAMP does not modulate the expression of A1AR. Alterations in A1AR density due to chronic agonist and/or antagonist exposure are not paralleled by substantive changes in receptor mRNA as measured by RNAse protection assay. These results indicate that down- or upregulation of A1AR levels are not mediated by transcriptional regulation. (Supported by NIDA grant F31 DA05631 to BHS).

415.5

ACTIVATION OF ADENOSINE A3 RECEPTORS REDUCES THE SENSITIVITY OF RAT HIPPOCAMPAL A1 RECEPTORS, L.H. Diao*, T.V. Dunwiddie, H.O. Kim, K.A. Jacobson. Depts. of Pharmacol. and Neurosci., Univ. Col. Hlth. Sci. Ctr., VA Med. Ctr., Denver, CO 80262, Molecular Recognition Section, Lab. of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD 20892.

The adenosine A3 receptor is expressed in rat brain, including the hippocampal formation. However, the effects of activation of this receptor on electrophysiological activity are unknown. To address this issue, the effects of a highly selective adenosine A3 receptor agonist, 2-chloro-N^6-(3-iodobenzyl)-adenosine-5'-N-methyluronamide (Cl-IB-MECA) were determined in the CA1 area of rat hippocampal slices. Superfusion with Cl-IB-MECA (0.1-1μM) had no direct effects on fEPSPs, population spikes, long-term potentiation, or paired-pulse facilitation. However, the A1 receptor-mediated inhibition of excitatory neurotransmission was significantly antagonized by Cl-IB-MECA in a concentration-dependent fashion, but not by the selective A2a receptor agonist, CGS 21680. The presynaptic inhibitory effects of baclofen and carbachol were unaffected by Cl-IB-MECA. The maximal response to adenosine was unchanged, suggesting that the primary effect of Cl-IB-MECA was to reduce the affinity of adenosine for the A1 receptor, rather than to uncouple it. The actions of Cl-IB-MECA were partially antagonized by chelerythrine, an inhibitor of protein kinase C, suggesting that presynaptic A3 receptors might be coupled to activation of phospholipase C.

Supported by NS 29173 and the VA Medical Research Service.

415.6

EFFECTS OF THE ADENOSINE BINDING ENHANCER, PD 81723, ON ELECTROPHYSIOLOGY & NEUROTRANSMITTER RELEASE IN THE HIPPOCAMPAL SLICE. G. Wang[1], R. F. Berman[1]* & R. M. Broad[2], [1]Wayne State University, Detroit, Michigan and [2]Karolinska Institute, Stockholm, Sweden.

Adenosine is an important inhibitory neuromodulator in the central nervous system. Adenosine depresses spontaneous neuronal firing in the mammalian CNS and inhibits evoked synaptic activity in the *in vitro* hippocampal slice. The adenosine receptor binding enhancer, PD 81723, enhances these inhibitory effects of adenosine *in vitro*. In the present study the effects of PD81723 alone, or in combination with 2-chloroadenosine on *in vitro* hippocampal synaptic activity were examined. Superfused hippocampal slices were prepared from Long-Evans rats. Schaffer collateral's were stimulated at 1 Hz and evoked field potentials were recorded.

As expected, the addition of 200 nM 2-chloroadenosine alone depressed population spike amplitude by approximately 43%. Subsequent addition of 10 uM PD 81723 produced a further 33% depression of synaptic activity. This enhancement of the effects of 2-chloroadenosine is consistent with our previously reported effects with this compound. The effects of 10 uM PD 81723 when added to 400 nM 2-chloroadenosine were similar, but the magnitude of the enhancement was greater (i.e., 73%). In contrast, addition of 50 uM PD 81723 partially reversed the 2-chloroadenosine-mediated depression of synaptic activity, again consistent with previous reports of antagonist-like effects of higher concentrations of PD 81723. The addition of 10 uM PD 81723 alone to the superfusion media produced a small but significant decrease in the population spike amplitude, and this effect was reversed when the concentration of PD 81723 was increased to 50 uM. These results were compared to the effects of PD 81723 on 2-chloroadenosine-mediated inhibition of evoked [^{3}H]-norepinephrine and [^{14}C]-acetylcholine release from perfused hippocampal slices. In contrast to the electrophysiological findings, PD 81723 had only limited effects on the ability of 2-chloroadenosine to affect neurotransmitter release from hippocampal slices. These results extend our previous findings that PD 81723 enhances adenosine activity, to the stable adenosine analog 2-chloroadenosine. (Supported by NIDRR H133A20016)

415.7

MODULATION OF GABA RELEASE IN RAT BASAL GANGLIA: ANTAGONISTIC ADENOSINE A_1/DOPAMINE D_1 RECEPTOR INTERACTION? R.D. Mayfield*, H.A. Miller, and N.R. Zahniser. Dept. Pharmacol., Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Behavioral and biochemical studies suggest that a negative interaction exists between adenosine (ADO) A_1 and dopamine (DA) D_1 receptors in the brain, which may contribute to the motor depressant effects of ADO agonists and the motor stimulating effects of ADO antagonists. We examined the functional significance of A_1 and D_1 receptor subtypes in modulating electrically-evoked endogenous GABA release from tissue slices of rat basal ganglia (striatum, globus pallidus, striatum containing globus pallidus, and substantia nigra reticulata) in the presence of a GABA uptake blocker. Selective stimulation of A_1 receptors with R-PIA (1-100 nM) resulted in a concentration dependent decrease in GABA release from globus pallidus and striatum containing globus pallidus. Maximum inhibition of release was approximately 40% in the presence of 100 nM R-PIA. NECA (10 nM) inhibited GABA release to the same extent. The selective A_1 antagonist DPCPX (10-100 nM) increased GABA release suggesting that endogenous ADO tonically inhibits GABA release. In contrast, consistent DA D_1 receptor modulation of GABA release was not observed in any brain region using a number of D_1 agonists (SKF-38393, fenoldopam, CY-208-243, and SKF-82958) or the D_1 antagonist SCH-23390. Finally, co-stimulation of A_1 and D_1 receptors in striatum containing globus pallidus with R-PIA (10 nM) and SKF-82958 (1 μM) resulted in a decrease in GABA release that was similar to that observed for R-PIA alone, thus, confirming the lack of D_1 receptor modulation under these conditions. These results do not support a role for an antagonistic A_1D_1 receptor interaction in modulating electrically-evoked GABA release; however, the possibility exists that this interaction might be observed in the absence of a GABA uptake blocker. (Support: NS 26851)

415.8

COCAINE WITHDRAWAL DOES NOT ALTER D1 RECEPTORS IN THE RAT BRAIN. C. M. Ohuoha*, M. H. Baumann, B. Emilien, R. B. Rothman. Clinical Psychopharmacology Section, CPB/DIR/NIDA, Baltimore MD 21224.

The role of dopamine (DA) receptor subtypes in drug abuse have been studied extensively with much of the investigation centered on the role of D2 receptors. D1 receptors are functionally and anatomically segregated from D2 receptors in the neostriatum. Unlike D2 receptor subtypes which occur as postsynaptic receptors or presynaptic autoreceptors, D1 subtypes are concentrated on postsynaptic sites in the nigrostriatal dopamine system. The measurement of D1 receptor density with the anatomical resolution of autoradiography may provide information regarding the effects of chronic cocaine on D1 receptors in cocaine withdrawal. In this study rats received 15mg/kg of cocaine or saline two times per day for seven days. A 42 hour withdrawal period was allowed prior to sacrifice and harvesting of the brains. Brains were stored immediately in -70^{0}C until processed. 14μm thick coronal sections through the striatum were mounted on acid-cleaned, gel coated slides. Tissue sections were preincubated in buffer (50mM Tris HCl, 120mM NaCl, 1mM $MgCl_2$, 5mM KCl and 2mM $CaCl_2$, pH 7.4) for 5 minutes at room temperature. Total binding was determined in buffer solution containing 2nM [^{3}H]SCH 23390 (44.2Ci/mmole NEN, Boston MA) and 20nM mianserin to block 5HT$_2$ receptors. Non-specific binding was assessed using 10μM (+)-butaclamol. Analysis of the autoradiograms did not show any significant difference in D1 receptor density in the two groups studied in the frontal cortex, striatum, substantial nigra, hippocampus and dentate gyrus. These results indicate that D1 receptors are unaltered during early cocaine withdrawal and are in contrast to some earlier studies in the striatum. (Work was supported by DIR/NIDA, NIH)

415.9

DIFFERENTIAL EFFECTS OF CLOZAPINE AND HALOPERIDOL ON DOPAMINE RECEPTOR mRNA EXPRESSION IN RAT STRIATUM AND CORTEX. S. P. Damask and J. H. Meador-Woodruff*. Mental Health Research Institute and Department of Psychiatry, University of Michigan Medical Center, Ann Arbor, MI 48109-0720.

The regulation of the dopamine (DA) receptors is of considerable interest, in part because treatment with antipsychotic drugs is known to upregulate striatal D_2-like receptors. While previous studies have focused on the regulation of striatal DA receptors, less is known about the pharmacological regulation of cortical DA receptors. The purpose of this study was to examine the regulation of DA mRNA receptor expression in the cortex compared to the striatum following treatment with antipsychotic agents. Adult male Sprague-Dawley rats were injected daily with haloperidol (2 mg/kg/day), clozapine (20 mg/kg/day) or a control vehicle for a period of 14 days. Following treatment, brains were subjected to *in situ* hybridization for the mRNAs encoding the five dopamine receptors; only D_1, D_2, and D_3 receptor mRNAs were detected in these regions. Haloperidol tended to either modestly upregulate or have no effect on dopamine receptor mRNAs detected in striatal structures, while clozapine generally downregulated these mRNAs. On the other hand, in the cortex, both drugs had striking effects on D_1 and D_2 mRNA levels. Cortical D_1 mRNA was upregulated by haloperidol, but this effect was primarily restricted to cingulate cortex; clozapine also upregulated D_1 mRNA, but primarily in parietal regions. Haloperidol downregulated D_2 mRNA in the majority of cortical regions, but most dramatically in frontal and cingulate regions; clozapine typically upregulated this mRNA, but primarily in regions other than frontal and cingulate cortex. These results indicate that clozapine and haloperidol each have regionally-specific effects, and differentially regulate dopamine receptor mRNA expression in striatal and cortical regions of the rat brain. This work was supported by a grant to Dr. Meador-Woodruff from NIMH (MH53327). This project was also supported by the Mental Health Research Institute and the Department of Psychiatry at the University of Michigan.

415.10

NEONATAL RAT MEDIAL PREFRONTAL CORTEX LESION: DOPAMINE AND LOCOMOTION G. Flores, G.K. Wood, J.J. Liang, R. Quirion, & L.K. Srivastava*. Douglas Hospital Research Centre, McGill University, Montreal, Quebec, Canada, H4H 1R3.

Adult rats with lesions to the medial prefrontal cortex (MPFC) demonstrate an increased behavioral response to stressful stimuli and altered mesolimbic/nigrostriatal dopamine (DA) levels (Jaskiw et al., Brain Res., **534**, 263). While this lesion relates to the MPFC pathology and presumed DAergic alterations seen in schizophrenia, it does not address the developmental aspect of this disease, namely, insult to the pre/neonatal brain causing postpubertal behavioral changes. Therefore, in order to determine if MPFC damage in a developmental period will manifest behavioral and DAergic enhancements postpubertally, neonatal (PD7) ibotenic acid lesions of the rat MPFC were performed and compared to adult MPFC lesions. Neonatal lesions to the rat MPFC caused increased novelty and amphetamine-induced locomotion postpubertaliy (PD56; postoperative week 7) but not prepubertally (PD35). Adult lesions to the rat MPFC produced significantly increased amphetamine-induced locomotion on postoperative week 7, but not to the extent of neonatal MPFC lesion. Autoradiographic and in situ measurement of DA receptor subtypes revealed a significant increase in D2 receptors in the shell of the accumbens and dorsal striatum in postpubertal rats neonatally lesioned in the MPFC. These results suggest that neonatal lesions to the MPFC can cause delayed onset of behavioral hyperactivity and concurrent increased mesolimbic/nigrostriatal DA D2 receptor density. (Supported by the FRSQ).

415.11

MUTATION OF SERINE 167 OF THE D2L RECEPTOR MODULATES RECEPTOR REGULATION. S. Starr*, V. J. Watts, M. N.Vu, and K.A. Neve. Oregon Health Sciences University and VA Medical Center, Portland OR, 97201

Molecular modeling studies of the D2L dopamine receptor have suggested that residues phenylalanine 110 and serine 167 are important for ligand binding and activation. These residues are found in transmembrane domains 3 and 4 respectively. Mutant D2L cDNA was generated in which phenylalanine 110 and serine 167 were changed to alanine (F110A) and (S167A) respectively, using the method of trans-PCR. HEK293 cells were stably transfected with D2L, F110A or S167A cDNAs. Saturation analysis using [3H]spiperone determined that receptor densities ranged from 300 to 3,000 fmol/mg protein for both D2L and mutant receptors. Ability to inhibit forskolin-stimulated adenylate cyclase activity was assessed using dopamine, (+)-3-PPP, and quinpirole. All three agonists were efficacious at both D2L and mutant receptors. However the EC50s for F110A and S167A were shifted slightly to the right, indicating decreased potency for the mutant receptors. Additional studies were carried out to examine agonist-induced regulation of D2L receptor density. Following an 18-hr treatment with agonist, there were modest changes in receptor density for D2L or F110A receptor. However the density of receptors was increased 4-5 fold for S167A, from 300 fmol/mg to 1580 and 1410 fmol/mg by treatment with NPA and quinpirole, respectively. Similar results were found with a second S167A clone. Apparent affinities for the these clones were somewhat decreased which may be the result of some residual drug. Further analysis of this mutant may provide insight into the mechanisms of agonist induced up-regulation of D2 receptors observed in some other cell lines. Supported by MH45372 and VA Merit Review Program.

415.12

LONG LASTING CHANGES IN CORTICAL EXCITATORY AMINO ACID RECEPTOR LEVELS AND DOPAMINE-INDUCED BEHAVIOR AFTER NEONATAL EXCITOTOXIC LESION OF THE VENTRAL HIPPOCAMPUS IN RATS Z. Albo*, G. Flores, R. Quirion and L.K. Srivastava. *Douglas Hospital Research Center, Departments of Psychiatry and Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H4H 1R3*

Bilateral excitotoxic lesions of the ventral hippocampus in neonatal rats result in hyperlocomotor behaviors induced by dopamine agonists, and hyperresponsiveness to stress induced behavior in postpuberal animals (Lipska et al., Neuropsychopharm. 9:67, 1993). A cortical maldevelopment has also been suggested to explain the behavioral changes observed but direct evidence is lacking to support this assumption (Weinberger and Lipska, Schizophr Res.16:87,1995). We have reported that the levels of cortical N-methyl-D-aspartate (NMDA) receptors increased in postpuberal (PD56) but not prepuberal (PD35) rats after neonatal excitotoxic lesion of the ventral hippocampus (Albo et al, Soc Neurosc. Abst. 21:1145, 1995). Now, we have compared the levels of NMDA receptors in cortical areas of 6 month-old rats that underwent either bilateral ibotenic acid lesions or sham-lesion of the ventral hippocampus at postnatal day 7 (PD7). Neonatally hippocampal-lesioned 6 month-old rats showed increased locomotor activities induced by D-amphetamine (1mg/kg). The density of NMDA receptors as measured by ligand autoradiography using [^{125}I] MK-801 (200pM) was found to be significantly increased in the frontal and cingulate cortices in the 6 month-old neonatally hippocampal-lesioned group. Other brain areas studied showed no significant change in [^{125}I] MK-801 binding. Taken together our results suggest that cortical glutamate abnormalities observed in hippocampal-lesioned rats are of enduring nature and may underlie some of the behavioral changes observed in these animals. (Supported by FRSQ).

415.13

PRE- AND POST- SYNAPTIC DOPAMINERGIC GENE EXPRESSION AFTER NMDA RECEPTOR BLOCKADE. Andrea de Bartolomeis*, Luigi Aloj, Carlo Contaldi, David Pickar, Giovanni Muscettola and Alan Breier *Section of Psychiatry, Department of Neuroscience. Univ. School of Medicine Federico II, Via Pansini 5, 80131 Napoli Italy; ETB NIMH, NIH ,Bethesda MD, USA; Nuclear Medicine, NIH, Bethesda, MD, USA.*

Combined dysfunction of dopaminergic and glutamatergic systems and NMDA hypofunction has been suggested to be involved in pathophysiology of schizophrenia and has been investigated in humans with sub-anesthetic doses of ketamine a dissociative agent and NMDA non competitive antagonist. The effects of sub-anesthetic doses of ketamine on presynaptic and postsynaptic dopaminergic gene expression as well as enkephalin gene expression were studied in an animal model by means of quantitative in situ hybridization. Male Sprague Dawley rats were treated with NaCl 0.9%, or 12 mg/kg or 50 mg/kg ketamine-HCl i.p. Brain sections were hybridized with ^{35}S radiolabeled synthetic oligonucleotides for dopamine receptors D_1 and D_2, dopamine transporter (DAT), and preproenkephalin. The computer assisted analysis of the autoradiograms showed increased DAT mRNA in ventral tegmental area (p= 0.019) and modulation of enkephalin gene expression in sub-regions of striatum in rats treated with ketamine compared to controls. These results suggest a potential regulation of dopamine transporter and enkephalin mRNA after NMDA blockade by sub-anesthetic doses of ketamine. *FIS -IRGASD grant to A.d.B. and G.M.*

415.14

MODULATORY EFFECTS OF THE NEUROSTEROID DEHYDROEPIANDROSTERONE (DHEA) ON NEUROTRANSMITTERS MEMBRANE RECEPTORS IN THE MALE RAT BRAIN. S. Lee Mei Si and T. Di Paolo*, School of Pharmacy, Laval University and Dept. of Molecular Endocrinology , C.H.U.Q. Research Center, Quebec, Que., Canada, G1V 4G2.

DHEA is a precursor of sex steroids and has been shown to decline in the blood of aging men and women. DHEA is considered as a neurosteroid and its neuromodulatory activity includes interactions with a variety of membrane receptors. The present study investigated the role of DHEA in male rats on the balance of numerous neurotransmitters receptors in the brain which are involved in central nervous system disorders. 30 animals were divided into 3 distinct groups: (1) Intact, (2) orchidectomized, (3) orchidectomized + DHEA. Castration was used as a model of decreasing gonadal function such as in aging. DHEA treatment consisted of a 15 mg subcutaneous injection, b.i.d for 2 weeks. D2 dopamine receptors in the striatum (D2Rs) and 5-HT2A serotonin receptors in the frontal cortex (5-HT2ARcf) were determined by saturation experiments on appropriate tissue, using [3H]Spiperone and [3H]Ketanserin respectively. [3H]Flunitrazepam ([3H]Flu) binding to the $GABA_A$ receptor complex in the substantia nigra pars reticulata was measured by quantitative autoradiography. Orchidectomy decreased 5-HT2Acf levels ($p<0.05$) and improved the affinity for its ligand ($p<0.05$). Those changes were not corrected with DHEA treatment. Gonadal steroid withdrawal did not alter D2Rs or [3H]Flu binding properties. Supplementation with DHEA decreased D2R density by 23.6% ($p<0.01$) and enhanced [3H]Flu binding by 29 % ($p<0.01$). Affinity was not affected by castration nor DHEA replacement therapy. DHEA could exert its action by itself or via its transformation into estradiol or testosterone. However, the different response of D2 and $GABA_A$ receptors to DHEA as compared to our previous data with estradiol suggest that DHEA activity is more likely androgenic. Our study provides evidence of DHEA influence on aminergic and gabaergic neurotransmission in the male rat brain. Supported by the MRC of Canada.

415.15

THE ROLE OF G-PROTEINS IN MEDIATING DOPAMINE RECEPTOR SUPERSENSITIVITY: THE USE OF IN VIVO ANTISENSE OLIGONUCLEOTIDES. E.R. Marcotte* and R.K. Mishra. Depts. of Psychiatry and Biomedical Sciences, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada.

Previously, studies of receptor supersensitivity have focused largely on changes in receptor density and ligand affinity. Although receptor upregulation provides an obvious and direct mechanism by which supersensitivity could occur, it has proven insufficient in explaining many aspects of dopamine receptor supersensitivity. G-proteins, as the next step in receptor-mediated signal transduction and amplification, provide a logical focus for further research. We have previously shown that the striatal stimulatory G-proteins Gs and Golf are elevated in an animal model of Parkinson's disease, the 6-hydroxydopamine lesioned rat. We now report direct evidence for the role of Golf in mediating dopamine receptor supersensitivity using *in vivo* Golf antisense oligonucleotides. Golf antisense infusion virtually abolished apomorphine-induced rotation in this animal model. Correspondingly, Golf levels were specifically reduced by antisense infusion. In the MPTP mouse model, a differential regulation of stimulatory G-protein levels by MPTP treatment was observed. We found an acute reduction in Gs and Golf shortly after the lesion, followed by a delayed increase in stimulatory G-proteins during recovery. This is in stark contrast to the consistent upregulation seen in 6-hydroxydopamine lesioned rats, and presumably reflects differences in the regulation of dopamine receptor supersensitivity in these two animal models of Parkinson's disease. Further study with *in vivo* antisense agents may help to resolve these issues. Funded by the Parkinson's Foundation of Canada, and the NIH.

CARDIOVASCULAR REGULATION: PERIPHERAL AUTONOMIC CONTROL

416.1

IDENTIFICATION OF PROTEIN KINASE C ISOENZYMES IN ADRENAL MEDULLA AND SYMPATHETIC GANGLIA OF RAT A. R. Wakade* Y. Watanabe & H. Hidaka Dept. of Pharmacology, Wayne State Univ. School of Medicine, Detroit and Nagoya University School of Medicine, Nagoya, Japan.

Protein kinase C (PKC) plays an important role in exocytosis and neuronal differentiation in adrenal medulla (AM) and sympathetic neurons. Using mono-and poly-clonal antibodies we have identified various PKC isoenzymes in adrenal medulla (AM) and superior cervical ganglia (SCG) of rat. Among cPKC, gamma was absent in AM and SCG. Beta form was sparsely detectable in AM but was abundant in cytosol fraction of SCG. Alpha was present in cytosol fractions of both tissues but was higher in SCG. nPKC isoforms, delta and epsilon were also detected in AM and SCG. Delta was weak but epsilon was high in SCG. aPKC zeta form was most abundant of all forms in SCG and AM cytosol fractions. Alpha PKC was translocated to AM membrane fraction by stimulation with excess K^+, muscarine (MUS), and pituitary adenylate cyclase activating polypeptide (PACAP). PACAP and MUS also translocated zeta form. In contrast, all the three agonists translocated beta isoform in SCG membranes. Zeta form was unaffected by these agents and phorbol 12, 13- dibutyrate. Excess K+ and muscarine translocated alpha cPKC and epsilon nPKC, respectively in SCG membranes. We conclude that alpha and beta cPKC are the major isoforms of PKC involved in signal transduction mechanisms in AM and SCG, respectively and the role of other isoenzymes remains unclear at this time.

416.2

MONOAMINE SYNTHESIZING ENZYMES WITHIN CARDIAC GANGLIA OF THE ADULT HUMAN HEART. S. Singh*, P. I. Johnson, A. Javed, T. S. Gray, and R. D. Wurster. Neuroscience Program and Departments of Anatomy, Physiology, Pharmacology and Neurosurgery. Loyola University Medical Center, Maywood, Illinois 60153-3500.

Classically, cardiac ganglia were thought to contain cholinergic neurons relaying information from preganglionic neurons in the brainstem to the heart's muscle and nodal tissue. More recently evidence suggests that cardiac ganglia contain a heterogeneous population of neurons capable of synthesizing several neurotransmitters and containing various neuropeptides. However, reports regarding catecholamine and indoleamine synthesizing enzymes within these ganglia are limited and conflicting. In addition, studies examining all of these enzymes in the human cardiac ganglia do not exist. Therefore, the purpose of this study was to determine if monoamine synthesizing enzymes are present within neurons of the human cardiac ganglia. Human heart tissue from near the sino-atrial and atrio-ventricular nodes was obtained from heart transplants and autopsies. Polyclonal antibodies previously shown to be specific for tyrosine hydroxylase (TH), aromatic amino-acid decarboxylase (AADC), dopamine β-hydroxylase (DβH), phenylethanolamine-N-methyltranferase (PNMT), tryptophan hydroxylase (TryH) and choline acetyltransferase (ChAT) were used to detect these enzymes within the cardiac ganglia. Primary antibodies were omitted from the incubation solution as negative controls while sympathetic nerve fibers and small intensely fluorescent cells, known to contain monoamine synthesizing enzymes, served as positive internal controls. Our results indicate that many neurons within the cardiac ganglia are immunoreactive for TH, AADC, DβH, PNMT, TryH and ChAT. To the best of our knowledge, this is the first demonstration of PNMT and TryH within neurons of any mammalian cardiac ganglia. These findings suggest that dopamine, norepinephrine, epinephrine, serotonin and acetylcholine can all be synthesized by neurons within the human cardiac ganglia. Furthermore, since most neurons are also immunoreactive for ChAT, a subpopulation of neurons within the cardiac ganglia expresses the enzymes needed to synthesize multiple neurotransmitters. Supported by NIH Grant HL 27595

416.3

NITRIC OXIDE AND CARBON MONOXIDE SYNTHESIZING ENZYMES WITHIN CARDIAC GANGLIA OF THE ADULT HUMAN HEART. C. L. Webber, Jr.*, S. Singh, P. J. Johnson, R. D. Wurster. Neuroscience Program and Departments of Physiology, Pharmacology and Neurosurgery, Loyola University Medical Center, Maywood, Illinois 60153-3500.

Nitric oxide and carbon monoxide are diffusible gases that can activate soluble guanylyl cyclase and act as neuromodulators within the central and peripheral nervous systems. In addition, nitric oxide and carbon monoxide have been linked with a variety of physiological and pathophysiological cardiovascular functions. Neuronal nitric oxide synthase (bNOS), which synthesizes nitric oxide, has been found in the cardiac ganglia of several mammalian species but has not been demonstrated in the human cardiac ganglia. Heme-oxygenase 2 (HO-2), which synthesizes carbon monoxide, has been demonstrated within certain autonomic ganglia but never within cardiac ganglia of any mammalian species. The purpose of this study was to determine if the enzymes involved with generation of nitric oxide and carbon monoxide are present within the human cardiac ganglia. Human heart tissue from near the sino-atrial and atrio-ventricular nodes was obtained from heart transplants and autopsies. Polyclonal antibodies previously shown to be specific for bNOS, HO-2 and choline acetyltransferase (ChAT) were used to detect these enzymes within the cardiac ganglia. Primary antibodies were omitted from the incubation solution as negative controls, while brain regions previously shown to express bNOS, HO-2 and ChAT were used as positive controls. NADPH-diaphorase histochemistry, which detects bNOS, was also used to confirm the immunohistochemical findings. Our results indicate that many neurons within the human cardiac ganglia are immunoreactive for bNOS and HO-2. Given that most of the neurons within the cardiac ganglia were also immunoreactive for ChAT, the data suggest that a subpopulation of neurons expresses the enzymes needed to synthesize nitric oxide or carbon monoxide along with acetylcholine. Furthermore, these observations suggest a possible role for nitric oxide and carbon monoxide in the integration of cardiac ganglionic function and cardiovascular regulation. Supported by NIH Grant HL 27595

416.4

LOCALIZATION OF NITRIC OXIDE SYNTHASE IN SENSORY FIBERS AND EFFECTS OF NITRIC OXIDE IN CULTURED PREVERTEBRAL SYMPATHETIC GANGLIA NEURONS. Z. L. Zheng, D. L. Kreulen, and R. A. Travagli*. Dept. of Physiology, Sch. of Med., West Virginia Univ. Morgantown, WV 26506-9229

Sympathetic neurons in prevertebral ganglia (Celiac ganglion, CG, and Inferior Mesenteric Ganglion, IMG) innervate the mesenteric vasculature and regulate intestinal blood flow. Mesenteric vessels are also innervated by capsaicin-sensitive primary sensory neurons which can evoke a nitric oxide mediated vasodilatation in mesenteric artery. The purpose of this project was: 1) to determine if NOS-containing fibers in the prevertebral ganglia originated in primary sensory neurons, 2) to study the effects of nitric oxide on neurons in prevertebral ganglia. Using primary anti-neuronal NOS (1:100) and anti-SP (1:200) antibodies, NOS was colocalized with SP in cell bodies of DRG and in fibers in the CG and IMG (n=3). Pretreatment with capsaicin (50, 100, or 350 mg/kg injected over 1, 2 and 3 days, N=5), a toxin known to induce a selective degeneration of sensory nerves, abolished all the SP immunoreactivity and significantly decreased the NOS immunoreactivity in fibers in the prevertebral ganglia. To investigate the actions of nitric oxide on sympathetic neurons, cultured CG cells were used for patch clamp studies. The NO-generating compound L-nitrosocysteine (100 μM-1 mM, LNC) depolarized >70% of CG neurons in a concentration-related fashion. LNC also reduced the amplitude of the afterhyperpolarization that follows the action potential. In voltage clamp, 300 μM LNC induced an inward current (-85±31 pA, N=4) which was accompanied by an increase in input resistance (+20±11%); these effects were antagonized by pretreatment with the NO scavenger, hemoglobin (10μM, N=3). In conclusion, the present study provides evidence that sensory nerves from DRG are a source of NO to sympathetic ganglia, NO also modulates sympathetic neuron activity in preverteberal sympathetic ganglion neurons *in vitro*.

416.5

LOCALIZATION OF NEURONS IN PREVERTEBRAL GANGLIA THAT INNERVATE INFERIOR MESENTERIC ARTERY AND VEIN. D.L. Kreulen* Z. L. Zheng, and R. A. Travagli. Dept. of Physiology, Sch. of Med., West Virginia Univ. Morgantown, WV 26506-9229

Prevertebral ganglia (Celiac, CG, and Inferior Mesenteric, IMG) innervate the intestine and the mesenteric blood vessels in guinea pig. Electrical stimulation of IMG evokes different response in mesenteric arteries (IMA) and veins (IMV), thus, suggested the probability of a diversified neuronal population providing innervation to the vasculature. The aims of this study are: 1) to determine whether sympathetic neurons in IMG that innervate the IMA comprise a separate population from those that innervate IMV. 2) to investigate if the IMG innervating the IMA and/or IMV contain NPY. Retrograde neuronal tracing in combination with immunohistochemistry were applied to an *in vitro* preparation. Rhodamine beads and Fluoro-Gold were injected into the lumen of IMA or IMV, respectively. After fixation, 16 μm thick sections of IMG were stained FITC-labeled NPY and analyzed for fluorescence. Neurons, filled with rhodamine beads, were located in central areas of IMG while the neurons providing venous innervation stained with Fluoro-Gold were distributed more laterally in the IMG. Neurons containing NPY were occasionally colocalized with rhodamine beads. Double labeling of rhodamine beads and Fluoro-Gold was not observed. The NPY-labeled nerve fibers were more prominent in IMV than IMA. The results provide evidence that there are neurochemically and functionally distinct subpopulations of neurons in guinea pig IMG that innervate IMA and IMV.

416.6

THE EFFECTS OF DEPOLARIZATION ON FROG CHROMAFFIN AND ADRENOCORTICAL CELLS IN CO-CULTURE. S.P.Shepherd* and M.A. Holzwarth. Neuroscience Program and Dept of Molecular and Integrative Physiology, Univ. of Illinois, Urbana IL 61801.

Based on previous morphological and physiological evidence of adrenal medullary modulation of adrenocortical function, we have developed co-cultures of amphibian (*Rana pipiens*) chromaffin and adrenocortical cells to study directly the cellular interactions. Many characteristics are retained in our co-cultures, including the expression and release of neurotransmitters by chromaffin cells and steroidogenesis by adrenocortical cells. Furthermore, chromaffin cells extend processes that project toward or onto adrenocortical cells, thereby providing the substrate for direct autonomic regulation of adrenocortical function and also mimicking the organization *in vivo*. In the process of investigating the effect of chromaffin cell activation on adrenocortical function, we have observed concomitant morphological changes in the chromaffin cells. Thirty-six hour chromaffin cell depolarization with veratridine (50 μM), a sodium ionophore, was followed by fixation on day 7 *in vitro* and identification of the chromaffin cells by immunocytochemistry of the neuronal marker for tau (*linc*). Chromaffin cell depolarization results in increased mean neurite length (70 μm to 150 μm, $p<.001$), increased mean number of neurites per cell (1.5 to 2.5, $p<.001$), and increased mean number of branches per neurite (0 to 1.5, $p<.005$). Time lapse microscopy confirms that veratridine increases neurite outgrowth and thus is likely to enhance cell-cell interactions in our co-cultures. Importantly, the veratridine-induced chromaffin cell depolarization activates adrenocortical cells as shown by increased Fos expression and increased steroidogenesis. These findings indicate that depolarization can affect neuronal morphology and provide further evidence that chromaffin cell activity modulates adrenocortical function. (Supported by NIH HD07333 and NSF IBN-9310866).

416.7

ADRENOCORTICAL CELLS AND GROWTH FACTORS AFFECT SYMPATHETIC NEURON GROWTH IN CULTURE. Ching-H Wu* and M. A. Holzwarth. Dept. of Molecular and Integrative Physiology and Neuroscience Program, Univ. of Illinois, Urbana, IL 61801.

In the process of establishing co-cultures of sympathetic neurons and adrenocortical cells to study sympatho-adrenal interactions, factors which affect the growth and differentiation of the sympathetic neurons have been identified. These include the presence of adrenocortical cells as target cells as well as growth factors. Sympathetic neurons from superior cervical and chain ganglia of 5-7 day old rat pups were dispersed and grown in supplemented DME/F12 media on poly-D-lysine- and laminin-coated glass coverslips in the absence or presence of adrenocortical cells. Sympathetic ganglion cells were cultured with 10ng/ml nerve growth factor (NGF), 10ng/ml basic fibroblast growth factor (bFGF), 10^{-8} M IL-1β, 5-10% fetal bovine serum (FBS) or media alone. Neuronal growth and differentiation were evaluated using ICC localization of neurofilament protein (2H3), tyrosine hydroxylase (TOH), bFGF, vasoactive intestinal peptide (VIP) and synaptophysin. Identification of adrenocortical cells was verified by 3-ß-hydroxysteroid dehydrogenase (3-ß-HSD) histochemistry and ICC. The presence of NGF, bFGF, IL-1 enhanced the expression of NOS, TOH, bFGF and also stimulated neurite outgrowth. The presence of adrenocortical cells, also enhanced neurite outgrowth, expression of synaptophysin bFGF, VIP and TOH. When adrenocortical cells were separated from the sympathetic neurons by plating in transwell, the effects of adrenocortical cells were less evident. These findings suggest that both the physical proximity of the adrenocortical cells as well as secreted factors contribute to the diffentiation and development of sympathetic neurons. (Supported by NSF Grant IBN-9310866)

416.8

PLASTICITY OF AUTONOMIC NERVES IN THE ADRENAL CORTEX. M.A. Holzwarth*[1], G.Wendelschafer-Crabb[2], W.R. Kennedy[2] and W.C. Engeland[3] [1]Dept. of Molecular and Integrative Physiology, Univ. of Illinois, Urbana IL 61801; [2]Dept. of Neurology and [3]Dept. Surgery, Univ. of Minnesota, Minneapolis MN 55455, USA

Autonomic innervation of the adrenal cortex includes fine plexuses of nerve fibers expressing catecholamines and a variety of neuropeptides. These nerves, hypothesized to participate in the regulation of adrenal blood flow, steroidogenesis and proliferation, are distributed primarily in the capsule and outer zona glomerulosa suggesting cell-specific functions. Since growth and secretory function of the zona glomerulosa are stimulated in rats on a low Na+ diet, we investigated whether adrenal innervation changes when the glomerulosa is expanded. Adrenals were collected at 1, 2 and 3 weeks from male rats maintained on low (0.04%) or normal (0.4%) Na+ diets. Adrenals were fixed, cryosectioned at 100 μm and immunostained for nerve fibers using antibodies against calcitonin gene-related peptide (CGRP), vasoactive peptide (VIP) and the pan-neuronal marker, protein gene product (PGP). With normal diet, the VIP(+) nerves show a more extensive distribution in the capsule and among outer cortical cells compared to the CGRP(+) nerves. The distribution of the nerve fibers was greatly expanded commensurate with the expanded glomerulosa in the low Na+ group. The nerve fibers appeared to loop inward within the glomerulosa and back out to the subcapsular region. Growth of nerve fibers was already evident after one week of low Na+ diet but was most prominent after 3 weeks. These findings demonstrate that the adrenal autonomic nerves show plasticity with their distribution being associated with the glomerulosa cell phenotype (Supported by NSF IBN-9310866 and IBN-9319097 and funds from the Minn. Med. Found.).

416.9

ENDOTOXIN INDUCES NEURONAL NITRIC OXIDE SYNTHASE IN THE ADRENAL IN AN AGE-DEPENDENT MANNER. J.R. Tobin*, L.C. Moore. Dept. of Anesthesia, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1009.

Nitric oxide (NO) is recognized as an important neurotransmitter and second messenger. Endotoxin (ENDO) elicits large increases in inducible (Type II) nitric oxide synthase (NOS) in many organs. This NOS isoform has a different molecular weight than neuronal NOS, and its activity is independent of Ca^{++}. Dexamethasone (DEX) inhibits inducible isoform expression. NOS in the adrenal inhibits steroidogenesis and has biphasic action on catecholamine secretion. Adrenal NOS increases following ENDO exposure.[1] This study examines age-related changes in NOS and isoform expression in the adrenal.

WKY rats of 5 age groups were studied (<48 hrs, 1, 2, 4, and 12 wks of age [n=8/group]). Animals were anesthetized and adrenals harvested. In a second experiment, ENDO (1 mg/kg ip), DEX 2 mg/kg + ENDO, or saline was administered to 1, 2, 4 and 12-wk-old rats (n=8/group) 4 hr prior to adrenal harvest. NOS activity was assayed by conversion of [^{14}C]-arginine to [^{14}C]-citrulline.[2] NOS activity was assayed for Ca^{++} dependency, and isoform analysis was performed by Western blotting, and immunocytochemistry. Data analysis was performed by ANOVA (*p<0.05).

Adrenal NOS is age-dependent with highest levels seen in young animals. ENDO elicits a significant increase in NOS at 2 and 4 wks of age (23±1 to 29.5±2.6* and 7.2±0.3 to 18±2* pmol/mg prot/min, respectively), but not 1 wk or 12 wk of age (7.5±0.6 to 7.8±0.3 and 3±0.3 to 3.2±0.3, respectively). DEX inhibits the increase in expression of NOS (4 wk control 7.2±0.3, DEX+ENDO 9.7±1.2, ENDO 18±2). Increased NOS activity by ENDO was Ca-dependent (activity without Ca=<1 pmol/mg prot/min) and Western blotting revealed increased neuronal isoform but minimal expression of Type II NOS. Immunocytochemical localization demonstrated heterogeneous staining of chromaffin and non-chromaffin cells. We noted age-related differences in NOS expression and age-related ENDO induced increases. These findings contrast with ENDO induced NOS expression primarily of Type II isoform in other tissues. Age-specific adrenal NOS responses to endotoxin may contribute to altered adrenal physiology.

1. Anesthesiology 83:A696, 1995; 2. Proc Natl Acad Sci USA 87:682-685, 1990

Supported by the Department of Anesthesia, Bowman Gray School of Medicine.

416.10

NONEXOCYTOTIC NORADRENALINE RELEASE FROM RAT CARDIAC SYNAPTOSOMES. M. Dumont* and S. Lemaire. Department of Pharmacology, University of Ottawa, Ottawa Ontario CANADA K1H 8M5.

Nonexocytotic noradrenaline (NA) release was examined in preparations of cardiac synaptosomes from male Wistar rats. Synaptosomes were prepared from rat hearts and labelled with [^{3}H]NA (300 nM; 1 hr at 37°C). Ischemic conditions (1 mM iodoacetate + 2 mM NaCN; 15 min at 37°C) caused a release of [^{3}H]NA from cardiac synaptosomes which represented 33.4% of total synaptosomal content. The [^{3}H]NA release was not diminished in the absence of extracellular Ca^{2+} or Na^+ but it was inhibited by preincubation of the loaded synaptosomes with the uptake$_1$ inhibitors, nisoxetine (10^{-4} M) and desipramine (10^{-4} M). Tyramine (10^{-6}-10^{-3} M), phencyclidine (10^{-6}-10^{-3} M) and rimcazole (10^{-6}-10^{-3} M) were also able to evoke the release of [^{3}H]NA from cardiac synaptosomes in the absence of extracellular Ca^{2+}. Among various drugs tested, dextrorphan (10^{-4} M) blocked the release of [^{3}H]NA induced by ischemic conditions and tyramine (10^{-4} M) but not by phencyclidine (10^{-4} M) and rimcazole (10^{-4} M). These data suggest that in all four experimental conditions, [^{3}H]NA release occurs via a nonexocytotic process involving most likely the reversal of the uptake$_1$ mechanism for ischemic conditions and tyramine and some other efflux mechanism for phencyclidine and rimcazole. Supported by the HSFO.

416.11

AGMATINE IS AN ENDOGENOUS MODULATOR OF NORADRENERGIC SYMPATHETIC NEUROTRANSMISSION IN BLOOD VESSELS. C. González, S. Ragunathan#, D.J. Reis#and C. Estrada*. Dpto. de Fisiología, Facultad de Medicina, UAM, Madrid, Spain, and #Div. of Neurobiology, Dep. of Neurology and Neuroscience, Cornell Univ. Med. Coll., New York, N.Y.

We investigated the vascular effects of agmatine (decarboxylated arginine), an endogenous ligand for α_2-adrenoreceptors and non-adrenergic imidazoline (I-) receptors, present in endothelium, smooth muscle and plasma, using the rat tail artery as a model. Isometric tension recordings were performed in isolated vascular rings maintained in an oxigenated physiological solution at 37°C and exposed to transmural nerve stimulation (TNS, 200 mA, 0.2 ms, 1 Hz, 10 s) and/or different drugs. By itself, agmatine (10 nM-1 mM) was without effect on isolated arterial rings, although at the highest concentration used (1 mM) it increased EC_{50} for contractions elicited respectively by the α_1- and α_2-adrenoreceptor agonists metoxamine and clonidine. Agmatine (0.03-1 mM) had a biphasic effect on contractions induced by TNS: an immediate reduction followed after ~10 min by a delayed facilitation. These effects were not replicated by agmatine on contractions induced by noradrenaline administration. The inhibition by agmatine of TNS-induced contractions was abolished by rawolscine or idazoxan, while the delayed facilitation was prevented by cocaine. Agmatine can regulate vascular function by actions at sympathetic nerve terminals wherein it acts as a prejunctional α_2-adrenoreceptor agonist and also by antagonizing noradrenergic transporter function. The results reveal the existence of a novel endogenous amine modulating noradrenaline release and re-uptake in the perivascular sympathetic terminals. Supported by grants 94/0388, and 95/1422 FIS, Spain.

416.12

EFFECT OF IRON (II) ON THE GENERATION OF HYDROXYL FREE RADICALS IN RAT MYOCARDIUM. T. Obata* and Y. Yamanaka. Dept. of Pharmacol. Oita Medical Univ., Hasama-machi, Oita 879-55, Japan

Using microdialysis technique, we examined the effect of iron (II) on the generation of hydroxyl free radicals (•OH) in the extracellular fluid of rat myocardium. Salicylic acid in Ringer's solution (0.5 $nmol \cdot \mu l^{-1} \cdot min^{-1}$) was directly infused through a microdialysis probe to detect the generation of •OH as reflected by the formation of dihydroxybenzoic acid (DHBA) in the myocardium of anesthetized rats. Cardiac dialysate was assayed for 2,3- and 2,5-DHBA by a high performance liquid chromatograph with an electrochemical (HPLC-EC) procedure. The average relative recovery of 2,3- and 2,5-DHBA by this system, tested *in vitro*, was 10.1±0.8 % and 10.5±0.9 %, respectively. Iron clealy produced a dose-dependent increase in the •OH formation. A positive linear correlation between the iron (II) and the formatioin of 2,3-DHBA (R^2=0.970) or 2.5-DHBA (R^2=0.983) was observed. However, when desferrioxamine (DES) was infused through dialysis probe, a marked increase in the DHBA formation was obtained.

416.13

GANGLION NEURONS ASSOCIATED WITH THE CAT LINGUAL NERVE AND ITS BRANCHES: DISTRIBUTION AND PARASYMPATHETIC SUPPLY TO THE ORO-MANDIBULAR REGION. S. Kuchiiwa[1]* and T. Kuchiiwa[2] Dept. Anat., Fac. Med., Kagoshima Univ., Kagoshima 890, Japan[1]; Dept. Life Science, Kagoshima Immaculate Heart College, Kagoshima 890, Japan[2].

Whole mount acetylthiocholinesterase (WATChE) histochemistry was performed to detect the localization of ganglion neurons associated with the cat lingual nerve and its branches with the aid of a dissection microscope. Besides the submandibular, sublingual and lingual ganglia, many WATChE-positive ganglia were found in the mandibular regions. Particularly constant were a single irregularly shaped prominent ganglion (oromandibular ganglion) and a few small ganglia (perilingual ganglia) in the mucous membrane of the oral cavity medial to the body of the mandible. The oromandibular ganglion consisted of two ganglion cell populations, large elliptical neurons which resembled the submandibular and sublingual ganglion neurons, and small irregularly shaped cells with an eccentric nucleus which were nearly comparable to the lingual ganglion neurons. The perilingual ganglia consisted of small cells similar to the lingual ganglion neurons. In the nicotinamide adenine dinucleotide phosphate- (NADPH-) diaphorase histochemistry, virtually all neurons in the oromandibular, perilingual and lingual ganglia were intensely stained, while the submandibular ganglion consisted of clusters of NADPH-diaphorase intensively labeled neurons and non-labeled neurons. In the animals which had received injections of WGA-HRP into the rostral half of the tongue in which no exocrine glands existed, retrogradely labeled neurons were observed in the lingual, perilingual and oromandibular ganglia. When the tracer was injected into the submandibular ganglion, labeled cells were found in the oromandibular ganglion. These findings suggest that the oromandibular ganglion plays a role in the vasomotor and secretomotor functions of the oro-mandibular region, and that the perilingual ganglia play some part in the vasomotor function of the tongue.

416.14

SYMPATHETIC NERVES CONTROL CEREBRAL VESSELS OPPOSITELY TO SYSTEMIC VESSELS. M. Maeda*, M. Inoue, H. Hirakawa, Y. Ikegami and Y. Hayashida. Dept. of Systems Physiology, Univ. of Occupational and Environmental Health, Kitakyushu 807, Japan

During an acute development of hypertension, cerebral vessels are constricted to maintain cerebral blood flow constant (autoregulation), on the other hand systemic vessels are dilated by a baroreflex. Denervation of the sympathetic nerves to control cerebral vessels impairs cerebral autoregulation. We, therefore, had a hypothesis that the sympathetic nerve activities (SNA) to control cerebral vessels may increase during hypertension in the opposite direction of the SNA to control systemic vessels. In order to test the hypothesis, we monitored the vertebral nerve activities, which are thought to be pure SNA to control cerebral vessels, during hypertension in anesthetized male rats. During hypertension by intravenous injection of phenylephrine (2 μg), the vertebral sympatetic nerve activities (VSNA) increased on the other hand the renal sympatetic nerve activities (RSNA) decreased. During hypotension by controlled hemorrhage, the VSNA decreased on the other hand the RSNA increased. The VSNA was completely undetectable after hexamethonium (4 mg) was intravenously injected, indicating this nerve activities were post ganglionic. The characteristics of the SNA, which are thought to be decreased and increase during baroreflex activation and inhibition, may have to be changed.

416.15

THE ROLE OF THE SYMPATHETIC INNERVATION IN THE REGULATION OF THE EYE BLOOD VESSELS IN FELINE. M.Razumovskij*, N.Balashov and A.Grigorian. Lab. of Vision Physiol., Inst. of Occup.Guidance and Decease, St.Petersburg, 195096, Russia.

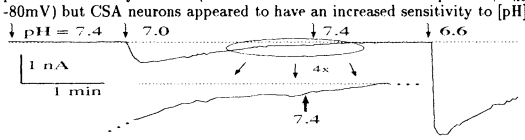
The functional significance of the sympathetic innervation in regulation of the arterial blood vessels tone in the eye bulb connective as well as in retina is still sufficiently far from clear understanding. With this aim in our experiments on adult male cats the diameter of fine vessels of the bulb connective and of eye retina was studied by means of special microsphere, the level of outflow intraocular liquid and contents of noradrenaline in arterial blood in iris membrane and ciliar body before and after desympathyzation were also determined. For control of the chronic desympathyzation a hystochemical method was used. It was shown that in the microcirculatory blood network of the feline eye there is a heterogenity of sensitivity to sympathetic influences. The vessels of the eye bulb connective as well as of episclera exist under predominantly tonic sympathetic influences. Meanwhile sympathetic denervation had no effect on the diameter of the arteriols of the central part of retina. The increase of the intraocular pressure was accompanied by vasoconstriction of the arterioles of the retina in desympathetized eye. In summary, the obtained data suggest, that in contrast to eye bulbar connective and episclera, the autoregulatory mechanism for vessels exists only in the arterial network of the retina.

416.16

DO IDENTIFIED CARDIAC SYMPATHETIC AFFERENT NEURONS EXHIBIT AN INCREASED SENSITIVITY TO $[pH]_o$?

J.H. Schild* Oregon Health Sciences University, Portland OR. (jhs@rice.edu)

Cardiac sympathetic afferent (CSA) fibers with cell bodies in the thoracic spinal ganglia are markedly activated during acute myocardial ischemia (MI) and are believed to be involved in cardiac related pain. One of the many consequences of MI is a decrease in extracellular pH ($\downarrow[pH]_o$) which can contribute to the activation of CSA fibers. **Objectives**: Is it possible to label CSA fibers in a manner that enables identification of CSA neurons from among a general population of isolated sensory neurons? Do CSA neurons respond any differently to $\downarrow[pH]_o$ than do cells from the general population of sensory neurons? **Procedure**: The fluorescent probe rhodamine was injected into the pericardial sac of 3-4 day old rat pups. After 3-7 days the rats were euthanized and the left T_3 ganglia was removed. The neurons were enzymatically dispersed in the conventional manner and prepared for patch clamp recording. Rhodamine labeling identified the functionally specific CSA neurons. A multibarrel pipette provided rapid exchange of perfusates which were closely matched in chemical composition, osmolarity and temperature but differed in pH (6.5-7.4). **Results**: A rather modest 0.4 $\downarrow[pH]_o$ from a background $[pH]_o$ of 7.4 elicited a large inward current. The time course of the response was similar to that reported in the literature for an unspecified population of sensory neurons (both transient and sustained components, V_{hold} = -80mV) but CSA neurons appeared to have an increased sensitivity to $[pH]_o$.



Conclusion: It is technically feasible to fluorescently label CSA fibers and study identified CSA neurons in isolation. These preliminary data support the intriguing possibility that, consistent with their physiological role, CSA neurons exhibit an elevated sensitivity to $\downarrow[pH]_o$. (support: NIH HL09242)

416.17

INCREASED EXPRESSION OF SP mRNA IN DORSAL ROOT GANGLIA NEURONS OF THE TWO KIDNEY-ONE CLIP OVARIECTOMIZED FEMALE RAT. L. P. Solano-Flores*, M. P. Rosas-Arellano and J. Ciriello. Department of Physiology, University of Western Ontario, London, ON, Canada, N6A 5C1.

It has been suggested that afferent renal nerves are involved in both the development and maintenance of the elevated arterial pressure in the two kidney, one clip (2K1C) model of experimental hypertension. However, the receptors and the neurotransmitters associated with the afferent fibers from these receptors involved in this hypertensive process are not known. Recently, we have shown that renal artery occlusion results in the induction of c-fos in dorsal root ganglion neurons that contain substance-P (SP). In this study, the possibility was examined that SP may be the putative neurotransmitter in afferent renal nerves activated during the development of 2K1C hypertension. Experiments were done in female ovariectomized rats. The left renal artery was clipped or sham-clipped. After arterial pressure had increased approximately 15-20 mmHg above sham-clipped control animals in the 2K1C animals, all animals were sacrificed and the dorsal root ganglia from T8 - L1 of both the clipped or sham-clipped side and the contralateral side were removed. The ganglia were processed for *in situ* hybridization using an oligonucleotide probe for SP mRNA labelled using terminal deoxynucleotide transferase and alpha-^{35}S dATP. Autoradiograms were exposed for 4 weeks and analyzed using light microscopy. A large number of neurons in the dorsal root ganglia from levels T11 - T13 on the side ipsilateral to the clipped renal artery expressed SP mRNA. On the other hand, few, if any neurons in dorsal root ganglia from the contralateral side or in sham-clipped animals at the same spinal levels expressed SP mRNA. These data suggest that SP may be one of the neurotransmitters involved in mediating renal sensory information during the establishment of 2K1C hypertension. (Supported by Heart and Stroke Foundation of Ontario).

416.18

INDUCTION OF C-FOS IN DORSAL ROOT GANGLION NEURONS CONTAINING NEUROPEPTIDE IMMUNOREACTIVITY AFTER ACTIVATION OF THE RENAL MECHANO- OR CHEMO-RECEPTORS. M. P. Rosas-Arellano,* L. P. Solano-Flores and J. Ciriello, Department of Physiology, University of Western Ontario, London, ON, Canada, N6A 5C1.

Although a variety of putative neurotransmitters have been identified in afferent renal nerves (ARN), it is not known whether ARN mediating certain functional information contain specific neurotransmitters. The region of the kidney where renal mechano- (MR) and chemo- (CR) receptors are found is innervated by nerve fibers that contain substance-P (SP) and calcitonin gene-related peptide (CGRP). The present study was done to determine whether MR and/or CR afferent fibers contain SP and/or CGRP and to identify the distribution of c-fos immunoreactive neurons in relation to SP and CGRP terminal fields in the spinal cord after MR or CR activation. The left renal vein was occluded (VO) to activate MC, and left renal artery occlusion (AO) was used to activate CR in the Inactin anesthetized rat. C-fos labelled neurons were found in the ipsilateral dorsal root ganglia (DRG) extending from T9 - L1, with most within the T11 - T13 DRG after either activation of MR or CR. None were found in the contralateral DRG or DRG from the same spinal levels of sham-occluded animals. After AO, most c-Fos labelled neurons in DRG were found to be immunoreactive to SP and a small number to CGRP. In contrast, in DRG of rats with VO, a few c-fos labelled neurons contained CGRP and a smaller number contained SP. Within the ipsilateral dorsal horn of the spinal cord, c-fos labelled neurons were found throughout laminae I-III in rats with VO, and predominantly along the medial edge of lamina III in AO rats. These c-fos labelled neurons were surrounded by SP and CGRP immunoreactive fibers. No c-fos labelled neurons were found in the contralateral dorsal horn or in the dorsal horn of the sham-occluded animals. These data suggest that specific neurotransmitters may be used by functionally different ARN. (Supported by Heart and Stroke Foundation of Ontario)

416.19

SEGMENTAL ORGANIZATION OF NERVE INNERVATING THE VERTEBRO-BASILAR ARTERIAL SYSTEM IN CATS. J. C. Liu, L. C. Lu, S. D. Wang and C. L. Cheng*. Depts. of Biology & Anatomy, and Surgery, National Defense Medical Center, Taipei, Taiwan, ROC.

In mammal, intracranial and extracranial blood vessels receive extensive afferent and efferent innervation that plays a very important role for physiological function. Only a few studies examined the origin of the vertebro-basilar arterial system. The present experiment is designed to extensive study the localization of the vertebro-basilar arterial system in cats by using retrograde HRP tracing method. HRP was applied on the initial segment, C2-C3 level and intracranial segment of the vertebral artery (VA), and basilar artery. HRP labeled cell bodies are existed in bilateral middle cervical and stellate ganglia, and ipsilateral C8-T3 dorsal root ganglia from the initial segment of VA. HRP labeled cell bodies are presented in bilateral superior cervical ganglia and ipsilateral C2-C3 dorsal ganglia from the C2-C3 level of VA. HRP labeled cell bodies are identified in bilateral superior cervical and ophthalmic branch of trigeminal ganglia and C1 dorsal root ganglia from intracranial segment of VA. HRP labeled cell bodies are existed in bilateral superior cervical and ophthalmic branch of trigeminal ganglia. The labeled neurons are identified more on extracranial arteries than intracranial arteries with ipsilateral predominance. There is no HRP labeled neuron in the ciliary, pterygopalatine, otic and nodose ganglia. These results suggest that segmental organization of nerve innervation is noted at the vertebro-basilar arterial system. (Suported by NSC-85-2331-B016-095, ROC)

416.20

INWARD RECTIFIER POTASSIUM CHANNELS (K_{ir}'s) IN THE RAT MIDDLE CEREBRAL ARTERY (MCA). **T.D. Johnson, M.L. Steenberg, R.G. Grossman*, and R.M. Bryan, Jr. Dept. of Anesthesiology, Baylor College of Medicine, Houston, TX 77030**

K_{ir}'s are voltage dependent channels (closed by depolarization) whose open state probability increases with increased extracellular K^+. We asked three questions: (1) Are K_{ir}'s present in the proximal portion of the rat MCA? (2) Do K_{ir}'s contribute to the resting tone of the MCA? (3) Are K_{ir}'s involved in the myogenic response in the MCA? Rat MCAs were isolated, cannulated at each end with a glass micropipet, and pressurized to 40, 85, or 100 mm Hg. The MCAs were immersed in a bath (37° C) containing physiological saline solution. Resting diameters for the MCAs at 40, 85, and 100 mm Hg were 209 ± 8, 198 ± 6, and 189 ± 6 μm (n=7 for each) respectively. Abluminal administration of 15 mM KCl produced a dilation of 18 ± 2, 19 ± 2, and 21 ± 4 % at 40, 85, and 100 mm Hg respectively. At a concentration of $BaCl_2$ (75 μM) specific for K_{ir} inhibition, the dilation to KCl was completely blocked at all three pressures. The dilations elicited by 15 mM KCl were not affected by TEA (1 mM), Glibenclamide (10 μM), or 4-aminopyridine (1 mM) indicating the dilation did not involve other types of K channels. The addition of $BaCl_2$ decreased the resting diameter by approximately 8% at all pressures. Upon increasing the pressure from 40 mm Hg to 85 and 100 mm Hg, MCAs demonstrated a myogenic response by significantly constricting 5 ± 1 and 10 ± 1 % respectively ($p<0.0001$). Blocking the K_{ir}'s with $BaCl_2$ did not affect the myogenic response. We conclude that (1) K_{ir}'s are present in the proximal portion of the rat MCA; (2) Some, but not all, of the K_{ir}'s are open during the resting conditions and are responsible for dilating the MCAs by 8%; and (3) The K_{ir}'s are not involved in the myogenic response. (PHS grant PO1 NS 27616)

416.21

HYPERPOLARIZATION-ACTIVATED CATION CURRENT (I_H) IN NODOSE SENSORY NEURONS. T. N. Doan, P. J. Reynolds, M. C. Andresen, and D. L. Kunze*. Baylor College of Med., Houston, TX 77030;Oregon Health Sciences Univ., Portland, OR 97201;Rammelkamp Research Center, Cleveland, OH 44109.

The role of I_H at the peripheral terminal of the baroreceptor neuron is unknown. When capsaicin-insensitive fibers of the aortic depressor nerve (ADN) were perfused with CsCl (a blocker of I_H) during a slow pressure ramp, there was a decrease in the threshold pressure (95.2%±2.1; means±SE) and a decrease in threshold frequency (79.4%±20.8) relative to control without any significant changes in the slope, saturation pressure, and saturation frequency (n=7). An ADN fiber that was capsaicin-sensitive (previously shown not to have I_H) was not affected by CsCl. To further investigate the effects of CsCl, isolated rat nodose sensory neurons were studied with the nystatin-perforated patch during current clamp. When neurons were given hyperpolarizing current injections, a time-dependent rectification occurred due to the slow activation of the non-inactivating inward current I_H. When CsCl was superfused, the time-dependent rectification was abolished, resulting in greater hyperpolarization. The input resistance of the neuron at membrane potentials more negative than -70mV was increased from 0.40±0.09 GΩ to 1.15±0.15 GΩ in the presence of CsCl (n=9; p<0.001). Unexpectedly, when depolarizing current ramps from the resting membrane potential (-60mV) were used to simulate pressure changes at the peripheral terminals, CsCl did not affect the interspike interval or the profile of the action potential (n=3; p>0.05). However, block of I_H with CsCl at hyperpolarized membrane potentials (-100mV) enabled nodose neurons to spike during subsequent depolarizing current injection to potentials at which there was no discharge in the absence of CsCl (n=3). The contribution of I_H to this response is currently under investigation. (Supported by HL36840, HL41119.)

416.22

THE EFFECT OF THE ANTICHOLINESTERASE EDROPHONIUM ON AUTONOMIC CHOLINERGIC TRANSMISSION. RD Stein*, C Polosa and SB Backman, Dept. Anaesthesia, Royal Victoria Hospital & Dept. Physiology, McGill University, Montreal, Quebec H3A 1A1.

In the absence of preganglionic input, the anticholinesterase edrophonium (EDRO) can produce autonomic responses. For example, in vagotomized, beta-blocked cats EDRO produces a dose-dependent bradycardia which is highly correlated to its inhibition of cholinesterase (Can J Anaesth 1996, 43 in press). Presumably, the bradycardia results from an anticholinesterase action, preventing the hydrolysis of ACh spontaneously released from pre- and postganglionic neurons. The present study examined the effect of EDRO on autonomic responses in the presence of preganglionic input. Cats were anaesthetized, mechanically respired, bilaterally vagotomized and beta-blocked. Continuous right vagus nerve stimulation decreased baseline heart rate from 162 ± 11 bpm to 101 ± 6 bpm (38 ± 1% decrease, n=8). EDRO produced a dose-dependent bradycardia with a max decrease to 81.4 ± 4.0 bpm (78.9 ± 2.6 % baseline) at a dose of 0.8 ± 0.2 $mg.kg^{-1}$. Unexpectedly, at doses greater than 1.5 $mg.kg^{-1}$ EDRO produced a dose-dependent tachycardia to a max of 147.3 ± 9.2 bpm (131 ± 3 % baseline) at a dose of 20 $mg.kg^{-1}$. The possibility that EDRO in higher doses interferes with cholinergic ganglionic transmission was investigated in an *in-vitro* rat cervical sympathetic ganglion preparation. In the presence of muscarinic and partial nicotinic block, EDRO produced a dose-dependent reduction in the compound action potential recorded from postganglionic axons to preganglionic stimulation (ED_{50} 147 ± 24 μM, n=3). In anaesthetized, mechanically respired, muscarinic- blocked rats EDRO (20 $mg.kg^{-1}$) abolished the excitatory response recorded from postganglionic axons to injection of ACh (100 μg) into the cervical sympathetic ganglion (n=4). These observations suggest that EDRO, in higher doses, may interfere with nicotinic receptor activation and thereby block autonomic ganglionic transmission.

Support: The Canadian Anaesthetists' Society (SBB) and MRC (CP).

GASTROINTESTINAL REGULATION: PERIPHERAL MECHANISM

417.1

EVIDENCE FOR LOCAL EFFECTOR ACTION OF VAGAL AFFERENT INTRAGANGLIONIC LAMINAR ENDINGS (IGLEs) IN RAT GASTRIC MYENTERIC PLEXUS. H.-R. Berthoud*, A. Lauve, K.A. Sharkey and L.M. Patterson, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808, USA, and Univ. of Calgary, Canada.

Intraganglionic laminar endings (IGLEs) are found throughout the myenteric plexus of esophagus and gastrointestinal tract, but no specific receptive function has been conclusively associated with this most prominent type of vagal afferent terminal. Since ultrastructural studies have shown the presence of numerous mitochondria and small clear vesicles in WGA-HRP labeled IGLEs of rat esophagus, it has been suggested that these terminals can release neurotransmitters or modulators to affect myenteric plexus neurons (Neuhuber et al., 1991), and the aim of the present experiment was, therefore, to provide functional evidence for such effector action.

Left supranodose vagotomy was performed in male SD rats to let vagal preganglionic fibers degenerate but leave vagal afferent fibers intact. Ten to 30 days later animals were anesthetized, and the peripheral end of the left cervical vagus was re-exposed and electrically stimulated for 30 min (16 Hz, 1 mA, 0.5 ms, 10s on, 10s off), or sham-stimulated (no current). One hour after termination of stimulation the rats were perfused and c-fos immunocyto-chemistry was performed on sections of the gastric corpus. On the ipsilateral (ventral) corpus, c-fos was expressed in 1.60±0.48% of myenteric plexus neurons, while in sham-stimulated animals 0.08±0.04%, and in animals with efferent vagal stimulation 27% of the neurons were activated.

The results support the hypothesis that vagal afferent IGLEs can act as effectors to execute local axon reflexes. However, only a fraction of the neurons anatomically associated with the IGLEs seems to be activated, and an additional sensory function as mechano- or chemoreceptors cannot be ruled out. Supported by NIH grant DK 47348.

417.2

LOCALIZATION AND CHEMICAL CODING OF NK3 RECEPTOR-IMMUNOREACTIVE ENTERIC NEURONS IN RAT GASTROINTESTINAL TRACT. A.M. Yunker†‡*, V.V. Karpitskiy‡, K.A. Roth†, and J.E. Krause‡. Depts. of †Pathology and ‡Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Tachykinins (TKs) bind and activate three distinct neurokinin (NK) receptors (NKrs) in gastrointestinal (GI) tissues, resulting in altered motility, secretion, and absorption. Although distribution of NK1rs has been elucidated, the cellular localization of NK3rs is unclear. Therefore, polyclonal antiserum and monoclonal antibodies generated against NK3rs were used on longitudinal sections and whole mount preparations of rat GI tract. Immunoreactive myenteric somata with Dogiel type I morphology and numerous varicose nerve fibers were observed in preparations of glandular stomach, small and large intestines, and caecum. Although NK3r-ir nerve fibers associated with arterioles, GI smooth muscle, and lamina propria, the density of innervation was not uniform, as more NK3r-ir nerves associated with circular muscle in stomach and caecum, whereas more NK3r-ir nerves associated with lamina propria in colon. NK3r-ir was not detected in epithelial, endothelial or smooth muscle cells, or in interstitial cells of Cajal. In preparations of small intestine, many submucosal and myenteric NK3r-ir nerve fibers contained vasoactive intestinal polypeptide (VIP)-ir or nitric oxide synthase (NOS)-ir, although some VIP-ir and NOS-ir nerves did not contain detectable NK3r-ir. Subpopulations of submucosal and myenteric TK-ir nerves also contained NK3r-ir, although TK-ir and VIP-ir were not co-localized. Finally, most NK3r-ir somata did not contain NK1r-ir. Taken together, these results suggest that NK3rs are distributed throughout the gut, and are likely found on both inhibitory and excitatory nerves. Supported by NIH grants GM07805 (JEK), NS35107 (KAR), and T32-DK07296.

417.3

CHOLECYSTOKININ SYNERGISTICALLY INTERACTS WITH LEPTIN TO ACTIVATE RAT GASTRIC VAGAL AFFERENT TERMINALS. Yu Hua Wang, Y Taché, AB Scheibel*, VLW Go and Jen Yu Wei. Dept. of Med., CURE Digest. Dis. Res. Cent. and BRI, UCLA Sch. of Med., Los Angeles, CA 90095.

Leptin and cholecystokinin (CCK) administered peripherally reduced food intake in rodents. CCK action is mediated via activation of gastric vagal afferent (GVA) terminals. We investigate the influence of leptin on GVA discharges and modulation of leptin action by CCK. Experiments were conducted on in vitro gastric vagus-stomach preparation (Gastroenterology 100:A507, 1991). The left gastric artery was cannulated for CCK and leptin intra-arterial injection (ia). The GVA unit activities were recorded from thin strands isolated from the gastric branch of the vagus. Three multi-units and 9 single units were analyzed from 3 experiments. Five of the 9 units displayed continue irregular ongoing activity (median 0.22 spike/sec), 3 with rhythmic and 1 intermittent activity. The mechanoreceptive fields were located at pylorus-antrum (n=5) and fundus-corpus (n=4) regions. The response magnitude of CCK-8 ia (100 pmol n=3, 10 pmol n=9) were 5 to 10 times greater than those of leptin ia (5, 10, 50 μg). Most of the response durations of CCK-8 ia were >30 min. Data indicate that prior CCK-8 ia enhances the leptin sensitivity of GVAs. For a confirmation, comparisons between the GVAs response to leptin before and after CCK-8 10 pmol ia were made by dividing the leptin-induced average spike count/bin (5 sec) after CCK-8 ia by that before ia. A quotient >1 indicates that the leptin sensitivity of GVAs increased. Six units have been analyzed from 2 experiments, and of those, three were tested with 5 and 10 μg leptin ia and the other with 50 μg. Seven quotients of the 9 trials were >1 (a range of 7.94 - 1.15 with median of 1.38), whereas the other 2 were 0.93. A similar enhancement effect of prior leptin ia on CCK-8 ia has also been noticed. (We thank Amgen Inc. for providing the leptin, Supported by NIH Grants NS 28433 & DK30110)

417.4

METABOTROPIC GLUTAMATE RECEPTORS IN THE ENTERIC NERVOUS SYSTEM (ENS) AND PANCREAS. A. Kirchgessner* and M.-T. Liu, Dept. of Anat.& Cell Biol., Columbia Univ., NY, NY 10032.

Glutamate appears to be an excitatory neurotransmitter in the ENS and pancreas. Prior studies have demonstrated NMDA and AMPA receptor subtypes in enteric and pancreatic ganglia. The present study was carried out (i) to determine whether metabotropic glutamate receptors (mGluRs) are involved in enteric glutamatergic neurotransmission, and (ii) to locate mGluRs in the gut and pancreas. Microejection of the mGluR agonist, 1S,3R-ACPD, induced a slow depolarization in a subset of myenteric AH/type 2 neurons. The slow response was associated with an increase in input resistance, inhibition of the afterhyperpolarization and an increase in the excitability of the cells. Immunocytochemistry, using antibodies (from Dr. R.J. Wenthold) which recognize mGluR1α and mGluR2/3 subtypes, was employed to identify the neurons on which these receptors are located. mGluR1α and mGluR2/3 immunoreactivities (ir) were found in the perikarya of subsets of submucosal and myenteric neurons in all regions of the rat and guinea pig bowel. In both plexuses, all mGluR2/3-ir neurons contained ChAT, and a subset were co-stained with antibodies to calbindin (marks AH/type 2 neurons). mGluR1α-ir neurons were rare in the guinea pig myenteric plexus; however, in the submucosal plexus, 5.0 ± 0.5 neurons/ganglion were mGluR1α-ir. In addition, mGluR1α-ir fibers were abundant in the circular muscle. In the pancreas, a subset of neurons and insulin-ir islet cells were mGluR2/3-ir. Moreover, mGluR2/3-ir was found on ductal epithelial cells and goblet cells in mucosal crypts and villi. These observations are consistent with the idea that mGluRs play a role in the function of the gut and pancreas. Supported by NIH grants NS01582, NS27645 and the American Diabetes Association.

417.5

ANALYSIS OF PURINERGIC PATHWAYS IN MYENTERIC PLEXUS OF THE GUINEA PIG ILEUM. K. J. LePard* and J. J. Galligan, Dept. of Pharmacology/Toxicology and Neuroscience Program, Michigan State University, E. Lansing, MI 48824.

ATP mediates fast excitatory postsynaptic potentials (fEPSPs) in the myenteric plexus *via* P_{2X} receptors. These studies examine axonal length and polarity of projection of purinergic neurons. Under ketamine/xylazine anesthesia, either extrinsic nerves were crushed (extrinsic denervation) or long myenteric pathways were interrupted (myotomy, single or double). Each treatment group consisted of 4 animals except sham (2). Effectiveness of surgical treatments was confirmed by histochemical methods. After severed axons degenerated (4-5 days), the relative proportion of purely cholinergic fEPSPs (blocked ≥83% by hexamethonium, 100 μM) *vs.* cholinergic/purinergic fEPSPs (noncholinergic fEPSP blocked ≥20% by pyridoxal phosphate-6-azophenyl-2',4'-disulphonic acid, PPADS, 10 μM) at the operated site was determined using conventional electrophysiology methods. Noncholinergic fEPSPs unaffected by PPADS were classified 'other'. Table lists percentage of each type fEPSP observed in operated tissues for each surgical treatment. (Kruskal-Wallis *p<0.05 as compared to sham.)

surgical treatment	# neurons	mixed cholinergic/ purinergic fEPSP	cholinergic fEPSP	mixed cholinergic/ other fEPSP
sham	16	75%	19%	6%
extrinsic denervation	23	61%	17%	22%
double myotomy*	27	30%	48%	22%
single myotomy				
oral side*	14	21%	79%	0%
anal side	15	53%	47%	0%

The proportion of mixed cholinergic/purinergic fEPSPs was reduced in the isolated segment of myenteric plexus created by the double myotomy. A similar reduction was observed in recordings from the oral side of a single myotomy. These data suggest that most purinergic pathways are long and orally directed while a smaller proportion come from shorter local projections. (Supported by DK40210, NS01738 NS33289, NS07279.)

417.6

ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL INVESTIGATION OF LONGITUDINAL MUSCLE MOTOR NEURONS IN THE GUINEA-PIG ILEUM. T.K. Smith and C.W.R. Shuttleworth*, Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV, 895570-0046.

During the peristaltic reflex, contraction of the longitudinal muscle layer precedes contractile activity of the underlying circular muscle layer.

The mechanisms underlying increased sensitivity of the longitudinal muscle are not understood. We have tested the hypothesis that this phenomenon is due to increased excitability of longitudinal muscle motor neurons (LMMNs).

Standard intracellular microelectrodes (containing 2% neurobiotin in 2M KCl) were used to impale myenteric neurons in isolated segments of guinea-pig ileum. Impaled neurons were subsequently visualized by confocal microscopy, after processing to couple FITC-streptavidin to injected neurobiotin.

LMMNs were small neurons (10 - 15 μm) whose axon formed a number of varicose collaterals that contributed to the tertiary plexus supplying the longitudinal muscle. LMMNs were either at the entrance of internodal strands or in microganglia. These cells formed a distinct electrophysiological class of neuron, having 1) low resting membrane potential (-47 ± 5 mV), 2) high input resistance (475 ± 50 MΩ), 3)an "off spike" following the termination of hyperolarizing voltage response 4) low threshold for action potential generation (0.02-0.04nA) and responded either tonically or phasically with a short burst of action potentials (1 to 8). 20% (n=58) of LMMN neurons fired action potentials spontaneously. Nerve stimulation produced fast and slow excitatory synaptic potentials. Inhibitory postsynaptic potentials were seen in some neurons.

In conclusion, these results suggest that LMMNs have a characteristic topagraphy within ganglia and distinctive electrophysiological characteristics. Their high excitability suggests that they may be activated before other muscle motor neurons during the peristaltic reflex. Support: NIH DK 45713 and DK 41315

417.7

LOCALIZATION AND FUNCTION OF GLUTAMATE TRANSPORTERS IN THE ENTERIC NERVOUS SYSTEM (ENS) AND PANCREAS. M.-T. Liu*, J. D. Rothstein and A.Kirchgessner, Dept. of Anat.& Cell Biol., Columbia Univ., NY 10032, Dept. of Neuro., Johns Hopkins Univ., MD.

Glutamate (Glu) mediates excitatory neurotransmission in the ENS and pancreas. In the CNS, Glu is inactivated by diffusion and uptake. The present study was carried out (i) to locate Glu transporters in the gut and pancreas, and (ii) to determine whether Glu transport is important in enteric glutamatergic transmission. Immunocytochemistry, using antibodies to the Glu transporters, EAAC1, GLT-1, and GLAST, was employed to identify the structures on which these transporters are located. All Glu-immunoreactive (ir) neurons in both plexuses of the rat and guinea pig gut and in the pancreas were EAAC1-ir; however, EAAC1-ir was also observed in non-glutamatergic neurons including myenteric calbindin-ir neurons (marks AH/type 2 neurons). GLT-1-ir was observed in varicose nerve fibers in both enteric plexuses, and in the circular muscle layer. Both EAAC1- and GLT-1-ir were found in 5-HT-ir enterochromaffin cells. GLAST-ir was found in interstitial cells of Cajal. In the pancreas, EAAC1- and GLAST-ir were found on insulin-ir islet cells. If the EAAC1 transporter is important in glutamatergic transmission then its inhibition would be predicted to potentiate responses to Glu. In the presence of THA (200 μM), a blocker of Glu transport, depolarizing responses to Glu and Glu-mediated fast EPSPs were enhanced in myenteric AH/type 2 neurons. These observations are consistent with the idea that Glu transporters play a role in enteric glutamatergic transmission. The postsynaptic expression of EAAC1 supports its function to clear Glu; its presence in non-glutamatergic neurons could serve to provide neurons with Glu as a precursor of GABA and/or an energy source. Supported by NS01582, NS27645 and the American Diabetes Association.

417.8

LOWER ESOPHAGEAL DISTENSION ALTERS THE SENSITIVITY OF CHOLECYSTOKININ RESPONSIVE GASTRIC VAGAL AFFERENTS: AN IN VITRO STUDY. Jen Yu Wei* and Yu Hua Wang. Dept. of Med., CURE Digest. Dis. Res. Cent. and BRI, UCLA Sch. of Med., Los Angeles, CA 90095.

Esophageal distension (ED) induces gastric relaxation through vagal dependent pathways (Dig Dis Sci 34:873, 1989). We reported that >40% of the relaxation response can be preserved after the central and enteric nervous system connection were interrupted while the vagal link between the lower esophagus and stomach was kept intact. This result suggests the possible involvement of a peripheral collateral reflex action (Neurosci Abst 21:1632, 1995; J Auton Nerv Syst, 52:83-97, 1995). CCK iv decreases intragastric pressure (Am J Physiol, 253:G165, 1987) and activates gastric vagal afferents (GVAs) which has been implicated in mediating CCK's satiety action. Using an in vitro gastric vagus-stomach preparation (Gastroent 100:A507, 1991), we investigate the influence of ED on the responsive of GVAs to CCK-8 ia (via a left gastric artery cannula). A ligature was made at the gastroesophageal junction, which keeps both gastric vagal nerves intact. The GVA unit activities were recorded from ventral vagus nerve strands at the lower thoracic level. A thin latex balloon was introduced into subdiaphragmatic esophagus for ED. Comparisons between the GVAs response to CCK-8 ia with vs without prior ED (0.1 ml saline) were made by dividing the CCK-induced average spike count/bin (5 sec) with prior ED by that without ED. A quotient >1 indicates that the CCK-8 sensitivity of GVAs increased. Three multi- and 6 single units were analyzed from 3 experiments, and of those, 2 multi- and 4 single units were tested with 5 pmol CCK-8 and the other with 10 pmol. Three quotients of multi units were 1.09, 1.48, 0.98, respectively. The quotients of 5 of the 6 single units were >1, with a range of 1.86-1.05 and a median of 1.47, the other one is < 1 (0.75). These results suggest that ED enhances the sensitivity of GVAs to CCK-8 ia. (Supported by NIH Grant NS 28433)

417.9

INTRACELLULAR RECORDING FROM NEURONS OF THE HUMAN GALLBLADDER. K. Hillsley, L. J. Jennings*, S. B. Backman† and G. M. Mawe. Dept. Anatomy and Neurobiology, University of Vermont, Burlington, VT, 05405 & †Dept of Anaesthesia, Royal Victoria Hospital, Montreal, H3A 1A1 Canada

Gallbladder neurons are important governors of gallbladder function. In animal models, gallbladder ganglia are regulated both by neural inputs from sympathetic fibres, vagal efferent fibres and sensory axon reflexes, and by hormonal input from cholecystokinin. The purpose of this study was to demonstrate feasibility of obtaining recordings from intact human gallbladder neurons.

Human gallbladders were obtained from liver transplant donors (n=3) and routine cholecystectomies (n=2). Preparations were bathed in oxygenated Krebs (37 °C) containing the vital fluorescent stain 4-(4-diethylaminostyryl)-N-methyl-pyridinium iodide to aid in localization of the ganglia. Cells were characterized using conventional intracellular recording techniques. A total of 8 cells, from both normal (n=5) and diseased (n=3) gallbladders which exhibited overshoot action potentials were studied.

The mean resting membrane potential of human gallbladder neurons was -52.3±2 mV (range=-45 to -59 mV; n=8). Depolarizing current pulses (≥ 250 ms) elicited only 1-3 spikes regardless of the amplitude or duration of the current pulse. Afterspike hyperpolarizations had a mean duration of 114±6 ms (range=77-181 ms; n=7) and an amplitude of 10.1±2.2 mV (range=4-15.9 mV; n=7). Anodal break excitation was recorded in 2/8 neurons in response to 0.2 nA negative current pulses. Spontaneous activity was recorded in 3 neurons, all from the same normal gallbladder. In 5 cells, fibre tract stimulation elicited fast EPSPs, resulting in spikes in 3 neurons. There was no detectable difference between neurons from healthy and diseased gallbladders.

These preliminary results are the first report of intracellular recordings of human gallbladder neurons. It appears from these results that human and guinea pig (Mawe; J. Physiol., 1990, 429, 323-38) gallbladder neurons have similar electrical properties and that electrophysiological studies of intact human gallbladder neurons are feasible. (Supported by NIH grants DK45410 & NS 26995).

417.10

IDENTIFICATION OF THE CHOLINERGIC NEURONS IN GUINEA PIG SPHINCTER OF ODDI (SO) GANGLIA. E.K. Talmage, K. Hillsley, A.L. Krystofolski, and G.M. Mawe*. Dept. Anatomy and Neurobiology, University of Vermont, Burlington, VT, 05405

Neurons in guinea pig SO ganglia can be divided into two populations on the basis of transmitter phenotype. The majority of cells express substance P (SP) and/or enkephalin and are presumed to be excitatory neurons. A distinct group of cells express nitric oxide synthase (NOS) plus vasoactive intestinal peptide or neuropeptide Y, and are presumed to be inhibitory neurons. The purpose of this study was to identify the neurons in these ganglia express choline acetyltransferase (ChAT) immunoreactivity (IR).

SO whole mount preparations were fixed 2-12 hr in 2% formaldeyde/ 0.2% picric acid in phosphate buffer, and immunostained for expression of putative neurotransmitters and related synthetic enzymes. Similar results were obtained with and without colchicine pretreatment. ChAT-IR was present in 70-80% of the neurons in the SO. Many, but not all, ChAT-IR neurons expressed SP-IR, while all SP-IR neurons expressed ChAT-IR. ChAT and NOS were not coexpressed; nor were ChAT-IR and NADPH-diaphorase activity. Together, the ChAT and NOS positive populations of cells accounted for all of the detectable SO neurons.

These data indicate that a large proportion of SO neurons are able to synthesize acetylcholine. Furthermore, these findings are consistent with the concept that the ganglia of the SO consist of two general populations of neurons, one excitatory and the other inhibitory. (Supported by NIH grants DK45410 & NS 26995).

417.11

INNERVATION OF THE SMALL AND LARGE INTESTINES BY PREVERTEBRAL AND PELVIC GANGLIA. G.B. Luckensmeyer and J.R. Keast* Dept. Physiol. & Pharmacol., Uni. Qld., 4072, Australia.

Our studies investigate the effects of sympathetic nerve stimulation on different regions of intestine. The ileum, proximal and distal colon and rectum with associated prevertebral and pelvic ganglia were removed from anaesthetised male Wistar rats and placed in an organ bath. The effects of sympathetic nerve stimulation on longitudinal (LM) and circular muscle (CM) were observed. Nerve stimulation caused frequency-dependent inhibition in the ileum, while in all regions of the large intestine a mixture of excitation (α-adrenergic, and non-adrenergic, non-cholinergic) and inhibition (mediated by all three types of β-adrenoceptors) was observed. In the LM this excitation was the dominant response for low frequency stimulation (0.1-2Hz), while the inhibitory response was dominant at 5-30Hz. In the CM the excitatory response was more prevalent (0.5 -20Hz). The sympathetic innervation of the lower bowel by the pelvic ganglia differed slightly to that by the prevertebral ganglia, with a weaker and less complex response in both LM and CM. Stimulation of the cavernous nerve (CN; connecting the pelvic ganglia with the caudal rectum) resulted in excitation of both LM and CM, most of which was atropine sensitive. An anatomical visualisation of the CN projections to the rectum was also carried out by anterograde labelling. These studies show that the sympathetic innervation of the large intestine differs to that of the rest of the bowel by predominantly causing excitation rather than inhibition (with implications for the effect of viscerofugal reflexes in the colon). There are subtle differences in the innervation of the different muscle lay-ers and by different ganglia. Finally, a functional connection with the rectum via the CN has now been demonstrated. Work supported by NHMRC Australia.

417.12

EFFECT OF COELIAC GANGLIONECTOMY IN THE LEVEL OF NGF OF THE RAT INTESTINE. M.Iwashita, K.Koyama, A.Miura* and M.Kawatani. Depts. of Surgery and Physiology, Akita Univ. Sch. of Med., Akita 010, Japan.

Nerve growth factor (NGF) is one of trophic factors for innervation of peripheral tissue. In contrast to well-investigated NGF release and it's receptors in somatic sensory systems, a little information was available for NGF in intestine. Coeliac and superior mesenteric ganglionectomy was performed on adult rats, after 24-h fast. The rats were sacrificed at 12h, 1, 2, 3, 4, 5 and 7days after ganglionectomy under deep anesthesia with Nembutal. Tissue specimens were excised from the small intestine: the jejunum 4 cm aborad to the ligament of Treitz. NGF content of each specimen was measured by ELISA. In control animals NGF measured and the average of contents was 10.8±3.5 pg/mg protein. A significant increase in NGF content in the jejunum occurred at 12h to 5days after ganglionectomy, as compared with the contents of control rats ($p<0.05$). The maximal increases occurred at 2 days after denervation (59.9±12.9 pg/mg protein; 555±119% of control, $p<0.001$). At 7 days after ganglionectomy the content of NGF returned to no significant level (25.7±5.8 pg/mg protein, $p>0.05$). These results suggest that NGF might play a role for maintaining innervation in the digestive tract following peripheral nerve damage.

417.13

SENSORY TRANSDUCTION STUDIED WITH A CLONAL STRAIN OF SEROTONERGIC EPITHELIAL CELL. P.R. Wade* and M.D. Gershon, Dept of Anatomy & Cell Biology, College of Physicians & Surgeons, Columbia University. New York, NY 10032

Enterochromaffin (EC) cells are enteric epithelial cells that secrete 5-HT, which initiates peristaltic and secretory reflexes by stimulating intrinsic sensory nerves. To study stimulus-secretion coupling, we developed, by serial dilution, a clonal cell strain that synthesizes, stores, and secretes 5-HT. This strain was derived from a heterogeneous parent cell line (BON [from a human carcinoid tumor]; donated by Dr. C. Townsend, Jr.). The BON cells were selected for uniformity in 5-HT immunoreactivity and the resultant clonal strain resembled EC cells in their basal accumulation of secretory granules and apical microvilli. Coincident expression of neurotensin, substance P, and/or CRF immunoreactivities was also observed. Secretion of 5-HT was evoked by ACh (10.0 μM). The effect of EC cell secretagogues on $[Ca^{2+}]_i$ was investigated in individual cells by imaging Fura-2 fluorescence. Agonists that raised $[Ca^{2+}]_i$ included ACh, isoproterenol (10 nM), and 5-HT (1-10 μM), but not KCl (50 mM). Differences between individual cells were detected in the magnitude of responses to ACh and 5-HT and the rates at which ACh receptors desensitized. Responses to 5-HT were not mimicked by the 5-HT_{1P} agonist, 6-hydroxyindalpine (10 μM) nor were they blocked by the 5-HT_{1P} antagonist, 5-HTP-DP (0.1 mM), or by the $5\text{-HT}_{3/4}$ antagonist, tropisetron (1 μM); thus, responses to 5-HT were not mediated by receptors that stimulate enteric neurons. The similarity between the cloned strain and EC cells in agonist responses suggests that the cloned cells are useful for studies of enteric sensory transduction. Supported by NIH grant NS12969.

417.14

ACTION OF NITRIC OXIDE ON NEUROMUSCULAR TRANSMISSION IN MOUSE CRURAL DIAPHRAGM J. Liu, C.C. Cheng, E.H. Middlekauff, C. Joo, P.L. Stevenson, T.J. O'Shaughnessy, Y. Yamamoto, R.K. Mittal and Y.L. Kim.* Departments of Biomedical Engineering & Neurology and Division of Gastroenterology & Hepatology, University of Virginia School of Medicine, Charlottesville, VA 22908.

The gastroesophageal junction pressure is maintained by two components: the lower esophageal sphincter smooth muscle and the crural diaphragm (CD) striated muscle. Both muscles relax during esophageal peristalsis. The mechanism for relaxation of the CD is not well understood. Peristaltic muscle relaxation of the CD can be blocked by a nitric oxide (NO) antagonist. The role of NO in neuromuscular transmission of the skeletal muscle is not known. In order to characterize the neuromuscular junction activities of the CD and to determine if NO plays a local inhibitory role at the neuromuscular junctions of the CD, intracellular microelectrode recording techniques were used to record spontaneous miniature end-plate potentials (MEPPs), neurally-evoked end-plate potentials (EPPs) and muscle action potentials (MAPs) from 20 intact phrenic nerve/CD preparations obtained from mice. MAPs with a mean peak-to-peak amplitude of 70.5 mV (n=10 fibers) were recorded from the CD. These MAPs were not significantly different in amplitude, duration or maximum dv/dt compared to those of the costal diaphragm. Spontaneous MEPPs exhibited a mean amplitude of 0.74 mV (n=33) and frequency of 1.27 Hz. Application of 10 mM sodium nitroprusside (NP; NO donor) did not alter these MEPP parameters. When measured in 1.2 mM Ca^{2+}/10 mM Mg^{2+} solution, the quantal content of EPPs (m) was 0.81 (n=35), which declined to 0.46 in the presence of 10 mM NP. This effect was significant ($p<0.001$) and found to be due to presynaptic inhibitory action of the drug. These findings indicate that NO causes inhibition of neuromuscular transmission in the CD, reducing the neurally-evoked release of ACh. (Supported by NIH and MDA).

UROGENITAL REGULATION: SEXUAL ORGANS

418.1

PENILE PATHWAYS IN THE CNS REVEALED BY THE TRANSSYNAPTIC TRACING WITH PSEUDORABIES VIRUS (PRV) IN THE CAT. V. Erickson, C. Tai, A.M. Booth, P. Card, W.C. de Groat and J.R. Roppolo* Dept. of Pharmacology, Univ. of Pgh. School of Medicine, Pittsburgh. PA 15261

The distribution of spinal efferent neurons and interneurons which control the penis, was examined using PRV. PRV (Becker strain) was injected bilaterally into the cavernous sinus of the cat penis under halothane anesthesia. Animals were perfused 60-98 hours later and the tissue processed with an antibody to the virus. Cholinergic preganglionic neurons were distinguished from PRV labeled (PL) interneurons by treating alternate sections, with an antibody to choline acetyltransferase (ChAT) which also delineated the sacral parasympathetic nucleus (SPN). PL neurons were identified in the S_1-S_3 and L_1-L_4 segments of the spinal cord with the majority of the PL neurons in the S_1, rostral S_2 and L_3 segments. PL neurons were found in the sacral parasympathetic nucleus (SPN), the sacral dorsal commissure (DC) and the ventral horn. In the SPN both preganglionic neurons and small interneurons were distinguished by comparing the size, shape and location of ChAT labeled and PL cells in adjacent sections. Processes from PL SPN neurons projected medially to the DC and ventrally along the lateral edge of the ventral horn. PL neurons in the lumbar cord were primarily in the intermediolateral cell column but a few cells were seen in the lumbar DC. Sacral and lumbar dorsal root ganglion cells were also labeled. Following longer survival times (>90 hrs) PL neurons were seen in the brainstem in the dorsolateral pontine tegmentum just medial to the brachium conjunctivum and locus coeruleus. These data taken together with previous tracing studies which identified bladder and external urethral sphincter (EUS) efferents and interneurons suggest that the penile preganglionic neurons are adjacent to, but somewhat rostral (mostly in S_1) to bladder preganglionic neurons (mostly in S_2) while EUS motoneurons are largely in S_1 at the base of the ventral horn in Onuf's nucleus. There was considerable overlap of penile, bladder and EUS interneurons in the DC.(Supported by NO1-NS-5-2332)

418.2

IDENTIFICATION OF RAT SPINAL NEURONS THAT INNERVATE THE PROSTATE: COMPARISON OF HYPOGASTRIC AND PELVIC INPUTS USING TRANSNEURONAL TRACING WITH PSEUDORABIES VIRUS L. Marson,* and R. Orr. Department of Surgery/Urology, University of North Carolina School of Medicine, Chapel Hill, NC 27599

The rat prostate is innervated by the hypogastric and pelvic nerves. Previous research has focused on the sympathetic system (primarily hypogastric nerve), however, the parasympathetic system (pelvic nerve) may also be important. McVary and colleagues have shown that unilateral pelvic nerve cuts increased prostate weight of the intact side, whereas, unilateral hypogastric denervation decreased prostate weight of the lesioned side. In this study, transneuronal tracing was used to identify the spinal inputs of a single nerve to the ventral prostate. Rats received either bilateral pelvic and unilateral hypogastric nerve cuts or bilateral hypogastric and unilateral pelvic nerve cuts. Bartha's K strain of PRV was injected into one side of the ventral prostate (2-3 μl, 10^4 pfu/ml, Lynn Enquist). Therefore, PRV was transported through only the ipsilateral hypogastric or the pelvic nerve. In addition, a group of rats were left intact, such that both nerve inputs were traced. Rats were perfused 65-72 hrs after their injections.

Spinal preganglionic neurons and interneurons were labelled in all groups (n=21). In intact rats, the majority of PRV neurons were found in the medial gray of L1-L6. Many cells were also labelled in the lateral gray of ipsilateral T12-L1 and L6, and contralateral L1 and L6. In rats with one hypogastric nerve intact, PRV cells were found primarily in the medial grey of L1-L4 and L6. In contrast, the majority of PRV cells in rats that had one pelvic nerve intact were in the medial and ipsilateral grey of L6. Thus, selective denervation results in differential labelling of spinal circuits that innervate the prostate. (Supported by NIH grants NS29420, DK49503 and Edwin Beer Award of the NYAM).

418.3

PERIPHERAL PATHWAYS OF THE SYMPATHETIC INNERVATION OF THE PENIS. V. Barba, R. Galindo, L. D. Partridge* and W. G. Dail. Dept. of Anatomy, Univ. of New Mexico, Sch. of Med., Albuquerque, NM 87131.

Previous functional studies suggest that branches of the pudendal nerve are the major route of sympathetic fibers to the penis but some contribution may be made by the cavernous nerves. The present study has used image analysis of catecholamine histofluorescence to further define sources of sympathetic innervation to rat erectile tissue. Interruption of the sensory branch of the pudendal nerve (SPN) in the gluteal area resulted in a severe loss (up to 90%) of adrenergic fibers of the intrinsic muscle of the penile crura. Some fibers remained on the helicine arteries (HA) but none were present on the deep penile artery (DPA). In the penile shaft, there were no adrenergic fibers to the intrinsic muscle near the cavernous vein or to the dorsal artery on the side of the lesion. Loss of adrenergic fibers from interruption of the motor branch of the pudendal (MPN) was more variable (5-30%). The DPA, HA and intrinsic muscle were still heavily innervated after this procedure. A near total reduction (to about 5% of control) in crural innervation was obtained when the SPN and MPN were cut and the pudendal artery and veins were treated with phenol. These results suggest that the SPN conveys the majority of adrenergic fibers to the penis while some fibers travel in the MPN and along penile vessels. By inference, the penis may not be a target of the adrenergic fibers in the rat cavernous nerves. Supported by NIH RO119839-13

418.4

MODULATION OF CAVERNOUS SINUS PRESSURE (CSP) BY MICROSTIMULATION OF THE SACRAL SPINAL CORD. C. Tai*, A.M. Booth, W.C. de Groat and J.R. Roppolo. Dept. of Pharmacology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261

The purpose of the present study was to determine the stimulation parameters and the location of sites within the sacral spinal cord where focal electrical stimulation produced penile erection in the cat. Since electrically evoked penile protrusion correlated well with increased CSP, pressure recordings from the cavernous sinus (CS) were used as a quantitative measure of penile tumescence or erection. Adult male cats anesthetized with pentobarbital (20-25 mg/kg IV) were used in this study. CSP was recorded via a 22 gauge intracath placed in the CS through a small incision at the tip of the penis. Each sacral ventral root (S_1-S_3) was stimulated with a hook electrode to determine the spinal segment which produced the largest amplitude CSP response. In most cases (5 of 6 animals) the S_1 root produced the largest CS response (100-160 cm H_2O peak pressure), while in one animal the S_2 root was more effective. The S_2 root, however, usually produced the largest bladder response. The segment which produced the largest CSP change was probed with fine tipped (200-400μ^2 surface) activated iridium microelectrodes advanced from the dorsal surface of the spinal cord in 200μ increments. Stimulation sites which produced the largest CS responses were in the middle of the ventral horn, 1.6-2.0 mm from the surface of the S_1 sacral segment and midway between the midline and the lateral edge of the grey matter. The area was 200-400μ wide (medial to lateral) and extended 1-2 mm in the rostrocaudal direction. The response evoked with spinal stimulation occurred at a latency of 8-40 seconds and had an amplitude ranging from 20-120 cm H_2O. The duration and frequency of stimulation was important to produce a maximal response. The stimulus was presented for 1-1.5 minutes. The optimal frequency was between 25-35 Hz using 0.2 msec pulses at an intensity of 50-150μA. These studies suggest that focal microstimulation of the sacral spinal cord may be a useful technique for producing penile erection. (Supported by NO1-NS-5-2332)

418.5

HYPOGASTRIC NERVE-INDUCED PENILE VASODILATION FOLLOWING PARASYMPATHETIC DENERVATION: CHARACTERIZATION AND INVOLVEMENT OF NITRIC OXIDE. J. Gonzales, F. Harji, and W. G. Dail*. Dept. of Anatomy, Univ. of New Mexico Sch. Med., Albuquerque, NM 87131.

Stimulation of the hypogastric nerve (HN) in the rat increases vasodilation in the corpora cavernosa penis (CCP) after chronic interruption of the pelvic nerve (PN). We further characterized this response by: (1) examining its stimulus characteristics, (2) determining whether it involves nitric oxide, and (3) asking if it also involves the corpus cavernosa urethra (CCU). Stimulation of the ipsilateral HN two weeks after unilateral interruption of the PN consistently resulted in penile vasodilation. This response was always greater on the injured side (26 ± 13 mmHg vs. 8 ± 7 mmHg) and showed graded characteristics with changing frequency and voltage. The enhanced HN vasodilation in the CCP was inhibited by systemic infusion of Nw-Nitro-L-Arginine (ave 57% of control after 45 min. with 50 μg L-NNA/min./kg). Two weeks after cutting the pelvic nerve, ipsilateral HN stimulation also increased CCU pressure, while the contralateral HN provoked minor changes (24 mmHg vs. 6 mmHg). These results show that the HN mediates a weak and inconstant vasodilation of the CCP and CCU. Responses in both tissues are markedly strengthened after cutting the PN. Evidence suggests that with regard to NO, mediators of penile erection remain the same in the enhanced response. Supported by NIH R0119839-13.

418.6

POOR SPERM MOTILITY IN SPINAL CORD INJURED (SCI) MEN IS RELATED TO FACTORS IN THEIR SEMINAL PLASMA N.L. Brackett*, S.M. Ferrell, T.C. Aballa, and C.M. Lynne. The Miami Project to Cure Paralysis and Department of Urology, University of Miami School of Medicine, Miami, FL 33136

Introduction: SCI men have sperm of normal concentration but below normal motility. Lifestyle factors alone (scrotal hyperthermia, infrequent ejaculations, bladder management) do not seem to account for this condition. We have recently shown that seminal plasma of SCI men inhibits sperm motility of normal men, indicating that factors within the seminal plasma of SCI men contribute to their poor sperm motility. (Brackett et al., J Urol (1996) 155:1632-1635). **Objective:** The present study investigated if the degree of inhibition was related to the starting sperm motility of SCI men. **Method:** Identical aliquots of sperm from normal men (n=19) were mixed with seminal plasma originating from each of two groups of SCI men: those with "low" (<10%) or "high" (>20%) sperm motility. The resulting motility of the normal men's sperm was analyzed 5 minutes after mixing with seminal plasma. **Results:** Motility of sperm from normal men showed significantly less inhibition if mixed with seminal plasma originating from SCI men with high compared to low sperm motility. **Conclusions:** The below normal sperm motility observed in SCI men is related in a dose-dependent manner to factors within their seminal plasma.

Support was provided by The Miami Project to Cure Paralysis, and State of Florida Specific Appropriations.

418.7

ENKEPHALIN-IMMUNOREACTIVE AFFERENTS ARE SEXUALLY DIMORPHIC IN RAT LUMBOSACRAL AUTONOMIC REGIONS. B.W. Newton* and W.A. Tate. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

The distribution of enkephalin-immunoreactivity (Enk-IR) has been described in the autonomic regions of the rat spinal cord, where a distinct "ladder-like" pattern was observed (Romagnano, et al., JCN, 266:319,'87). However, former studies have not examined this Enk-IR pattern with regard to potential sex differences. This study used 12 male and 12 female, adult (P90-120), Sprague-Dawley rats (Sasco, Omaha, NE). The rats were anesthetized and perfused for IHC. The entire spinal cord was sectioned. The Enk antibody (IncStar, Corp., Stillwater, MN) recognizes m-Enk (>99%) and l-Enk (<1%). Determination of sexual dimorphisms were made by observers who were "blind" as to the sex of the rat. These data indicate that, by far, the most pronounced sexual dimorphism for Enk-IR occurs in laminae VII and X of the lumbosacral spinal cord: a region which provides the autonomic control of the pelvic viscera. Enk-IR afferents are found in greater numbers surrounding male lumbosacral autonomic neurons than females. The sexual dimorphism is most pronounced in the lumbar region, and is less pronounced in the sacral region. One of the functions of Enk is to decrease sensitivity to pain. The increased amounts of Enk in male rats provides anatomical evidence supporting data which indicates that male rats have a higher tolerance for pain than females (c.f., K.J. Berkley, Behav. Brain Sci., In press, Nov. '96). Furthermore, the lesser amounts of Enk in the female rat may "sensitize" the spinal cord to sensory input from the cervix which is necessary for induced ovulation to occur. Supported by NSF Grant IBN-9211368 to B.W.N.

418.8

HISTOAUTORADIOGRAPHICAL EVIDENCE FOR OXYTOCINERGIC (OT) RECEPTORS IN THE LUMBOSACRAL SPINAL CORD OF THE MALE RAT. F. Véronneau-Longueville, F. Giuliano*, O. Rampin, M-J. Freund-Mercier and A. Calas. Dept. Urology and Lab. Exp. Surgery., Fac. Méd. Paris-Sud, F-94270 Bicêtre; Lab. Neurobio. Fonctions Végétatives, INRA, F-78352 Jouy-en-Josas; CNRS URA 1446, ULP, F-67000 Strasbourg; CNRS URA 1488, Univ. Paris 6, F-75005 Paris.

In the rat, the sacral parasympathetic nucleus (SPN) located in the intermediolateral gray matter of the L6-S1 spinal cord, contains procrectile parasympathetic preganglionic neurons. Reflexive erections are elicited by activation of dorsal penile nerve (afferent limb) and procrectile SPN neurons (efferent limb). This reflex response is organized at the L6-S1 spinal cord and likely involves interneurons. OT is a candidate for the spinal regulation of reflexive erections. Aim of our study was to search for OT binding sites in the SPN. Four adult male SD rats (250g) were anesthetized, decapitated, and the spinal cords were removed and frozen. Serial transverse sections (16μm) of the L6-S1 spinal cord were treated with highly specific radioionidated OT antagonist ($d(CH_2)_5$[Tyr(Me)^2,Thr^4,Tyr-NH$_2^9$]OVT) for 24 hours, processed for autoradiography for 4-5 days, then for histoautoradiography for 20 days. Non specific binding was assessed by treating adjacent sections with an excess of OT. Histoautoradiography permitted light microscope detection of OT binding sites. They were found in layers I-II and the inner edge of the dorsal horn, the dorsal gray commissure, the intermediate gray matter and the SPN at the L6-S1 levels. No labeling occurred in adjacent control sections. We previously described synaptic contacts between OT fibers and SPN preganglionic neurons. The present study provides further evidence for the supraspinal regulation of parasympathetic efferent activity by OT either directly or via interneurons.

418.9

EFFECTS OF THE NMDA GLUTAMATERGIC RECEPTOR ANTAGONIST MK-801 ON REFLEXIVE ERECTIONS IN THE RAT. O. Rampin*, J. Bernabé and J.P. Rousseau. Lab. Neurobio. Fonctions Végétatives. I.N.R.A., F-78352 Jouy-en-Josas.

Electrical stimulation of the dorsal penile nerve elicits an increase of the intracavernous pressure (ICP) in the anesthetized spinalized rat (Pescatori et al., J. Urol. 1993; Rampin et al., Neurosci. Lett., 1994). The role of glutamatergic transmission was evaluated in this model by determining the effects of the glutamate antagonist MK-801 on the ICP increase and the cavernous and motor pudendal nerves responses elicited by dorsal penile nerve stimulations (5V, 5Hz, 0.1ms, 30s). The non-competitive NMDA antagonist MK-801 dose-dependently (0.1-3 mg/kg i.v.) increased the latency of ICP increase by 61-108%, decreased its maximal value by 24-65% and lowered the filling rate of the corpora by 31-78% (n=4). In contrast MK-801 did not affect ICP increases elicited by pelvic nerve stimulation (n=4). MK-801 produced a dose-dependant (0.1-3mg/kg) inhibition of the complex evoked potentials recorded on the cavernous nerve; their amplitude and surface were decreased by 21-63% and 17-87%, respectively (n=5). Reflex potentials recorded on the motor pudendal nerve were decreased by 28-70% in amplitude and 23-38% in surface following injections of 0.1-10mg/kg MK-801 (n=5). The data suggest that glutamate acting at NMDA receptors, may play a role in the regulation of reflexive erections in the anesthetized, spinalized rat. Because MK-801 has no peripheral action, glutamate appears to mediate the response at the spinal cord level. Both autonomic and somatic reflex responses were affected by MK-801. It suggests that glutamate is present either in dorsal penile nerve afferents or in interneurons projecting onto parasympathetic preganglionic neurons and pudendal motoneurons.

418.10

THE DISTRIBUTION OF CHOLECYSTOKININ-8 AFFERENTS SURROUNDING LUMBOSACRAL AUTONOMIC NEURONS IS SEXUALLY DIMORPHIC AND ALTERED BY LACK OF ANDROGEN RECEPTORS. D.C. Phan* and B.W. Newton. University of Arkansas for Medical Sciences, Little Rock, AR 72205

The distribution of cholecystokinin-8 immunoreactivity (CCK-IR) has been previously described in the rat lumbosacral (L-S) spinal cord (Schrøder, JCN, 217:176,'83). While L-S autonomic neurons innervate the sexually dimorphic pelvic viscera, the potential sexually dimorphic distribution of this peptide has not been examined. Other studies have shown that CCK in lumbar motoneurons is regulated by testosterone (T) titers; such that absence of T causes a decrease in CCK mRNA (Popper & Micevych, Neurosci., 50:87,'92) This study used 6 male and 6 female, adult, Sprague-Dawley/King-Holtzman rats, as well as SD/KH Testicular feminization mutation (*Tfm*) rats. After anesthesia, the rats were perfused for IHC. The CCK-8 antiserum was purchased from IncStar (Stillwater, MN). The results indicate that there is a pronounced sexually dimorphic distribution of CCK-IR afferents surrounding L-S autonomic neurons: males have greater numbers of CCK-IR fibers than females. In male *Tfm* rats (which produce T, but lack androgen receptors), the levels of CCK-IR in the L-S spinal cord is dramatically reduced and presents with a distinctly female-like CCK-IR pattern. These results suggest that CCK plays a variable role in modulating autonomic neurons which innervate the pelvic viscera in males vs. females. In addition, CCK-IR within afferent fibers responds in a similar fashion to decreased T as has been demonstrated for CCK mRNA in sexually dimorphic motoneurons. Supported, in part, by NSF Grant IBN-9211368 to B.W.N.

418.11

ESTROGEN RECEPTOR IMMUNOREACTIVITY IN THE LUMBOSACRAL SPINAL CORDS OF INTACT, OVARIECTOMIZED, AND PREGNANT RATS. **S.J. Williams* and R.E. Papka. Department of Anatomical Sciences, University of Oklahoma HSC, Oklahoma City, OK 73190.**

Throughout the reproductive cycle the uterus is influenced by estrogen. Little is known about the effects of estrogen on spinal cord neurons which might be involved in neural mechanisms conveying sensory and autonomic information regarding pregnancy, parturition, pain, and genital stimulation. We identified estrogen receptor (ER) containing neurons by immunostaining and determined if the mean number of ER neurons varied with serum estradiol (SE2) levels which occur at different reproductive states. Intact, ovariectomized, and pregnant rats were perfusion fixed, and sections of the lumbosacral cords were immunostained for ER using H222 antibody. SE2 levels were determined using radioimmunoassay. ER containing neurons were present in the dorsal half of the spinal cord. The mean number of ER neurons/section was significantly higher in ovariectomized rats. The ovariectomized rats had a mean of 15.15 ± 0.8 with SE2 levels ranging from <5 - 12 pg/ml. During estrus, metestrus, and diestrus, the mean number of ER neurons/section and SE2 levels were respectively 12.47 ± 3.9 and 24 pg/ml, 7.46 ± 2.4 and 31 pg/ml, and 6.94 ± 2.2 and 45 pg/ml. During proestrus a.m. the mean number of ER neurons/section was 6.35 ± 2.0 and significantly decreased to 1.43 ± 0.5 when SE2 levels rose from 58 to 73 pg/ml by the afternoon of proestrus. The 10 day pregnant rat had a significantly higher mean of ER neurons/section at 6.5 ± 0.8 with SE2 levels of 31 pg/ml than did the 20d (4.6 ± 0.3 and 75 pg/ml) or 22d (3.0 ± 0.2 and 80 pg/ml) pregnant rats. Our studies show that there is a significant change between ER neurons/section and SE2 levels of female rats in different reproductive states. (Supported in by NIH NS22526 & Presby. Health Fnd.)

418.12

ARE NEUROGENIC INFLAMMATORY PROCESSES INVOLVED IN CERVICAL RIPENING PRIOR TO PARTURITION? R.E. Papka*, K.E. Miller and B. Srinivasan. Department of Anatomical Sciences, University of Oklahoma HSC, Oklahoma City, OK, 73190

Mechanisms for initiating "ripening" of the uterine cervix at parturition are not known. However, they likely involve multifactorial processes and a variety of agents such as: hormones, eicosanoids, cytokines, and cervical neurogenic inflammatory responses. The idea of a localized inflammatory response has received support but little research attention. Primary afferent nerves can release transmittters from their peripheral terminals which help orchestrate local inflammatory responses including vasodilation, increase of vascular permeability with tissue edema & protein extravasation, and activation and migration of inflammatory immune cells. Intense stimulus of uterine cervical primary afferents at term could lead to release of such neuropeptides as substance P (SP). SP, working through NK1 receptors, could be a component of neurogenic inflammation. Our preliminary studies sought to determine if uterine neurons influence cervical ripening near parturition through release of transmitters that contribute to neurogenic inflammation. We employed immunohistochemistry to study innervation of cervical venules in pregnancy and at parturition, the presence of NK1 receptors, and changes in extravascular inflammatory cells. Preliminary results suggest that all of the elements for neurogenic inflammation are present in the cervix of pregnant and parturient rats. The cervix from these rats show the hallmark signs of an inflammatory reaction including inflammatory cells (e.g., neutrophils & eosinophils) in the connective tissue, edema (reflected in increased spaces in the connective tissues), and dilated vascuature. Primary afferent nerves containing tachykinins innervating venules and NK1 receptors (for SP and neurokinin A) are apparent in endothelial cells of venules in cervixes from parturient rats (Supported by Presbyterian Health Fnd. and NIH NS-22526).

418.13

EFFECTS OF SUBSTANCE P (SP) AND SP ANALOGS [Sar^9,$Met(O_2)^{11}$]-SP, [$Cys^{3,6}$,Tyr^8,Pro^{10}]-SP AND [D-Pro^4,D-$Trp^{7,9}$]-SP ON RAT UTERINE ACTIVITY IN VITRO. R.L. Shew*, R.E. Papka, R.H. Goertz and C.W. Gatto. Department of Cell Biology & Neuroanatomy, University of Minnesota and Children's Health Care-St. Paul, Minneapolis, MN 55455 and St. Paul, MN 55102 and Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Substance P and related tachykinins have been localized in nerves in the uterus and stimulate uterine contraction in a dose-related manner. SP has been reported to bind preferentially to the NK1 receptor in peripheral tissues. Highly selective agonists and antagonists for NK-1 receptors have been developed. Thus, we investigated the effect of various analogs of SP ([Sar^9,$Met(O_2)^{11}$]-SP, [$Cys^{3,6}$,Tyr^8,Pro^{10}]-SP and [D-Pro^4,D-$Trp^{7,9}$]-SP) on rat uterine tissue in vitro. Mature Sprague-Dawley rats (200-225 g) were treated with diethylstilbestrol and uterine horns removed 14-16 hours later and examined using an in vitro uterine assay. SP (10^{-6}-10^{-9} M), [Sar^9,$Met(O_2)^{11}$]-SP, and [$Cys^{3,6}$,Tyr^8,Pro^{10}]-SP (10^{-5} -10^{-9} M) stimulated uterine contraction in a dose-related manner. Contractile responses ranged from 0-4 grams (n= 3-10). However, [D-Pro^4,D-$Trp^{7,9}$]-SP, a SP antagonist in the guinea pig ileum and rabbit mesenteric vein assay, demonstrated weak agonist activity at high concentrations (10^{-5} M; n=5, 1.1 ± 0.18 grams). These data demonstrate that, in our uterine assay, the SP analogs [Sar^9,$Met(O_2)^{11}$]-SP and [$Cys^{3,6}$,Tyr^8,Pro^{10}]-SP, stimulate uterine contraction in a dose-related manner similar to SP and these analogs are probably acting through a NK-1 receptor. (Supported in part by NIH grant 1RO1HD30817)

418.14

NITRIC OXIDE SYNTHASE LEVELS IN CHRONIC DIABETIC RATS. R.E. Brannigan, K.T. McVary and K.E. McKenna* Depts. Physiology and Urology, Northwestern Med. School, Chicago, IL 60611

Nitric Oxide (NO) is the nonadrenergic, noncholinergic mediator of penile erection, and it is produced by the conversion of L-Arginine to L-Citrulline by Nitric Oxide Synthase (NOS). In the penis, release of NO results in penile smooth muscle relaxation, corpora cavernosal engorgement, and erection.

In humans, diabetic males have a high incidence of erectile dysfunction. Our previous studies of chronic diabetic BB/Wor rats revealed profound deficits in the sexual behavior of diabetic rats as well as deficits in sexual reflexes and penile erections. These results suggested the possibility of concurrent CNS dysfunction and peripheral neuropathic processes.

We undertook this study to semi-quantitatively evaluate NOS isoforms in the penile and spinal cord tissue of chronic diabetic BB/Wor rats . Tissue was harvested from both chronic diabetic and diabetes resistant (control) rats. The lumbosacral spinal cord and penises were homogenized, and Western blots were performed. NOS I and NOS III isoforms were identified with specific antibodies (Transduction Laboratories, Lexington, KY) . Subsequently, semi-quantitative densitometry was used to evaluate our results. In the penis, NOS III staining was significantly reduced in the diabetic rats to approximately 40% of the controls. NOS I levels in the spinal cord were reduced by about 20%, but did not reach statistical significance.

Our results suggest that diabetic impotence may be related to NOS deficits in the penis and the central nervous system.

419.1

EXPANSION OF CORTICAL CONNECTIVITY IN THE BARREL FIELD FOLLOWING PARTIAL VIBRISSECTOMY IN ADULT MICE. M. Kossut* and S.L. Juliano. Nencki Institute, Warsaw, Poland and Anatomy and Cell Biology, Neuroscience, USUHS Bethesda, MD 20814.

A vibrissectomy that spares row C in adult mice results in changes in the areal extent of the cortical representation of the spared row. The anatomical substrate of the changes in cortical functional response observed after peripheral denervation in adult animals is not well documented. In the visual cortex of adult cats, partial denervation triggers growth of layer III axons into the denervated area. We examined changes in cortical connectivity in the barrel field 6-8 weeks after a vibrissectomy in young adult mice, sparing row C on both sides of the snout. We traced connections between the spared and denervated rows of the barrels in living cortical slices, 300 μm thick, cut in a plane perpendicular to the rows of barrels. Small volumes of dextrans conjugated to either fluorescein or rhodamine were injected into visually identified barrels in transilluminated slices. Injections were also placed in layers II/III and V, over or under selected barrels. After 6 hours of incubation in a slice chamber the slices were fixed, cryoprotected and resectioned at 40 μm thickness. The fluorescent label was examined using a confocal microscope. The injection sites ranged from 120 to 180 μm diameter. The extent of resulting label was estimated in vibrissectomized and control animals. The distribution of labeled cell bodies and the fibers was more widespread when the injections were centered on the barrel of the spared row C (mean diameter 1000 μm) than in the control animals (mean diameter 650 μm). This occurred after both the supragranular and granular layer injections, but not for those located in layer V. Counts of dendritic spines revealed a 30% increase of spine density on neurons in the spared vibrissal column compared to control mice.

Support: Senior Fulbright Grant (M.K) and the Alzheimer's Association (SLJ).

419.2

FIBROBLAST TRANSPLANTS GENETICALLY ENGINEERED TO SECRETE NGF RESTORE FUNCTIONAL ACTIVITY IN BARREL CORTEX OF BASAL FOREBRAIN LESIONED RATS. O. Rahimi*, G. Hill III, O. Olaghere, J. Coulombe and S.L. Juliano. Anatomy, USUHS, Bethesda, MD 20814.

Stimulus-evoked 2-deoxyglucose (2-DG) uptake is reduced in barrel cortex of rats after basal forebrain (BF) lesions that deplete the neocortex of acetylcholine (ACh). We previously showed that intraventricular injections of NGF restore stimulus-evoked 2DG activity toward normal, despite sustained ACh depletion. To more clearly understand the site of NGF action, we used a more easily located form of NGF delivery consisting of fibroblasts genetically engineered to secrete NGF (NGF+). Since the most likely sites of NGF action include cerebral cortex or BF, NGF+ fibroblasts were transplanted in both places (in different rats receiving unilateral BF lesions). After survival times of 1-4 weeks, rats received 2DG injections during bilateral whisker stimulation. Controls included transplants of fibroblasts without the NGF gene (NGF-) into BF or cerebral cortex. NGF+ fibroblasts transplanted into the somatosensory cortex of BF-lesioned rats resulted in increased 2DG uptake in response to whisker stimulation, 36% above that evoked in the opposite control hemisphere. NGF+ transplants into the BF, or NGF- in either site, resulted in decreased (46%) 2DG uptake compared to opposite control hemisphere, similar to animals with BF lesions alone. These results support the notion that NGF is an important factor in mediating neocortical plasticity and suggest that NGF acts directly in the cerebral cortex, since transplants of NGF+ cells into BF had little effect. The mechanism of action used by NGF however, is unclear. Since recent studies indicate that NGF can increase expression of Bcl-2 protein and overexpression of Bcl-2 can rescue neurotrophin-deprived neurons *in vitro*, we assessed the immunoreactivity of Bcl-2 in normal and NGF-treated brains. Our results indicate increased Bcl-2 protein in the site of NGF treated cortex.

Support: Alzheimer's Association and USUHS RO7064.

419.3

VIBRATION-INDUCED ACTIVATION OF THE PRIMARY SOMATOSENSORY CORTEX AND SEVERE PERIPHERAL DEAFFERENTATION OF THE LEMNISCAL SYSTEM. CONTRIBUTION OF A SINGLE-CASE PET ACTIVATION STUDY. P. Remy*, P.F. Pradat, P. Belin, B. Aymard, , M. Zilbovicius, E. Fournier, L. Naccache, T. Maisonobe, J.C. Willer, Y. Samson. SHFJ-CEA, Orsay and La Salpêtrière, Paris, France.

PET activation studies show that high-frequency vibration (>100 Hz) induces a marked rCBF increase in the corresponding contralateral primary sensorimotor cortex (SM1). It remains however unknown if the extra-lemniscal system participates to this SM1 activation. We have previously reported a patient having a spinal cord section (T3 level) sparing only a part of the left anterolateral quadrant and in whom left foot vibration, although not perceived, still activated the right foot SM1. Here, we report a PET activation study in a 35-y.o. patient with a rare hereditary ganglionopathy with a selective disappearance of the large-diameter somatosensory fibers as demonstrated by neuromuscular biopsy. This patient had a marked deficit of discriminative touch and proprioception senses. rCBF measurements were performed using a high-resolution tomograph during rest; right hand vibration; and right foot vibration (vibrator: 130 Hz, 2-mm amplitude). Regions-of-interests (ROIs) were defined in hand and foot SM1 on the coregistered MRI. In these ROIs, the rCBF variations were compared to those obtained in 9 healthy volunteers (28.3 ± 10.4 y.o., mean ± sd) using the same methods. In the patient, right foot vibration which was not perceived induced a +5.8 % rCBF increase in contralateral foot SM1 (controls: +15.8 ± 9.6%). The right hand vibration of the patient induced a slight feeling of touch and was associated with a +7.7% rCBF increase in the contralateral hand SM1 (controls: +10.9 ± 3.2 %). Thus the extra-lemniscal system may participate to the activation of SM1 induced by vibration. This result also show that conscious perception of a sensory stimulus may disappear despite remaining mild activation of the primary sensory cortex.

419.4

AN ANATOMICAL ISOMORPH OF THE HAND IN SOMATOSENSORY CORTEX OF OWL MONKEYS AND ITS IMMUTABILITY FOLLOWING PERIPHERAL DEAFFERENTATION. N Jain*, K C Catania and J H Kaas. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

We have observed a pattern of myelin dense ovals separated by myelin light septa in brain sections cut parallel to the surface in area 3b of owl monkeys *(Aotus trivirgatus)*. Using microelectrode recordings to identify the zones of activation within the pattern, we determined that each of a lateromedial ovals in the rostral row corresponded to a specific digit of the hand, D1-D5. More caudal ovals represented specific pads of the palm. Thus there was a complete correspondence between the morphological and physiological maps. Furthermore, the components of morphological maps, which could be precisely measured, varied little across cases. Nevertheless, the physiological maps, as previously shown, could be modified by peripheral deafferentation. In two monkeys that had accidentally lost one or two digits, the morphological maps were completely normal, but neurons in ovals corresponding to the missing digits were activated from the remaining inputs from digits or palm. Likewise, the morphological map was normal in a monkey with long standing dorsal column section at the C3/C4 level even though this led to a profound reorganization of the physiological map of the hand. Thus an anatomical substrate for the hand representation is immutable in adult monkeys while the evoked activity pattern is plastic. Supported by NINDS NS-16445.

419.5

CHARACTERISTICS OF NEURONAL ACTIVITY IN THE REGION OF THE THALAMIC NUCLEUS VC IN PATIENTS WITH AMPUTATIONS. A. Zirh, F.A. Lenz, I.M. Garonzik, R.T.Richardson, M. Ringkamp*, L.H. Rowland, P.M. Dougherty. Dept. Neurosurg., Johns Hopkins University, Baltimore MD 21287.

Thalamic activity was studied during the physiologic exploration which precedes stereotactic surgical procedures for stump pain (3 patients) and movement disorders. One patient exhibited an enlarged thalamic representation of the stump covering an area of approximately 7mm^2. The stump representation in the 15mm lateral plane was adjacent to the perioral representation which is usually found next to the thumb representation. Consistent results were found in two other patients suggesting that there has been reorganization of somatosensory thalamus.

The mean interspike intervals (ISI) was significantly shorter in the representation of the stump than in Vc region of movement disorder patients (control) or in the non-stump representations in the amputation patients (stump control, P< 0.01, Dunn multiple comparison test). Cells firing with a bursting pattern were much more common in stump control (41%) and stump areas (33%) than in control areas (15% - chi square tests, P<0.00001). Recent studies have demonstrated the presence of an area posterior to Vc with activity related to pain. In the stump and stump control areas there were large differences in the number of bursting cells by location in anterior (29%) or posterior (55%) regions (Chi square, P < 0.002). Bursting cells were found to have patterns consistent with low threshold calcium spikes (LTS). LTS bursting occurred more commonly for cells in the stump region with RFs than for cells of any other type. Therefore stump pain may be related to LTS bursting in cells with receptive fields in the posterior zone of patients with amputations. Supported by grants to FAL from the NIH (NS28598, K08 NS01384, P01 NS32386-Proj. 1).

419.6

REORGANIZATION OF INTRACORTICAL PROJECTIONS TO SOMATOSENSORY CORTEX AFTER NEONATAL THALAMIC ABLATION IN THE HAMSTER. M. A. Kingsbury *, N. A. Lettman, B. Miller, and B. L. Finlay. Developmental Neuroscience Group, Cornell University, Ithaca, New York, 14853.

Thalamic afferents arrive at the cortex prior to the establishment of mature cortical projection systems. These axons may be important in directing the development of specific connections between cortical areas in the visual system. Previously, the removal of thalamic input to visual cortex resulted in an increase in the callosal and intracortical projections to the deafferented cortex. This expansion was manifested as a substantial increase in the number and tangential distribution of labeled cells. Here, we examine the organization of intracortical connections to somatosensory cortex that has been denervated of thalamic input.

Hamster pups were given unilateral electrolytic ablations of somatosensory thalamic nuclei on the day of birth. To visualize intracortical connections, thirty day old hamsters were given an injection of HRP into somatosensory cortex ipsilateral to the thalamic ablation. The distribution of retrogradely labeled intracortical cells was plotted. The experimental animals were compared to a control matched for placement of injection site. Animals with ablations displayed an increase in the number of labeled intracortical cells in temporal and visual cortices (T1, T3, V1 and V2) relative to the control. In addition, the horizontal connections within primary somatosensory cortex (target of VB afferents) increased after thalamic ablation. The normal projection has a comparable number of labeled cells in the supragranular and infragranular layers. In contrast, the increase in cells in experimental animals is manifested as an increase in the number of infragranular cells.

These results demonstrate that the development of normal intracortical projections depends upon the presence of thalamic afferents, which may provide genetic instruction or organizing activity to cortical areas.

Supported by NIH R01 NS 19245.

419.7

A PRIMATE GENESIS MODEL OF FOCAL DYSTONIA AND REPETITIVE STRAIN INJURY: II. THE EFFECT OF MOVEMENT STRATEGY ON THE DE-DIFFERENTIATION OF THE HAND REPRESENTATION IN THE PRIMARY SOMATOSENSORY CORTEX IN OWL MONKEYS N.N. Byl*, M.M. Merzenich, S. Cheung, P Bedenbaugh, S.S. Nagarajan, W.M. Jenkins, Keck Center for Integrative Neurosciences, Sch of Med, Grad Prog in Physical Therapy, Depts. of Otolaryngology and Physiology, Univ of California San Francisco, SF, CA 94143-0736

The purpose of this study was to document the neural consequences of an active, repetitive handclosing/opening behavioral paradigm using two Owl monkeys, one of whom trained once a day using an articulated *hand squeezing strategy* (A) and the other who trained twice a day using a *proximal arm pulling strategy* (B). After 20 weeks of training, the investigators analyzed motor control and then generated an electrophysiologic map of the hand representation within the primary somatosensory (SI area 3b) cortical zone. Monkey A showed statistically significant motor deterioration and degradation of the normally sharply segregated areas of the hand representation in the primary somatosensory cortex (3b) while Monkey B did not exhibit motor dysfunction and the hand representation was only mildly de-differentiated. Degraded representational features included: 1) unusually large receptive fields (RFs) on the skin of the hand; 2) emergence of RFs covering the entire glabrous surface of an individual digit; 3) multiple RFs including the surfaces of two or more digits; 4) multiple RF's extending to the glabrous and the dorsal skin; and 5) signs of degradation of the hand representation on the untrained hemisphere. Highly stereotypical, repetitive, *articulated hand squeezing* was associated with learned, cortical changes with measureable loss of motor control, but proximally controlled hand closing was associated with only a mild degradation of the hand representation without loss of motor control. Treatment of RSI must restore the normal cortical sensory representation of the hand and patients must adapt a stress free, proximally controlled hand strategy to perform repetitive work without injury.

419.8

EFFECTS OF LARGE-SCALE LIMB DEAFFERENTATIONS ON THE MORPHOLOGICAL AND PHYSIOLOGICAL ORGANIZATION OF RAT FOREPAW BARREL SUBFIELD (FBS) ARE DEPENDENT ON THE TIME OF DEAFFERENTATION. P.P. Pearson , C.X. Li, E.J. Johnson, and R.S. Waters* Dept. Anatomy and Neurobiology, UT, Memphis, Col. of Medicine, Memphis, TN 38163.

We and others have described the organization/reorganization of barrels and barrel-like structures in layer IV of rat somatosensory cortex associated with the representation of the forelimb/forepaw. Pons et.al, (1991) reported the physiological results of large-scale limb deafferentations in monkeys physiologically mapped more than twelve years later and reported that cortical regions formally served by the deafferented limb were subsequently responsive to new inputs from the face. The face representation in the intact monkey is represented many millimeters away from the forepaw representation and their findings reflect wide scale cortical reorganization.

As part of our ongoing work on mechanisms of cortical reorganization we reexamined the consequences of large-scale deafferentations using the rat barrel-field model system. Deafferentations were made in one group of rats on post-natal day 3 (PND-3); prior to the end of the critical period for development of the forelimb barrels (PND-5), while a second group of rats was deafferented in adulthood. One month after deafferentation we mapped the body representation in the deafferented animals using extracellular recording methods. Following mapping, the barrel field cortex was examined using cytochrome oxidase, and the following results were noted:

1. Morphological and physiological maps of controls were aligned with one another.
2. Adult deafferents had normal morphological maps, but the shoulder and trunk representations expanded into the FBS as revealed by physiological mapping.
3. Deafferented neonates had altered morphological maps as well as an expanded representation of the shoulder and trunk in the FBS.
4. Large-scale and restricted deafferentations reflect different mechanisms of reorganization. (Supported by NSF Grant IBN-9400318 to R.S.W.)

419.9

FIRING CHARACTERISTICS OF NEURONS IN THE FOREPAW BARREL SUBFIELD (FBS) CORTEX IN ADULT RAT : AN *IN-VIVO* INTRACELLULAR RECORDING AND LABELING STUDY. C.X. Li* , R.S. Waters. Dept. of Anatomy and Neurobiology, UT, Memphis, Col. of Medicine, Memphis, TN 38163.

The intrinsic firing pattern of somatosensory (SI) cortical neurons has been characterized in the *in-vitro slice* preparation. In rat, three classes of cortical spiking neurons have been identified: regular spiking (RS), intrinsic bursting (IB), and fast spiking (RFS). The laminar location(s) of each class and cell morphology have been described. Recently, Zarzecki and colleagues reported similar classes of spiking neurons in raccoon SI cortex using an *in-vivo* preparation; however, differences in laminar location and mode of thalamic input were observed between the preparations.

As part of our ongoing studies of synaptic input in the forepaw barrel subfield (FBS), we describe the firing properties of SI neurons (n=39) in rat FBS using an *in-vivo* intracellular recording and labeling technique. Adult rats were anesthetized with Nembutal, the head was placed in a stereotaxic apparatus, and the SI cortex was exposed. The digit representation was mapped using a carbon-fiber extracellular electrode. Following mapping, an intracellular electrode containing 2M K acetate and 1% biocytin was used to impale neurons within the FBS forepaw representation. Mechanical and electrical stimulation applied to the digit was used to activate SI neurons. Latency to fire and intrinsic firing properties were measured using standard techniques, and when possible (n=20), the cell was labeled with biocytin. Our results:

1. Neurons in laminae II-V were activated by mechanical/electrical stimulation.
2. Latency differences were observed for cells in each lamina; shortest latencies (6-7 ms) were always observed for neurons in layer IV.
3. Three firing patterns were observed: RS (51%), IB (41%), FS (7%); subclasses of RS and IB were also seen. RS and IB neurons were found in cortical layers II-V.
4. Multiple patterns of discharge were observed in RS and IB neurons as a function of membrane potential. (Supported by NSF Grant IBN-9400318)

419.10

DIFFERENTIAL TIME COURSE OF USE-DEPENDENT PLASTIC REORGANIZATION OF CORTICAL AND THALAMIC HINDPAW REPRESENTATIONS BY MODIFICATION OF WALKING IN ADULT RATS. R.F. Zepka, M. Jürgens and H.R. Dinse (SPON: Europ. Neurosci. Assoc). Institut für Neuroinformatik, Theoret. Biol., Ruhr Univ. Bochum, 44780 Bochum, Germany.

Numerous studies have revealed that subject-environment interactions have significant impact on the reshaping of the cutaneous representation of somatosensory cortex (SI). We have recently demonstrated that also age-related modifications of the walking patterns that are paralleled by changes of sensory inputs, in turn affect the hindpaw representations in the ventral posterolateral nucleus (VPL) of the thalamus and in the somatosensory cortex (Spengler, Godde, Dinse 1995 Neuroreport 6: 469).

Here we report experiments designed to evaluate the influence of systematic modification of walking behavior of the hindlegs on the timecourse of reorganization of the cutaneous hindpaw map in the SI-cortex and thalamus by unilateral cutting the tendon plantaris in adult rats. Receptive field (RF) measurements were performed starting a few days up to several months after the surgery. Thalamic and cortical RFs were derived from the contra- and ipsilateral hemisphere, the latter serving as intra-individual control. Significant enlargement of the RF-size in the contralateral hemisphere (impaired limb) compared to the ipsilateral control was found in SI as well as in VPL. However, there were significant differences in the onset and in the degree of RF-size changes. The reorganizations recorded in VPL of thalamus were smaller and began substantially later than those found in cortex. The results indicate that modifications in the patterns of the sensory stimulation on the paws induced by tendon plantaris cutting, which lead to an impaired locomotion similar to that described for old rats, influence within a few days the representations of the cutaneous cortical and thalamic maps. The clear temporal delay in the thalamic reorganizations provides further evidence for a predominant cortical role in representational postontogenetic plasticity.

Supported by CAPES-Brazil, DFG and the Institut für Neuroinformatik, Germany

419.11

EFFECTS OF MEDIAN NERVE CUT, REPAIR AND REGENERATION IN INFANT MACAQUE MONKEYS. II. CORTICOCORTICAL CONNECTIONS. S.L. Florence* & J.H. Kaas, Dept. Psychology, Vanderbilt Univ. Nashville, TN 37240.

The basis for recovery of tactile sensation in children with nerve cut and surgical repair presumably involves structural or functional reorganization within the ascending somatosensory pathway. In an ongoing effort to establish how and where this recovery occurs, we studied the pattern of corticocortical connections in macaque monkeys (*Macaca mulatta*) that had median nerve cut and repair soon after birth. At 12 - 18 months of age, each monkey received an injection of 2% WGA-HRP into the median nerve representation of cortical area 1, using AAALAC-approved surgical procedures. Approximately twenty hours later, the monkeys were sacrificed and sections through cortex were processed to visualize the injected tracer. The present report focuses on the distribution of label in area 3b. The results indicate that after early postnatal nerve cut, repair and regeneration, corticocortical connections are widespread and virtually non-topographic. For example, a single injection spanning the representations of D2 and D3 in area 1 labeled the entire hand representation of area 3b. Thus, there appears to have been extensive growth of cortical connections or retention of an immature widespread distribution. We suggest that such disperse connections allow greater potential for synapse selection so that nearly normal receptive fields can be generated through use-dependent processes. Presumably, this is one of the major mechanisms of recovery after nerve injury early in life. Supported by NIH grants NS16446 and NICHDHD15052.

419.12

EFFECTS OF MEDIAN NERVE CUT, REPAIR AND REGENERATION IN INFANT MACAQUE MONKEYS. I. THALAMOCORTICAL CONNECTIONS. Taub, H.B., S.L. Florence, & J.H. Kaas*, Dept. Psychology, Vanderbilt Univ. Nashville, TN 37240.

Recovery of tactile sensation is much better in children with nerve cut and surgical repair than in adults, and the basis for this recovery presumably involves structural or functional reorganization within the ascending somatosensory pathway. In an effort to establish how and where this recovery occurs, we studied thalamocortical connections in macaque monkeys (*Macaca mulatta*) that had median nerve cut and repair soon after birth. At 12 - 18 months of age, monkeys received an injection of 2% WGA-HRP into the median nerve representation of cortical area 1, using AAALAC-approved surgical procedures. Later, the monkeys were sacrificed and sections through cortex and thalamus were processed to visualize the injected tracer. The early sensory deprivation had little effect on the architectural appearance of the ventroposterior nucleus (VP), although cytochrome oxidase staining revealed an apparent reduction of metabolic activity in the region where the digit representations are located. Plots of the label in VP demonstrated a remarkably normal distribution despite the deprivation. Both labeled cell bodies and axon terminals were confined to a narrow dorsoventral column lateral to the cell free zone separating the face and hand representations, with an occasional labeled neuron just medial to this border. These findings suggest that reorganization of thalamocortical connections does not significantly contribute to the recovery after nerve injury early in life. Supported by NIH grants NS16446 and NICHDHD15052.

419.13

CHANGES OF RESPONSIVENESS OF VPL THALAMIC NEURONS FOLLOWING TEMPORARY DEAFFERENTATION OF IPSILATERAL HOMOLOGOUS RECEPTIVE FIELD (RF) S.H. Park[1], J.H. Sohn[1], I. Yi[1], C.K. Won[2], B.K. Kim[2], B.R. Park[3]*, J.W. Leem[4], H.C. Shin[2] Dept. Physiol.[2], Hallym Univ., Chunchon, Dept. Psychology[1], Chungnam Nat'l Univ. Taejeon, Dept. Physiol.[3], Wonkwang Univ., Dept. Physiol.[4], Yonsei Univ., Korea

Peripheral sensory deafferentation induces immediate unmasking of new RFs at multiple levels of the somatosensory system of adult animals. It has also been reported that plasticity induced in one cortex is immediately mirrored in the other hemisphere (Calford and Tweedale, 1990). In this study, we tested the possible presence of interhemispheric transfer of the plasticity in the VPL thalamus of rats after peripheral deafferentation. After determining the stability of RF for 30 min of control period, denervation of an ipsilateral digit, which is the counterpart of the contralateral digit RF, were performed by subcutaneous injection of lidocaine (2%, 0.03 ml) to the ipsilateral digit. Afferent sensory transmissions from the contralateral RF were significantly facilitated (25.66±10.7%, 15-20 min, $p < 0.01$) during 5-25 min following temporary deafferentation of ipsilateral homologus RF. This result suggests that plasticity-induced in one hemisphere by temporary sensory deprivation may immediately induce plastic chnages in thalamus of other hemisphere (supported by the 95' Basic Medical Science Grant from the KME to HCShin and in part by the 93' Nondirected Research Fund from the KRF to JHSohn).

419.14

DIVERGENCE OF CONNECTIONS FROM SECONDARY SOMATOSENSORY (SII) AND INSULAR CORTEX TO ANTERIOR PARIETAL CORTEX (APC) IN MACAQUES. M.M. Glasier*, E.R. Ergenzinger, K.A. Findlay, V.J. O'Boyle Jr. & T.P. Pons, Department of Neurosurgery, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

APC cortex in macaques contains topographic representations of the contralateral body surface. The goal of this study was to determine the degree of divergence of projections from SII and insular cortex onto body representations in APC. Retrograde tracing techniques were used in combination with microelectrode mapping procedures to determine the regions of efferent neuronal connections to APC from SII and insular cortex. Injections of different tracers were made into three separate regions of APC, corresponding to face, hand and occiput/neck/shoulder representations, using diamidino yellow, fluororuby and fast blue dyes, respectively. In the granular insular cortex, neurons labeled by different dyes were observed in Layers V and VI. In SII, labeled neurons were seen in supragranular and infragranular layers, forming two bands that followed the contours of the lateral fissure. Some of the labeling in SII and the insula was discrete, where neurons labeled with different dyes occupied separate zones, indicating independent regions give rise to projections to APC. In other portions of SII or the insula, the location of labeled neurons overlapped, indicating a divergence of projections onto different body part representations in APC. The pattern of projections from SII and the insula to APC could form the anatomical substrate for the expansion of the chin and jaw representation seen in APC after dorsal rhizotomies, via utilization of existing neuronal connections.

T. P. Pons was an Alfred P. Sloan Fellow. (Supported by NIMH grant RO1 MH53369-02.)

419.15

SEGREGATED THALAMIC LABELING AFTER TRACER INJECTIONS INTO SOMATOSENSORY CORTEX OF MACAQUES. E.R. Ergenzinger*, K.A. Findlay, M.M. Glasier, V.J. O'Boyle Jr., and T.P. Pons. Department of Neurosurgery, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1029.

While large-scale reorganization of anterior parietal cortex (APC) has been demonstrated in macaques following long-standing dorsal rhizotomies (Pons et al., Science, 1991) the mechanism which explains the extent of this reorganization is unknown. Two possible mechanisms have been proposed: 1) that the reorganization occurs along pre-existing connections that are normally silent, or 2) that new connections are formed at some point along the ascending somatosensory pathway. Lund et al. (Science, 1994) proposed that thalamic input to the occiput/neck/shoulder region of APC, which is located medial to the upper limb representation, and input to the lateral face representation, might overlap across the entire upper limb representation. To directly test this possibility, we injected the fluorescent tracers diamidino yellow (DY), fluoro ruby (FR), and fast blue (FB) into electrophysiologically defined face, hand, and occiput/neck/shoulder regions of APC, respectively, and looked for overlap of label or double-labeled cells in the ventroposterior lateral and medial (VPL and VPM) nuclei of the thalamus. FB and FR labeled cells were observed in non-overlapping portions of VPL and DY labeled cells were observed in VPM. Virtually no overlap of label was observed, nor were there double-labeled cells in VPL or VPM resulting from any of the injected zones. Our results demonstrate that overlap of thalamocortical arbors is insufficient to account for the extent of the reorganization we previously reported after dorsal rhizotomies.

Supported by NIMH RO1 MH53369-01. T.P. Pons was an Alfred P. Sloan Fellow.

419.16

SHORT-TERM VIBRISSA DEPRIVATION IN JUVENILE RATS DOES NOT ALTER RESPONSIVITY OF VIBRISSA PRIMARY AFFERENTS. S. Glazewski*, K. Fox, J.A. DeMaro & M.F. Jacquin. Neurology & Center for the Study of Nervous System Injury, Washington Univ. Sch. Med., St. Louis, MO 63110; Molecular & Medical Biosci., Univ. Wales, Cardiff, UK.

An accompanying paper (Fox & Glazewski) describes the effects of daily removal of all of the whiskers on the responses of supragranular barrel cortex neurons to deflections of the regrown whiskers. As a first step in identifying a possible subcortical source(s) for the observed suppression of cortical responses, identical single unit recording and electromechanical whisker deflection methods were applied to trigeminal ganglion cells after an identical deprivation regimen. Tungsten microelectrodes and urethane anesthesia were used to record responses from 335 ganglion cells in 12 age-matched control rats and 155 cells in 7 cases whose whiskers were plucked daily for 1 week 4-7 days prior to the recording experiment. For all cells, stimuli were 50 vertical whisker deflections of 1° amplitude and 10 msec duration delivered at 1 Hz. Responses in the 0-50 msec post-stimulus interval were analyzed. All cells had single-whisker receptive fields and they rarely displayed spontaneous activity. Nonparametric ANOVA indicated that deprived and control groups did not differ ($p = 0.09$) in their total numbers of responses to single stimuli (mean of animal means ± SD: 2.6 ± 0.7, 4.0 ± 1.9, respectively), whereas deprived cells had significantly ($p = 0.03$) longer latencies to first response (3.6 ± 1.1 msec) than control cells (2.5 ± 1.0). Posthoc comparisons of those cells displaying only an "on" response or only an "off" response or both are in progress to determine the cell type(s) responsible for this latency effect. These data suggest that deprivation-induced reductions in the numbers of cortical unit responses to whisker deflection are not due to altered primary afferent responses.

Support: NIH DE07662, DE07734, NS17763, NS27759.

419.17

EVIDENCE FOR HETEROSYNAPTIC DEPRESSION IN THE RAT BARREL CORTEX Fox, K* and Glazewski, S. *Molecular and Medical Bioscience, U. of W., Cardiff, U.K. and Washington University School of Medicine, St. Louis MO*

Vibrissae deprivation not only causes potentiation of spared vibrissae responses but also depression of deprived vibrissae responses. In a deprived barrel-column, depression is greater for neurons located close to an active barrel-column. One possible explanation is that the spared vibrissa produces heterosynaptic depression of the principal vibrissa response [Glazewski and Fox, J. Neurophysiol., 75:1714]. To test this idea further, we compared the effect of depriving all vibrissae (11 rats) with depriving one single vibrissa (10 rats). Vibrissae were removed without injury unilaterally for 7 days and regrew for 6 to 8 days before recording single unit responses to standard vibrissae deflections. Animals were 28-35 days old. The positions of all recording penetrations were recovered within the barrel field. Vibrissae responses were quantified using post-stimulus time histogram analysis.

In animals deprived of all vibrissae, principal vibrissae responses were depressed to 38% of control levels in layers II/III (deprived 167, control 46 cells) and 78% in layer IV (deprived 89, control 40 cells). Depression was significant for cells in layers II/III (0.46±0.06 spikes per stimulus vs 1.22±0.12 for undeprived controls [$p<0.005$; df=13; t-test]) but not for those in layer IV (0.98±0.18 sp/stim vs 1.25±0.15 [$p>0.1$, df=13]). This indicates that a component of vibrissae response depression is homosynaptic in layers II/III.

However, depression was far greater if animals were only deprived of a single vibrissa. In the deprived barrel-column, principal vibrissa responses were depressed to 16% of control in layers II/III (100 cells) and 41% in layer IV (48 cells). Deprived vibrissa responses were significantly smaller for the "single-deprived vibrissa" paradigm than for the "all-deprived" cases for both layer II/III (0.2±0.06 sp/stim [$p<0.01$; df=19; 2-tail t-test]) and IV cells (0.51±0.14 sp/stim [$p=0.05$, df=19]). This indicates that a component of vibrissae response depression is heterosynaptic in origin.

Supported by NIH NS27759 to KF.

419.18

GENE EXPRESSION IN SOMATOSENSORY CORTEX OF MONKEYS WITH LESIONS OF VENTROBASAL THALAMUS. T.M. Woods, P.R. Manger, E. Rausell*, K.Trinh & E.G. Jones. Dept. Anatomy and Neurobiology, Univ. California Irvine, Irvine, CA 92717-1280.

Thalamocortical projections from the ventrobasal nuclear complex (VB) of the thalamus to the somatosensory cortex of the postcentral gyrus in monkeys exhibit substantial divergence and overlap. This property of thalamocortical divergence may be one basis of somatosensory cortical plasticity. This study addresses the question of the extent to which this divergence can maintain a normal pattern of gene expression for molecules that may be involved in plasticity of 3b representational maps in the presence of diminishing VB input. Lesions of varying size were made in VB as defined by extracellular multi-unit mapping either electrolytically or with kainic acid. After one to five weeks recovery, the thalamus and somatosensory cortex were sectioned and stained for Nissl, cytochrome oxidase (CO), immunocytochemically for gamma-aminobutyric acid (GABA), NMDA receptor 1 (NR1), type II calcium/calmodulin dependent protein kinase α (CAMIIKα), parvalbumin, β2/β3 $GABA_A$ receptor subunits, and glutamate receptor 2/3 (GluR2/3), or hybridized with radioactive cRNA probes for glutamic acid decarboxylase (GAD), CAMIIKα, NR1, NR2a, NR2b, NR 2d receptors, the α1, β2 and γ2 subunits of the $GABA_A$ receptor and the GluR2 receptor. Both electrolytic and kainic acid lesions produced a complete loss of VB neurons within the area of the lesion. Regions of decreased CO staining and neurotransmitter related molecule staining and hybridization in area 3b were dependent on the magnitude of the lesion and could only be detected with lesions that caused loss of VB neurons beyond a critical level of approximately 10% of the population.

Supported by NIH grant NS21377

419.19

EFFECTS OF SENSORY DEPRIVATION ON GLIAL FIBRILLARY ACIDIC PROTEIN IN THE RAT BARREL CORTEX. I. Tzimou and D. A. Weldon.* Dept. of Psychology, Hamilton College, Clinton, NY 13323.

The rodent barrel cortex shows plasticity in neuronal receptive fields and cell chemistry following alterations of sensory input from the whiskers. In the present study we investigated whether tactile deprivation from removal of whiskers would affect barrel cortex glial cells. Anesthetized rats had vibrissae on one side of the face removed with forceps. One week later, tangential sections were taken through the somatosensory cortex. Adjacent sections were stained with cresyl violet and processed for glial fibrillary acidic protein (GFAP) using immunocytochemistry. After identifying the barrel cortex location in the nissl-stained sections, we examined the GFAP-containing cells in the equivalent location of the immunocytochemically processed tissues. Cells contralateral to the intact vibrissae showed heavier and less patchy GFAP immunoreactivity than the cells located in the barrel cortex receiving information from the side of the face in which the whiskers had been removed. The results suggest that changes in glial cell chemistry can reflect neuronal plasticity in the barrel cortex.

Supported by Hamilton College.

419.20

WIDESPREAD 2DG LABELING IN BARREL CORTEX FOLLOWING REPEATED WHISKER TRIMMING IN ADULT HAMSTERS- A 2DG/IMMUNOSTAINING STUDY. D.L. Maier and J.S. McCasland*. *Dept. of Anatomy, SUNY HSC at Syracuse, Syracuse, NY 13210.*

We have previously demonstrated a widespread, whisker-dependent activation of the entire barrel field in adults subject to ablation of most whisker follicles at postnatal day 7 (P7), after the fate of the barrel map is set (Soc. Neurosci. Abstr. 21: 124). Such strong activation of all large-whisker-related columns by as few as two whiskers is never observed in acutely-trimmed adults. Acute removal of the spared whiskers greatly reduces the intensity of metabolic labeling in all barrels, indicating that their presence was indeed responsible for the widespread labeling observed in the two-whisker animals. We interpret these data to mean that a large-scale synaptic reorganization of barrel cortex in hamsters occurs in response to P7 follicle ablation.

Physiological studies have shown plasticity in single-unit responses to whiskers "paired" for several days in adults (Diamond et al., Science '94). We tested for similar plasticity in our 2DG/immunostained materials following repeated trimming of most whiskers. Beginning around P40, animals had all whiskers except Row C trimmed on alternating days for periods ranging from 1 day to 2 weeks, after which they were administered ^{3}H 2DG IP and allowed to explore a fresh empty cage.

Autoradiograms from these animals showed a clear expansion in the zone of heavy 2DG labeling with continued whisker trimming. Hamsters with Row C spared overnight showed markedly higher labeling in the Row C barrels as expected. After 2 weeks of repeated trimming, the pattern of 2DG labeling in the barrel field varied among the 4 cases we have examined, ranging from complete activation of all large-whisker columns reminiscent of that seen following follicle ablation, down to a more localized activation of Rows B, C, and D. More subtle effects were observed after six or seven days of trimming.

Our data suggest that a large-scale synaptic reorganization occurs in both neonatally- and adult-deprived barrel fields. The results provide a convenient model system for testing plasticity mechanisms operative in development, in adults, or in both. Supported by NIH grant NS31829.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS VI

420.1

GLOBAL TRAVELING WAVE AND LOCALIZED OSCILLATIONS IN NEOCORTICAL SLICE J.-Y. Wu*, L. Guan, and Y. Tsau Department of Neurology and Institute for Cognitive and Computational Sciences, Georgetown University Medical Center, Washington DC, 20007

Coherent activity in neocortex may be important in information processing and storage. Here we report that localized oscillations were triggered by an initial traveling wave in neocortical slices. Spontaneous epoch oscillations in neocortical slices in a nominally zero magnesium medium (Silva et. al. Science 251:432, 1991) were examined by voltage sensitive dye (RH 479) imaging and electrode array (field potential). Each epoch of spontaneous oscillations was composed of a global initial traveling wave followed by localized loci of oscillation. The initial wave spread over the entire preparation (coronal slice of half hemisphere between Bregma -3 to -7 mm, from 20 to 30 day old SD rat). Its propagation velocity (40-120 mm/sec) appeared to vary significantly from epoch to epoch but complicated traveling patterns revealed by optical recordings suggesting that the velocity difference may due to multiple initiation sites or variations in the pathway. After each initial wave, oscillations emerged from different loci. At 27°C, each locus oscillated for 2 to 20 cycles at 2 to 8 hz. Optical imaging showed that some loci were highly localized, covering a narrow strip (0.2 to 1 mm) of the cortical section but encompassing all cortical layers. Overlap of activity from different loci was observed optically but the activity from different loci appeared independent or only weakly coupled. These segregated loci of oscillations may represent modular neural assemblies or oscillation attractors formed by local synaptic interactions (Supported by NIH NS 31425).

420.2

DYNAMIC REORGANIZATION IN RAT NEOCORTICAL SLICE. Y. Tsau*, L. Guan, and J.-Y. Wu. Georgetown Institute for Cognitive and Computational Sciences, Georgetown University Medical Center, Washington D.C. 20007

Neurons in the neocortical slice tend to oscillate under conditions of zero Mg^{2+}. We report that these oscillations underwent dynamic reorganization over time and was distributed unevenly over the neocortical slice. Experiments were carried out on SD rat coronal neocortical slices using electrode array and optical recording with voltage-sensitive dye (RH479). Spontaneous coherent rhythmicity was recorded electrically and optically, composed of a global initiation wave followed by localized oscillations. The initiation waves originated in temporal cortex area and propagated to parietal cortex when they started spontaneously 40 min to 1 hour after the slice was bathed in zero Mg^{2+} medium. After 2 to 4 hours in zero Mg^{2+}, the propagation velocity gradually increased from 44 mm/sec up to 110 mm/sec. After then the time delay between two recording sites became random in both sign and value, suggesting that the site of initiation waves became variable. The propagation velocity of an initiation wave also depended on time interval following the previous wave. The evoked initiation waves by electrical stimuli propagated at 57 mm/sec 20 sec after the previous waves and at 28 mm/sec 2.5 sec after. Simultaneous recordings of localized oscillations from different sites appeared similar but the oscillations were actually coincident. However, the oscillation patterns and locations seemed unchanged over time. The data suggest that the system is reorganized for generating initiation waves but the local circuit for oscillations remains stable after the medium changes. (Supported by NIH NS 31425)

420.3

IONIC MECHANISMS GENERATING THE REFRACTORY PERIOD IN THALAMIC SYNCHRONIZED OSCILLATIONS. A. Lüthi* and D.A. McCormick. Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

Recent studies have shown that spindle wave oscillations in the ferret lateral geniculate nucleus are followed by a refractory period associated with a slowly decaying afterdepolarization (ADP) that involves the activation and subsequent deactivation of the inwardly rectifying cation current I_h. Here we examined the physiological factors contributing to this persistent activation of I_h. The intracellular injection of rhythmic short duration hyperpolarizing current pulses (5-20 pulses, 200-400 pA, 120 ms, 4 Hz), mimicking the arrival of IPSPs during synchronized oscillations, was associated with the generation of an ADP whose amplitude was larger than that following a single, long-duration hyperpolarizing pulse. This result suggests that the rebound Ca^{2+} spikes that occur during repetitive hyperpolarization may contribute to enhanced activation of I_h. Indeed, reduction of Ca^{2+} bursts with local application of Ni^{2+} ions resulted in a substantial reduction of the ADP. We suggest that both voltage-dependent cumulative activation of I_h and Ca^{2+} influx contribute to the development of the ADP and the generation of the refractory period. It remains to be investigated whether or not Ca^{2+} modulates I_h with respect to voltage-dependence, kinetics and/or maximal conductance. Sponsored by NIH and the Swiss National Science Foundation.

420.4

RELATION BETWEEN OPTICAL IMAGING MAPS AND SPIKING AND NON-SPIKING POINT-SPREAD FUNCTIONS RECORDED IN SUPRAGRANULAR AND GRANULAR LAMINAE IN RAT SOMATOSENSORY CORTEX. T. Hilger, T. Berkefeld, B. Godde, K. Behrend* and H.R. Dinse. Institut für Neuroinformatik, Theoret. Biology, Ruhr-Univ. Bochum RUB, Bochum, Germany

We investigated the relationship between optical imaging data and the width and the slope of cortical spiking and non-spiking point-spread functions (PSFs) recorded in the supragranular and granular layers of the hind- and forepaw representations of the somatosensory cortex of urethane anaesthetized rats. As a rule, all different types of maps were always recorded for each animal. Spiking PSFs were obtained by measuring representational maps based on cutaneous receptive field (RF) mapping. Non-spiking PSFs were obtained by recording local field potential (LFP) maps. Each individual electrophysiological map was reconstructed from 60 to 80 electrode penetrations. Optical maps were obtained by means of optical recording of reflectance changes of intrinsic signals (LaVision Lightstar II imaging and acquisition system) using a 546 nm light source. Optical imaging maps and LFP maps were separately calculated for 25, 50 and 75 % of their maximal amplitudes.

We found a close correspondence between the size and the slope of the optical maps and the non-spiking PSFs recorded in layers IV. While the slopes of the non-spiking PSFs recorded in layers (II/III) were also comparable to the optical imaging data, the size of the layer II/III non-spiking PSF was significantly smaller than the optical maps and the layer IV LFP maps. Spiking PSFs based on RF measurements revealed a considerable resemblance with the optical and LFP maps calculated for the 50 % amplitude level. Combined, the data demonstrate that in rat somatosensory cortex optical imaging using a 546 nm light source reveals activity distributions that are in close correspondence with electrophysiologically obtained maps. The correspondence of optical imaging maps with layer IV PSFs suggest a substantial contribution of layer IV to the overall layout of optical maps.

Supported by DFG and the Institut für Neuroinformatik - RUB, Germany

420.5

DUAL WAVELENGTH OPTICAL IMAGING OF INTRINSIC SIGNALS IN RAT CORTICAL ACTIVITIES. M. NAKASHIMA*[1], M. YANAURA[1], S. YAMADA[1], S. SHIONO[1] and M. TANIFUJI[2]. Advanced Technology R&D Center[1], Mitsubishi Electric Corp., Hyogo 661, Japan and PRESTO[2], JRDC, Tokyo 100, Japan

Simultaneous optical imaging with two wavelengths has the potential advantage of allowing efficient noise reduction and selective signal amplification, and achieving real-time imaging of cortical activities. One wavelength was set to be more sensitive to the cortical activities than the other, but both recorded the noise associated with cortical movement. In the optical system, two CCD cameras were used to visualize the same cortical surface. The combination of 570 and 600 nm band-pass filters was chosen to detect intrinsic hemoglobin signals relating to the cortical activities.

A SD strain rat was anesthetized with urethane and kept at the level of surgical anesthesia. The animal was placed in a non-traumatic head holder, and its somatosensory or gustatory cortex was exposed with the dura intact. To evoke the cortical response, a single whisker, and tongue region were stimulated by mechanical touch, and by taste solutions (sucrose, NaCl, HCl), respectively.

We obtained the optical signals responding to the contra-lateral whisker stimulation. The significant optical changes were observed mainly at a time of 2 - 5s after the stimulus onset, and their extent was localized at about 1x 1mm^2 area of somatosensory cortex. The responding area to the whisker stimulation was topologically correlated, depending on the whisker location. On the other hand, significant optical signals of gustatory cortex were observed at the area of about 4mm x 3mm dorsal to rhinalis sulcus, approximately at the level of crossing with the middle cerebral artery.

420.6

STIMULUS-EVOKED LOW FREQUENCY MAGNETIC FIELD OSCILLATIONS IN SOMATOSENSORY CORTEX OF AWAKE HUMANS, D. Poeppel*, S.S. Nagarajan, H.W. Mahncke, T.P.L. Roberts, H.A. Rowley, and M.M. Merzenich, University of California, San Francisco, CA 94143-0732.

Low-frequency oscillations (8-12Hz) have been observed in several regions of sensorimotor cortex in awake humans. However, the role of these oscillations in sensory processing remains unclear. Studies in anesthetized animals show that stimulus-evoked oscillations of local field potentials and multi-unit activity in this frequency range clearly affect the response to subsequent stimuli (Sameshima et al., Soc. Neurosc. Abst., 644.4, 1993). The purpose of this study was (1) to characterize oscillations evoked by a single somatosensory stimulus in awake humans, and (2) to determine the effect of such oscillations on responses to stimuli occurring at various delays after the first stimulus.

We recorded evoked magnetic fields (37-channel biomagnetometer, Magnes, Biomagnetic Technologies Inc., San Diego, CA) from somatosensory cortex in 5 human subjects passively attending to suprathreshold stimuli delivered to the distal tip of the index finger. Stimuli were single or paired air-puffs (25 PSI) separated by 50-250ms. The frequency spectrum, amplitude, and total power of the response elicited by each stimulus were measured.

We observed (1) oscillations that lasted between 200 and 400ms in the range of 8-12 Hz in response to single air-puffs. (2) The amplitude and total power of the response depended on the position in time of the second stimulus relative to the phase of the ongoing oscillation evoked by the first stimulus. Both the amplitude and total power plotted as a function of the inter-stimulus delay appear to be oscillatory in the frequency range of 8-12Hz. This is inconsistent with monotonic recovery from inhibition. The data suggest that these oscillations play a role in the processing of time-varying stimuli in the range of hundreds of milliseconds. [Supported by HHMI, the Charles A. Dana Foundation, and Biomagnetic Technologies Inc.]

420.7

OPTICAL IMAGING OF NEURONAL ACTIVITY AND COUPLED METABOLIC ACTIVITY IN RAT BARREL CORTEX. I. TAKASHIMA* and T. IIJIMA, Electrotechnical Laboratory, 1-1-4, Umezono, Tsukuba, Ibaraki, 305, Japan.

We studied the relationship between the functional map of whisker barrel field of the rat obtained with extrinsic optical signal recordings using a voltage-sensitive dye and the map obtained with intrinsic optical signal recordings with near infrared light. The functional map was very similar in the both recordings, if we compared the intrinsic signal map at the initial phase (600 ms) with the extrinsic signal map at the maximal response. However, the functional map at the maximal response of each signals were significantly different. The maximal "hot area" detected with intrinsic signals occupied twice as large area as the maximal "hot area" detected with extrinsic signals. The domain of the intrinsic signals finally moved to the superior cerebral veins. The intrinsic signals were recorded at the wavelength of 570, 580 and 610 nm, respectively. The changes in regional cerebral blood volume (rCBV) and hemoglobin oxidation-reduction state were thought to be involved in the intrinsic signal as the major source at these wavelength. Indeed, the optical signal changed in size and polarity according to the wavelength used, reflecting their origin of signal source. We tried to separate these two components based on the wavelength-absorption characteristic of oxy-deoxy hemoglobin. The results showed that the oxygen consumption of neurons preceded 200-400 ms to the increase of rCBV, and that the functional map obtained with this component, not the rCBV, was almost identical to the map obtained with the extrinsic signal recordings.

420.8

HIGH RESOLUTION IMAGING OF RAT NEOCORTICAL NEURONS *IN VIVO* USING TWO-PHOTON EXCITATION LASER SCANNING MICROSCOPY. D. W. Tank*, K. Svoboda, R. A. Stepnoski, D. Kleinfeld, W. Denk. Bell Laboratories, Lucent Technologies, Murray Hill, NJ 07974.

Severe scattering of visible light by neural tissue has precluded high resolution optical imaging of neurons by conventional methods in intact nervous systems. The infrared wavelengths used to excite visible fluorophores by two-photon excitation penetrate much deeper into brain tissue. In addition, light absorption in Two Photon Laser Scanning Microscopy (TPLSM) occurs only in the focal volume, eliminating out-of-focus photobleaching and photodamage, while providing optical sectioning. Fluorescence light can be collected with high efficiency due to the absence of a confocal pinhole, reducing photodamage in the focal plane. These features suggest that TPLSM offers significant advantages in high-resolution imaging of neurons in the mammalian neocortex *in vivo*. To test this idea, we filled neurons in the somatosensory cortex of anesthetized rats with fluorescent calcium indicators and imaged them using a custom-made two-photon excitation laser scanning microscope. The excitation source was a pulsed Ti:sapphire laser (λ = 850 nm; 250 mW; 100 fs; 82 MHz). A skull-mounted chamber over a cranial window allowed simultaneous optical (40x 0.75 NA water immersion objective) and microelectrode access. Individual cortical neurons were filled iontophoretically with Calcium Green 1 using sharp microelectrodes. Dendritic processes were resolved with single spine resolution down to 500 µm below the pial surface. The complete 3D dendritic morphology could be reconstructed from stacks of optical sections. Dendritic calcium transients evoked by sensory stimulation or current injection through the recording electrode were repeatedly (> 100 times) measured with high temporal (up to 1 ms in line scan mode) and spatial (< 1µm) resolution. Our results demonstrate that TPLSM offers a new way to probe the dynamics and structure of neocortical neurons *in vivo*. (Supported by Bell Laboratories)

420.9

IN VIVO OPTICAL IMAGING OF DENDRITIC CALCIUM DYNAMICS IN NEOCORTICAL NEURONS UNDER SENSORY STIMULATION. K. Svoboda, W. Denk, D. Kleinfeld*, D.W. Tank. Bell Laboratories, Lucent Technologies, Murray Hill, NJ 07974.

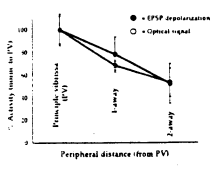
We used the high resolution provided by two-photon laser scanning microscopy (see accompanying abstract) to measure spatio-temporal dendritic calcium dynamics in rat barrel cortex pyramidal neurons. Experiments were performed on 4-6 week old rats under urethane anesthesia (2mg/g). Neurons in layers 2 & 3 were impaled with beveled sharp microelectrodes containing 3 mM Calcium Green 1 and 400 mM K-Acetate (resistance ~ 60 - 120 MΩ). Periodic mechanical whisker deflections (5 Hz) produced two types of short latency (~ 15 ms) response: i) Unitary 'slow' membrane potential transients (width ~ 40 ms), typically at the beginning of stimulus trains, which produced bursts containing 1- 3 Na^+ action potentials; ii) Sub-threshold slow transients with highly variable amplitudes. Sensory stimulation evoked rapid calcium transients (rise time < 10 ms) in the apical dendrite, synchronous with electrically recorded Na^+ spikes. Calcium transients produced by two spikes were twice as large as those produced by one spike. The fractional change in fluorescence first increased with distance from the soma (up to 40 - 200 µm) and then decreased, vanishing in the distal apical tuft in layer 1. Calcium transients were not observed during sub-threshold slow potentials. Hyperpolarization eliminated both Na^+ spikes and calcium transients but increased stimulus-induced slow potentials. Intracellular QX-314 (30 mM in the electrode) blocked Na^+ spikes but produced spontaneous regenerative potentials that could also be evoked with depolarizing current injections, consistent with increased calcium electrogenesis. Only under these conditions did sensory stimulation evoke calcium transients in the apical dendrite in the absence of Na^+ spikes. Under normal conditions apical dendritic calcium transients are always associated with Na^+ action potential invasion of the dendrite. (Supported by Bell Laboratories)

420.10

WHAT IS THE NEURAL CORRELATE OF THE OPTICAL IMAGING SIGNAL? INTRACELLULAR RECEPTIVE FIELD MAPS AND OPTICAL IMAGING IN RAT BARREL CORTEX C. I. Moore*, B. R. Sheth, A. Basu, S. Nelson & M. Sur Dept. of Brain & Cog. Sci., M.I.T., Cambridge, MA 02139

Changes in metabolic activity are co-opted in several techniques (fMRI, PET) to examine the organization of sensory cortex. Intrinsic signal optical imaging similarly relies on changes in the oxygenation state (and subsequent changes in the reflectance) of hemoglobin to provide a measure of activity in neuronal populations. The electrophysiological correlate of this metabolic signal is still being determined; either action potential firing or subthreshold neuronal activity is the more accurate correlate of the optical signal.

We examined this issue directly, with *in vivo* intracellular rf mapping in supragranular (SG) neurons (Moore and Nelson, 1994) and intrinsic signal optical imaging in the rat barrel cortex (Masino et al., 1992). We found a close correlation between the amplitude of subthreshold-EPSPs evoked by vibrissae that are 1- or 2-vibrissae away from the principle vibrissa of an SG neuron (78% and 52% of the optimal input, respectively), and the amplitude of the optical signal evoked by vibrissae 1- or 2-away from the principle vibrissa for a cortical activation zone (rad. 178m: 68% and 53% of the optimal input, respectively). Work in our laboratory and from others (Armstrong-James et al., 1992) demonstrates a much sharper fall off in the amplitude of SG extracellular receptive fields (i.e., 1-away inputs drive a neuron with <= 50% the efficacy of the principle vibrissa). We conclude that subthreshold inputs provide an accurate correlate of the optical signal generated in somatosensory neocortex. *Support: EY07023.*



421.1

TOPOGRAPHIC PATTERNS OF V1 AND V2 CORTICAL AND SUBCORTICAL CONNECTIONS IN TREE SHREWS. D.C. Lyon*, N. Jain, and J.H. Kaas Dept of Psychology, Vanderbilt University, Nashville, TN, 37240.

V2 connections were used to define and characterize extrastriate visual areas in tree shrews *(Tupaia belangeri)*. Tree shrews are of special interest because they are highly visual, and they are closely related to primates. Injections of distinguishable fluorochromes were placed in different visuotopic locations along the length of V2. For comparison, WGA-HRP was injected into primary visual cortex. The results indicate that at least four extrastriate areas have topographic patterns of connections with V2. Three of these areas have been previously identified along the lateral border of V2 as the temporal anterior (TA), temporal dorsal (TD), and temporal posterior (TP) areas, since they also have inputs from V1 (Sesma et al., JCN, 230: 337, 1984). A small oval of cortex lateral to TA and TD has less dense V2 connections. Both V1 and V2 have inputs from the lateral geniculate nucleus and pulvinar. We conclude that tree shrews have several areas, TA, TD, TP, rather than a single area, V3, along the lateral border of V2, and they have at least one further visual area in temporal cortex. Because these close relatives of primates do not have a V3, we question the premise that V3 is a valid visual area in primates. Supported by NEI grant EY-02686.

421.2

CONNECTIONS OF VISUAL CORTICAL AREA 18 (V2) IN FLATTENED CORTEX OF GALAGOS. M.W. Pospichal* and J.H. Kaas. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

Connections of the second visual area, V2 or area 18, of adult galagos were investigated by injecting different fluorochromes and WGA-HRP into the field. Later, the cortex was separated from deep structures, manually flattened, and cut parallel to the surface. Some sections were reacted for cytochrome oxidase or myelin, and areas V1, V2, and MT were identified. Results show that injections in V2 labeled neurons and terminations in V2, V1, the middle temporal area (MT) and regions of cortex presumed to contain dorsolateral (DL), dorsomedial (DM), and middle superior temporal (MST) visual areas, as well as inferotemporal cortex (IT). The largest zones of V2 inputs arose from V1 and from cortex rostral and adjacent to V2 (proposed DM and DL). V2 connections with MST and IT were relatively sparse and inputs from MT and intrinsic connections of V2 were intermediate in density. Separate injections in V2 led to partially overlapping regions of label in areas outside V2 that decreased with more widely spaced pairs of injections. The results support the conclusion that major cortical connections of V2 in prosimian primates closely resemble those of simian primates. (Supported by EY-02686 and EY-07135)

421.3

NEUROFILAMENT AND CALCIUM-BINDING PROTEINS DEFINE VISUAL CORTICAL AREAS IN HUMAN. M.M. Adams[1], L.G. Ungerleider*[1], G. Eden[1], C. Bouras[2], G. Bertini[1], J.H. Morrison[3] and P.R. Hof[3]. [1]Lab. Psychol. Psychopath., NIMH, NIH, Bethesda, MD 20892; [2]IUPG Belle-Idée, Univ. of Geneva, Switzerland; [3]Dept. of Neurobiol., Mt. Sinai Sch. Med., New York, NY 10029.

Visual cortex in the macaque monkey consists of at least 30 areas that can be distinguished on the basis of differential cytoarchitecture, patterns of connectivity, visual topography, and physiological properties. It has also been shown that many of these functional cortical regions can be delineated using neurochemical markers. In particular, nonphosphorylated neurofilament protein (NFP) has specific regional and laminar distributions in each visual area. To investigate the chemoarchitectonic organization of visual areas in the human, we used antibodies to NFP, which is found in a subset of pyramidal cells, and to the calcium-binding proteins (CaBP) calbindin, parvalbumin, and calretinin, which occur in subsets of GABAergic neurons. Immunohistochemistry revealed labeling patterns that defined at least six distinct visual areas which we have identified as homologues of monkey areas V1, V2, VP (V3v), V3d, V4, and MT. In area V1, strongly labeled NFP-positive neurons were found in layers IVA, IVB, and VI. Area V2 had more prominently labeled NFP-positive neurons in the superficial than in the deep layers. Area VP showed many NFP-positive neurons in layers III, V, and VI. In contrast, area V4 had light NFP label in layers II, III, V, and VI. Area V3d displayed strong NFP label in layers II and III, with a few neurons in layer V extending their apical dendrite up to layer III. The most easily distinguishable area was MT, which had dense NFP label in layers III, V, and VI. Differences between visual areas were also observed using CaBP immunohistochemistry; however, the borders between these areas were not as clearly defined as those seen with NFP. These results show that, as in the macaque monkey, the human visual cortex can be subdivided into distinct areas on the basis of specific neurochemical characteristics. *Supported by NIH AG06647 and MHDA52145 (JHM and PRH) and NIMH-IRP (MMA, LGU, GE, and GB).*

421.4

PARCELLATION OF VISUAL CORTICAL AREAS IN THE CHIMPANZEE USING NEUROFILAMENT AND CALCIUM-BINDING PROTEINS. R. Gattass*[1], M.M. Adams[2], P.R. Hof[3], and L.G. Ungerleider[2]. [1]Inst. Carlos Chagas Filho, Univ. Fed., RJ, Brazil; [2]Lab. Psychol. Psychopath., NIMH, NIH, Bethesda, MD 20892; [3]Dept. Neurobiol., Mt. Sinai Sch. Med., New York, NY 10029.

Over 30 distinct visual areas have been described in the macaque cortex based on physiological properties and patterns of connectivity. Many of these regions have also been distinguished on the basis of neurochemical characteristics. Functional imaging and lesion studies have demonstrated that the human visual cortex displays comparable, functionally specialized regions. Preliminary results have shown that these visual areas in the human exhibit neurochemical specialization patterns similar to those in the monkey. To investigate whether the chimpanzee visual system exhibits comparable organization, we used antibodies to neurofilament protein (NFP) and to the calcium-binding proteins (CaBP) calbindin, parvalbumin, and calretinin. We found at least 8 visual areas based on different patterns of immunostaining, which we regard as homologues of monkey areas V1, V2, V3, V4, MT, TEO, LIP, and VIP. Area V1, V2, and MT were comparable in appearance in the monkey, chimpanzee, and human. In V1, darkly stained large pyramidal neurons were found in layers IVA, IVB, and VI. Area V2 had stongly labeled NFP-positive neurons in the superficial layers. Area MT was easily distinguishable by the intensely stained NFP labeled neurons in layers III, V, and VI. Areas V3, V4, LIP, and VIP were more similar to the monkey. Area V3 and V4 were characterized by many neurons in the superficial portion of layer III. TEO had many darkly labeled neurons in layers II and III, and an occasional large pyramidal neuron in layer V. Areas LIP and VIP displayed intensely stained neurons in layer III and occasional large pyramidal cells in VA. CaBP also showed regional differences but the borders between areas were more subtle than those defined by NFP. These results indicate that the chimpanzee visual cortex exhibits a comparable organization scheme to that of both monkeys and humans, although the location of the visual areas in relation to the sulci pattern in the chimpanzee is more similar to humans. The chimpanzee brain was donated by the Yerkes Regional Primate Center. *Supported by CAPES, CNPq[1] and NIMH-IRP[2].*

421.5

RETINOTOPIC ORGANIZATION BEYOND THE EARLY VISUAL AREAS IN HUMANS. M.K.Hasnain, M.Woldorff*, P.T.Fox. Research Imaging Center, UTHSCSA, San Antonio, TX 78284-6240

Evidence was found for retinotopic organization extending well beyond the early visual areas in the human visual cortex. A retinotopic organization existing in the cerebellum was also identified. Striate and extrastriate areas were functionally mapped with PET (H_2O^{15}). Bow-tie-shaped random dot patterns, changing at 8 Hz were presented with a centrally placed fixation point, oriented along the horizontal meridian in one task condition and along the vertical meridian in another. In the control condition the fixation point was presented without any surrounding patterns. Each condition was performed three times, for a total of nine scans. PET images within each task were averaged and averaged control images were subtracted from each of the averaged task images. Z-statistics were performed on the subtraction images and the significantly activated regions were overlaid onto a 3-D surface model of the occipital lobe generated from co-registered MRI images of the same subject. Significant activations were observed from the calcarine sulcus outward along the cortical surface, alternating between those caused by the horizontal meridian stimulus and those caused by the vertical meridian stimulus. Based on the alternating pattern of the activation sites, up to four extrastriate regions were discernible on the medial occipital lobe and up to five extrastriate regions were discernible on the lateral aspect of the occipital lobe. The study identifies at least three more retinotopically organized areas than have currently been reported in the occipital lobe. In addition, the horizontal and vertical meridian stimuli produced different activation sites in the inferior temporal lobe and in the cerebellum.

UTHSCSA, RIC, Act # E-XOKO-30-000-2-17

421.6

VISUAL PROCESSING MEMORIES OF HUMAN CEREBRAL CORTEX. M. A. Uusitalo[a]*, S. J. Williamson[b]. [a]Brain Research Unit, Low Temperature Laboratory, Helsinki University of Technology, FIN-02150 Espoo, Finland. [b]Department of Physics, New York University, New York City, NY 10003-1113.

Magnetic source images (MSIs) obtained with an array of 122 planar sensors revealed 12 cortical areas that responded within 0.5 s to a brief (70 ms) presentation of a checkerboard stimulus. The response amplitude of each source, modeled as a current dipole, could be characterized as a function of interstimulus interval (ISI) by the expression A(1-e^[ISI/tau]), where A is the saturated response amplitude for long ISI, and tau describes the shape of this "ISI curve". The parameter tau can be interpreted as the lifetime for the neural activation trace. These results provide evidence that every responding cortical region supports a memory function, for which we use the term "processing memory". The responding cortical areas fell into two groups: the earliest in occipital lobes with lifetimes ranging from 0.2 to 0.6 s and the later ones in temporal, parietal, and frontal lobes with lifetimes ranging from 7 to 30 s.

These data suggest that the participation of local memories is an essential component of visual information processing.

This study was supported by the Academy of Finland, the Magnus Ehrnrooth Foundation and Vilho, Yrjö, and Kalle Väisälä Foundations in Finland, and the Horace W. Goldsmith Foundation in the USA by a grant to New York University.

421.7

OXIDATIVE METABOLISM IN HUMAN VISUAL CORTEX DURING PHYSIOLOGICAL ACTIVATION STUDIED BY PET. M. Vafaee, S. Marrett, T. Paus, A. Gjedde[1], A.C. Evans, A. Ptito*, and E. Meyer. McConnell Brain Imaging Centre, Montreal Neurological Institute, Montreal, Canada H3A 2B4, [1]Aarhus, Denmark.

For the purpose of studying oxidative metabolism in individual human subjects, we tested a previously developed activation paradigm that was shown to increase brain oxidative metabolism in a group of normal controls (Marrett et al.; 1993). We used positron emission tomography (PET) to measure cerebral metabolic rate of oxygen (CMR_{O_2}) during visual stimulation. Six healthy normal volunteers (age 19-33) were studied with the Scanditronix PC-2048 15B PET scanner. A yellow and blue checker board (5^0-20^0 of visual angle), reversing at a frequency of 8 Hz, was presented for a total of 7 min (starting 4 min before the onset of a 3-min scan). In the baseline condition, subjects began fixating a cross-hair 30 s before the scan and continued throughout the 3-min scan. Baseline and activation scans were repeated (n=12) for better statistics. CMR_{O_2} was measured using the two-compartment, weighted integration method of Ohta et al. (1992). Individual PET images were co-registered with the corresponding MRI scans. We found consistent foci in V1/2, V4 and parieto-occipital areas of visual cortex. CMR_{O_2} significantly increased in V1/2 and parieto-occipital areas ($t>4.0$) in both the averaged and in all individual images. We conclude that this particular visual stimulus not only increases CMR_{O_2} in the striate cortex but also consistently elevates CMR_{O_2} in the extra-striate cortex. Moreover, this paradigm could be used to evaluate CMR_{O_2} in visual cortex in individual patients suffering from defects in oxidative metabolism such as mitochondrial disorders.

This work was supported by MRC (Canada) Grant SP-30.

421.8

SURFACE-BASED COORDINATE SYSTEM FOR A CANONICAL HUMAN NEOCORTEX. M.I. Sereno*, A.M. Dale, A. Liu, and R.B.H. Tootell. Cognitive Science Department 0515, University of California at San Diego, La Jolla, CA 92093-0515 and MGH NMR Center, Charlestown, MA 02129.

The cerebral cortex makes up the largest part of the human brain. It has the topology of a folded, 2-D sheet. Nevertheless, most approaches to analyzing and displaying human brain imaging data have relied upon a 3-D coordinate system. To allow surface-based techniques to be applied routinely, our cortical surface reconstruction process has been further automated. The method devised several years ago for recovering and unfolding the complete cortical surface of each hemisphere from high resolution MRI images can now be applied in a turnkey fashion to a single 5 minute scan.

To make it possible to average data in a surface-based format, however, we need to make a "canonical" cortical surface. This is done by averaging 2-D surfaces rather than 3-D volumes. The brains are first individually unfolded ("inflated") to remove minor and major sulci. This reconstruction and unfolding process has now been applied to a large number (~100) hemispheres. The brains are then morphed into each other, pair by pair, using a "shrink-wrapping" algorithm similar to that used for surface refinement and unfolding. A single, unfolded, curvature-marked (sulcus/gyrus) brain surface is obtained with a binary tree, and then completely flattened (or conversely, re-folded). Each point of this surface accesses a unique surface point on each of the hemispheres in the database and, hence, any of their associated functional (fMRI/EEG/MEG) data (see http://cogsci.ucsd.edu/~sereno).

A 2-D coordinate system is needed to refer sensibly to locations on the unfolded cortex. The obvious choice is latitude and longitude (two angles measured from a center point), applied after deforming the average cortical surface into an exact ellipsoid. This parameterization of the unfolded cortex has the attractive property that points with similar coordinates will refer to nearby points on the cortical map—not generally true of a 3-D coordinate system. Nevertheless, the 3-D (Talairach) coordinates of every 2-D surface point can be easily obtained.

Supported by ONR N00014-94-1-0856, NIH MH47035, EY07980, MH50054, HL39810, P01 CA48729, and the UCSD McDonnell-Pew Center.

421.9

THE TIME-RESOLVED CODE BEHIND IMAGES FROM CORTICAL ISO-ORIENTATION MAPS.

G.Bernroider*, Institute of Zoology, University of Salzburg, Austria

Intrinsic optophysiological images from cat Area 18 of visual cortex can reproduce conditions of orientation specific, moving gratings in terms of light reflections within a given wavelength and exposed cortical patch (*Bonhoeffer, et al, J. Neurosci., 13, 4157-4180, 1993*). Traditionally, these 'maps' are combined and analyzed *post hoc* by correlating gray-level coded ensemble activities with specific sensory stimuli. However, revealing the neural code underlaying sensory configurations requires approaches from the opposite perspective: reconstructing unique stimulus conditions from the spatially resolved layout of activity maps. Here I propose a computational approach that uniquely deduces a time-advanced population code from the space-time layout of neural ensemble activities obtained from single-condition, iso-orientation maps. Computations are based on the description of neuronal charge propagation and link the spatio-temporal organization of post-synaptic charges along dendritic trees to an action-potential phase-time code. The composition of these signals applied to iso-orientation maps shows, that 'orientation' can become coded by the temporal evolution of population signals.

VISUAL CORTEX: EXTRASTRIATE—FUNCTIONAL ORGANIZATION II

422.1

SPATIOTEMPORAL PATTERNS OF SYNAPTIC ACTIVATION EVOKED IN CORTICAL PYRAMIDAL CELLS BY DIFFUSE STROBOSCOPIC VISUAL STIMULI

David M. Senseman*
Div of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX 78249

Voltage-sensitive dye (VSD) signals recorded from the turtle visual cortex, *in vitro*, have waveforms that are similar to the complex polysynaptic potentials recorded concurrently in spiny pyramidal cells with intracellular microelectrodes (Senseman, *Vis. Neurosci.*, in press). In the current series of experiments, I characterized the time-course of cortical VSD signals in order to better understand the spatiotemporal patterns in which local populations of pyramidal cells are activated by diffuse visual stimuli. Each element in a 464 element silicon photodiode array monitored activity in a 150 μm x 150 μm cortical area with near millisecond temporal resolution. Diffuse stroboscopic visual stimulation of the attached eye initiated a cortical response that appeared at the junction between the lateral and medial visual cortical subregions in a small area rostral to the eminence of Fowler. Onset latency ranged from 100 ms to more than 300 ms depending on the intensity of the visual stimulus. With monochromatic light at either 450nm or 650 nm, the relationship between stimulus intensity and onset latency followed a power function. From its site of initiation, the cortical response spread in rostral, caudal and dorsomedial directions with the greatest spread caudally. Velocity of the caudal propagation ranged between 0.02 and 0.04 m/s. Larger cortical responses evoked by more intense light flashes generated cortical waves with faster propagation velocities. Since cortical activity evoked by focal electrical stimulation propagates at ~ 0.1 m/s, it seems unlikely that the slow rate of cortical spread can be explained simply by the conduction velocity of the intrinsic cortical fibers. A more likely possibility is that the rate of spread reflects the balance of excitatory and inhibitory interactions between local populations of cortical pyramidal cells. Efforts are underway to examine this idea with a medium scale neuronal model.

422.2

BEHAVIOURAL VISUAL ACUITY OF WILD-TYPE AND *BCL-2* TRANSGENIC MOUSE. L.Gianfranceschi[1], A. Fiorentini[2], L.Maffei[1,2], V.Porciatti[2]. [(1)]Scuola Normale Superiore,[(2)]Istituto di Neurofisiologia CNR, Via S.Zeno 51; Pisa, ITALY. (SPON: European Brain and Behaviour Society).

Since the advent of gene manipulating techniques the mouse has become the favourite experimental subject for studying functional neural development. The *bcl-2* gene has a powerful inhibitory action on naturally occurring cell death. In consequence of that the brain of a transgenic mouse overexpressing *bcl-2* only in neurons is 1.5 times bigger than the brain of a wild type (*wt*) mouse (Martinou *et al.*, 1994), and its retina has more than twice as many ganglion cells than normal. This fact opens the question of evaluating the possible functional differences between the nervous systems of *wt* and *bcl-2* mice. Behavioural visual acuity was evaluated by a two-alternative, forced choice procedure on hybrid mice derived from breeding the C57BL/6J strain with the transgenic line NSE73a. The presence of the transgene was assessed by PCR. Mice were trained in an apparatus modified from the Wiesenfeld and Branchek maze (1976) and containing a start box, a choice area with swinging doors, and two parallel alleys 30 cm long, at the end of which stimulus cards were placed. The task consisted of discriminating between pairs of high-contrast vertical and horizontal gratings of the same spatial frequency. The correct choice (vertical) was rewarded by 15 mg of powdered mice pellets. Following the wrong choice (horizontal) the food reward was omitted and the animal was put back into the start box. Visual acuity was then measured with a staircase procedure in which blocks of four forced-choice trials for each spatial frequency were run. Spatial frequency was increased if more than two correct choices per block were made and decreased otherwise. Daily estimates of visual acuity were obtained from at least two oscillations in the staircase and found comparable with the 70% correct level of choice in frequency-of-seeing curves. Visual acuity was found to be of the same order (about 0.7 c/deg) in the 3 *wt* and the 2 *bcl-2* mice tested so far. (Supported by Institute for Paraplegia).

422.3

SPATIOTEMPORAL STRUCTURE OF STIMULUS INDUCED ELECTRICAL ACTIVITY IN TURTLE VISUAL CORTEX. JC Prechtl*[1,2] L.B. Cohen[1,3] P.P. Mitra[4] & D. Kleinfeld[1,4]. (1) Marine Biological Laboratory, Woods Hole, MA 02543; (2) University of California San Diego, La Jolla, CA 92093; (3) Yale University School of Medicine, New Haven CT 06520; (4) Bell Laboratories, Murray Hill, NJ 07974.

In turtles, visual stimuli induce oscillations in the local field potential (LFP) in dorsal cortical areas D1 and D2 (Prechtl 1994). High resolution electrical recordings with linear arrays of electrodes showed that these responses include systematic phase differences. Here we examine the spatial structure of the fast electrical activity (DC to 50 Hz) across D1 and D2 cortical areas in response to a behaviorally relevant visual stimulus, a looming object, or to a simple step change in diffuse illumination.

To probe the large-scale structure of the response in greater detail, we used wide-field fluorescence microscopy (24 by 24 pixels at 1000 frames/s) in conjunction with voltage sensitive dyes (VSDs) to image the electrical activity from a roughly 3x3 mm region of visual cortex that was stained with the VSD RH795. To eliminate movement and blood flow artifacts, the spinal cord and cranial nerves IV-XII were sectioned and the animal was perfused with CSF+O^2. The LFP near the surface of D1 was measured concomitantly with the optical measurements. All results are based on single trials.

The optical response began as a spread of depolarization in geniculocortical visual area D2 and was followed by undulations of depolarization and hyperpolarization in areas D2 and D1. The spatiotemporal structures of the responses were complex, but support several conclusions: (1) The frequency spectrum of the temporal response has a ~"1/f" character, suggesting the involvement of many oscillators, in addition to a broad peak near 20 Hz. (2) The spatial and temporal aspects remain coupled. A singular value decomposition revealed that the looming stimulus response required ~6 modes for characterization while that for the diffuse light required only 2 to 3 modes. The spatial modes remained essentially invariant between trials with the same stimulus, while the temporal modes varied. (3) We estimated the propagation rate of various fronts to be ~ 0.1 mm/ms, an order of magnitude slower than axonal transmission. (4) The spatial modes are largely confined to D1, as determined from anatomical reconstruction of sectioned tissue. (5) Lastly, the appropriately denoised signal in the 3 to 50 Hz range correlated well with the LFP. The relationship between the spatiotemporal structure of the response and behavioral variables is under study.

(Supported by The MBL, AT&T, and NIH grants NS08437 and MH26149.)

422.4

CENTER AND SURROUND RESPONSES IN EXTRASTRIATE AREAS L AND ML IN THE CALIFORNIA GROUND SQUIRREL. M. Paolini* and M.I. Sereno. Cognitive Science Department, University of California San Diego, La Jolla, CA 92093-0515.

The classical excitatory center and non-classical surround of direction selective units have often been demonstrated to have an antagonistic relationship. The response to motion in the classical receptive field (RF) in a cell's preferred direction (PD) has often been shown to be depressed by similar motion in the surround and enhanced by dissimilar surround motion. We analyzed the local direction selectivity to translational motion at many locations in and around the classical RF to more quantitatively determine how direction-selective RFs are organized.

We previously reported (Paolini and Sereno, 1995) that extrastriate areas L and ML (lateral and middle lateral) in ground squirrels contain many strongly direction-selective units. We studied the responses in the center and surround of 140 cells in areas L and ML, during acute extracellular recording sessions. Excitatory RFs were mapped on the display monitor, using moderately fast (3-6/s) sequences of stationary flashing squares presented at 100 locations in the visual field in and beyond the hand-mapped RF. Then, small moving stimuli (high-contrast 2D textured shapes; random dot patterns moving through a fixed aperture) were presented to the same cells at the same rate at 36 or 100 visual field locations to characterize local translational direction selectivity. The location of the flashed receptive field, the area spanned by the path of individual stimuli, and local direction selectivity measures were superimposed to analyze the direction selectivity in the center and in the surround of the receptive field. In most of the cells (59%), the PD of the flashed-defined surround was the same as that of the center (37%) or changed smoothly across space from that of the center (25%). The surround direction selectivity was markedly different from the center in a smaller number of cells (37%). Responses to motion in an aperture were similar. These data show that when the center and the surround are separately stimulated, in most cases, the PD in the surround is not markedly different from the PD of the center.

Supported by MH47035, ONR N00014-94-1-0856, and the UCSD McDonnell-Pew Cognitive Neuroscience Center.

422.5

DIVERSITY OF THE W-CELL SYSTEM IN THE GENICULO-CORTICAL PROJECTIONS REVEALED BY A WGA-HRP ANTEROGRADE TRACING STUDY IN THE CAT.

J. Kawano[1], M. Kudo*[1] and S. Kawamura[2]. [1]Dept. of Anatomy, Sch. of Medicine, Kanazawa Univ., Kanazawa 920, JAPAN & [2]Div. of Morphological Neural Science, Kumamoto Univ., Graduate Sch. of Medical Sciences, Dept. of Neuroscience and Immunology, Kumamoto 860, JAPAN.

To examine the central visual pathways of the W-cell system, of which precise organization is as yet unknown, injections of WGA-HRP were placed in the parvocellular laminae C (Cparv) of the dorsal lateral geniculate nucleus in the cat, since the Cparv in this species is said to exclusively receive W-cell information from the retina, the superior colliculus, and the parabigeminal nucleus.

The results show that the cortical targets of the Cparv are areas 17, 18, 19, 20a, and 21a and the posteromedial (PMLS) and ventral (VLS) lateral suprasylvian areas. In area 17, the projection fibers terminate in the superficial half of layer I, the lower two-thirds of layer III - the upper quarter of layer IVa, and the lower quarter of layer IVb - layer Va, forming triple bands. The projection terminals in layer I are continuous, while those in layers III, IV, and Va distribute periodically, exhibiting patchy appearance. In areas 18 and 19, the projection fibers terminate in the superficial half of layer I and the full portions of layers III - IV, forming double bands. In these areas, the projection terminals in layer I are continuous, while those in layers III - IV distribute periodically, exhibiting patchy appearance. In areas 20a, 21a, PMLS, and VLS, projection fibers terminate in the superficial part of layer I, a part of layer III - the full portion of layer IV, although they are much fewer than those seen in areas 17, 18, and 19.

Thus, the present results document the existence of localized projections from the Cparv to various cortical areas. Those projection fibers may constitute a W-cell system of pathways in the visual thalamocortical organization in a fashion comparable to the X-cell and Y-cell systems. The multiplicity of the projections may further imply functional divergence of the W-cell system.

Supported by a Grant-in-aid for Scientific Research C (07680817).

422.6

RESPONSES OF CELLS IN EXTRASTRIATE CORTEX OF CATS AND MONKEYS TO BOUNDARIES INDUCED BY DIFFERENT CUES. Y-C. Wang[1]*, M.T. Schmolesky[1], T-D. Shou[2] and A. G. Leventhal[1]. [1]Dept. of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT 84132. [2]Dept. of Biology, USTC, Hefei 230027, P.R.China.

Some cells in monkey area V2 and cat area 18 respond selectively to boundaries induced by a variety of cues such as luminance (LIBs), motion (MIBs), texture (TIBs), gratings (GIBs) and illusory contours (ICs). We refer to these cells as cue-invariant (CI). In this study we compared the responses of CI cortical cells to boundaries induced by different cues.

CI cortical cells exhibit length summation when tested with light bars (LIBs). In contrast, the responses of these cells to TIBs, GIBs and ICs vary as a function of the number of elements inducing the boundary. Thus, a short GIB containing many inducing elements evokes a greater response from a CI cell than does a long GIB containing fewer inducing elements. The velocity tuning of CI cells varies depending upon the cue inducing the boundary employed as a stimulus. CI cells respond to rapidly moving LIBs, but not to rapidly moving MIBs, TIBs, GIBs and ICs. Some CI cells respond selectively to the orientation of flashing boundaries induced by various cues. The latency of visual response to flashing LIBs is almost 15 ms shorter than is the latency of response to flashing TIBs and GIBs.

The responses of CI cortical cells are consistent with a number of psychophysical phenomena indicating that 1) the detection of GIBs and ICs is dependent upon the number of inducing cycles defining the stimulus, and 2) rapidly moving GIBs and ICs are difficult to detect. The present results suggest that different connections mediate the responses of CI cells to LIBs and equiluminant boundaries (GIBs, TIBs and ICs).

Supported by EY04951 to A.G.L.

422.7

INTRINSIC CONNECTIONS OF THE LATERAL SUPRASYLVIAN CORTEX (LS) IN THE CAT. M. Kase, K. Hoshino and M. Norita* Dept. Neurobiology and Anatomy, Niigata Univ. Sch. Med., Niigata, 951, Japan.

LS is known to have numerous interconnections with visual cortical areas as well as with subcortical structures implicated in visually-guided behaviors. In contrast, little data is available regarding connections within LS itself. To gain information about intrinsic connections, an anterograde tracer (biocytin or biotinylated dextran amine) was deposited within various cortical laminae along the medial and lateral banks of LS of the cats. Injections in all laminae produced label contained within the respective bank that extended up to 8-10 mm rostrally or caudally from the injection site . Following medial bank injections, considerable label was distributed throughout the fundus and, to a lesser extent, in the lateral bank. In contrast, no label could be detected in the medial bank after lateral bank injections, and, although label was observed in the fundus, it was restricted to the most lateral aspects. However, the lack of medial bank label that was evident following centrally placed lateral bank injections was not evident in the most rostrally or caudally placed lateral bank injections. In these cases, moderate label could be observed in the medial aspect of the fundus as well as in the medial bank. Following an injection into the fundus, anterograde label was distributed in both rostral and caudal directions and into both banks as well. Injections in the supragranular laminae (I-III) yielded patchy label mainly within the supragranular laminae whereas injections in the infragranular laminae (IV-VI) gave rise to patchy label largely in laminae III-IV. The size of the patches varied, ranging from 0.2-0.5 mm. Supported by a Grant-in-Aid (06680731) from the Japanese Ministry of Education, Science and Culture and NISSAN SCIENCE FOUNDATION.

422.8

COMPENSATION OF DIRECTION AND ORIENTATION SENSITIVITY IN CAT POSTEROMEDIAL LATERAL SUPRASYLVIAN (PMLS) EXTRASTRIATE CORTEX AFTER NEONATAL VISUAL CORTEX DAMAGE. Y.P. DANILOV*, V.R. KING, A. AHMAD, K.R. ILLIG and P. D. SPEAR. Dept. Psychology and Ctr. Neuroscience, Univ. Wisconsin, Madison, WI 53706, USA.

Purpose. Damage to areas 17 and 18 (visual cortex, VC) in adult cats produces a loss of orientation (OS) and direction (DS) sensitivity among PMLS neurons. In contrast, qualitative studies suggest that functional compensation occurs following neonatal VC damage so that there is no loss of DS among PMLS neurons. The present experiment used quantitative methods to re-examine the effects of neonatal VC damage on DS and to examine the effects on OS. **Methods.** Single-unit recordings were made in the PMLS cortex of anesthetized and paralyzed normal cats (n=4), cats that received VC damage on the day of birth and survived to adulthood (n=4), and cats that received VC damage as adults (n=4). Stimuli were moving or flashing sine-wave gratings (20) at the optimal spatial frequency for each cell. **Results.** Adult VC damage caused a significant decrease of both OS and DS among PMLS cells compared to normal adult cats. Specifically, 85% of 108 PMLS cells in normal adult cats showed DS, compared with only 32% of 69 PMLS cells in cats that received VC damage during adulthood. Similarly, 79% of PMLS neurons in normal adult cats showed OS, compared with only 12% in cats that received VC damage during adulthood. In contrast, among PMLS neurons recorded in cats that received VC damage on the day of birth (n=88), 67% showed OS and 76% showed DS, which is not significantly different from normal. **Conclusions.** These results demonstrate that there is functional compensation for both DS and OS among PMLS neurons following neonatal VC damage.

Supported by NIH grants RO1 EY01916 and RO1 EY0 2545 (PDS) and F32 EY06385 (VRK) None

422.9

PROPERTIES OF NEURONS IN THE CAT'S PMLS CORTEX: RESPONSES TO MOVING TEXTURE PATTERNS. K. Minville*, L. Merabet, A. Desautels and C. Casanova. École d'Optométrie et Centre de Recherche en Sciences Neurologiques, Université de Montréal; Montréal, Québec, Canada.

Neurons in the posteromedial lateral suprasylvian cortex (PMLS) of the cat are sensitive to moving stimuli and the vast majority of these cells are highly direction selective. Previous studies (*e.g., von Grünau and Frost, E.B.R.: 49, 1983*) have reported that cells in PMLS do not respond to fine textured patterns (visual noise) despite evidence that response properties can be influenced by the presence of a visual noise outside the classical receptive field. Our group among others have previously demonstrated that area 17 and the striate recipient zone of the LP-pulvinar complex both respond to the motion of a fine texture field. Given that both these regions project to the PMLS, we reinvestigated the response properties of this cortical area with noise stimuli as used to characterize area 17 and LP-pulvinar. Experiments were performed with anesthetized normal adult cats. Receptive fields of PMLS units were first characterized using 60% contrast drifting sine-wave gratings then with moving visual noise presented at different directions and velocities (minimum texture element size: 0.07°). To date, 96 units have been recorded in PMLS and the large majority (77 cells, 80.2 %) responded to a varying extent to visual noise. For 75% of these cells, the responses to drifting gratings were more vigorous than those evoked by the motion of noise (dot index; mean=0.66± 0.43, median=0.53). The preferred directions for noise of PMLS cells were distributed evenly across 360° (X^2=8.9, p=0.6, df=11). Generally, the optimal direction for noise was similar to that of gratings but the noise tuning function was broader (mean: 62.6°± 40 for noise vs 40.4°± 22.7 for gratings). Of the texture sensitive cells, 76% were band pass tuned to noise velocity (mean bandwidth of 2.4 octaves). The preferred velocity ranged between 5 to 65°/sec with a mean of 23.4°/sec (the remaining cells were low pass tuned). In contrast to previous studies, our results suggest that most PMLS neurons are responsive to moving textured patterns and support the notion that PMLS cortex is strongly involved in the analysis of moving visual scenes. *Supported by MRC and FRSQ.*

422.10

AUDITORY TAKE OVER OF EXTRASTRIATE VISUAL AREAS IN EARLY BINOCULARLY DEPRIVED CATS: PHYSIOLOGICAL EVIDENCE R. Yaka[1,2], U. Yinon[2] and Z. Wollberg[1]* Dept. Zool.[1], George S. Wise, Fac. Life Sci., and Physiol. Lab[2]., Goldschleger Eye Res. Inst., Fac. Med., Sheba Med. Ctr, Tel - Aviv University, Tel - Aviv, 69978, Israel.

Visual and auditory activity in non-primary anterolateral - and anteromedial - lateralsuprasylvian cortices (ALLS & AMLS, respectively) have been studied in eight normal and six neonatally visually deprived cats. Our working assumption was that an early loss of sight would induce a takeover of visual areas by the auditory system. Three of the deprived cats were binocularly enucleated (BE), and in the other three both eyelids were sutured (BD). About a year later single unit activity was extracellulary recorded. The major findings, regarding the changes in the responsiveness to various visual and auditory stimuli, in our cats, are summarized in the following table:

group	% out of total cells					
	ALLS			AMLS		
	auditory	bimodal	visual	auditory	bimodal	visual
NORMAL	13.5	1.5	58.0	1.5	26.0	20.0
BD	38.5	15.5	0.0	32.0	2.0	18.0
BE	65.0	-	-	not tested		

In the ALLS of the deprived cats, as deprivation became more severe, there was a remarkable increase in the proportion of auditory cells. In the BD cats, a similar trend was found in the proportion of bimodal cells. Intriguingly, while bimodal cells were found in the BD cats, visual cells were not found in this group. In the AMLS of the BD cats there was a marked increase in the proportion of auditory cells. However, the proportion of bimodal cells dropped significantly, with no change in the proportion of visual cells. It is evident that in visually deprived cats there is a take over of visual areas by auditory input, and that the degree of take over depends on the severeness of deprivation. *Supported by M. Waldman Ph.D. Fellowship and by the Israeli Ministry of Health (Grant no. 2397).*

422.11

AUDITORY SENSORY RESPONSES IN AREA LIP?

J.F. Linden*, A. Grunewald and R.A. Andersen, Division of Biology, California Institute of Technology 216-76, Pasadena, CA 91125.

Neurons in the macaque lateral intraparietal area (LIP), long considered a visual area, respond during delayed saccades to remembered auditory targets (Mazzoni *et al.*, 1996). During the stimulus period of the auditory delayed-saccade task, when the monkey is required to maintain fixation through presentation of an auditory target, 36% of LIP neurons respond. Are these responses dependent on the performance of the auditory saccade task? To answer this question we trained a monkey to maintain fixation during presentation of behaviorally irrelevant visual and auditory stimuli. Of 78 LIP neurons tested, 37 (47%) responded to localized presentation of visual stimuli, while only 3 (4%) responded to auditory stimulation (ANOVA, $p < 0.05$). The proportion of cells with auditory responses in this task was therefore significantly less than the previously reported proportion of cells responding during the auditory stimulus period of a delayed-saccade task. Two possible explanations for this task dependence are being investigated. First, training the monkey to perform an auditory delayed-saccade task may introduce long-term changes in the responses of area LIP neurons to auditory stimuli. Alternatively, auditory signals may be gated into LIP only when they are behaviorally relevant, e.g. when they serve as targets for saccadic eye movements.

Supported by the Howard Hughes Medical Institute, the McDonnell-Pew Foundation, the Sloan Foundation and the National Institutes of Health.

422.12

CONVERGENCE OF Y AND NON-Y INFORMATION CHANNELS ONTO SINGLE NEURONS IN THE ECTOSYLVIAN VISUAL CORTEX OF THE CAT. B. Dreher[1], M. Pitto*[2], C. Wang[1], N. Assaad[1] and W. Burke[1]. Institute for Biomedical Research[1], The University of Sydney, N. S.W. 2006, Australia and Groupe de Recherche en Neuropsychologie Expérimentale[2], Université de Montréal, Montréal, QC H3C 3J7, Canada.

The ectosylvian visual area (EVA) is a "higher-order" motion cortical area which receives its principal visual inputs via cortical motion areas located within the lateral suprasylvian region. EVA neurons respond optimally at very high stimulus velocities (200-990°/s) and tend to be strongly direction-selective. Furthermore, EVA is reciprocally connected with the "frontal eye field" and the "premotor" cortex. In the present study we have examined the visual receptive field properties of single neurons recorded from EVA of normal cats and cats with selective pressure-blocked Y-information channel. As in the normal cats, in the cats in which the Y-channel in one optic nerve has been selectively blocked, virtually all EVA neurons could be activated by photic stimuli presented via either eye. Thus, virtually all EVA neurons receive excitatory inputs from both Y and non-Y channels. Furthermore, discharge field sizes and direction selectivity indices of EVA neurons were not significantly affected by selective blockade of the Y-channel. These results challenge the idea of strictly selective excitatory links between the large-fibre channel (Y-channel) in the mammalian retino-geniculo-cortical pathway and the motion-processing stream in the visual cortex. On the other hand, the Y-channel clearly provides the principal excitatory drive to EVA, since the responses of EVA neurons to photic stimuli presented via the Y-blocked eye were usually substantially weaker (mean peak discharge rate: 18.5 spikes/s) than those to the stimuli presented via the normal eye (mean peak discharge rate: 41.5 spikes/s). Furthermore, the excitatory input from the Y-channel underlies the responsiveness of EVA neurons to high stimulus velocities (above 200°/s). Supported by Australian NH&MRC to BD and WB and NSERC (Canada) to MP.

AUDITORY, VESTIBULAR, AND LATERAL LINE: HAIR CELL PROPERTIES

423.1

EFFECTS OF LIPOPHILIC IONS ON OUTER HAIR CELL VOLTAGE-DEPENDENT CAPACITANCE AND MOTILITY Min Wu*, J. Santos-Sacchi. Sections of Otolaryngology and Neurobiology, Yale Medical School, New Haven, CT 06510.

The outer hair cell (OHC) from the mammalian organ of Corti possesses a bell-shaped voltage-dependent capacitance function. The ratio of peak voltage-dependent capacitance to linear capacitance ranges from about 1 to 2 depending upon cell length. The nonlinear capacitance reflects the activity of membrane bound voltage sensors associated with membrane motors which control OHC length. We have studied the effects of the lipophilic ions, tetraphenylborate (TPB^-) and tetraphenylphosphonium (TPP^+), on OHC capacitance and motility. Effects on supporting cells were also investigated. Capacitance and motility were evaluated under whole cell voltage clamp using a Windows based program, jClamp. See Kakehata and Santos-Sacchi (Biophys J, 68, 2190-2197, 1995) for experimental details. TPB^- produced an increase in the OHC peak capacitance (Cm_{pk}) and shifted the voltage at peak capacitance (V_{pkcm}) to hyperpolarized levels. Washout reversed the effects. Perfusion of 1 μM TPB for two minutes caused an average increase in Cm_{pk} of 20.8 pF and V_{pkcm} shift of -9.3 mV (n= 17-22). With salicylate intracellularly (10 mM) to block OHC nonlinear capacitance, TPB^- treatment caused an increase in Cm_{pk} of 36.8 pF (n=16). This may indicate that either TPB^- reversed the effects of salicylate block or is more effective under these conditions. Preliminary results of mechanical response studies indicate little effect of TPB^- on OHC motility. TPB^- induced a voltage dependent capacitance in supporting cells whose characteristics were similar to those of the OHC, but no indication of mechanical responses were noted. TPP^+ (0.5-3 mM) did not change Cm_{pk}, but did shift V_{pkcm} to depolarized levels. The average shift was 34.7 mV (n=13). Preliminary mechanical response studies indicate that TPP^+ enhanced OHC motility and shifted its voltage dependence. These results indicate that OHC mechanical responses are not simply related to quantity of nonlinear charge moved within the membrane.

(Supported by NIDCD grant # DC00273 to JSS)

423.2

ROLE OF ION CHANNEL PHOSPHORYLATION IN OUTER HAIR CELL FUNCTION. D.J. Jagger and J.F.Ashmore*.Dept. of Physiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK.

Voltage-dependent ion channels play a significant role in the generation of the outer hair cell (OHC) motility which is believed to power the cochlear amplifier. Such channels determine the receptor potential and the cell resting potential and consequently set the contribution of OHCs to cochlear mechanics. We have been investigating long term channel regulation using whole-cell patch clamp recordings from isolated OHCs from the guinea-pig cochlea and in particular the hypothesis that channel phosphorylation can modulate whole cell currents in OHC's.

Bath application of 10 μM okadaic acid (OA) reversibly enhanced (by up to 40%) an outward K^+ current (measured at 0 mV) and an inward current (measured at -120 mV) suggesting that the underlying currents are regulated by a protein phosphatase. 10 nM OA gave similar results. This identifies phosphatase 2A as one enzyme involved (Biochem. J. 256:283-290, 1988). These effects of OA were also observed when pipette K^+ was replaced by Cs^+ suggesting that one target current is I_{Kn}, a low voltage-activating potassium current (J.Physiol. 448, 73-98 1992). In order to reduce K^+ currents further the cells were dialysed with a Na^+ based pipette solution. In 4 out of 10 cells exposure to OA reduced the amplitude of the residual inward current (at -60 to -80mV), suggesting that the OHC cation current is down-regulated by phosphorylation.

The data show that OA has distinct effects on the different ionic currents present in OHCs and that the channel phosphorylation state may have a significant influence on OHC force generating mechanisms.

Supported by the MRC and the Wellcome Trust.

423.3

THE VOLTAGE-DEPENDENT CAPACITANCE FUNCTION OF THE OUTER HAIR CELL HAS MEMORY J. Santos-Sacchi*, Seiji Kakehata and Shin Takahashi. Sections of Otolaryngology and Neurobiology, Yale Medical School, New Haven, CT 06510.

The outer hair cell (OHC) from the mammalian organ of Corti possesses a bell-shaped voltage-dependent capacitance function., which reflects the activity of membrane bound voltage sensors associated with membrane motors that control OHC length. The voltage at peak capacitance (V_{pkcm}) which corresponds to the voltage at maximal mechanical gain of the OHC is affected by a variety of treatments, including membrane stress and lanthanides. Here we report that initial voltage conditions affect V_{pkcm}. OHC capacitance or nonlinear gating charge movement was evaluated under whole cell voltage clamp, after blocking ionic conductances (see Kakehata and Santos-Sacchi, Biophys J, 68, 2190-2197, 1995 for experimental details). In the present experiments, capacitance or gating charge was evaluated following prepulses of variable duration and/or magnitude. Prepluses to negative voltages shift V_{pkcm} to depolarized levels. The change in V_{pkcm} across the prepulse (1 sec duration) range of -150 to 50 mV is sigmoidal, with the magnitude of V_{pkcm} shift being about 10-15 mV (n=4). The greatest rate of shift is near the OHC's normal resting potential of -70 mV. The effect depends on the duration of prepulse, and is essentially absent below 30 ms duration. Recovery from prepulse effects appears on the same time scale as onset. We are currently working on determining the mechanism of this phenomenon. Nevertheless, these results indicate that the motility of the OHC may be dynamically modulated by factors which affect the resting potential in vivo. For example, the slow time course of efferent activation of the OHC would cause not only a hyperpolarization of the OHC, but a depolarizing shift of the motility function. The consequences of both actions would tend to reduce the gain of the cochlear amplifier.

(Supported by NIDCD grant # DC00273 to JSS)

423.4

HYPERPOLARIZATION-ACTIVATED CURRENT IN GUINEA PIG SPIRAL GANGLION NEURONS. Chu Chen*, Kresge Hearing Research Lab., Dept. Of Otorhinolaryngology, LSU Medical Center, New Orleans, LA 70112.

The ionic mechanisms underlying relaying and encoding of auditory signals in primary afferent nerve fibers are not clear. In heart, a cationic inward current activated upon hyperpolarization (I_h), called the pacemaker current, plays a dominant role in diastolic depolarization that elicits the spontaneous firing in sinoatrial node cells and Purkinje fibers. In the central neurons, I_h is believed to provide the pacemaker depolarization during spontaneous rhythmic firing. The purpose of the present study was to determine whether such an anomalous rectification is present in mammalian spiral ganglion neurons (SGNs). The experiments were performed in SGNs acutely isolated from adult guinea pig cochlea using the whole-cell patch-clamp technique. A large inward current was seen in response to hyperpolarizing steps negative to the resting membrane potential. The current activation and deactivation showed voltage- and time-dependence. The activation curve derived from tail currents was fitted to the Boltzmann equation. The current demonstrated no inactivation and was reversibly blocked by 1 mM Cs. Results suggest a hyperpolarization-activated inward current exists in SGNs. This current may contribute to the membrane depolarization during firing of the afferent nerve fibers.

Supported by Deafness Research Foundation, Kam's fund for Hearing Research, and the Louisiana Lions Eye Foundation.

423.5

COCHLEAR DOPAMINE RECEPTORS: cDNA QUANTITATION AND IMMUNOHISTOCHEMISTRY. A.A. Karadaghy[1], M.J. Drescher[1]*, J.S. Hatfield[3] and D.G. Drescher[1,2]. Lab. of Bio-otology, Depts. of [1]Otolaryng. and [2]Biochem., Wayne State Univ. Sch. of Med., Detroit, MI 48201; and [3]Veterans Admin. Med. Center, Allen Park, MI 48101.

Previous investigations have demonstrated that dopamine is present in the cochlea where it may function as an efferent neurotransmitter (Jones et al., Hearing Res. 30: 33-38, 1987; Gil-Loyzaga and Pares-Herbute, Devel. Brain Res. 48: 157-160, 1989; Drescher et al., Soc. Neurosci. Abstr. 20: 972, 1994). In the current study, expression of dopamine receptor isoforms was examined in the mouse cochlea and brain by RT-PCR analysis and nucleotide sequencing, utilizing specific primers. Cochlear cDNA corresponding to dopamine $D_{2(long)}$ and D_3 receptors was amplified, whereas cDNA for D_{1A}, D_{1B}, $D_{2(short)}$, and D_4 receptors was not detected. Utilizing quantitative competitive PCR analysis, the relative levels of dopamine receptor transcripts (per total RNA) were found to be 0.002, 0.014, 0.016, and 1.000 for $D_{2(long)}$ cochlea, D_3 cochlea, D_3 brain, and $D_{2(long)}$ brain, respectively. Localization studies of D_2 receptor-like immunoreactivity in rat cochleas were performed utilizing a rabbit polyclonal antibody to a rat D_2 receptor peptide (Chemicon). Fixed cochleas were decalcified, embedded in parafffin, and the deparaffinized sections were blocked, reacted with primary antibody, and visualized with biotinylated secondary antibody and streptavidin-peroxidase-diaminobenzidine (Vectastain). Reaction product was found in a discrete region below the inner hair cells. Taken together, these results are consistent with the presence of dopaminergic lateral cochlear efferents targeting cochlear receptors of the D_2 subfamily, and provide key evidence necessary to establish that dopamine is a neurotransmitter in the auditory inner ear. (Supported by NIH Grants R01 DC00156 and T32 DC00026.)

423.6

4-AMINOPYRIDINE EFFECTS ON COCHLEAR SUMMATING POTENTIALS IN THE GUINEA PIG. M. G. van Emst*, S. F. L. Klis and G. F. Smoorenburg. Lab. of Exp. Audiology, Utrecht University, Utrecht 3584 CX, The Netherlands.

Sound-evoked DC receptor potentials in cochlear hair cells, and the associated extracellular DC potential known as summating potential (SP), are due to nonlinearities in the mechanoelectrical transduction chain. Voltage- and time-dependent K^+ channels sensitive to 4-aminopyridine (4-AP), which have been found in the basolateral membrane of both inner hair cells (IHC) and outer hair cells (OHC), might contribute to these nonlinearities. We perfused the perilymphatic spaces of the guinea pig cochlea with the K^+ channel blocker 4-aminopyridine (4-AP). Sound-evoked (2-12 kHz) intracochlear potentials were recorded from the basal turn of both scala vestibuli and scala tympani. Bidirectional frequency- and level-dependent effects of 4-AP on the magnitude of the SP were found. In both scalae a decrease in SP was found for low and moderate levels of 8 and 12 kHz stimuli where the SP presumably depends mainly on contributions from basal turn IHCs. The decrease in SP in both scalae indicates that 4-AP prevented the 4-AP sensitive K^+ channels in the IHC membrane from increasing their conductance, which normally could serve to prevent excessive depolarizations of the membrane during stimulation and quicken repolarization after stimulation. In both scalae an increase in SP was found for high levels of 8 and 12 kHz stimuli and all levels of 2 and 4 kHz stimuli where the SP is assumed to depend mainly on contributions from basal turn OHCs. Blocking the 4-AP sensitive K^+ channels in the OHC may lead to an increase in SP because of membrane depolarization and subsequent mechanical alterations.

423.7

MEASURING MECHANICALLY AND ELECTRICALLY EVOKED RESPONSES IN THE ORGAN OF CORTI. D.C. Mountain[1], K.D. Karavitaki[1], R.C. Naidu[1] and A.R. Cody[2]. Boston University[1], Hearing Research Center, Boston, MA 02215, The University of Queensland[2], St. Lucia, 4072 QLD, Australia.

The electromechanical characteristics of outer hair cells (OHC) are believed to significantly enhance hearing sensitivity in the mammalian cochlea. We address this issue by studying the motion of the organ of Corti (OC) in the excised gerbil cochlea in response to mechanical and electrical stimuli.

Point stiffness of the cochlear partition was determined by measuring the restoring force generated in response to a sinusoidal displacement. The measurements were performed using a piezoelectric force probe. The preparation was mounted on the stage of an inverted microscope so that the measurement location could be simultaneously viewed from the opposite side using a CCD camera. Images from the camera were processed off-line to study OC deformation. Data obtained from the top and bottom sides of the OC indicate that the stiffness of the pillar cell region is significantly greater than that of the OHC region.

Electrically-evoked micromechanical movements of the OC were elicited by delivering AC current near the scala vestibuli of the cochlear turn of interest using a low impedence glass electrode. The return electrode was placed toward the modiolus near scala tympani of the following turn. Motion synchronized to the stimulus frequency was captured using stroboscopic illumination. Images at specific stimulus phases for all frequencies were collected using a CCD camera and digitized. Animations of the observed motion were created for each stimulus frequency. Fourier analysis was used to estimate the magnitude and phase of motion at each frequency. The magnitude of the electrically stimulated motion was greater in the OHC region reaching up to 1.4 um peak-to-peak OHC length change in the apical turn.

Based on the data from both experiments we conclude that the pillar cells mechanically isolate the IHC from the OHC. This work was supported by NIDCD.

423.8

REGENERATION OF HAIR-CELL TIP LINKS FOLLOWING CALCIUM-CHELATOR RUPTURE IN THE CHICKEN BASILAR PAPILLA. Y.-D. Zhao, E.N. Yamoah, and P.G. Gillespie.* Dept. Physiology, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Tip links are 5 × 150-nm extracellular filaments that run from the tip of a stereocilium to its tallest neighbor's side, and are thought to be the gating springs that open and close transduction channels. Although previous experiments showed that tip links can be broken by calcium chelators (Assad *et al. Neuron* 7, 985-994; 1991), regeneration of tip links has not been investigated previously.

We broke tip links of newborn chicken basilar papillae with the calcium chelator BAPTA, cultured the papillae in serum-free M199 for 0-24 hours, then observed tip links using scanning electron microscopy. We took top-view photomicrographs of hair bundles, and four individuals blindly counted tip links in normal and angled (60° from the mirror-symmetry axis) positions. Although BAPTA treatment eliminated tip links, their numbers returned to control by 24 hours. The recovery curve was fit well by $[A+B*(\exp(1-t/\tau)^n)]$. With n=1, the time constant for recovery was ~7 hours; fits were better with n=2 or 3, suggesting that two or three identical subunits may come together to form a tip link. After BAPTA treatment, angled tip links increased dramatically at 2 hours, then fell to 2- to 3-fold higher than control. Although cycloheximide did not block tip-link regeneration, indicating new protein synthesis is not required, the calcium ionophore ionomycin effectively blocked regeneration.

BAPTA treatment fully abolished mechanoelectrical transduction, yet transduction currents recovered after 24 hours in culture. Adaptation was nearly absent. These results show that tip links are dynamic structures.

(Supported by the NIH and the Pew Foundation.)

423.9

POSTNATAL DEVELOPMENT OF VOLTAGE-GATED CONDUCTANCES IN TYPE I AND TYPE II HAIR CELLS OF THE MOUSE UTRICLE. A. Rüsch and R.A. Eatock*. Otolaryngology, Baylor College of Medicine, Houston, TX 77030.

The mature vestibular organs of amniotes have two types of hair cell, I and II, which have different complements of voltage-gated channels. We report that in mouse utricles these differences arise during the first postnatal week. Whole-cell currents were recorded from 360 hair cells in utricular epithelia that were excised and either put into denervated orgnanotypic culture or studied acutely between the day after birth, postnatal day (PD) 1, and maturity.

On PD 1-3, all hair cells expressed a delayed rectifier, $g_{DR,II}$, and a fast inward rectifier, g_{K1}. $g_{DR,II}$ had a mean half-maximal activation voltage (V_{half}) of -30±2.7 mV (SD; n=35) and a mean maximal value (g_{max}) of 42±24 nS. For g_{K1}, g_{max} was 4±1.7 nS (n = 107) and V_{half} was -88±5.3 mV (n = 5). 42% of hair cells also expressed a voltage-gated Na^+ conductance, g_{Na}. Between PD 4 and 8, an increasing percentage of cells expressed a large delayed rectifier conductance, $g_{K,L}$ (g_{max} = 73±42 nS, n = 57), with a very negative and variable range of activation (V_{half} between -88 and -62 mV). $g_{K,L}$ is typical of mature type I, but not type II, hair cells. From PD 8 on, $g_{K,L}$ was present in 65% of cells. g_{K1} and g_{Na} were not found in cells with $g_{K,L}$, implying that these conductances were lost as $g_{K,L}$ was acquired. Cells with $g_{K,L}$ also had a large delayed rectifier, $g_{DR,I}$ (g_{max} = 114±61 nS, n = 42), that resembled $g_{DR,II}$ but was less sensitive to external Ba^{2+} and 4-AP. Also beginning on PD 4, an increasing percentage of cells expressed a slow inward rectifier, g_h (g_{max} = 1±0.9 nS, n = 79; V_{half} = -101±5 mV). By PD 8, 88% of cells without $g_{K,L}$ and 34% of cells with $g_{K,L}$ expressed g_h.

Thus, hair cells in the neonatal mouse utricle express $g_{DR,II}$, g_{K1} and often g_{Na}. After PD 4, they divide into two groups that resemble mature type I and II cells: one with $g_{K,L}$, $g_{DR,I}$ and sometimes g_h, and the other with $g_{DR,II}$, g_{K1}, g_h and sometimes g_{Na}. These developmental changes affect the cells' voltage responses to injected current.

Supported by NIDCD grant DC02290.

423.10

POSTNATAL MORPHOLOGICAL DIFFERENTIATION OF TYPE I AND TYPE II HAIR CELLS OF THE MOUSE UTRICLE. A. Lysakowski†*, A. Rüsch‡ and R.A. Eatock‡. Anatomy & Cell Biology, University of Illinois, Chicago, IL 60612† and Otolaryngology, Baylor College of Medicine, Houston, TX 77030 ‡.

The mature vestibular organs of amniotes have two types of hair cell, I and II, upon which afferent neurons make calyx and bouton terminals, respectively. Although the afferent terminal has been the primary criterion for classifying cell type, the two types also differ in secondary attributes. We have developed criteria, which include stereociliary and mitochondrial diameters, nuclear location and heterochromatin pattern, for classifying cell type in developing mouse utricles before calyx formation is complete. Utricular epithelia were fixed *in situ* on postnatal day (PD) 0 (birth), 4, 7, 10 and 28. We studied one sample each from 2-3 animals at each age; each sample comprised 32 consecutive ultrathin sections cut from the center of the epithelium. We classified each cell in a sample as a *supporting cell* or one of the following kinds of hair cell: *type I, type II, immature* or *undefined*, and counted all cells in each class.

At PD 0, cell division is almost complete (Ruben, *Acta Otolaryngol.* Suppl. 220, 1967). 17% of hair cells were immature, with features that appeared transitional between supporting and hair cells. 16% were type II. 33% were type I: two-thirds of these bore immature, partial calyces and the remainder were identified by secondary attributes. 34% of hair cells were not clearly in one of the other classes (undefined). The striolar region was more advanced than the extrastriolar region. By PD 4, 36% of hair cells bore partial or full calyces, and only 2% had type I secondary attributes but no calyx. Complex calyces were first seen after PD 4. By PD 28, 90% of hair cells could be typed as I (63%) or II (27%). 1% were immature and 9% were undefined.

Some utricles were put into denervated organotypic culture on PD 1 and fixed 3, 6 and 9 days afterwards. As there were no calyces, we used the secondary attributes to classify cell type. Similar cell types were present and differentiation followed a similar time course to that *in vivo*. (Supported by NIDCD grants DC02290 and DC02521.)

423.11

CALCIUM TRANSIENTS IN HAIR CELLS: MATHEMATICAL MODELING OF CONFOCAL MICROSCOPY OBSERVATIONS. F. Jaramillo*, S. Betarbet and J. D. Hall. Dept. of Physiology. Emory Univ. Sch. of Med., Atlanta, GA 30322.

Hair cell synapses in the auditory and vestibular systems faithfully relay sensory information to the CNS. These synapses respond to graded receptor potentials by admitting Ca++ into the hair cell's basal pole, where most synaptic active zones are located. The purpose of this study was to develop a model for the dynamics of Ca++ inside the hair cell, following a depolarizing stimulus. We monitored the activity of Ca++ with the help of the Ca-sensitive dye Fluo-3. The dye was introduced into the cell either through the whole cell recording pipette or by using the membrane permeant form, and fluorescence was recorded with a confocal microscope in the line-scan mode. To model the dynamics of intracellular Ca++ near an active zone we consider the phenomenon as a diffusion-reaction of Ca++ from 80 point sources into the cell. Three species diffuse and react concurrently; Ca++, a buffer and a Ca-buffer complex. Cellular volume is divided into "cells" of a given size. The cells at the membrane surface which surround the point sources are allowed to integrate the incoming current and thus develop an elevated Ca++ concentration when the channels are open. A three dimensional solution of this problem accounts for the observed Fluorescence vs. Ca++ current relationship, as well as for the fluorescence rise-time, time course, decay-time, and relative intensity. Supported by NIDCD Grant DC02235.

423.12

PLASMA-MEMBRANE CALCIUM ATPASE MAINTAINS A VERY LOW STEREOCILIARY CALCIUM CONCENTRATION. R.A. Dumont, E.N. Yamoah, E.A. Lumpkin†, A.J. Hudspeth†*, and P.G. Gillespie. Dept. Physiology, Johns Hopkins School of Medicine, Baltimore, MD 21205; †Rockefeller Univ., New York, NY 10021.

Ca^{2+} entering stereocilia through open transduction channels imposes a significant load on hair-bundle Ca^{2+}-control mechanisms. Previous results demonstrated expression of the plasma-membrane Ca^{2+}-ATPase (PMCA) by cochlear hair cells (Crouch & Schulte, *Hearing Res.* **92**, 112; 1995). Using the 5F10 antibody, we find that bullfrog saccular stereocilia also possess large amounts of PMCA, some concentrated at stereociliary tips. Triton X-100-soluble, calmodulin-binding bundle proteins of 145 and 170 kD are precipitated with 5F10; their abundance suggests that PMCA is present at a density of ~2000 μm^{-2}.

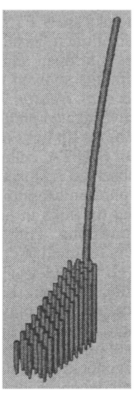
Measurements with a vibrating Ca^{2+}-selective electrode show that hair bundles, but not cell bodies, extrude substantial amounts of Ca^{2+} at rest. From hair cells clamped at -80 mV and dialyzed with Cs^+ and with Cl^- at a concentration similar to that externally, we observe a transduction-activated outward current of ~10 pA. This current disappears when extracellular Ca^{2+} is replaced with Sr^{2+} or when cells are dialyzed with vanadate. These data suggest that the outward current originates from electrogenic PMCA pumps, whose density is ~1600 μm^{-2}.

Vanadate and ADPβS, inhibitors of PMCA, increase the resting Ca^{2+} concentration in stereocilia from ~50 nM to >500 nM. After Ca^{2+} elevation, modulations of Ca^{2+} concentration due to influx through open transduction channels can no longer be visualized. These results indicate that PMCA plays a major role in maintaining low bundle Ca^{2+} levels.

(Supported by the NIH, HHMI†, and the Pew Foundation.)

423.13

FINITE ELEMENT MODEL OF UTRICULAR CILIARY BUNDLES IN A TURTLE, *Trachemys (Pseudemys) scripta*. J.R. Cotton[1], J.W. Grant[1], and E.H. Peterson*[2]. [1]Dept. Engineering Science & Mechanics, Virginia Polytechnic Inst., Blacksburg, VA 24061, [2]Neurobiology Program, Ohio U., Athens, OH 45701.



Ciliary bundles in the turtle utricle exhibit orderly differences in structure. We are using finite element analysis (FEA) to explore how these structural differences may affect the hair cells' mechanical characteristics. This report describes a 3-dimensional model of type II hair cells on the medial (neural) side of the striola. These mechanoreceptors have 4-6 columns of cilia with 9-13 cilia per column. Maximum ciliary heights are short (2-4 μm) relative to the kinocilium (≤ 10 μm). Ciliary heights increase systematically with nearness to the striola.

We took dimensions and geometry of ciliary bundles from light (DIC) and electron micrographs of turtle utricle, and we estimated material properties of stereocilia, kinocilium, and connecting links from the literature. Stereocilia were modeled as transversely isotropic using Timoshenko (shear deformable) beam theory so that during bundle deflection their internal structure exhibits significant shear as described experimentally (Tilney et al., 1983). Force was applied to the tip of the 9 μm kinocilium. The stiffness of a representative ciliary bundle (see figure) was 1.4×10^{-4} N/m, which is within the range of stiffness values observed experimentally (Szymko et al., 1992).

Supported by NSF IBN-9319630 and OUCOM

423.14

VOLTAGE-GATED CURRENTS IN HAIR CELLS OF THE TURTLE POSTERIOR CRISTA. A.M. Brichta†*, A. Aubert ‡, J. M. Goldberg§ and R.A. Eatock‡. Otolaryngology† and Pharm. Physiol. Sci.§, Univ. of Chicago, Chicago, IL 60637 and Otolaryngology, Baylor Coll. Med., Houston, TX 77030‡.

The voltage-gated currents of hair cells isolated from the turtle posterior crista were studied with the whole-cell tight-seal method. Most hair cells were classified as type I or type II by their morphology. The goals were to determine if type I hair cells have a distinctive outwardly rectifying current, $I_{K,L}$, and to compare the electrophysiology of type II hair cells from the central zone and from the parts of the peripheral zone near the torus and near the planum. The last comparison is of interest as afferents innervating the two parts of the peripheral zone differ in their discharge properties.

$I_{K,L}$ is recognized as an instantaneous current that is evoked by voltage steps from a holding potential of -65 mV and that deactivates negative to -100 mV. Cells without $I_{K,L}$ had outward rectifiers that activated only above -55 mV. $I_{K,L}$ was present in >75% of type I hair cells, but in <20% of type II hair cells. $I_{K,L}$ activated above -75 mV and was almost fully activated by -50 mV. Activation was sigmoidal and slow. The mean reversal potential was –81 mV, close to E_K. Maximal conductances were large, averaging 40 nS. A second outward rectifier was also present in type I hair cells. Like $I_{K,L}$ it had slow activation kinetics and large maximal conductances. Unlike $I_{K,L}$, it activated only above -55 mV. In these respects, type I hair cells from the turtle posterior crista resemble those from other vestibular organs.

Type II hair cells had outwardly rectifying currents activating positive to –55 mV and two inward rectifiers (I_h and I_{K1}). Type II hair cells from different regions differed in three ways: 1) Activation of outward rectifiers was slower for central than for peripheral hair cells. 2) Inactivation of outward rectifiers was fastest near the planum. 3) I_{K1} was largest near the torus. The differences in voltage-gated currents from the planum and torus were too small, however, to explain the large differences in discharge properties of afferent fibers innervating the two regions. (NIDCD grant DC 02058)

423.15

MODELING OF SYNAPTIC VESICLE CAPTURE BY THE SYNAPTIC BODY IN FROG SACCULAR HAIR CELLS. D. Lenzi*, and W.M. Roberts. Institute of Neuroscience, University of Oregon, Eugene OR 97403-1254.

Afferent synapses in hair cells from the sacculus of *Rana pipiens* belong to the class of ribbon synapses. They feature a 400 nm spherical synaptic body that hovers above the active zone, covered by a shell of 40 nm vesicles. Since the synaptic body is of unknown function, we tested the idea that it increases the surface area with which the synapse captures vesicles from the cytoplasm. We compared the steady-state rate at which vesicles could reach the active zone, with and without a 400 nm synaptic body, assuming that diffusion to the active zone or synaptic body was rate-limiting, and that the entire surface of the active zone or synaptic body was absorbent. We modeled a 7 μm radius hemisphere of hair cell cytoplasm, bordered by the plasma membrane, and centered over a 300 nm active zone. The concentration at the active zone or synaptic body was set to zero, and that at the far boundary to a constant. The cytoplasm was parsed into 50 nm x 1 degree compartments (polar coordinates), and the steady-state concentration of vesicles in each compartment calculated by iteration. The computation was checked against the analytical solution (without the synaptic body), and converged independently of the starting concentrations. The rate of vesicle absorption by the active zone or synaptic body was computed from the concentrations in the adjacent compartments, and was found to be four fold greater with the synaptic body present, showing that the synaptic body can improve vesicle capture. Although this ratio is independent of a vesicle's diffusion coefficient, it is smaller than the 10 fold ratio in surface areas available for vesicle binding. However, the diffusion-limited rates far exceeded the maximal rate of vesicles release observed in physiological experiments, suggesting additional roles for the synaptic body. Supported by NIH grant NS27142 to WMR, and a DRF grant and MDA postdoctoral fellowship to DL.

423.16

IONIC MECHANISMS ASSOCIATED WITH THE DISCHARGE REGULARITY OF VESTIBULAR AFFERENTS NEURONS. A.Flores, J.Cebada and E.Soto*. Dept. de Fisiol, Biofis. y Neurosc. CINVESTAV-IPN, Mexico, D.F. Facultad de Medicina e Instituto de Fisiología. BUAP, Puebla, México.

Vestibular afferent neurons differ in their discharge regularity, which is determined by the coefficient of variation (CV) of interspike intervals. It was found (Manjarrez and Soto, unpublished results) that the correlation between the slope of action potential repolarization and CV was positive (r=+0.75) for the most irregular afferents (CV>1) and negative (r=-0.30) for the intermediate afferents (0.5<CV<1). In order to explain which are the ionic mechanisms involved in these correlations, we have examined the effects of 4AP and TEA on the slope of repolarization and CV, and studied the outward ionic currents present in these neurons. Two methods of conventional recording in an *in vitro* preparation of the semicircular canal ampullae of axolotl (*Ambystoma tigrinum*) were employed: 1) intra-axonal recording of basal spike discharge of the afferent fibers, and 2) patch-clamp (whole-cell recording) in dissociated afferent neurons (Scarpa's ganglion). Using intra-axonal recordings (n=8) we have found that 4AP (0.1mM) or TEA (3mM) decreased the slope of repolarization in all the tested fibers. In the 4AP experiments the CV increased (0.65 to 1.30) and in TEA experiments decreased (0.94 to 0.42). These results suggests that the slope of repolarization is causally related to discharge regularity (CV) rather than merely correlated with it. Patch-clamp recordings of vestibular afferent neurons (n=9) indicated that there are two types of outward currents: 1) I_A (transient potassium current) and 2) I_K (delayed rectifier), which suggests that these currents possibly have an important role in determining the discharge regularity in vestibular neurons. Supported by CONACyT fellows 88420 (AF) and 91894 (JC) and grant 2133-N (ES).

423.17

NON-L-TYPE ALPHA-1 CALCIUM CHANNEL SUBUNIT IN A VERTEBRATE HAIR CELL SHEET. S.A. Sheikhali[1], A.A. Karadaghy[1], G.E. Green[1] and D.G. Drescher[1,2]*, Lab. of Bio-otology, Depts. of [1]Otolaryng. and [2]Biochem., Wayne State Univ. Sch. of Med., Detroit, MI 48201.

We have previously presented evidence for the expression in teleost saccular hair cells of both an α_{1D}-like (L-type) voltage-gated calcium channel subunit message and a non-L-type α_1 message, the latter with similarities to both α_{1A} (P-type) and α_{1B} (N-type) sequences (Green et al., Assoc. Res. Otolaryngol Abstr. 19: 151, 1996). Bullfrog saccular hair cells have been reported to utilize L-type and non-L-type calcium channels as well (Su et al., Hearing Res. 87: 62-68, 1995). To further elucidate the genotype of the α_1 pore-forming subunit of the non-L-type calcium channel in the teleost saccular hair cell, we have utilized RT-PCR and nucleotide sequencing.

Saccules from rainbow trout were rapidly removed and placed in saline at 2 °C. Under magnification, hair cells were dissected as an intact sheet and homogenized in 4M guanidinium thiocyanate solution. Total RNA was obtained by phenol-chloroform extraction and treated with DNase prior to reverse transcription. Resulting cDNA was amplified by PCR employing primers designed to the doe-4 α_1 sequence of the marine ray (Horne et al., Proc. Natl. Acad. Sci USA 90: 3787-3791, 1993), a homolog of the mammalian α_{1B} calcium channel subunit gene. A targeted 3.0-kb product was obtained which was partially sequenced. A sequence representing the intracellular loop between domains II and III of the voltage-gated calcium channel α_1 subunit was found to be most homologous to the α_{1B} (N-type) gene sequence. The above results confirm that the teleost saccular hair cell expresses calcium channel mRNA corresponding to a non-L-type α_1 subunit, and suggest that this form is an N-type homolog. (Supported by NIH Grant RO1 DC00156 and T32 DC00026.)

423.18

Modulation of calcium and potassium channels in the mechanosensory hair cells from the statocyst of the Octopus, *Eledone cirrhosa* A. Chrachri*[1] and R. Williamson[1,2] [1]MBA, Citadel Hill, Plymouth, PL1 2PB, UK & [2]Department of Biological Sciences, Univ. of Plymouth, Drake Circus, Plymouth, PL4 8AA, UK.

Cephalopods, such as sepia, squid and octopus, show a well developed and sophisticated control of balance, particularly during prey capture and escape behaviours. This control is mediated by mechanosensory hair cells in their statocysts. This equilibrium system receives a large efferent innervation that can depress or enhance the statocyst output and that has been shown to contain acetylcholine (Auerbach & Budelmann, *Cell Tissue Res.* 243, 429-436), noradrenaline (NA) and/or dopamine (DA) (Budelmann & Bonn, *Cell Tissue Res.* 227, 475-483). The aim of this study is to determine whether ACh and DA modulate the calcium currents (I_{ca}) and potassium currents (I_A, I_K).

Using whole cell patch clamp technique, Williamson (*J. Comp. Physiol. A* 177, 261-271, 1995) identified an inward calcium current, a transient outward current resembling the A-current (I_A) and a sustained outward current resemling the delayed rectifier (I_K) in statocyst sensory hair cells.

We now show that bath application of ACh reduces both the voltage-dependent, inward calcium current and the outward potassium currents in a dose dependent manner. Bath application DA increases the calcium current in a dose dependent manner and decreases the potassium currents in a dose dependent manner. No change in the time course of the currents was observed during these applications.

ACh and DA have been shown previously to modulate electrical coupling between some groups of statocyst hair cells, with ACh decreasing the coupling and DA increasing coupling. The observations reported here are consistent with the view that intracellular calcium levels act to modulate the electrical coupling and hence alter the sensitivity of the cells to mechanical stimulation.
Supported by the Wellcome Trust

AUDITORY SYSTEMS: CENTRAL PHYSIOLOGY—FOREBRAIN

424.1

EFFECTS OF CENTRAL MUSCARINIC BLOCKADE AND INTERSTIMULUS INTERVAL ON HIPPOCAMPAL AUDITORY EVOKED POTENTIALS IN THE RAT. T.R. Gregg* and K.A. Campbell. Dept. of Psychology, Univ. of Delaware, Newark, DE 19716.

The auditory evoked field potential (AEP) recorded from microelectrodes targeted to dentate gyrus (DG) in 8 awake freely-moving rats displayed a sharp negativity (N1) at 34 ms after onset of a 1-s, 80-dB, 10-kHz tone. Interstimulus intervals of 1 s or less suppressed N1 amplitude.

Scopolamine (0.5, 1.0, 2.0, and 5.0 mg/kg, subcutaneous) suppressed N1 peak amplitude in the first hour after injection, in a non-dose-dependent manner. However, the time required for N1 amplitude to recover to control levels after injection was dose-dependent: at 3 h, recovery was complete at 0.5 mg/kg but still suppressed at 5.0 mg/kg. Control injections of methscopolamine did not affect N1; thus central, not peripheral, muscarinic blockade suppressed N1 amplitude.

Motor activity temporarily increased after scopolamine but not after methscopolamine. Motor activity can suppress AEPs, so scopolamine may have suppressed N1 indirectly through the increase in motor activity. To test this possibility, AEPs were recorded in 4 anesthetized, isothermic, acutely-prepared rats. Preliminary data indicate that N1 declined after scopolamine, suggesting that scopolamine's effect on N1 may be due to a direct effect of scopolamine on auditory input to the hippocampus. (Supported by University of Delaware)

424.2

THE EFFECT OF BRAINSTEM STIMULATION ON NEURONS OF RAT AUDITORY THALAMUS IN VITRO. D. M. Mooney* and B. Hu. Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada K1Y 4E9.

Non-auditory input from the midbrain reticular formation (MRF) to the medial geniculate body (MGB) mediates behavioural state-dependent changes in sensory signal processing and is believed to be mostly cholinergic. To examine the synaptic influence of MRF input on MGB neurons, brain slices preserving the MGB and efferent tracts of the MRF were employed. 50 μM of picrotoxin was continuously present in the bathing medium. Repetitive electrical stimulation of putative MRF efferents produced a slow afterdepolarization or sADP in 35 of 53 responding MGB cells. The sADP was associated with an increase in membrane conductance (3/4) by an average of 160%. The sADP was abolished by tetrodotoxin (4/4). It was not, however, blocked by a CNQX/(±)-AP-5 mixture (5 μM/50-200 μM) or by the metabotropic GluR blocker (S)4CPG (100-200 μM). Neither was it blocked by methylscopolamine (5-50 μM) or hexamethonium (500 μM), suggesting a noncholinergic, nonglutamatergic mechanism. In another 13 of the responding MGB cells, MRF efferent stimulation elicited a slow afterhyperpolarization (sAHP). The remaining 5 cells had mixed or varying responses. The neurotransmitters underlying the sADP and the mechanisms of the other responses remain to be defined. These data suggest that MRF efferents provide a noncholinergic, nonglutamatergic input to the rat MGB. *Supported by grants from MRC of Canada and the Ontario Graduate Scholarship Program.*

424.3

DISTRIBUTION OF NADPH-DIAPHORASE AND CYTOCHROME OXIDASE IN RELATIONSHIP TO FUNCTIONAL ORGANIZATION OF THE MEDIAL GENICULATE BODY IN GUINEA PIG. J. Syka, R.Druga, J. Popelár, E. Kvasnák, D. Suta (SPON: European Neuroscience Association) Institute of Experimental Medicine, Academy of Sciences, 142 20 Prague, Czech Republic

The detailed functional organization of the medial geniculate body (MGB) was studied in guinea pigs with morphological and electrophysiological methods. Animals anaesthetized with pentobarbital were perfused with aldehyde fixation, and sections through the hemispheres and brainstem were cut on a freezing microtome and processed for NADPH-diaphorase (NADPH-d) according to Scherer-Singler *et al.* (J. Neurosci. Meth., 9: 229, 1983) and for cytochrome oxidase (CyOx) according to Wong-Riley (Brain Res., 171: 11, 1979). In another group of guinea pigs anaesthetized with ketamine, the MGB was penetrated with a microelectrode and parameters of neuronal response to acoustical stimuli, such as the characteristic frequency (CF), Q_{10} and latency, were evaluated. The MGB in the guinea pig (as in other mammalian species) can be divided into primary ventral division and secondary divisions such as dorsal, medial and suprageniculate division. The histochemical staining for CyOx was most pronounced in the ventral division of the MGB (mainly in its basal part), while weaker CyOx activity was present in the secondary divisions of the MGB. Intensely positive NADPH-d neurons were localized only in the caudal part of the medial and suprageniculate divisions. The ventral division is tonotopically organized. During penetration of the electrode in the dorso-ventral direction, the characteristic frequencies of recorded neurons increased from low to high CF. The tonotopy of other divisions of the MGB is more complex and less expressed. The majority of neurons in the ventral division have narrow tuning curves, whereas many neurons in other MGB divisions are broadly tuned or respond with multiple frequency ranges. The onset type of response is present about twice as frequently in the ventral division as in secondary divisions. Several units localized throughout the MGB respond with two or three reverberating discharges with a period of 150-250 ms.

Supported by grant 309/940/0735 of the Grant Agency of the Czech Republic.

424.4

THE SPATIO-TEMPORAL RESPONSES TO TWO-TONE STIMULATION IN GUINEA PIG AUDITORY CORTEX

K.Fukunishi*, R.Tokioka, N. Murai and T.Miyashita. Advanced Research Lab., Hitachi, Ltd., Hatoyama, Saitama 350-03 Japan

Spatio-temporal neural activities in response to tones as the simplest sound and vocalizations as complex sounds in the auditory cortex (field A) of guinea pig observed by optical imaging with dye have revealed a neural encoding structure that the coding of the complex sounds in the cortex temporally correlates to the dominant spectrum at each time of the onset phase. Here, neural responses to a sound synthesized from tone bursts are observed optically in the guinea pig auditory cortex and discussed.

An optical recording system of 12 by 12 photo-diode array with voltage sensitive dye (RH795) and a high N/A and low noise microscope were used for measuring spatio-temporal neural response in the auditory cortex (field A) of anaesthetized animals. The measured area was 3 mm in diameter with a spatial resolution of 0.05 mm^2. Simple sounds synthesized from tone bursts (rising time: 10 ms, plateau: 30 ms, falling slope: 10 ms) of, for example, 2 kHz and 9 kHz. Sound pressure levels were just above response thresholds. The responses in the cortex to the synthesized sound and each tone are compared. The tonotopical areas for 2 kHz are suited rostrally and caudally for 9 kHz as previously recorded. The response amplitudes for 2 kHz were larger than those for 9 kHz and the latency is shorter for lower frequencies than for higher ones. The response area for the synthesized sound covered over both tonotopical areas and the latency was entrained by the response to the lower frequency. The response area of the responses to the synthesized sound subtracted by the responses to either tone almost overlaps to the response area to another tone. The results that the tonotopical area is linearly additive to the tone stimulus supports the previous experiment regarding to the coding of the complex sound temporally by its spectral characteristics.

424.5

EFFECTS OF TETRODOTOXIN ON AUDITORY AND VISUAL EVOKED POTENTIALS IN THE RAT. E.L. Moore*, D.J. Carter, and C.E. Kling. Neurotoxicology Branch, Pathophysiology Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425

The consumption of puffer fish is the leading cause of tetrodotoxin poisoning and represents a significant public health threat in many parts of the world. Tetrodotoxin is a potent neurotoxin, which is known to block voltage-activated Na^+ channels, resulting in a reduction in action potential initiation. The present study was designed to examine possible central actions of sublethal doses of TTX using auditory and visual evoked potentials recorded from rats. Male, Long-Evans rats (250-350 grams) were anesthetized with sodium pentobarbital (42 mg/kg; i.p.) and implanted with recording electrodes. Electrodes were secured to the skull with dental acrylic. Animals were allowed to recover from surgery for approximately one week. Rats were restrained and placed in a sound attenuating chamber for recording brainstem auditory evoked potentials (BAEPs) or in front of a screen for pattern reversal visual evoked potentials (PREPs). All evoked potentials were recorded before and after injection of TTX (2, 4, or 8 μg/kg; i.p.). TTX decreased the amplitude and increased the latency of BAEP peak III (representing superior olivary complex activity). This is strong evidence of a specific central effect of the toxin, since peak I (representing the peripheral component of the BAEP corresponding to auditory nerve input) was unaffected. Significant increases in latency and changes in PREP peak amplitudes were found after TTX exposure. No changes were found in BAEPs or PREPs prior to TTX administration or with saline controls. These results suggest that TTX, given at sublethal doses, impairs auditory and visual processing in a selective manner. In summary, auditory and visual evoked potentials appear to be sensitive tools for assessing central nervous system effects of TTX and may have possible diagnostic applications.

424.6

THE EFFECTS OF SUBCORTICAL LESIONS ON EVOKED POTENTIALS AND SPONTANEOUS HIGH FREQUENCY (GAMMA-BAND) OSCILLATING POTENTIALS IN RAT AUDITORY CORTEX. B. Brett* and D.S. Barth. Department of Psychology, University of Colorado, Boulder, CO 80309-0345.

Functional subdivisions of auditory cortex in the rat were identified based on the distribution of temporal components of the mid-latency auditory evoked potential (MAEP) recorded with a multichannel epipial electrode array. Spontaneous data collected from the same location exhibited spindle-shaped bursts of oscillations in the gamma-band (20-40 Hz) whose location and spatial distribution were similar to that of the MAEP complex in that the bursts were localized to primary and secondary auditory cortex, the principle targets of thalamocortical projections. This suggested that the neural generators of these electrophysiological events may be similar. However, ablation of the medial geniculate nucleus (MG) of the thalamus revealed that while this nucleus is required for the generation of MAEPs, it is not required for the generation of spontaneous gamma-band oscillations. Ablation of subcortical cholinergic nuclei revealed that cholinergic input via the thalamus or the basal forebrain is not necessary for the generation of either MAEPs or spontaneous gamma-band oscillations recorded in this study. These results indicated that there may be networks of cells in sensory cortical areas endowed with an intrinsic capacity to oscillate independently of sensory or cholinergic input, but that may be modulated by this input.

Supported by NSF Grant IBN-9119525 and NIH Grant 1-R01-NS2575

424.7

TEMPORAL PROPERTIES OF STIMULI DETERMINE THE RATE OF RECOVERY FROM ADAPTATION IN THE AUDITORY CORTEX. H.W. Mahncke*, C.E. Schreiner, and M.M. Merzenich. Keck Center for Integrative Neuroscience, University of California, San Francisco, SF, CA 94143-0732

Most auditory cortical neurons respond to the onset of a stimulus with a burst of activity that subsides to substantially lower levels during the remainder of the stimulus. Recovery from this form of adaptation has not been closely examined to date; it bears important implications for the processing of temporally complex stimuli by the cortex. The goals of this experiment were (1) to measure the time course of recovery from adaptation in auditory cortical field A1, and (2) to determine if the recovery of excitability is dependent on the temporal structure of the conditioning stimulus.

We recorded multiple-unit activity from the auditory cortex of nembutal anesthetized rats and presented click trains of positive-going 250µs square waves at frequencies between 125pps and 500pps. Click trains of equal duration were interrupted by variable duration silent gaps. The onset response to the second click train was used as a measure of recovery from adaptation to the first train. At each recording site, responses to single and paired click trains at 125pps and one other frequency were recorded. Responses to the 125pps click trains were used to normalize across different recording sites and different animals.

We found that (1) a 100ms long 125pps click train required, on average, a 49±10ms (s.d.,n=43) silent gap for a subsequent click train to evoke 50% of the activity generated by the first train. (2) The recovery time from adaptation was dependent on the frequency of the click train; click trains of higher frequency yielded shorter recovery times. The recovery time relative to the 125pps case was a linear function of the inter-click interval of the conditioning click train with a slope of 4.3ms/ms. Control experiments demonstrated that the difference in recovery times between click trains of different frequencies could not be attributed to the differing number of clicks in either the conditioning or probe click trains.

These effects may be related to a form of auditory psychophysical adaptation observed by Hafter and colleagues (Hafter and Buell, 1990) and suggest specific physiological mechanisms by which sequentially occurring stimuli can be segregated and integrated in auditory cortex. [Supported by HHMI, NIH Grant NS-10414, and Hearing Research, Inc.]

424.8

ANATOMY AND PHYSIOLOGY OF IDENTIFIED RAT AUDITORY CORTICAL NEURONS. B. J. Hefti* and P.H. Smith, Neuroscience Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706

Cortical neurons have been classified based on anatomical and physiological features in rodent somatosensory, visual, and motor cortices, but the rat auditory cortex remains largely uncharacterized. We have begun to characterize neurons according to existing classifications primarily based on intrinsic firing patterns and responses to synaptic stimulation, and to correlate these properties with anatomical features. Using neurobiotin-filled sharp microelectrodes, we record intracellularly from neurons in an *in vitro* slice preparation. Auditory cortical cells respond to current injection in the three basic patterns described by Agmon and Connors (1992) for somatosensory cortex. Layer II-VI pyramidal cells can show regular spiking (RS) behaviour with little (RS1) or significant (RS2) spike frequency adaptation. Supragranular cells show predominantly RS1 responses, infragranular can be either. Lowering Ca^{++} has little effect on the initial rapid spike rate of RS1 or RS2 responses but eliminates the buildup of adaptation in RS2 units. Other pyramidal cells in layer V and deep layer IV show intrinsic burst (IB) responses with an intial rapid burst of 3 to 4 spikes followed by single spikes at regular intervals. In low Ca^{++} the later single spikes were converted to multispike bursts. Cell bodies of IB cells tend to be larger than RS cells in these layers with thicker apical dendrites which extend to layer I, where they branch profusely. Fast spiking (FS) cells are rarely seen, scattered through the layers. In RS units, white matter (wm) shock stimulation could usually elicit an early epsp, a subsequent higher threshold depolarizing $GABA_A$ ipsp (at rest), followed by a long duration $GABA_B$ ipsp. For two RS cells, theta burst stimulation of wm inputs elicited pathway-specific LTP. Layer I shock stimulation typically evoked a longer latency epsp and was less likely to activate inhibition. IB units could show large suprathreshold responses to either wm or layer 1 simulation with little sign of inhibition. Supported by PHS grant RO1 DC-01999.

424.9

MULTIPLE AUDITORY CORTICAL FIELDS IN THE RAT. N. Doron, J.E. Ledoux and M.N. Semple*. Center for Neural Science, New York University, New York, NY 10003.

Previous anatomical studies have shown that rat auditory cortex is composed of a central core and a diffuse surrounding belt. The core is innervated heavily by the primary thalamic relay (the ventral medial geniculate) and the belt receives its predominant subcortical input from non-primary auditory areas. Physiological studies have demonstrated a single tonotopic field identified as primary auditory cortex (AI), but it is unclear whether additional tonotopic fields are contained within the core. Nothing is known about the functional properties of the belt. We studied responses of single neurons recorded extracellularly throughout core and belt areas of auditory cortex in the anesthetized Sprague-Dawley rat. Acoustic stimuli (tones, noise, and temporal modulations) were delivered dichotically via sealed systems. Consistent with previous findings, AI was characterized by an anterior to posterior tonotopic progression from high (>40 kHz) to low (<1 kHz) frequencies. A frequency reversal at the posterior border of AI marked entry into a second core tonotopic region (field P) with progressively higher frequencies encountered further posteriorly. Frequency tuning was relatively sharp in both fields. In contrast, responses in the ventral belt were often broadly tuned or unresponsive to tones, with no clear indication of tonotopic order. Broadband noise was an effective stimulus throughout rat auditory cortex, but bandpass noise was often even more effective in belt regions. Although a few neurons in AI responded to stimulus offset, discharge patterns in all cortical areas were more typically transient at stimulus onset, with shortest minimum latencies in AI. In all areas, response magnitude was most commonly a monotonic function of stimulus level, and most neurons were binaurally influenced. The discovery of multiple auditory cortical fields in the rat is consistent with auditory cortical organization in other mammals. Supported by a grant from the W. M. Keck Foundation, and by PHS grants R37MH38774, K02MH00956 & R01MH46516.

424.10

RESPONSES TO SOUND MOTION OF SINGLE NEURONS IN THE RAT PRIMARY AUDITORY CORTEX, D. E. Doan and J. C. Saunders*, Departments of Bioengineering and Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA 19104.

Single-unit recordings were made from the left primary auditory cortex of Long Evans rats. Computer synthesized sounds were generated by dynamically manipulating doppler shifts and interaural phase and intensity differences. Each sound mimicked motion of a sound source along one of 4 radial or 3 circumferential sound source trajectories in the front contralateral quadrant of the horizontal plane. In addition, simulated motion along each trajectory was presented at one of two speeds, and either in forward or reverse direction. Sounds were presented bilaterally via hollow ear bars and the total battery of 28 different waveforms (7 trajectories x 2 speeds x 2 directions) was repeated five times for each cell at its characteristic frequency. The activity of the cell was recorded throughout.

Preliminary results from 10 animals indicate that about 20% of all cells encountered showed a statistically significant increase in activity for one of the 28 conditions. This suggests that some cells within the rat primary auditory cortex respond not only to a preferred auditory spatial location, but that they also respond preferentially to a particular direction and speed of motion within that spatial extent.

Research supported by the Pennsylvania Lions Hearing Research Foundation

494.11

THE EFFECT OF PRIMARY AND SECONDARY AUDITORY CORTICAL LESION ON TEMPORAL AUDITORY PROCESSING IN RATS. M.G. Clark, Y.H. Liu, and T.M. Perney, P. Tallal, R.H. Fitch*. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Functional localization of complex auditory processing to primary and secondary auditory cortices of the human brain is well established. Damage to these regions is known to produce severe deficits in speech perception and, to a lesser extent, non-linguistic auditory temporal processing. Moreover, in cats and monkeys it has been demonstrated that unilateral and bilateral ablation of auditory cortex produces severe impairments in certain aspects of auditory discrimination, including the ability to discriminate temporally patterned stimuli. Additionally, monkeys with auditory cortical ablation exhibit deficits in discriminating both human speech sounds and species-specific vocalizations. In contrast, little data exists regarding functional deficits following auditory cortical ablation in a rodent model. Most studies of which we are aware employed relatively low-level tasks (e.g., sound localization). It has been hypothesized (Kelly, 1990) that a temporally complex auditory task would elicit a deficit following such lesions. We trained adult male rats in an operant conditioning paradigm to discriminate between temporally patterned tone sequences, e.g. high-low vs. high-high, low-low, and low-high. The rats were randomly assigned to one of four lesion treatment groups: left (n=5), right (n=5), bilateral (n=4) and sham (n=5). Following aspiration lesion surgery and recovery, the rats were retested. Prior to surgery, all animals were able to discriminate the temporally patterned stimuli ($p<.05$). Post-surgically there was no significant change in the discrimination abilities of the shams, but lesion subjects performed near significantly worse than shams ($p=.054$). Furthermore, shams showed significant discrimination post-surgically ($p<.05$), while lesion subjects did not. These results confirm that the auditory cortex of the rat is functionally specialized for complex temporal auditory processing.

424.12

EFFECTS OF DIFFERENT ANESTHETICS ON THE RESPONSES TO BROAD-BAND SOUNDS IN THE FERRET PRIMARY AUDITORY CORTEX AND INFERIOR COLLICULUS. A.Z.Kohn, D.A.Depireux and S.A.Shamma*, Institute for Systems Research, University of Maryland, College Park, MD 20742.

We have previously shown that neuronal responses in Primary Auditory Cortex (A1) of barbiturate-anesthetized Ferret to broad-band sounds are fully characterized by their responses to ripples, i.e. sounds whose spectral envelope is a sinusoid on a log frequency axis. This is analogous to the local spatial frequency analysis used in visual cortex, with ripple spectrum, stationary or moving, corresponding to visual gratings. We have also previously presented Inferior Colliculus (IC) recordings in the Pentobarbitol anesthetized Ferret. To understand the effects of anesthetics on the response characteristics, we have repeated our experiments using Ketamine and Xylazine as anesthetics. Our results show that, in AI, neurons respond essentially as before, i.e., maximally to ripples of specific ripple frequency, specific phase for stationary ripples, and specific ripple velocity for moving ripples. Some of the tuning characteristics are slightly different under Ketamine; e.g., ripple frequency tuning tends to be lower in the Ketamine preparation, possibly corresponding to a less effective inhibition of the inhibitory sidebands of the response area. Also, whereas under barbiturates, IC cells tend to be tuned to ripples with the lowest ripple frequency we used, or about .2 cycles per octave, under Ketamine we see more of a variety of tunings to higher ripple frequencies. Other response characteristics, including temporal properties of cells, will be presented.

Single units also exhibit periodicities in their responses that did not correspond to the spectral envelope of the stimulus, but rather to interactions between component tones present within the response area of the cell. While this substructure is common in IC recordings, it is uncommon in cortical ones for the barbiturate preparation, and present in 50% of recordings in the Ketamine preparation.

Supported by grants from the Office of Naval Research, the Air Force Office of Scientific Research and the National Science Foundation.

424.13

SPATIO-TEMPORAL PATTERNS OF EVOKED POTENTIALS IN FERRET PRIMARY AUDITORY CORTEX. A.L. Owens, H. Versnel*, S.A. Shamma. Institute for Systems Research, University of Maryland, College Park, MD 20742.

The work presented here is motivated by the question of how the topographic distribution of activity in primary auditory cortex (AI) varies temporally during presentations of non-stationary sounds. To address this question auditory evoked potentials (EPs) are measured at the surface of AI in barbiturate-anaesthetized ferrets with a flexible electrode array. The array consists of 25 recording electrodes arranged in a square grid with 200 micron spacing between sites (Owens, A. et al, Journal of Neuroscience Methods, (58) 209-220, 1995). EPs are recorded in response to tones, clicks and frequency-modulated (FM) sweeps. Responses to pure tones determine the range of frequencies represented within the recording area. FM sweeps are generated by sweeping a tone frequency across this range of frequencies. The frequency is swept both upwards and downwards at rates ranging from 30 octaves/s to 300 octaves/s. Spatial maps presented in video format are generated at 1-2 ms intervals during the sound presentation. The results indicate a temporal progression of evoked activity along both the tonotopic and isofrequency axes which depends on sweep rate and direction.

(Supported by a grant from the Office of Naval Research)

424.14

INHIBITION AND DISINHIBITION OF AUDITORY CORTEX UNIT RESPONSES BY FORWARD MASKERS. L.M. Kitzes* Dept. of Anatomy & Neurobiology, University of California Irvine, Irvine, Ca 92717

Acoustic stimuli commonly occur in quick succession, providing both synchronous input to the two ears and sequential stimulation of each ear. Relative to stimulation of one ear, interactions between the activity evoked by synchronous stimulation of the two ears can result in facilitation, suppression or in a response when stimulation of either ear alone is totally ineffective. The response to a given stimulus can suppress the response to a subsequent stimulus. Such forward masking of unit responses in auditory cortex has been studied using only monaural stimuli. The present study assessed reciprocal influences between binaural interactions and forward masking on unit responses in primary auditory cortex of pentobarbital anesthetized cats. Stimuli consisted of two pairs of 50-ms duration binaural tonal stimuli, at the CF of the unit, separated by a 50-ms interval.

For units excited by stimulation of either ear, increasing levels of the first stimulus to the more effective ear increased the masking of the response to the second pair of stimuli, i.e., decreased the response to the second stimulus pair. This was true also for units excited by stimulation of one ear and inhibited by stimulation of the other ear. For these units, increasing levels of the first stimulus to the inhibitory ear increased, i.e., disinhibited, responses to the second pair of stimuli. The binaural interaction of the initial stimulus pair always preceded the forward masking.

Supported by DC-00450

424.15

EFFECTS OF BACKGROUND NOISE ON NEURAL RESPONSES TO CONSONANT-VOWEL (CV) STIMULI IN THE PRIMARY AUDITORY CORTEX OF THE CAT. S.W. Wong* and C.E. Schreiner. Coleman Laboratory, W.M. Keck Center for Integrative Neuroscience, University of California, San Francisco, CA 94143.

The representation of speech at the level of the primary auditory cortex challenges our understanding of how complex sounds are encoded in the central auditory nervous system. Our understanding of the functional organization of A1 for basic receptive field properties, such as intensity and bandwidth, to simple stimuli may provide a framework whereby spatial-temporal patterns of distributed neural activity to complex sounds such as speech may be classified. In studies on anesthetized cats, multi-unit neural tuning from a low-frequency patch of A1 was mapped and neural spike train responses to binaurally presented CV syllables were recorded. Responses were recorded for a repertoire of CV stimuli in a quiet background and in backgrounds of continuous noise at several levels. Data obtained in the quiet listening condition revealed a double-on response similar to that reported by Eggermont (JASA, 1995 Aug 98 (2 Pt.1): 911-920) for CV stimuli with unvoiced consonants. In addition, A1 topography of spike responses depicted spatially selective temporal activity patterns in accordance with slight spectral-temporal differences in the stimuli. The addition of continuous background noise exerted differential effects upon the spike response topography of A1 neurons along the dimensions of noise level and CV stimulus type. Suppressive effects of the noise were manifest along both spatial and temporal domains. Findings in this study further emphasize the prominent effects of background noise on the representation of complex sounds in the primary auditory cortex.

Work supported by ONR N00014-94-1-0547 and the Coleman Fund

424.16

INFORMATION-BEARING ELEMENTS OF SPIKE TRAINS IN THE CAT'S AUDITORY CORTEX. J.C. Middlebrooks* and L. Xu. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109-0506

We have shown previously that the temporal firing patterns of cortical units carry substantially more information about sound-source location than is carried by spike counts alone (*Science* 264:842-844). In the current study, we have begun to identify specific elements of the spike patterns that carry information. We represent averaged spike patterns as weighted sums of principal components. For 114 of 118 single units, the spike count correlates closely ($r>0.8$) with the weight on the first principal component (PC1). This means that, given only one number with which to represent a firing pattern, spike count usually is the best to choose. Nevertheless, PC1 accounts for an average of only 55.4% of the variance across all the spike patterns of each unit. We evaluated mean post-stimulus-times (PST's) and a measure of the temporal dispersion of spikes as possible contributors to the additional variance. For some units, those variables correlated with spike counts and, thus, contributed little additional information. For the majority of units, however, mean PST and temporal dispersion were practically independent of spike count. Among those units, mean PST correlated with the weight on PC2 or PC3 in the majority of cases, and temporal dispersion often correlated with the weight on PC3. We now are testing the extent to which mean PST and temporal dispersion carry information specifically about sound-source azimuth, elevation, and SPL. (supported by NIDCD)

424.17

LONG-LATENT NEURONS IN THE CAT DORSAL AUDITORY CORTEX IN RELATION TO TEMPORAL INTEGRATION J. He*, T. Hashikawa & Y. Kinouchi, Laboratory for Neural Systems, Frontier Research Program, RIKEN, Saitama, and Department of Electrical and Electronic Engineering, The University of Tokushima, Tokushima, Japan

The present study examined the response dependency of cortical neurons on the duration length of noise burst. We recorded 150 neurons with latency longer than 30 ms to auditory stimuli in the cat dorsal auditory cortex. 78 out of 150 neurons were classified to temporal summation neurons which showed increasing responses to noise bursts of longer duration (type I neuron), and 72 neurons showed temporal suppression processing in one or two phases of the stimulus time domain, and were classified to temporal suppression neurons (type II neurons). Of 78 type I neurons, 30 showed no response to noise burst of duration shorter than a threshold, and showed increasing responses only when we prolonged the duration longer than the threshold and shorter than their latency. Each of these neurons seemed to have an integrating period and remained their maximum responses when the duration of noise burst were longer than their integrating periods. Of 72 type II neurons, 41 showed good responses to noise burst of short duration, but poor responses to that of longer duration, their responses were induced by the beginning period of the noise burst, but were suppressed by the later coming period of the noise burst. 13 type II neurons showed maximum responses to noise bursts of a certain duration and were classified to duration-tuned neurons, as which have been previously found in the frog and the bat.

These results suggest that the long-latent neurons are related to temporal integration on the time domain before their responses. This integration includes temporal summation and temporal suppression.

424.18

INTRA-CORTICAL INHIBITORY CONNECTIONS REFINE THE SPECIFICITY OF CAT A1 NEURON RESPONSIVITY. S. Taha, B. Bonham*, S. W. Wong, and C. Schreiner. Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143.

In the mammalian auditory system, ascending auditory information passes from the medial geniculate nucleus (MGN) of the thalamus to the primary auditory cortex (A1) as well as associated auditory fields. In this ascending circuit, while the broad organization of tonotopy is maintained, individual neuronal response properties are considerably altered. The degree to which these differences can be ascribed to ascending thalamic projections or to intra-cortical connections is largely unknown. Using the $GABA_A$ inhibitor bicuculline, we investigated the contribution of the intra-cortical inhibitory connections in shaping cortical neuron frequency response areas (FRAs) and modulation transfer functions (MTFs).

Multi-unit extracellular recordings were made in A1 of the cat; using tone burst and wide-band click stimuli, tuning curves and MTFs for all units were derived before and after bicuculline application. Five microliters of bicuculline were applied directly to the exposed surface of the brain at a concentration of 1 mM. Bicuculline consistently altered three aspects of unit responses. 1) Loss of $GABA_A$ inhibition expanded tuning curves to include both higher and lower frequency regions, leaving the best frequency for the unit unchanged. 2) The threshold of the best frequency response was reduced; neurons became more sensitive to low intensity stimuli. 3) Bicuculline addition increased the monotonicity of firing in response to increasing sound intensity. Initially non-monotonic units responded monotonically after bicuculline addition. Comparison of MTFs before and after bicuculline application did not indicate a consistent change in the phase-locking ability of recorded cells. These results suggest that inhibitory sidebands created in the cortex via lateral inhibitory connections play a significant role in shaping the spectral but not the temporal response properties of cortical neurons. (Supported by NIDCD 02260)

424.19

OPTICAL IMAGING OF TOPOGRAPHY OF FREQUENCY AND INTENSITY ORGANIZATION OF CAT AUDITORY CORTEX USING INTRINSIC SIGNALS. H.R. Dinse, B. Godde, T. Hilger and C.E. Schreiner* Institut für Neuroinformatik, Theoret. Biol. Ruhr-Univ. Bochum RUB, D-44780 Bochum, Germany and Keck Center for Integrative Neuroscience., Univ. California, San Francisco, CA 94143

We studied the functional organization of cat auditory cortex (AI) by means of optical imaging of reflectance changes of intrinsic signals (LaVision Lightstar II) using a 546 nm light source.

We were able to measure up to 70 different maps for a variety of stimulus conditions in one individual animal. Following tone burst stimulation at various stimulus intensities we found topographically restricted response areas. However, these areas were large and highly overlapping, which is in accordance with results recently obtained in somatosensory cortex (Godde et al. 1995, Neuroreport 7: 24-28). Variation of the stimulation frequencies between 0.5 and 28 kHz revealed a systematic and corresponding shift of the response areas revealing an overall tonotopic frequency organization. Two-tone presentations revealed severe suppressive and excitatory interactions involving large portion of AI. Systematic variation of the sound level over a range of 50 dB evoked different spatial patterns of reflectance changes that appeared patchy. Increasingly higher levels recruited increasingly larger response areas resulting in a decreasing spatial selectivity. This was paralleled by positional shifts of activity zones indicating that increasing intensity involves the additional engagement of previously unresponsive areas and the retreat from previously activated zones.

The results suggest that optical imaging intrinsic signals which corresponds to the measurement of the cortical point spread function reveals a profound tonotopic organization compatible with single cell data. On the other hand new aspects such as the substantial overlap between isofrequency domains could be visualized that bears important implications for stimulus interactions and plastic reorganizations. Changes in other signal dimensions, such as azimuthal location or fundamental frequency, also resulted in distinct spatial patterns of activation that may hold important information regarding the functional organization of primary auditory cortex.

Supported by DFG, NATO, and ONR

424.20

EFFECTS OF ELECTRICAL STIMULATION OF A MULTI-CHANNEL COCHLEAR IMPLANT ON CAT AUDITORY CORTICAL ORGANIZATION REVEALED BY OPTICAL IMAGING. B. Godde*, G. Reuter, T. Hilger, S.M. Cords, T. Lenarz and H.R. Dinse. Institut für Neuroinformatik, Theoret. Biol. Ruhr-Univ. Bochum RUB, Bochum and Med. Hochschule, Exp. HNO, Hannover, Germany

We measured reflectance changes by means of optical imaging (LaVision, Lightstar II imaging and acquisition system) of intrinsic signals to study the effects of acute electrical cochlear stimulation on the topography of the cat auditory cortex. Multichannel implant electrodes consisting of several pairs of platinum-iridium balls were inserted into the scala tympani. The total length of the inserted electrode portion was about 8 mm, the mean distance between electrode sites was about 1.8 mm.

Following single pulse electrical stimulation at selected sites of a multichannel implant device, we found topographically restricted response areas representing mainly the high frequency ranges of the primary auditory cortex (AI) and of the anterior auditory field (AAF). Systematic variation of the stimulation pairs and thus of the cochlear frequency sites revealed a systematic and corresponding shift of the response areas that matched the underlying frequency organization. Based on estimates on average sizes of the organ of corti and the corresponding frequency localization, the stimulating electrode sites were likely to cover a frequency range of 6 to maximal 50 kHz. Intensity functions were usually very steep. Increasingly higher stimulation currents evoked increasingly larger response areas resulting in decreasing spatial, cochleotopic selectivity. Using high frequency periodic stimulation between 100 and 5000 Hz evoked similar spatial patterns as did single pulse stimulation.

The results suggest that the method of optical imaging intrinsic signals can be used to map response areas evoked by electrical cochlear stimulation and that the electrical stimulation in fact produces a profound cochleotopic selectivity. The implications of these findings are discussed in respect to underlying mechanisms of sound sensation mediated by cochlear implants and in respect to the information processing of normal acoustic signals.

Supported by the DFG Neurovision, the Institut für Neuroinformatik and a BMFT grant

425.1

THE GABAergic ORGANIZATION OF THE CAT MEDIAL GENICULATE BODY. C.L. Huang*, D.M. Huchton, D.T. Larue, and J.A. Winer. Department of Molecular and Cell Biology, University of California, Berkeley, California 94720.

We studied the GABAergic cells and axon terminals (puncta) in the MGB with antisera to GABA or GAD. Neurons were classified in 50 μm-thick sections, and 1 μm-thick sections were used for quantitative purposes. We found that (1) each division had a different concentration of GABAergic neurons. The ventral division (V) had 34%, the dorsal division (D) 26%, and the medial division (M) 21%. (2) In V and M, GABAergic cells had a drumstick shaped perikaryon (~12-14 μm in diameter) and sparse dendrites. D had small (10-12 μm × 15-18 μm) and large (12-15 μm × 18-21 μm) cells, the former with a flask-shape perikaryon, the latter with a triangular or ovoid soma; each had a stellate or radiate dendritic branching pattern. (3) There were caudal-to-rostral spatial gradients in the proportion of these cells: V ranged from 29% (caudally) to 38% (rostrally), D from 32% to 16%, and M from 9% to 39%. Others (Rouiller et al., *Hearing Res.*, 1990, **49**:249-258) found the opposite gradient in V. (4) The form of puncta were unique to each division. V neuropil had many globular, grape-like clusters; D puncta were denser and more variable in shape; M had small, flattened puncta and some large and prominent axosomatic endings. Thick axons (3-4 μm in diameter) in the brachial neuropil may arise from the IC (J.A. Winer et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1996, **93**: in press). (5) GAD+ somata received few endings, while most GAD- cells had many axosomatic puncta.

Each division shares a similar distribution of axosomatic puncta, and each has more than 20% GABAergic cells. Divisional differences in their proportion, qualitative differences in puncta, and the two GABAergic cell types in D suggest that each division also has unique inhibitory features.

Supported by United States Public Health Service grant R01 DC02319-16.

425.2

GABAergic ORGANIZATION OF THE MAMMALIAN MEDIAL GENICULATE BODY: A COMPARATIVE STUDY. D.T. Larue* and J.A. Winer. Department of Molecular and Cell Biology, University of California, Berkeley, California 94720.

GABAergic neurons and axon terminals (puncta) were studied in the medial geniculate body (MGB) of mustache bats, albino rats, cats and monkeys. Antisera to GABA were used on thick Vibratomed (50 μm) or semithin (0.5-2 μm) sections, while frozen sections 25 μm thick were immunostained with antisera to GAD. The results were the same for each method.

The proportion of GABAergic neurons ranged from <1% in the bat and rat to 25% or more in the cat and monkey. In all species, the chief type of MGB GABAergic neuron had a flask-shaped soma ~10 μm in diameter, thin dendrites, and a fine axon. In the cat and monkey a second, larger and rarer GABAergic cell was also seen. While the proportion of GABAergic MGB cells varied, their number in the inferior colliculus and auditory cortex was ~15-20% in all species.

In the bat, these cells were most numerous in the dorsal division, and rare or absent in the medial division. In the rat, they were concentrated in the ventral and dorsal divisions. In the cat and monkey, each division had many.

All four species had many GABAergic puncta that arise from the thalamic reticular nucleus and intrinsic sources. A third origin in the cat is the inferior colliculus (J.A. Winer et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1996, **93**: in press).

Dendrodendritic glomerular arrangements common in the cat (and perhaps in the monkey) should be rarer in the bat and rat. The number of MGB GABAergic cells is similar to that in the ventrobasal complex, while the proportion of GABAergic cells in the lateral geniculate body is largely conserved. Perhaps the number of MGB GABAergic neurons relates to the forebrain's role in vocalization and speech-like communication.

Supported by United States Public Health Service grant R01 DC02319-16.

425.3

GABA AND GLYCINE IN THE AUDITORY THALAMUS AND MIDBRAIN OF THE BARN OWL (*Tyto alba*). J.A. Winer* and D.T. Larue. Department of Molecular and Cell Biology, University of California, Berkeley, California 94720.

We surveyed the distribution of GABA, GAD, and glycine (Gly) in the owl's auditory thalamus (nucleus ovoidalis; Ov) and midbrain (nucleus mesencephalicus lateralis, pars dorsalis; MLD). Neurons and axon terminals (puncta) were analyzed in 50 μm thick sections (GABA and Gly), 25 μm sections (GAD), and in postembedded material 0.5-2 μm thick (GABA and Gly).

In Ov, the presumptive homologue of the mammalian MGB, GABAergic neurons were rare (<1%) and these were concentrated in the rostral pole. Both features are characteristic of the GABAergic organization of the mustache bat's MGB. There were many GABAergic puncta in the neuropil, on GABA-negative perikarya, and many preterminal axons in the fiber tract beneath Ov. The Gly arrangement in Ov was entirely different, with a sparse, widespread network of fine preterminal axons and puncta, including axosomatic endings.

In MLD, there were many GABAergic neurons and numerous puncta. A few Gly neurons were immunostained, and Gly puncta were plentiful. Glycinergic cells are entirely absent in the IC of the bat, rat, and cat.

A third finding was the apparent absence of brain stem glycinergic centers in the owl that correspond to the medial nucleus of the trapezoid body. Perhaps the neurons that represent this nucleus do not form a discrete group.

Despite global parallels between avian and mammalian auditory system organization, there are substantial differences in chemical architecture that may reflect species differences in sound processing or phylogenetic distance between orders.

We thank Dr. Catherine E. Carr for kindly providing the owls, and we are grateful to her and to Dr. E.I. Knudsen and Dr. L.E. Proctor for helpful advice. Supported by United States Public Health Service grant R01 DC02319-16.

425.4

CONTRASTING AUDITORY CORTICOFUGAL PROJECTIONS TO THE INFERIOR COLLICULUS AND THE MEDIAL GENICULATE BODY. J.J. Diehl* and J.A. Winer. Department of Molecular and Cell Biology, University of California, Berkeley, California 94720.

We compared the corticocollicular (CC) projections to the cat inferior colliculus (IC) with the corticothalamic (CT) input to the medial geniculate body (MGB). Axoplasmic transport (WGA) and axonal bulk filling (BDA) methods were used.

1. *Divergence.* All 10 fields are part of the CT system, while three nonprimary ones had no CC input. Tonotopic areas had more limited MGB targets than nontonotopic areas; in the IC, input from tonotopic fields dominated.
2. *Convergence.* Single MGB nuclei received input from 3-10 areas; IC nuclei were the target of 2-7 areas. The central nucleus of the IC received only sparse input.
3. *Topography.* All projections were focal and clustered. The input from nontonotopic fields was as orderly as that from tonotopic areas.
4. *Axonal structure.* CC axons had a narrow size range (0.3-2 μm in diameter), a simple terminal architecture, and strong similarities despite their many areal origins. CT axons spanned a wider size spectrum (0.3-4 μm), and had diverse terminals ranging from simple configurations to massive (~5 μm) endings that can form clusters of several hundred terminals in the dorsal division of the MGB.

Our main conclusions are:

- The CC system has more limited origins and targets than the CT projection, and fewer types of axons. Perhaps it has a more specific functional role.
- Cortical input to the MGB reaches both auditory and polysensory nuclei.
- Association and limbic-related areas have the most widespread input to the MGB, and far less to IC.

Supported by United States Public Health Service grant R01 02319-16. We thank D. Tran, F.A. Naseer and B.J. Hefti for assistance.

425.5

Selective labelling of GABAergic inputs to the central nucleus of the inferior colliculus using retrograde transport of γ-vinyl GABA. M.W. Spitzer*, M.B. Calford, D.V. Pow. Department of Physiology and Pharmacology, Univ. of Queensland, Brisbane, QLD, 4072, Australia.

Previous investigations have demonstrated selective uptake of vinyl GABA by GABAergic neurons in the retina. We investigated the potential use of γ-vinyl GABA as a neurotransmitter-specific retrograde tracer in the central nervous system. Pressure injections of γ-vinyl GABA (4 mM in saline) were placed in the central nucleus of the inferior colliculus (IC) of adult pigmented guinea pigs anesthetized with ketamine/xylazine. Following a 48 hour recovery period animals were sacrificed and perfused with glutaraldehyde. Labelled neurons were visualized immunocytochemically using a polyclonal primary antibody raised against glutaraldehyde conjugates of γ-vinyl GABA. Immuno-positive cell bodies, axons and terminals were always present within the injected IC. Immunocytochemical labelling of alternate series of semithin sections demonstrated that all γ-vinyl GABA immuno-positive neurons were also immunoreactive for GABA, but only a fraction of all GABA-immunoreactive neurons were labelled with γ-vinyl GABA. Following some injections labelled neurons were also located in the dorsal nuclei of the lateral lemniscus, bilaterally, but not in other auditory brainstem nuclei. These results suggest that uptake and retrograde transport of γ-vinyl GABA is specific for GABAergic neurons.

Supported by NHMRC (Australia), ARC and The University of Queensland.

425.6

NEURONS OF THE RAT INFERIOR COLLICULUS WITH GABA-LIKE IMMUNOREACTIVITY CAN PROJECT TO THE MEDIAL GENICULATE BODY. D. Peruzzi* and D.L. Oliver. Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06030.

To determine if neurons that contain GABA in rat inferior colliculus also project to the medial geniculate body, two techniques were combined. Projection neurons from the inferior colliculus were retrogradely labeled by making injections of red latex microshperes (Lumifluor) into the medial geniculate body. After two to four days survival, 50 μm thick slices were taken through the inferior colliculus. The presence of GABA was assessed by immunostaining these slices with a monoclonal antibody to GABA (Sigma) and an FITC labeled secondary. Data were collected from four rats.

Roughly one-quarter to one-half of neurons that project from the ipsilateral inferior colliculus to the medial geniculate body displayed GABA-like immunoreactivity. When the inferior colliculus was parcelled into the subdivisions of central nucleus, dorsal cortex, and lateral cortex, regional differences were noted. For instance, the percentage of GABA-positive projection neurons in the central nucleus was usually higher than that of those neurons in the dorsal cortex.

The presence of GABA-positive projection neurons suggests that neurons in the inferior colliculus may participate in GABAergic synapses in the medial geniculate body. Thus, ascending projections from the inferior colliculus may produce inhibitory potentials in thalamic neurons as well as excitatory potentials.

Supported by NIH grant DC00189.

425.7

COMPARATIVE ANALYSIS OF CALCIUM-BINDING PROTEIN-POSITIVE NEURONAL POPULATIONS IN VISUAL AND AUDITORY SYSTEMS OF CETACEAN, UNGULATE, CARNIVORE AND PRIMATE BRAINS . Ilya I. Glezer*[1], Patrick R. Hof[2], Gundela Meyer[3] and Peter J. Morgane[4]. [1]Dept. Cell Bio. & Anat Sci. CUNY Med. Sch.NY, NY 10031, [2]Dept. of Neurobiol., Mount Sinai Sch. Med..NY, NY 10029, [3]Dept.of Anat., Univ. La Laguna Sch. Med., Tenerife, Spain and [4] Dept. Pharmacol. Univ. New Engl., Biddeford, ME 04005.

Immunocytochemical analysis, combined with computerized imaging, revealed that in the visual and auditory systems (V1, A1 of cortex, LGN, MGN, superior and inferior colliculi of tectum) of odontocete whales calretinin (CR) and calbindin (CB) are the major calcium-binding proteins (CaBP), whereas parvalbumin (PV) is present in significantly fewer neurons. The prevalence of CR and CB-positive neurons is especially prominent in the auditory system of cetaceans. Among studied species of terrestrial mammals only in ungulates this cetacean-like pattern of distribution of CaBP is found. In ungulates CR-positive neurons are found, not only in cortical layers II and IIIa, but also in layer I. In auditory and visual systems of Old World monkeys and carnivores CR and CB-positive neurons are either equal in numbers or significantly fewer than PV-positive neurons. The prevalence of PV-positive, over CR- and CB-positive neurons is particularly obvious in visual system of primates. Thus, the dominant sensory systems in both aquatic and terrestrial mammals are enriched with a specific phenotypes of calcium-binding protein-positive neurons. The similarity between cetacean and ungulate cortex in ratios of different populations of CaBP neurons within specific neocortical areas correlates with paleontological and genetic data showing evolutionary linkage of these mammalian orders (McKenna, 1975, Eisenberg, 1981). Significant differences between cetaceans, on the one hand, and primates and carnivores on the other in ratios of CaBP neuronal populations may indicate different directions in biochemical evolution of sensory systems in totally aquatic (cetaceans) and terrestrial (primates, carnivores) mammals. [Supported by grants HD 22539 - NIH, Research Foundation of CUNY # 662232].

425.8

CORRELATIVE IMMUNOCYTOCHEMICAL AND ELECTRO-PHYSIOLOGICAL STUDIES ON JAPANESE MONKEY AUDITORY CORTEX H.Kosaki*[1,2], T.Hashikawa[1], J.He[1] & E.G.Jones[1,3]
1;Lab.Neural Sys., RIKEN, Saitama, Japan 2;Dept. Otolaryngology, Univ.Tokyo, Tokyo, Japan 3; Dept. Anatomy & Neurobiology, Univ.California, Irvine, CA

The superior temporal plane of Japanese monkeys (*Macaca fuscata*) was studied to confirm the location and subdivision of the auditory cortex by immunocytochemical staining for the calcium binding protein parvalbumin coupled with single unit recording. Anesthetized monkeys were exposed to white noise bursts and pure tone bursts for stimuli.

In parvalbumin staining, an intense staining area, which appears to be primary auditory cortex, is surrounded by a rather-weak staining zone. In the intensely stained central area neurons showed sharp tuning curves for pure tone bursts and were arranged in order of best frequency. The best frequency varied along the rostrocaudal axis. This axis was divided in two part : in the rostral parts, the best frequency of the most rostral site was the highest, then decreased caudally , but further caudally the best frequencies increased gradually again. Neurons of the surrounding zone also showed responses to acoustic stimuli, but sharp tuning curves were not observed. This surrounding zone is thus likely to be a non-primary auditory area. The lateral part of this surrounding area showed some degrees of tonotopicity. The axis of this was parallel to that of primary cortex. The neurons of other parts of the surrounding area responded poorly to pure tone stimuli.

These results suggest that the density of parvalbumin staining is coincident with electrophysiological features that delineate different functional areas of auditory cortex. And the neurons of non-primary areas which showed poorer responses to the simple pure tone stimuli than those of primary areas were expected to integrate information over a large range.

425.9

ACTIVATION OF HUMAN AUDITORY CORTEX DURING PASSIVE LISTENING TO FM TONES DEMONSTRATED WITH FUNCTIONAL MRI J. Wang*, M.S. Cohen S.Y. Bookheimer and M. Dapretto. Div. of Brain Mapping, UCLA School of Medicine, Los Angeles, CA 90095-1769.

The invasiveness of many brain mapping techniques has prevented the study of the function of human auditory cortex. A number of previous studies have used PET or functional MRI to measure responses in human auditory cortex to acoustic stimuli. However, most of these studies made use of acoustic stimuli of either language or musical context, or the subjects were required to make discriminations. The present study investigated activation of human auditory cortex in subjects who were passively listening to frequency-modulated tones with functional MRI.

Adult human volunteers with normal hearing were used as subjects. Sound stimuli were 500-ms FM tones, with 5 Hz modulation rate and deviation of 1/5 of the center frequency. FM tones were produced by a Macintosh software (SoundEdit 16, 8 bit, 22.05 kH sampling rate). Tones were presented to the subjects through tightly sealed earphones at a rate of 1 Hz. Five FM tones (at center frequencies of 250 Hz, 500 Hz. 1 kHz, 2 kHz and 4 kHz) were used in this study. The experimental series began with a rest block (no sound presentation) followed by alternating blocks of passive listening (presentation of FM tones at one of the five center frequencies). Each block lasted 25 seconds. Multi-slice functional images were acquired on a GE 3 Tesla scanner using EPI (Advanced NMR). A total of 110 images at each slice location were collected in a 4:35 time period (2.5 secs/image). The functional data were analyzed using within-subject Student's t-test of pixel intensity during lateralization vs. that during rest (not including the initial rest period). The functional data were analyzed using within-subject Student's t-test of pixel intensity during each passive listening period vs. rest period. Maps of pixels of t-statistic (t>2.66, df=68) were produced and superimposed onto the corresponding flow-enhanced image for localization of the auditory cortex.

Activation by FM tones of all the five center frequencies were demonstrated in all the subjects tested. Areas that showed significant signal increase also included superior temporal gyrus and association auditory cortex (posterior transverse temporal gyrus) in addition to the primary auditory cortex (anterior transverse temporal gyrus).

425.10

TONOTOPIC ORGANIZATION OF HUMAN AUDITORY CORTEX DEMONSTRATED WITH BOLD FUNCTIONAL MAGNETIC RESONANCE IMAGING C.L. Kussmaul[1,2], C.M.Wessinger[1,5*], M.H. Buonocore[4], G.R. Mangun[1,3] [1]Center for Neuroscience, Depts [2]Computer Science, [3]Psychology, Univ of Calif, Davis CA 95616; [4]Dept of Radiology, UC Davis Medical Center, Sacramento, CA 95817; [5]Georgetown Institute for Cognitive and Computational Sciences, Washington, DC 20007

Tonotopic organization refers to a systematic mapping of frequency within the auditory system, and can be observed beginning in the cochlea and continuing in various structures of the ascending auditory pathway to include the cortex. In the macaque monkey, microelectrode recordings have shown tonotopy in auditory cortex. Previous investigations of tonotopy in humans have used positron emission tomography and inverse dipole modeling of electric and magnetic field potentials recorded at the scalp. The present study investigated tonotopic organization in human auditory cortex using functional magnetic resonance imaging (fMRI). Functional MRI permits the direct visualization of human cortical organization without the necessity of either intersubject averaging or inverse modeling. Despite potential masking effects from the auditory environment inherent to the blood oxygenation level dependent (BOLD) technique we used, whole brain volume imaging demonstrated bilateral functional activity in individual subjects (N= 7). Furthermore, there was a tendency for high-frequency tones to produce activation more posterior and medial than low-frequency tones, especially in the left hemisphere. This is consistent with descriptions of tonotopy obtained with other methodologies. This research was supported by funding from NIMH, NINDS and the McDonnell-Pew Foundation.

425.11

CLAUSTRAL NEURONS PROJECTING TO AUDITORY CORTEX ARE ARRANGED TOPOGRAPHICALLY. M. Beneyto*, J.J. Prieto, M.L. Picó, M.L. Sala, and J.A. Winer. Department of Histology, University of Alicante, 03080-Alicante, Spain (MB, JJP MLP, MLS) and Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720-3200 (JAW).

The claustral neurons projecting to the limbic (Witter et al., 1988, *Neuroscience* **24**: 519-539), prefrontal and motor cortex (Clascá et al., 1992, *J. Comp. Neurol.* **326**: 402-422) are arranged topographically with regard to their cortical targets along the dorsoventral axis. We wondered if input to auditory cortex obeyed this principle. Auditory areas AI, AAF, PAF, VPAF, AII, SF/daz, EPD, EPI, TE and INS were injected with WGA-HRP. Three days later the animal was perfused, and frozen sections were reacted for TMB.

There was a rostrocaudal segregation of the claustrocortical neurons. Afferents to AAF were in the rostral claustrum, those innervating AI occupied the middle sections, and those projecting to PAF were caudalmost in the nucleus. There was also a dorsoventral segregation from injections in SF/daz (dorsally), through AI, EPD, AII, INS to TE (ventrally). Areas at similar dorsoventral levels, such as AAF, AI, and PAF, on one hand, and AII and EPD, on the other, preserved this dorsoventral topography within the claustrum.

We conclude that claustrocortical neurons are segregated spatially within the nucleus. This pattern precisely recapitulates, in two dimensions, the location of every cortical area on the hemisphere. Some overlap among these projections suggests that the segregation is not absolute. The claustrocortical system therefore follows similar principles in its affiliations with limbic, motor, and sensory fields and may play analogous functional roles in each.

Supported by DGICYT grant PB 93-0928 of the Spanish Government, United States Public Health Service grant R01 DC02319-16, and a personal fellowship of Generalitat Valenciana (M.B.).

425.12

DISTRIBUTION OF THE NEURONES PROJECTING FROM THE VENTRAL DIVISION OF THE MEDIAL GENICULATE BODY TO AUDITORY CORTEX IN THE RAT. F.Collia*,J.L. Lanciego, M.A. Pérez de la Cruz, J.A. Aboy,and J.A., García-Méndez.Dpto Anat. e Histol. Hum. Fac.Med.Univ.Sal. Salamanca 37007. España

The organization of the thalamo-cortical projections of the rat auditory cortex, mainly studied with degeneration or HRP techniques, has afforded information about the cortical targets of the medial geniculate body (MGB).However, data concerning the numerical proportions and distribution of neurons projecting to the different areas of the auditory cortex are lacking.

In this study, 20% HRP was injected iontophoretically into different areas of the left temporal cortex of 50 Wistar rats. After 48 hours the animals were perfused and the brain was removed and processed to visualize the neurones labeled in the MGB. The localization and extent of the areas of injection were mapped, as were the thalamic neurones projecting to the temporal cortex.Subsequent countings render the numerical distribution of labeled neurons in different territories of the ventral division of the MGB.

Our results show that neurones of the ventral division of the MGB project to the three areas of the temporal cortex(Te1,Te2 and Te3).The central area of Te1 and the rostroventral portion of Te2 receive projections from the largest number of neurons. An axial correspondence (rostro-caudal and ventro-dorsal) can be established between areas of the MGB, and the cortical divisions (Te1,Te2, and Te3)receiving projections these areas.Our results also confirm the "banded" model proposed by Cipolloni and Peters(1979) to interpret the auditory geniculo-cortical projections in the rat.

425.13

ANATOMICAL CONNECTIVITY OF THE AUDITORY FOREBRAIN IN THE BARN OWL. Y.E. Cohen* and E.I. Knudsen. Dept. Neurobiology, Stanford Univ. School of Med. Stanford, CA 94305.

The contribution of the forebrain to sound localization has been well documented in the barn owl and other mammalian species. In this study, we used retrograde and anterograde pathway tracing techniques to identify a forebrain pathway in the barn owl that is involved in sound localization and gaze control.

The results of this study indicated that auditory thalamic nucleus ovoidalis efferents terminate in a cytologically-distinct region of the primary auditory field (PAF) that corresponds to Field L of Rose. This region of the PAF, in turn, projects to adjacent regions of the PAF, located dorsally and ventrally. PAF efferents terminate in a variety of telencephalic structures, including the caudal neostriatum, the corpus striatum, and the archistriatal gaze fields (AGF). The AGF also receives input from other regions of the lateral neostriatum. Feedback from the AGF to the PAF is provided through a direct projection and an indirect projection via midline septal nuclei and the lobus parolfactorius. The AGF, in turn, projects to the optic tectum and tegmental structures involved in gaze control.

These results indicate that auditory spatial information is mediated, at least in part, by a direct and indirect projection from the PAF, a general processor auditory information, to the AGF, a structure that plays a critical role in sound localization and gaze control. A comparison of the binaural sensitivity of PAF and AGF sites suggests that AGF sites are more selective for binaural cue values than PAF sites. This transformation of spatial information appears to be comparable to the transformation that occurs in the midbrain sound localization pathway.

Supported by grants from the NIH and the NRSA.

425.14

THALAMOCORTICAL AXONS OF LORENTE DE NO: A DESCRIPTION OF AFFERENT AXONS FROM THE MGV TO LAYER I WITH DESCENDING PROJECTIONS TO LAYERS III/IV. J. S. Cetas, R.K. de Venecia and N.T. McMullen.* Dept. of Cell Biology and Anatomy, Univ. of Arizona College of Medicine, Tucson, AZ 85724.

In his seminal description of the "acoustic cortex" of the mouse in 1922 (transl by Fairen et al, 1992, Somatosens. Motor Res., 9:3-36), Lorente de No described at least three types of cortical afferent fibers that could be traced from the internal capsule and were thought to originate from the thalamus. The first two types constitute the classic categories of "specific" and "nonspecific" cortical afferents introduced in his famous chapter in Fulton's Physiology of the Nervous System (1938). A third type of afferent axon (not fully described in 1938) was unusual because it ascended unbranched to layer I and then descended to terminate within the glomerular afferent plexuses in layer IV. It was thought by Lorente de No to represent a separate route for "specific sensory impressions" to neocortex. We describe similar axons arising from the ventral division of the medial geniculate body (MGV). Focal injections of the anterograde tracers biocytin or biotinylated dextran amine (BDA) were made into the MGV of young adult rabbits. In addition to thalamocortical patches of "specific" axons in layers III/IV and tangential axons in layer I, these injections labeled a third class of axon that were reconstructed from 100 um thick serial sections. This class of thalamocortical axon ascends relatively unbranched to layer I where it turns and becomes a tangential fiber. After a variable distance in layer I, they descend into the supragranular layers, begin to branch and terminate within the "specific" thalamocortical patches in III/IV. Their morphology is remarkably similar to axons illustrated in Lorente de No's original article (Fig. 25, axon b; Fig 26, axons e and f). The significance of these axons as a parallel thalamocortical pathway for sensory information will be discussed. (Supported by NIH/NIDCD Grant 02410)

425.15

THALAMIC CONNECTIONS OF THE AUDITORY PARABELT REGION IN MACAQUE MONKEYS. T. Hackett*, I. Stepniewska and J.H. Kaas. Dept of Psychology, Vanderbilt University, Nashville, TN 37240.

As the result of previous studies, we have divided auditory cortex of macaques (*Macaca*) into a core of three primary-like areas, a narrow belt of surrounding areas, and a lateral parabelt of cortex along the upper half of the superior temporal gyrus. The purpose of present study was to determine the pattern of thalamic connections of the auditory parabelt region. Multiple injections of up to six different tracers (fluorescent dyes and WGA-HRP) were placed in the parabelt cortex in 4 monkeys. After 7-12 days survival, the brains were fixed and frozen, and thalamic tissue was cut into 40-50 mm sections. Patterns of label were related to architectonic subdivisions delineated in adjacent sections stained for Nissl, cytochrome oxidase, acetylcholinesterase, calbindin, and parvalbumin.

Patterns of dense label were found with the medial geniculate complex (MGC) and medial pulvinar (PM). The dorsal and magnocellular MGC nuclei projected most heavily to the auditory parabelt, whereas the ventral nucleus was almost free of label. Caudorostral rows of injections in the parabelt labeled rostral ventrolateral to caudal dorsomedial locations in MGNm and MGNd, and lateral to medial locations in PM. Foci of sparser label were found in the suprageniculate and limitans nuclei, as well as the peripeduncular, intralaminar nuclei, and the posterolateral part of the mediodorsal nucleus. The present results indicate that a third level of auditory processing in the auditory belt can be importantly influenced by widely projecting divisions of the auditory thalamus and the medial pulvinar. (Supported by NS-16446).

425.16

NUCLEUS SAGULUM: A POSSIBLE SUBSTRATE FOR CORTICAL CONTROL OF THE AUDITORY MIDBRAIN. J.J. Prieto*, M. Beneyto, M.L. Picó, J.A. Merchán, and J.A. Winer. Department of Histology, University of Alicante, 03080-Alicante, Spain (JJP, MB, ML, JAM) and Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720-3200 (JAW).

The sagulum is affiliated with many auditory centers, including the DNLL, IC, and MGB. However, the nature of its input from the auditory cortex is unknown. We mapped the distribution of corticofugal axons from areas AI, AAF, PAF, VPAF, AII, EPD, EPI, TE and INS. We injected WGA-HRP, and processed frozen sections with TMB as the chromogen.

AI and AII project throughout the rostrocaudal axis of the sagulum. AI labeling was moderate and concentrated in the central region of the nucleus, while the AII input was heavier and located laterally. SF/daz labeling was light, especially in caudal sections, and like the AI projection, chiefly in the central sagulum. The EPD projection was comparatively stronger and in the dorsolateral part of the nucleus. EPI had a light input situated dorsally. Finally, TE projected to the most lateral sagulum, spanning the entire dorsoventral region, while INS axons ended diffusely in the dorsolateral part.

This suggests that the secondary auditory and polymodal association areas provide the dominant cortical input to the sagulum. The sagulum may be part a hub through which the cortex propagates inhibitory influence to the IC and MGB. Such projections are independent of, yet parallel to, those from auditory cortex to IC.

Supported by DGICYT grant PB 93-0928 of the Spanish Government, United States Public Health Service grant R01 DC02319-16 and a personal fellowship from Generalitat Valenciana (M.B.).

425.17

DiI TRACING OF CENTRAL AUDITORY PATHWAYS IN THE BULLFROG. V.Kumaresan, M.Molina and A.Megela Simmons.*
Department of Neuroscience, Brown University, Providence, RI 02912.

DiI has been used to trace central auditory pathways in *Rana catesbeiana*. Adults, recently postmetamorphic juveniles (snout-vent length 2.5-4cm) and tadpoles (Gosner stages 28-43) were used. In ranids, metamorphosis involves loss of the lateral line system and acquisition of the ability to perceive airborne sounds. We have re-examined the reorganization of octavo-lateralis pathways that accompanies this change in sensory modality and have examined the presence of synaptic domains within the inferior colliculus, an area of multisensory integration. Small crystals of DiI were placed in tissue that was fixed in methanol-free,10% formalin.Tissue was incubated at room temperature in the dark. At the end of five weeks, 50 um thick sections were obtained using a vibratome, and viewed with an Olympus epifluorescence microscope equipped with a rhodamine filter. The transport pattern obtained using DiI is consistent with previous reports that have used horseradish peroxidase as the tracer.Some transneuronal dye transfer occurs in tadpoles. Placement of dye in the auditory nerve branchlets innervating inner ear sensory epithelia in tadpoles (Gosner stage 28-31) results in labeling of the lateral group of presumptive acoustic nucleus (AcN) cells in the medulla. In the midbrain, the caudal torus superior to the nucleus isthmi is labeled. In tadpoles (Gosner stage 38-43) however, there is a reduction of labeling in the caudal midbrain along with an increase of label within the principal nucleus. Initially, the central region of the principal nucleus, a low frequency sensitive region is labeled. Increased labeling of the central principal nucleus occurs concurrent with increase in label of the ventral area of the dorsally located AcN of the medulla. This suggests that there may be a changes in the projection to the midbrain concurrent with the development of an AcN that is typical to the adult structure.

NS28565(AMS) and Individual NRSA-NIDCDfellowship DC00192-02(VK).

426.1

REPRESENTATION OF ODORANT INFORMATION BY SPATIAL AFFERENT ACTIVITY PATTERNS IN THE ZEBRAFISH OLFACTORY BULB. R. W. Friedrich and S. I. Korsching[1]*. Max-Planck-Inst. für Entwicklungs-bio., Spemannstr. 35/I, D-72076 Tübingen, Germany. Present address: [1]Inst. für Genetik der Univ. Köln, Zülpicherstr. 47, D-50674 Köln, Germany.

To examine how odorant stimuli are represented within the olfactory bulb (OB) we traced olfactory receptor neuron (ORN) axons of the zebrafish *in vivo* with voltage- or calcium-sensitive dyes, thereby labeling afferent axons and glomeruli in the olfactory bulb. Neuronal activity in the olfactory bulb was then imaged with a CCD camera in a tissue explant. Experiments with voltage-sensitive dyes revealed that different classes of natural odorants elicit signals in different regions of the OB: amino acids (AAs) in a lateral neuropil region that is not parcellated into distinct glomeruli, bile acids in medial and ventral regions. Calcium-sensitive dye signals from nerve terminals were used to examine activity patterns induced by AAs in detail. Each of 17 L-amino acids tested (at 100 µM) elicits a unique pattern of foci of activity. The total number of distinguishable foci is usually >40 per fish. The degree of similarity between activity patterns correlates with the similarity in chemical structures of the odorants. Interindividually, patterns obtained with the same AA are similar. Individual foci of activity often have different response spectra and dose-response characteristics. No signal is elicited by D-Ala and Pro, indicating a high selectivity and stereospecificity of the underlying odorant receptor molecules.

These data show that olfactory stimuli are encoded within the OB by spatial activity patterns in a combinatorial fashion. A molecular and anatomical basis for this coding strategy could be the convergence of ORNs expressing the same odorant receptors onto common glomeruli in the olfactory bulb (Ressler et al., Cell 79, 1994; Vassar et al., Cell 79, 1994).

This work was supported by the Max-Planck-Gesellschaft. R.W.F. was supported by fellowships from the Boehringer Ingelheim Fonds and the Studienstiftung des deutschen Volkes. H. Baier was involved in an early phase of the experiments with calcium-sensitive dyes.

426.2

OLFACTORY RECEPTOR CELL MORPHOLOGY CORRELATES WITH SITE OF AXON TERMINATION IN THE OLFACTORY BULB. Y. Morita and T.E. Finger*. Rocky Mountain Taste & Smell Ctr., Dept. Cellular & Structural Biology, U. Colorado Med. Sch., Denver, CO 80262.

Olfactory receptor cells are bipolar neurons situated in the pseudostratified nasal epithelium. Thus, the nucleus of a receptor cell may lie nearer or farther from the basal lamina although the cell is thought to extend the full height of the epithelium. Our recent studies using dil as a postmortem retrograde tracer from injections into different sites in the olfactory bulb of catfish reveal that olfactory receptor cells do not have a uniform morphology but vary from short, superficially-situated cells to tall, slender bipolar cells more typical of classical descriptions. Further, a class of short microvillar receptor cell projects preferentially to the dorsal posterior bulb while tall ciliated receptors connect preferentially to the ventral posterior bulb. In the current experiments, dye applications to two more sites have revealed additional morphological classes of receptor cells. Following dil application to the rostral, ventral olfactory bulb, a unique class of short receptor cell is labeled. These short receptors have a distinctive apical specialization appearing as a bright elongate fluorescence perpendicular to the epithelial surface but contained within the apical part of the cell. Another unique receptor cell type was found after dil application directly to the olfactory tracts. The resulting retrogradely-filled receptor cells presumably give rise to the extra-bulbar olfactory pathway (EPOB) described by others. These EPOB receptors are broad, tall receptor cells not observed following dye application to the olfactory bulb proper. In addition, numerous labeled pseudounipolar and bipolar neurons are scattered along the olfactory nerve from the rostral tip of the epithelium up to the rostral end of the olfactory bulb. The peripheral processes of these cells enter the olfactory rosette, but the site and mode of termination could not be determined.

Supported by Grant PO1DC00244 from NIH

426.3

OCCURRENCE AND VOLUMETRIC PLASTICITY OF DISTINCT OLFACTORY AFFERENT FIELDS IN THE SALMON BULB. H.E. Jarrard*, B.B. Kang, & T.T. Takahashi, Inst. of Neurosci., Univ. of Oregon, Eugene, OR 97403.

In the adult trout, differential binding of the lectin *pokeweed agglutinin* (PWA) reveals nine biochemically and anatomically distinct projection fields in the glomerular layer of the olfactory bulb (Riddle et al. [1993] J.Neurosci. 13:3018). Using a double-labeling technique with PWA and anti-keyhole limpet hemocyanin (see Riddle & Oakley [1992] JCN 324:575), we have replicated this result in the coho salmon (*Oncorhynchus kisutch*), identifying nine regions that also differ distinctly in their PWA-binding capabilities and cytoarchitecture. In addition, we have compared the individual volumes of these fields, relative to that of the olfactory bulb (% OB), during juvenile life (1,3,9,12, and 14 months of age; n=3 at each). In preliminary results, two fields show a clear increase in % OB across these ages: anterior medial (AM) increases from a mean of 13.5 % OB (S.E. .3 %) to 22.1 % OB (.4 %) between 1 and 14 months of age, and dorsal lateral (DL) also increases from 4.1 % OB (.4 %) to 6.9 % OB (.2 %) across this period. In contrast, posterior lateral (PL) *decreases* in % OB from .88 % (.1 %) to .27 % (.009 %) between 1 and 14 months. The remaining fields, while identifiable, did not show a consistent trend. While the functional significance of specialization in these fields remains to be determined, they may represent segregation in the processing of olfactory information. Plasticity in projection field relative volume in juvenile salmon may reflect changes in the processing capabilities of the bulb, and have important behavioral implications in the phenomenon of olfactory imprinting. (Support: NIH GM07257, NSF IBN-9410637).

426.4

MORPHOLOGICAL CHANGES OF RECIPROCAL SYNAPSES INDUCED BY URINARY STIMULATION IN THE HAMSTER ACCESSORY OLFACTORY BULB. M. Matsuoka[1,2], Y. Mori[2], and M. Ichikawa*[1] [1]Tokyo Metoropoli. Inst. for Neurosci., Fuchu, Tokyo 183, Japan, [2]Univ. of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

The dendrodendritic reciprocal synapses in the accessory olfactory bulb may have important functions in the processing of pheromonal information. The effects of urine stimuli were studied on the reciprocal synapses in the accessory olfactory bulb of adult male hamsters by examining two different synaptic complexes; asymmetrical synapses, characterized by a marked postsynaptic thickening, are oriented in the polarity from mitral/tufted to granule cells, and symmetrical synapses, characterized by symmetrical pre- and postsynaptic thickenings, show the polarity from granule to mitral/tufted cells. Thirty-day old male hamsters were divided into five groups and exposed to one of following five materials: distilled water, female hamster urine, ovariectomized hamster urine, female rat urine, and male hamster urine. Each material was renewed daily. After fifteen days, the length of synaptic active zone, was characterized by the postsynaptic thickening, was measured as an indicator of synaptic size in both asymmetrical and symmetrical synapses by electron microscopy. The exposure to female hamster urine induced a reduction in the size of asymmetrical synapses, whereas that to ovariectomized hamster urine induced an increment in the size of symmetrical synapses. On the other hand, female rat urine and male hamster urine had no effect. These differential morphological changes in the reciprocal synapses can be ascribed to differing pheromonal components contained in each urine preparation.

426.5

ALTERED TRANSCRIPTION FACTOR EXPRESSION IN OLFACTORY BULB AFTER UNILATERAL NARIS CLOSURE IN ADULT MICE. E. Cigola, B.K. Jin, L. Franzen, C. Tinti, B. Conti, T. H. Joh and H. Baker*. Cornell Univ. Med. Coll. at Burke Med. Res. Inst., White Plains, NY 10605.

Sensory deprivation, produced by two months of unilateral naris closure in adult mice, resulted in a profound reduction in tyrosine hydroxylase (TH) expression in periglomerular (PG) dopamine neurons in the ipsilateral olfactory bulb. The upstream region of the TH gene contains consensus sequences for two cis-acting elements, CRE and AP-1, known to regulate TH expression *in vitro*. Previous studies demonstrated that expression of cFos, an immediate early gene which binds to the AP1 site, decreased in parallel with TH in PG neurons in the sensory-deprived olfactory bulb. The current experiments investigate regulation of transcription factor expression in the olfactory bulb *in vivo* using immunocytochemical and gel shift analyses. Two different antisera confirmed the previously reported extensive decrease in cFos expression in PG cells ipsilateral to the closure. Sensory deprivation, also produced a small decrease in FosB-positive PG cells. In the absence of novel odor stimuli, cFos and FosB exhibited differential distribution. cFos stained only PG cells while FosB labeled both granule and PG cells. Little or no change occurred in PG cell staining with antisera to CREB, phosphoCREB, CREM, cJun, JunD, FRA1 and SP4. Gel shift analysis showed that sensory deprivation did not alter the binding patterns of nuclear proteins to either AP-1 or CRE consensus sites. However, odor deprivation reduced AP-1 binding activity, as predicted by the change in cFos immunoreactivity, while protein binding to the CRE site increased. These data suggest that CRE and AP-1 sites may participate in the regulation of TH expression in the olfactory bulb. (Supported by AG09686)

426.6

APOLIPOPROTEIN E IN OLFACTORY BULB. R.G. Struble*, Z. Nie, V. Ramkumar, Alzheimer Center and Department of Pharmacology. Southern Illinois University School of Medicine, Springfield, IL 62794.

A possible function of apolipoprotein E (APOE) in the central nervous system is binding lipoproteins for processing either during degeneration or regeneration. The ability of the olfactory nerve to regenerate following an acute lesion presents an *in vivo* system for examining both of these processes. To evaluate the role of APOE in degeneration and regeneration, we initially determined the distribution and cellular locatization of APOE-like immunoreactivity in both human and rat olfactory bulb.

In human olfactory bulb APOE immunoreactivity was consistently found in processes surrounding the olfactory nerve and glomeruli. Only seldomly were somata stained, but when they were, their location was consistent with ensheathing glia. A slightly different pattern was seen in rats. APOE immunoreactivity was found both in ensheathing glia processes and also in somata of astroglia of the external plexiform layer. In normal animals, somata within glomeruli were not stained. The granule cell layer displayed little or no immunoreactivity.

To examine degeneration, we injected diethyldithiocarbamate in rats, which lesions the olfactory epithelium. In this preliminary study, APOE immunoreactivity substantially increased throughout the olfactory bulb three days after injection during olfactory nerve degeneration. Immunoreactive somata were clearly visible throughout the bulb and particularly apparent surrounding glomeruli. These observations suggest that APOE is substantially upregulated during degenerative changes following lesion of the olfactory nerve. We hypothesize that APOE is present in the olfactory bulb to support the continued degeneration and regeneration that occurs in this structure. Supported by a SIU-CRC grant to RGS.

426.7

IN VITRO MAGNETIC RESONANCE MICROIMAGING ALLOWS DETAILED RESOLUTION OF OLFACTORY BULB MICRO-STRUCTURE IN ADULT AND DEVELOPING RATS. Saied Agahi*, Essie Meisami and Paul C. Lauterbur (Dept. Molecular and Integrative Physiology & Biomedical Magnetic Resonance Laboratory, University of Illinois, Urbana, IL 61801).

We have recently reported that application of *in vivo* magnetic resonance imaging (MRI), utilizing a SISCO 4.7T/33 cm bore imaging spectrometer, permits a fair degree of visualization of rat olfactory bulb (OB) structure (Agahi et al, *Soc. Neurosci. Abst* 20, 328, 1994 & *ibid, Soc. Neurosci. Abst.* 21, 1185, 1995). To further improve resolution, we used, in the present study, an *in vitro* microimaging approach, utilizing a Varian NMR spectrometer system (11.7T/50 mm vertical bore) with a Doty microimaging probe. This method allows microimaging of a single detached rat OB (<5 mm in each dimension) with a high resolution of 35-50 μm compared to 300 μm in the *in vivo* images. The results revealed a high definition of OB's laminar organization: the mitral cell layer and some glomeruli were clearly visible, in contrast to the *in vivo* images; also the accessory olfactory bulb and its laminae were visualized. We have begun to microimage the OBs of newborn and developing postnatal rats to visualize, using this method, the changes taking place in the OB's cytoarchitecture during development, a feat which was not possible with the *in vivo* MR-imaging. In *in vitro* microimaging, the higher magnetic field and the smaller, more efficient radiofrequency coil, provide a higher signal-to-noise ratio than in *in vivo* studies, permitting smaller volume elements (voxel) and higher resolution for better visualization of OB's laminar structure. *Supp:* NSF DIR 89-20133- COOP, NIH PHS 5 P41 RR05964, Servants United Foundation, UIUC Research Funds.

426.8

CALRETININ, CALBINDIN D-28K, AND PARVALBUMIN IMMUNOREACTIVITY IN THE MACAQUE MONKEY OLFACTORY BULB. C. Crespo, J.R. Alonso*, A. Porteros, R. Arévalo, J.G. Briñón, and J. Aijón. Universidad de Salamanca, Departamento de Biología Celular y Patología, 37007 Salamanca, Spain.

The distribution and morphological characteristics of calcium-binding proteins (calbindin D-28k, calretinin, and parvalbumin) immunoreactive elements were studied in the macaque monkey olfactory bulb by using specific monoclonal antibodies and the avidin-biotin-immunoperoxidase method. A characteristic laminar pattern of immunoreactivity was observed for all three proteins.

Calretinin showed the highest number of immunoreactive elements with abundant labeled periglomerular cells and granule cells. In addition, scarce heterogeneous cells were observed in the external plexiform layer, horizontal cells in the internal plexiform layer, deep short-axon cells in the granule cell layer and fusiform cells in the white matter. The labeling for calbindin D-28k was qualitatively similar to that of calretinin in the deep layers but more scarce in all layers of the olfactory bulb. Positive neurons in the superficial layers were identified as superficial short-axon cells and superficial stellate cells. Parvalbumin-immunoreactivity revealed the lowest number of positive elements. They were identified as superficial short-axon cells and small interneurons of the external plexiform layer, granule cell layer and white matter.

This distribution of calbindin D-28K-, calretinin- and parvalbumin-immunoreactivity showed substantial differences to what has been reported in the olfactory bulb of rodents (rat, mouse, and hamster) and in insectivores, all of them macrosmatic animals.

Supported by the DGICyT (PB94-1388) and Junta de Castilla y León.

426.9

STRUCTURAL FEATURES OF INTERNEURONS IN THE GLOMERULAR LAYER OF THE RAT MAIN OLFACTORY BULB: I. CALBINDIN D28K-CONTAINING NEURONS. K. Toida*, K. Kosaka and T. Kosaka. Department of Anatomy and Neurobiology, Kyushu University Faculty of Medicine, Higashiku, Fukuoka, 812-82, Japan.

Our recent studies have revealed chemically defined subpopulations of interneurons in the glomerular layer of the rat main olfactory bulb and their heterogeneity in intraglomerular dendritic arborizations (K.Kosaka et al '95, 96). In the present study, we analyzed structural features of a group of periglomerular interneurons containing a calcium-binding protein calbindin D28k (CB-neurons). The vast majority of CB-neurons were identified as a type of the periglomerular cell by three-dimensional observations with a confocal laser scanning light microscope (CLSM) and a high voltage electron microscope, although they exhibited some variations in their dendritic arborizations.

Electron microscopic (EM) analyses of CB-immunoreactive (CB-IR) profile in and around glomeruli revealed two major types of synapses on them; CB-IR processes made symmetrical synapses onto and received asymmetrical ones from mitral/tufted cells' dendrites (M/Td), which occasionally formed reciprocal pairs. Furthermore CB-IR somata received symmetrical and asymmetrical synapses from CB-immunonegative (CB-IN) profiles, both of which contained numerous pleomorphic vesicles and the latter contained a few dense-cored vesicles. So far we encountered neither presynaptic sites on CB-IR somata nor synaptic contacts from olfactory receptor terminals (OR) to CB-IR elements.

Further EM reconstruction studies of a CB-periglomerular cell from 310 serial-sections, which was identified with CLSM beforehand, clearly indicated the followings; (1) The CB-neuron received 49 asymmetrical synapses from and made 21 symmetrical ones onto M/Td, and 11 of these synapses were reciprocal pairs. (2) It received 14 synapses from CB-IN profiles of unknown origins containing numerous pleomorphic vesicles. (3) So far no synaptic contact with OR has been observed. (4) Direct contacts with no structural specialization such as gap junctions and chemical synapses were observed between the CB-neuron and 4 CB-IR processes, which were not continuous each other within this reconstruction. The present study indicated that CB-neurons made synaptic contacts mainly with M/Td including reciprocal synapses, but not with OR.

This work was partially supported by Grants-in-Aid for Scientific Research from Japanese Ministry of Education, Science and Culture, and grants from Uehara Memorial Foundation, Mitsubishi Foundation and The Kazato Research Foundation.

426.10

THE PRIMARY SENSORY OLFACTORY PROJECTION IS DISRUPTED IN MICE LACKING THE N-CAM-180 ISOFORM.

H.B. Treloar*[1], T. Magnuson[2], H. Tomasiewicz[3] and B. Key[1]. [1]Dept. Anat. and Cell Biol., Univ. of Melbourne, Parkville, Vic. 3052, Australia. [2]Dept. Genetics, Case Western Reserve Univ., Ohio, 44106-4955, USA. [3]Dept. Anat., Emory Univ., Atlanta, GA, 30322, USA.

The recent generation of transgenic mice lacking the 180 kDa neural cell adhesion molecule (N-CAM) revealed that these mice had a grossly normal CNS apart from a smaller olfactory bulb. Because N-CAM is expressed by primary olfactory neurons (PONs) we hypothesised that this molecule is critical in the growth and guidance of developing primary olfactory axons. Previous studies using lectin staining have revealed the mosaic nature of the olfactory pathway. That is, juxtaposed neurons in the olfactory epithelium project axons to disparate sites in the olfactory bulb. We examined the organisation of the olfactory projection in mice lacking the N-CAM-180 specific exon using both immunohistochemical and lectin staining techniques. Olfactory glomeruli were smaller and irregularly shaped in mutant mice compared to the control animals. These glomeruli were not clearly separated from the underlying nerve fibre layer. Lectin staining revealed that the normal mosaic projection was perturbed in the mutant mice. A subset of axons expressing a ligand for the lectin *Dolichos biflorus* agglutinin generally innervated the correct regions of the olfactory bulb. However, the distribution of glomeruli within target regions was abberant. These results indicate that N-CAM plays a critical role in axon growth and guidance during the development of the olfactory system.

Supported by an NH&NRM grant # 950631

426.11

CALCIUM IMAGING OF MOUSE OLFACTORY SENSORY NEURONS THAT PROJECT TO DISCRETE REGIONS OF THE DORSAL OLFACTORY BULB. T. C. Bozza* and J. S. Kauer. Department of Neuroscience, Tufts University School of Medicine, Boston, MA 02111.

Previous electrophysiological and optical recording studies have suggested that there may be odorant-related projection patterns from the olfactory epithelium to the olfactory bulb. Recent molecular investigations have shown that individual glomeruli receive inputs from distributed olfactory sensory neurons that express the same or similar putative olfactory receptor mRNAs. There are no data available, however, comparing odorant responses from different olfactory sensory neurons that project to one or a few neighboring glomeruli. We are presently asking whether olfactory sensory neurons that project to neighboring glomeruli have similar odorant-response profiles. To do this, we have injected retrograde tracers into small groups of visualized glomeruli in the mouse olfactory bulb, and isolated labeled cells for physiological analysis. Small injections of fluorescent latex microspheres into clusters of 2–5 glomeruli in the dorsal olfactory bulb label sensory neurons found predominantly in the anterior dorsal recess and dorsal surface of endoturbinate IId of the olfactory epithelium. Labeled neurons have been identified in acutely dissociated preparations and responses to 3 odorant mixtures are being examined using fura-2 calcium imaging techniques. These methods will allow us to describe the functional convergence of olfactory sensory neurons and to test the idea that the molecular convergence described by others correlates with a convergence of afferents with the same responsiveness to odorants.

Supported by research grants from the NIDCD and the ONR.

426.12

FUNCTION AND MORPHOLOGY OF OLFACTORY EPITHELIUM AND BULB NEURONS IN RELATION TO THEIR PROJECTION TARGETS STUDIED BY RETROGRADE LABELING WITH CALCIUM SENSITIVE AND REGULAR FLUORESCENT PROBES. Z.Xiang*, R.Farooqui, and A.R.Cinelli. Dept. Anatomy & Cell Biol., SUNY Brooklyn, NY 11203.

In the salamander olfactory system, recent evidence shows that there is not a simple chemotopic mapping between the mucosa and the bulb. Although there is a broad topographical relationship between sectors of the epithelium and the bulb, most connections between these structures consist of complex convergent and divergent projections, and this pattern may reflect an important component for the integration of simple molecular determinants in complex odor compounds. In the olfactory bulb, mitral/tufted dendrites receiving projections from different glomeruli may gather this pattern in particular ways giving them response specificity. In the present study we have labeled retrogradely olfactory receptor neurons and mitral/tufted (M/T) cells with calcium indicators (Fura-2 and Calcium Green) and regular fluorescence probes (Lucifer Yellow and FITC) to correlate their morphology, spatial distribution and function in relation with their axonal targets. In the olfactory epithelium, receptor cells labeled from restricted bulb regions were densely located in particular epithelial regions but also found in other distal sites. In the olfactory bulb, M/T cells were labeled from different secondary olfactory region including the primordial hippocampal formation. Calcium transients evoked by olfactory nerve stimulation were recorded *in vivo* and exhibited similar characteristics and components than those obtained in slices by intracellular dye injections. These data show that this approach is highly useful not only for correlating the morphology and response characteristics of olfactory neurons with their axonal projection targets, but also to study odor response in the whole animal and the effects of centrifugal fiber and contralateral olfactory bulb activities on these responses. Supported by NIH Grant RO1-DC01804-04 and Dept. Anat. & Cell Biol., SUNY, Brooklyn.

426.13

EPITHELIUM-TO-BULB PROJECTIONS OF SALAMANDER OLFACTORY NEURONS EXPRESSING IDENTIFIED ODORANT RECEPTOR TYPES. R. Farooqui , Z. Xiang and A.R. Cinelli,* Dept. Anatomy & Cell Biol. , SUNY Brooklyn, NY 11203.

Recent evidence provides support to the notion that olfactory coding involves some degree of topographical organization. Whether similar receptor types converge to single restricted sites in the bulb are still matter of controversy. In this study, we have correlated the expression of particular receptor-specific probes in the salamander olfactory epithelium with their projection target in the bulb. Several cDNA-clones of the putative seven-transmembrane G-protein linked odor receptors were isolated using regular PCR with primers from the odorant receptor families. Digoxigenin-labeled antisense probes from these clones were used for whole mount *in situ* hybridization. Similar technique was used in the olfactory bulb, but after performing *in situ PCR* to amplify the odorant receptor mRNAs from the receptor axons. In the epithelium, most of our receptor probes were expressed in rather broad zones, but with clustered expression maps. Both dorsal and ventral epithelia were labeled, but labeling was usually more dense in the ventral epithelium. In the olfactory bulb, expression maps were also clustered. For each probe, however, there was more than a single, restricted labeled region. By correlating the expression maps of particular receptor probes with the activity patterns monitored with voltage sensitive probes (RH-795) evoked odorant stimulation, it was possible to isolate in the epithelium and the bulb a receptor probe that was related with one of the odorants. This probe was expressed in the region of the olfactory epithelium that showed stronger responses to amyl acetate. In the olfactory bulb, it was expressed in the region corresponding to one of component evoked by the same odorant. These data suggest that in the salamander olfactory system, odor compounds are encoded predominantly in distributed patterns involving several bulb locations, and specific odor receptor cells project to more than a single bulb location. Supported by NIH Grant RO1-DC01804-04 and Dept. Anat. & Cell Biol., SUNY, Brooklyn.

426.14

DEVELOPMENTAL MORPHOMETRICS OF OLFACTORY RECEPTOR AXONAL ARBORS. Jason R. Klenoff and Charles A. Greer*, Sec. Neurosurg. & Neurobiol., Yale Univ. Sch. Med., New Haven, CT 06510

The mechanisms subserving the mature distribution of afferent axonal arbors in olfactory bulb (OB) glomeruli remain unknown. Elsewhere in the CNS a common theme is early axonal exuberance followed by activity-dependent pruning. This led to the hypothesis that afferent axons in the OB may follow a similar developmental scheme. To test this hypothesis we studied morphological features of olfactory receptor neuron (ORN) axons on postnatal days 0, 3, 6, 9, 12, and 21. Sprague-Dawley rats were anesthetized, sacrificed, and the OBs processed with a Golgi-Kopsch technique that impregnated ORN axons. Twenty axons from each age were reconstructed using camera-lucida at 100X, oil immersion. Axons were evaluated for the number of branches (NB), varicosities (V), length of branches (LB), and area occupied (AO). Mean values for adults were: NB = 17, V = 9.6, LB sum = 169.67μm, AO = 797.78μm^2. The values from the younger age groups were not statistically different from those in the adult. Because our data suggest that ORN axons do not follow the hypothesized scheme, it would appear that the ORN axon, upon innervating a glomerulus, does not arborize beyond the mature limits. This argues strongly for the presence of highly specific guidance and trophic markers that the ORN employs during innervation. These data are the first to suggest that highly specific molecular markers underlie ORN arborizations rather than activity dependent mechanisms as seen in other sensory systems.
Supported in part by NIH NS10174 and DC00210.

426.15

SEMAPHORINS IN OLFACTORY NEURON AXONAL GUIDANCE, L.C. Williams-Hogarth*, X. Cai, N. Davarpanah, A.L. Kolodkin, and G.V. Ronnett. Dept. of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205 .

The olfactory neuroepithelium is a good model system for examining axonal guidance mechanisms involved in the developing nervous system. The adult olfactory receptor neurons (ORN's) have the ability to constantly regenerate during the lifetime of most animals, including rat and man. The regenerating ORN's have demonstrated the capacity to not only extend new axons, but to relocate and successfully reinnervate their target with functional synaptic contacts. What is not known, however, is whether the adult ORN axons use a mechanism for regeneration that is comparable to that used by developing ORN axons for ontogenetic pathfinding.

Semaphorins, a newly discovered gene family, have been shown to function in growth cone guidance by providing signals that guide growing axons to their appropriate destinations. A murine diffusible Semaphorin, M-sema III, has been shown to mediate inhibitory effects on the axons from a subclass of primary sensory neurons in E14 rat embryos (E.K. Messersmith et al., Neuron 14: 949-959, 1995). We localized M-sema III expression in E15 rat embryos by *in situ* hybridization techniques using a ^{35}S-labeled cRNA probe. M-sema III expression is seen in the olfactory epithelium and surrounding the nasal septum. These findings suggest the possibility that M-sema III may play a role in directing ORN's out of the epithelium and to their target structure, the olfactory bulb.

OLFACTORY SYSTEMS: INVERTEBRATES

427.1

CHRONIC RECORDING OF ODOR-INDUCED ACTIVITY IN THE PROCEREBRAL LOBE OF A TERRESTRIAL MOLLUSC. I.R.C. Cooke*, P.P. Mitra[1] and A. Gelperin[1],School of Biological and Chemical Sciences, Deakin University, Geelong, Vic 3217, Australia and [1]Biological Computation Research Department, Lucent Technologies Bell Laboratories, Murray Hill, NJ 07974, USA.

The procerebral (PC) lobes of the cerebral ganglia of limacid slugs process olfactory information detected by the superior tentacles and may be involved in the robust olfactory learning exhibited by these animals. Each PC lobe contains of order 10^5 highly interconnected interneurons and receives substantial input from primary sensory neurons and higher order olfactory interneurons located in the digitate ganglion in the superior tentacle. Isolated PC lobes exhibit spontaneous oscillatory network activity which is detected as regular oscilations in the PC field potential. These oscillations in vitro are interrupted by stimulation of olfactory afferents. In the present study we determined that oscillatory activity in the PC lobes occurs as part of the normal behaviour of intact slugs. Using general anaesthesia we implanted a pair of fine wire electrodes into the cell body layer of one PC lobe of large Limax. After allowing several days for recovery from surgery, we recorded the PC field potential from freely moving slugs. The PC field potential oscillated continuously with a frequency of 0.5-2 Hz as slugs explored their environment in the absence of strong olfactory stimuli. Abrupt presentation of strongly aversive olfactory stimuli (amyl acetate) caused substantial increases in the amplitude and frequency of the PC lobe oscillation which returned gradually to baseline levels when the stimulus was removed. New spectral analysis techniques will be used to quantify the oscillatory responses to odors.

Supported by Bell Laboratories and the Australian Research Council.

427.2

MINIMAL MODEL OF OSCILLATIONS AND WAVES IN THE LIMAX OLFACTORY LOBE WITH TESTS OF THE MODEL'S PREDICTIVE POWER. B. Ermentrout, J. Flores[1] & A. Gelperin[1]* Dept. Math., Univ. Pittsburgh, Pittsburgh, PA 15260 and [1]Biological Computation Res. Dept., Bell Labs, Lucent Technologies, Murray Hill, NJ 07974

To simulate the procerebral (PC) lobe of Limax, we created a minimal model which incorporates several known facts of PC anatomy and dynamics and assumptions about connectivity. We use a one-dimensional network of bursting (B) cells with each cell modeled as a single compartment having a persistent Ca current, a leak, and a delayed rectifier. Model B cells are coupled with graded synapses and interactions between B cells are kept fairly local. We impose an apical-basal gradient in B cell frequency by making the conductance of the delayed rectifier a function of position along the apical-basal axis. With appropriate choices of parameters the model produces regular traveling waves. The input from odor sensors is modeled as a depolarizing current applied uniformly across the PC, so that odor-elicited input shortens the apical-basal phase-lag to nearly zero, as previously measured. The apical-basal gradient of B cell frequency was tested experimentally by embedding the desheathed PC in agar and making a series of 4 150 μm slices normal to the apical-basal axis. The frequency of local field potential oscillation was highest in the most apical slice and decreased in each successive more basal slice. Visualization of cells in the oscillating slice by DIC-IR microscopy will facilitate recording from B cells and allow further tests of the PC model.

Supported by Bell Laboratories and NSF DMS-93-03706 to B.E.

427.3

SPIKE TIMING IN THE *LIMAX* OLFACTORY SYSTEM MEASURED WITH CALCIUM IMAGING IN NEURITES OF PROCEREBRAL INTERNEURONS. A. Gelperin, J. Flores & W. Denk* Biol. Comp. Res.Dept., Bell Labs, Lucent Technologies, Murray Hill, NJ 07974.

The olfactory system of the terrestrial mollusc *Limax maximus* is a model system for studies of information processing in olfactory systems which share the almost universal property of spontaneous or odor-evoked coherent network oscillations. The procerebral (PC) lobe receives direct input from olfactory receptors and displays a coherent 0.7 Hz oscillation and wave-like propagation of excitation from distal to proximal poles. Calcium changes evoked by single action potentials were measured, using 2-photon excited fluorescence microscopy, with high spatial and temporal resolution (J. Neurobiol. 30:110, 1996) in processes of bursting (B) and nonbursting (NB) PC interneurons loaded by extracellular application of Ca-green 10K dextran. B and NB processes fire at different phases of the oscillation. In addition, application of NO increases and decreases activity in B and NB cells, respectively. Individual B cell processes could be traced in the cell body layer for 10-20 soma diameters. Spike timing in B cells has a constant phase relation to the local field potential (J. Neurophys. 72:1402, 1994). Simultaneous meaurements of spike timing in adjacent B cell processes revealed differences between the 2 cells in the timing of burst onset that remained constant over many cycles. It will be interesting to see how the relative timing of activity in B cells is altered by odor-elicited inputs to the PC.

Supported by Bell Laboratories

427.4

FUNCTIONAL HETEROGENEITY WITHIN OLFACTORY GLOMERULI OF THE SPINY LOBSTER. M. Wachowiak*, C.E. Diebel and B.W. Ache, Whitney Lab and Dept. Neuroscience, Univ. Florida, St. Augustine, FL 32086.

Olfactory glomeruli in the lobster olfactory lobe (OL) are columnar in shape and consist of three anatomically distinct regions: a peripheral cap region, an underlying subcap and a proximal base. Recent studies showing that neurons can preferentially innervate one or more of these regions suggest that olfactory processing within glomeruli may be functionally heterogeneous. Here we have morphologically and physiologically characterized four classes of local interneurons involved in intrinsic processing within OL glomeruli. One class branches only in the cap region and responds to electrical stimulation of the olfactory nerve with a burst of 1 - 3 spikes followed by a slow-onset hyperpolarization lasting 0.5 - 1 sec. The cap neurons also show spontaneous, 1 - 2 Hz oscillations in membrane potential that sometimes give rise to spikes. A second class passes through the base but terminates only in the subcap, and responds to electrical stimulation with a burst of 1 - 4 spikes followed by a depolarization lasting 0.5 - 4 sec. A third class branches exclusively in the base and responds to electrical stimulation with a burst of 1 - 3 spikes followed rapidly by a hyperpolarization lasting approximately 1 sec. Finally, a fourth class innervates all three glomerular regions and responds to electrical stimulation with a burst of 1 to 20 spikes followed by a depolarization lasting 3 to 10 seconds, depending on stimulus intensity. Thus, each of the four interneuron classes has a distinct morphology with respect to the three glomerular zones and a different pattern of response to electrical stimulation of olfactory receptor cell axons. These findings confirm that the cap, subcap and base represent functionally distinct regions for olfactory processing within glomeruli of the lobster OL.

Supported by NSF-IBN9222765.

427.5

OPTICAL IMAGING OF OLFACTORY-EVOKED ACTIVITY IN THE BRAIN OF THE SPINY LOBSTER. L.B. Cohen*, M. Wachowiak and J. Fang, Dept. Physiology, Yale University, New Haven, CT 06520; Whitney Lab and Dept. Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

We have used voltage-sensitive dyes to optically record activity in the brain of the spiny lobster following electrical and odor stimulation of olfactory receptor cells. Di-4-ANEPPS (.05 mg/ml) or JPW-2081P (provided by Joe Wuskell and Leslie Loew) (.2 mg/ml) was bath applied or perfused into the brain. In the olfactory lobe (OL), the first synaptic level of the olfactory pathway, electrical stimulation evoked short-latency signals having an initial fast component followed by a slow long-lasting component. Paired pulse stimulation reduced the fast component by 30 - 50% and eliminated the slow component. Signals in the accessory lobe (AL), a second-order olfactory neuropile, were longer in latency and were sometimes expressed as a series of spike-like transients which traveled rapidly across the lobe. Similar transients were also observed in the lateral soma cluster, which contains somata of OL and AL projection (output) neurons. The transients had short latency in the region containing OL projection neuron somata and long latency in the region containing AL projection neuron somata, suggesting that signals in the soma cluster reflect the synchronized firing of action potentials in olfactory and accessory lobe projection neurons. Paired pulse stimulation eliminated all signals in the AL and soma cluster. These results agree with those obtained using intracellular recordings, confirming that olfactory processing occurs first in the OL and then in the AL. Odor-evoked signals in the OL were recorded in two preparations. While odors elicited activity across the entire lobe, the temporal pattern of activity varied slightly with spatial location and odor identity. Since activity in the lobster brain can be monitored simultaneously in three distinct regions of the olfactory pathway, this system should be useful in further studies on olfactory processing in the CNS.

Supported by NSF IBN-9222765 and NIH NS08437.

427.6

BILATERAL SAMPLING AND CHEMOTAXIS IN TURBULENCE WITH A LOBSTER INSPIRED AUTONOMOUS UNDERWATER ROBOT. F. Grasso*[1], T. Consi[2], J. Dale[1], D. Mountain[3] and J. Atema[1]. [1]Boston University Marine Program, Woods Hole, MA. 02543, [2]MIT Sea Grant, Cambridge MA 02139 and [3]Biomedical Engineering, Boston University, Boston MA 02215.

In the marine environment smooth concentration gradients that could guide an animal to an odor source over distances greater than a few centimeters do not exist on the time scale that the lobster makes its orientation decisions (Grasso, Basil and Atema 1996, Basil, Grasso and Atema 1996). We have been exploring alternative orientation strategies to concentration gradient ascent with a lobster-sized autonomous robot that uses the dynamics of turbulent odor dispersal as its sole cue to the odor source. The robot has speed and maneuverability which match or exceed those of the lobster. It senses its environment with two chemical sensors which sample the external concentration of tracer chemicals with a temporal resolution which exceeds that of lobster chemoreceptor cells. Using two algorithms which compare the signals arriving at the sensors to guide the robot we tested the effects of starting orientation, starting distance, and sensor separation on the accuracy of robot orientation in a turbulent odor plume. Depending on the performance measure: (hits, average closest approach, time to closest approach or path tortuosity) the two orientation strategies are more or less effective close (< 60 cm) to the odor source. Close to the source increased sensor separation (up to 7 cm from 1 cm) increases "hit" accuracy. This result is surprising as the lobster's primary distance chemoreceptor organs are separated by approximately 3 cm. However, farther from the source (60-100cm) all biologically reasonable sensor separations are equally poor at providing information on the direction to the source. The lobster's 3 cm sensor separation, though sub-optimal close to the source, may facilitate processing of cues at greater distances including sequential analysis of odor encounters. Experiments are now underway investigating algorithms that make use of the spatial organization of the odor dispersal dynamics in the 60-100 cm source distance range.

This work was supported by NSF award BES-9315791 to J.A.

427.7

POPULATION CODES FOR ODORS AND THEIR MIXTURES IN THE ANTENNAL LOBES AND MUSHROOM BODIES OF THE HONEYBEE REVEALED BY IN VIVO CALCIUM IMAGING.

Jasdan Joerges, Giovanni Galizia* and Randolf Menzel. Institut für Neurobiologie, Königin-Luise Str. 28-30, 14195 Berlin, FRG

The neuronal representation of odors and their mixtures in olfactory neuropiles of the bee was assessed using calcium imaging in the intact brain during natural odor stimulation. Following incubation of the brain with the cell permeant dye Calcium-Green-2-AM for 1h, fluorescence signals were recorded for up to 2h.

Odor evoked activity patterns in the antennal lobe (AL) showed the existence of distinct population codes for odors and their mixtures. Single activity centers could be related to specific glomeruli of the AL. The qualitative patterns were conserved over a wide range of odor concentrations but signal sizes increased with higher concentrations. Mixtures led to activity patterns that contain activity centers of both single components. However the patterns cannot be predicted by the linear sum of the two single component patterns, indicating nonlinear interactions between glomeruli.

The mushroom bodies (MBs) are important in the context of associative learning and memory processes. We recorded odor specific patterns in the the MBs with the same techniques. Activated regions were more spatially restricted compared to activity patterns in the AL, and the time courses of the signals were faster. This may be due to recurrent inhibition within the MBs. In some cases signal size increased during repetitive stimulation indicating possible effects of neuronal plasticity.

Supported by DFG Leibniz award no. Me 365/16-1 to RM.

427.8

DIFFERENT INSECTS EMPLOY SIMILAR OLFACTORY PROCESSING MECHANISMS. M. Stopfer* and G. Laurent, Division of Biology, 139-74, California Institute of Technology, Pasadena, CA 91125.

Presentation of non-pheromonal odorants evokes synchronous neural activity in the locust (*Schistocerca americana*) brain. This coherent activity can be detected extracellularly as large amplitude, oscillating local field potentials in the mushroom bodies, and intracellularly as oscillating membrane potentials in Kenyon cells and antennal lobe (AL) projection and local interneurons. We have now observed several key features of the locust olfactory response in the cockroach (*Periplaneta americana*) and honeybee (*Apis mellifera*) as well.

Odor-evoked oscillating field potentials recorded extracellularly from the alpha-lobes of both cockroach and honeybee reveal, as in the locust, the presence of synchronous, coherent neural activity. Furthermore, intracellular recordings from antennal lobe neurons of the cockroach reveal olfactory response patterns similar to those observed in the locust in two interesting respects. First, individual neurons tend to respond to more than one odorant, suggesting that specific odor encoding is achieved by an overlapping ensemble mechanism. Second, a given neuron often responds to different odorants with different firing patterns, suggesting that slow temporal dynamics play a role in odor encoding.

To further characterize the cockroach olfactory apparatus, AL neurons were visualized by intracellular cobalt fills. Neurons responding to odorants arborize broadly to a dozen or more glomeruli. The suitability of these insects for combined cellular and behavioral experiments will enable a mechanistic analysis of odor perception and memory.

Supported by an NSF-PFF and an NSF grant to G.L.

427.9

Inhibitory Mechanisms Underlying Oscillatory Synchronization of Projection Neurons in the Locust Olfactory System. K. M. MacLeod* and G. Laurent, Division of Biology, 139-74, California Institute of Technology, Pasadena, CA 91125.

Odors appear to be represented in the locust (*Schistocerca americana*) olfactory system by specific but overlapping ensembles of synchronously firing neurons causing fast, odor-evoked oscillations that can be recorded in local field potentials (LFP) from the mushroom body (MB). We showed that the GABA antagonist picrotoxin (PTX) can block these fast oscillations when pressure injected into the antennal lobe (AL), but not the MB (MacLeod and Laurent, 1995, Soc. Neurosci. Abstr. 58.10.) Electron microscopy and GABA-like immuno-gold labelling show reciprocal synaptic connections between GABA-positive and -negative profiles (Laurent and Leitch, J. Comp. Neurol., in press.) These data together suggest that the LFP oscillations are the result of synchronous action potentials from the projection neurons (PN) of the AL, whose synchronization arises from reciprocal interactions between the PNs and the non-spiking, inhibitory, local interneurons (LN) in the AL. Studies in other olfactory systems and in the visual system of insects have shown that histamine may also mediate post synaptic inhibition. We attempted to better characterize the nature of inhibitory interactions in the locust AL by using known GABA-antagonists (PTX, bicuculline, and gabazine (SR95531)) or histamine antagonists (cimetidine, pyrilamine) and testing their effectiveness in eliminating synchronization. PTX was found to effectively reduce the power of the LFP oscillations at the lowest concentrations (0.1mM in bath). Gabazine reduced oscillations at higher concentrations, but seemed to slightly enhance them at lower, while bicuculline was completely ineffective. The histamine antagonists were effective at 10-fold higher concentrations than PTX (cimetidine > pyrilamine.) While PTX abolished synchronization of PN firing, it did not greatly affect their slow temporal firing patterns whose modulation thus appears to be under the control of different synaptic mechanisms than those underlying synchronization. *Supported by NSF-PFF award and NSF grant to GL.*

427.10

ANALYSIS OF ODOR-EVOKED TRANSIENT SYNCHRONIZATION OF PROJECTION NEURONS IN THE LOCUST OLFACTORY SYSTEM. Michael Wehr and Gilles Laurent,* California Institute of Technology, Biology Division, Computation and Neural Systems Program 139-74, Pasadena, CA 91125.

Fast odor-evoked field potential oscillations in the mushroom bodies of the locust result from the formation of odor-specific ensembles of coherently firing projection neurons in the antennal lobe (Laurent & Davidowitz 1994, Science 265:1872-1875). We recorded simultaneously from small (2-5) ensembles of projection neurons in addition to the mushroom body field potential *in vivo*, using extracellular electrodes. Subsets of projection neurons transiently synchronize their firing to each other and to the mushroom body field potential, at precise and odor-specific epochs of the response to an odor. We have quantified this synchronization and developed methods to analyze the statistical significance of these synchronized patterns and of the correlation functions calculated from simultaneously recorded spike trains. Because synchronization provides a means to encode signals via time and phase delays, we are currently investigating the stimulus specificity of phase relationships between synchronized projection neurons and between these neurons and the mushroom body field potential. Preliminary evidence suggests that phase relationships between synchronized neurons may vary in a stimulus-specific fashion. *Research supported by an NSF-PFF and NSF grant to GL.*

427.11

OPTICAL RECORDING OF EVOKED ACTIVITY IN NEURAL CIRCUITS OF THE COCKROACH ANTENNAL LOBE USING A VOLTAGE-SENSITIVE DYE. J. Inouchi* and H. Ai Lab. of Neurophysiology, Nat'l. Inst. Sericul. and Entomol. Sci., Tsukuba, Ibaraki 305, Japan.

Optical recordings with high spatial (128x128 photodiodes) and temporal (0.6 ms) resolution of transmembrane activities were used to characterize the properties of the electrical signals in the antennal lobes (ALs) of the adult cockroach (*Periplaneta americana*) *in vivo*. After electrical stimulation of the antennal nerve (AN), activity spreads centripetally from the sites of synaptic input to generate nonhomogenous response patterns that are presumably mediated by circuits within the glomerular organizations. The activities showed a characteristic sequence of depolarizing and hyperpolarizing responses both at macroglomerular (MG) and ordinary glomerular (OG) area. Increasing the intensity of stimulation enhanced the size and duration of the depolarizing and hyperpolarizing responses. The size of the MG responses was always larger than that of OG responses. The optical events were correlated with field-potential waves recorded simultaneously at MG and OG regions, respectively.

These optical signals were practically abolished after 1μM TTX application on the AL. A low Ca^{2+} (1mM), high Mg^{2+} (50mM), in the Ringer solution reduced the depolarizing and hyperpolarizing activities. The depolarizing activities consist of a fast and a slow component. The low Ca^{2+} medium suppressed the slow component of the activity. This suggests that the fast component is a fast presynaptic response representing synchronous compound action potentials from AN fibers and the slow one is a postsynaptic response representing synchronous compound EPSPs and action potentials from neurites of AL neurons. A $GABA_A$ receptor antagonist, picrotoxin (1μM), enhanced the size and duration of the depolarizing components, and the opposite effect was observed in the hyperpolarizing components. Probably, inhibitory actions of local interneurons in the AL contribute to the regulation of these depolarization.

Supported by a Grant-in-Aid from MAFF, Japan.

427.12

SEX-SPECIFIC AND TEMPORAL ANALYSIS OF OLFACTORY CELL BIRTH IN THE ANTENNA OF THE MOTH MANDUCA SEXTA : SPECIFIC MITOTIC PATTERN. M. D. Franco, J. P. Poppy, P. Pierret and R. G. Vogt* University of South Carolina, Columbia, SC 29201 and Departement de Pathologie, Universite de Montreal, Montreal, Quebec, Canada*

The olfactory epithelium of the male moth *Manduca sexta* expresses two predominant classes of sensillum; the long and the short hairs. The long hairs contain the pheromone binding proteins (PBPs) and the short hairs contain one of two classes of general odorant binding proteins (GOBP1 or GOBP2). Female antennae have only short hairs which contain primarily the GOBP1 and GOBP2 proteins. These odorant binding proteins (OBPs) are expressed in the support cells of the sensillum and are released into its lumen. The olfactory cells associating with a sensillum derive from a common sensory mother cell early in the adult development. After a series of cell division, the mother cell gives rise to its progeny: support cells and neurons. Using an *in vivo* labeling of DNA synthesis, we have studied the mitotic activity of the epithelial cells during the larval and adult antennal development. fWe have shown sex-specific and temporal patterns for the birth of PBP and GOBP cells in the pupal antenna. In both male and female, birth of the olfactory cells occurs at stage 3 (preapolysis) in the pupal development. However, the pattern of expression is different: two stripes per annulus for the male and homogeneous pattern for the female, thus corresponding respectively to the morphological organization of PBP and GOBP sensory hairs in the adult. The termination of mitotic events is different for the two classes of sensory hairs in the male: long hair mitoses end by stage late-5 (post apolysis) while short hair mitoses end at stage 6 after the long hairs have begun to form. In contrast, in the female antenna, the mitoses which give rise to the short hairs ends 1 day earlier than in the male, by stage medium-5 (post apolysis). All together, these results suggest a differential spatio-temporal development in the birth of the sensory cells in the male and female antennae of the moth *Manduca sexta*. Support: NIH NIDCD DC-00588

427.13

EFFECTS OF ABNORMAL DEVELOPMENT ON THE ANATOMY OF THE PHEROMONE PROCESSING CENTER OF MALE MOTH BRAINS AND THE BEHAVIOR IT SUPPORTS. M.A. Willis*, J.D. Floyd, R.S. Kellar and L.P. Tolbert. ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Virtually all vertebrates and higher invertebrates have the first synapses between olfactory receptor cells and central neurons organized into discrete spherical knots called glomeruli. In some animals, a small subset of glomeruli are specialized for processing information about specific social odors, pheromones. In a recent study we have focused on one such group of specialized glomeruli, the macroglomerular complex (MGC), which is responsible for processing information about the female sex-attractant pheromone in the male moth *Manduca sexta*. Previous studies have indicated that the degree of glomerulus formation can be affected by surgically reducing the number of sensory axons growing from the antennae into the antennal lobes of female brains during metamorphosis. When similar experiments are done on developing male moths, a distorted neuropil develops in the area normally occupied by the MGC. These surgically manipulated animals, possessing a very distorted "MGC-like" structure, have been shown to be capable of exhibiting pheromone-modulated behavior in a manner similar to that observed in normally developed moths. This suggests that the normal MGC structure is not necessary for processing reception of the female pheromone. It is possible that the MGC-specific olfactory neurons are still forming functionally specific synapses in the MGC region despite the anatomical disruption of the region. We have tested this hypothesis using florescent labeling to trace male-specific olfactory axons to their destinations. Our results reveal that, even though the gross anatomy of the MGC is disrupted, the pheromone-sensitive receptor neurons continue to form MGC-like projections in the antennal lobe. [This study was supported by NIH grants NS-20040 and DC-00348, and NSF grant IBN-9216532.]

428.1

HEAT-SHOCK REDUCES THERMOSENSITIVITY OF NEURAL PARAMETERS IN THE LOCUST FLIGHT SYSTEM J. R. Gray* and R. M. Robertson. Department of Biology, Queen's University, Kingston, Ontario, CANADA K7L 3N6.

Pretreatment of tissues or whole animals with high, sublethal temperatures (heat-shock) induces thermotolerance to normally lethal temperatures. Thermotolerance can be manifested as increased survival or maintenance of physiological processes. We are interested in whether heat-shock induces protection of neuronal function at normally lethal temperatures. To this end we have investigated effects of heat-shock on the temperature sensitivity of neuronal parameters in the locust flight system.

We measured the rhythm frequency of the deafferented flight motor as well as the conduction velocity and amplitude of extracellularly recorded action potentials conveyed along the forewing stretch receptor axon. Measurements were made at temperatures ranging from 15°C to 50°C in heat-shocked and control animals.

The deafferented rhythm was less sensitive to temperature changes above 35°C in heat-shocked animals. The conduction velocity and relative amplitude of action potentials conveyed along the stretch receptor axon were less sensitive to temperature increases above 20°C in heat-shocked animals.

These data suggest that heat-shock conserves the operation of the flight system at high temperatures. This may be accomplished by a decrease in the thermosensitivity of the conduction velocity and amplitude of action potentials within the central flight circuitry. The latter effect may serve to protect synaptic interactions and thus allow the circuitry to operate within optimal parameters.

This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to RMR.

428.2

NEURAL SEGMENTS SPECIALIZED FOR SEXUAL BEHAVIOR: COMPARISON OF MALE AND FEMALE GRASSHOPPERS. K.J. Thompson* and H.R. Campbell. Dept. of Biology, Agnes Scott College, Decatur, GA 30030.

In a continuing study of the neural basis of dimorphism in sexual behavior of male and female grasshoppers, motorneurons (MNs), efferent dorsal unpaired median neurons (DUMs), and DUM interneurons are being studied. In females cobalt backfills of the 8th tergal nerves stain 24 MNs in the 7th abdominal segment (A7) including 8 ovipositor closer MNs, and 5 contralateral MNs in A8 including 3 ovipositor protractor MNs. The identical pattern of MNs is present in stains of males, with the matched 8 MNs in A7 supplying the male's median internal ventral muscle (247) and the matched 3 contralateral MNs in A8 supplying the male's paradorsal muscle. EMG recordings from male muscle 247 reveal a bursting pattern of activity that is elicited by the same experimental procedures that elicit the oviposition motor pattern in females. Backfills of the 8th sternal nerves of females stain MNs in 2 ipsilateral clusters in A8. The anteriormost cluster of 7 MNs contains the ovipositor retractor MNs and the more posterior group contains the 5 ovipositor opener MNs group. In addition, 6 efferent DUMs send axons to the ovipositor opener muscle, and one also supplies the ovipositor retractor. Male MNs backfilled from the sternal nerve fall into clusters similar to the female pattern, but with several of the male MNs notably smaller. The male's sternal nerve contains the axon of only 1 efferent DUM neuron. In both sexes neural segment A8 also contains the cell bodies of DUM interneurons. Their number is approximaltely 22 in females and 3 in males. The DUM neurons thus show dimorphism of cell number and of primary neurite structure in contrast to the minimal differences discovered when MNs are compared.

Supported by Whitehall #AF94-01 and National Science Foundation #IBN-9514559.

428.3

LEG DESIGN AND RESPONSE SPECIFICITY OF CUTICULAR STRAIN DETECTORS. S. N. Zill*, R. A. DiCaprio and S. F. Frazier. Dept. Anat., Marshall Univ. Sch. Med., Huntington, WV 25755 and Dept. Biol. Sci., Ohio Univ., Athens, OH 45701.

As part of a project to incorporate principles of sensory-motor integration into the design of legged robots, we are studying the morphological and physiological properties of the cockroach trochanteral campaniform sensilla. These sense organs are the largest collection of force sensitive mechanoreceptors in the limb and provide critical information for the control of walking. Our hypothesis is that the receptors act as arrays of strain gauges encoding stresses from leg loading and muscle contractions. We have begun testing the effects of load by applying bending forces to the leg, both in the plane of joint movement and in planes perpendicular to the joint axis, while monitoring sensory activity extracellularly. Loads applied in any of these planes produce discrete patterns of discharges that may encode the direction and magnitude of leg loading in the firing of individual or subgroups of sensilla. We also are examining whether the responses of sensilla are delineated by their location relative to muscle insertions and the architecture of the exoskeleton. We have assembled 3D reconstructions of the anterior groups of sensilla (Groups 2-4) from confocal images of DiI filled sensory neurons and have generated isocontour projections of the cuticular walls of the trochanter. The exoskeleton has a system of thickenings or buttresses with specific orientations relative to the principal sources of strain. These include a dorso-ventral buttress (perpendicular to the trochanteral long axis and distal to the Group 2 sensilla) which is linked to a cuticular 'gasket' at the trochanteral flexor muscle insertion, surrounding Group 3. We are extending these experiments to determine the specific information that individual sensilla provide during the interactions of internal and external forces that occur during walking.

Support: ONR N00014-93-1-0088 and N00014-96-1-0694.

428.4

ANALYSIS OF PROTHORACIC LEG MOVEMENT DURING WALKING AND CLIMBING IN THE COCKROACH. J.T. Watson*, A.K. Tryba and R.E. Ritzmann. Department of Biology, Case Western Reserve University, Cleveland, OH 44106.

Our laboratory is involved in collaborative efforts to design robotic systems based upon the neuromuscular and mechanical properties of arthropods. The current robot design is intended to include agile movements that enable turning and climbing over complex terrains. Observations that we have made on freely behaving cockroaches (*Blaberus discoidalis*) suggests that the movements of the prothoracic (T1) legs are crucial to directing and executing these types of movements.

During walking movements, specific joints of the T1 leg are different from those in the T2 or T3 legs. Extension of the T1 femur-tibia (FT) joint occurs during swing phase, preceding the coxa-femur (CF) joint by about 40 msec. This difference is also seen in the EMG records. The slow extensor of the tibia (SETi) fires a high frequency burst in antiphase with the slow depressor of the femur (Ds). A low frequency burst in SETi occurs during FT flexion and coincides with Ds activity. These movements of the CF and FT joints occur along with a rotation of the coxa around the thorax-coxa (TC) joint.

The animal also takes advantage of the mobility of the T1 legs during climbing. It typically rears up on its hind legs by rotating the TC joint of the T2 leg. The T1 legs then reach up to seek a foothold and the T3 legs extend to launch the climb up the obstacle. Electrically, Df activation in the mesothoracic leg is associated with the rearing up phase, whereas high frequency activity in Ds is associated with the actual climb. It appears that once the prothoracic leg has located the goal, the T2 and T3 legs simply use a follow-the-leader strategy to reach the same location.

Supported by ONR grant N00014-90-J-1545.

428.5

SIMULATED DYNAMICS OF WALKING AND CLIMBING IN THE COCKROACH. G.M. Nelson, R.D. Quinn, J.T. Watson, A.K. Tryba, R.E. Ritzmann, H.J. Chiel, R.D. Beer*. Departments of Mechanical Engineering, Biology, and Computer Science, Case Western Reserve University, Cleveland, OH 44106.

We are designing and constructing biologically based robots. Our previous robots have demonstrated that neurobiologically inspired control systems can generate a range of movements over moderately rough terrain and slatted surfaces. The goal of our current research is to develop a robot that is more closely patterned on detailed observations of cockroach locomotion (see abstract of Watson, Tryba and Ritzmann).

We have developed a dynamic simulation tool that uses digitized video images of walking and climbing cockroaches to estimate joint torques and ground reaction forces. Our simulation indicates that straight walking on a level plane requires front and middle legs with three degrees of freedom each, and hind legs with two degrees of freedom. However, because we want this robot to achieve climbing capabilities similar to those of cockroaches, we have also simulated video records of climbing animals. Preliminary analysis of these data suggest that the front and middle legs require an additional degree of freedom. Ongoing investigations are demonstrating the degree of motion at each joint for both running and climbing, and providing insight into the power requirements for each joint in order to size joint actuators.

The simulation is also capable of integrating electrical records that have been recorded in conjunction with the digitized kinematics. These data can then be used to relate electrical activity to joint torque during complex motion in order to generate appropriate control algorithms.

Supported by ONR grant N00014-90-J-1545.

428.6

THE EFFECT OF NOISE ON SIGNAL DETECTION AND TIME PRECISION IN A PARALLEL NEURAL NETWORK. X Pei[1]*, L. A. Wilkens[2] and F Moss[1, 2]. Center for Neurodynamics, Depts. of Physics[1] and Biology[2], Univ. of Missouri-St. Louis, St. Louis, MO 63121.

Noises are present in many sensory nerve systems, induced internally or externally. It has been demonstrated recently that noise can improve the coherence of the responses when stimulus amplitudes are below a threshold, a process known as *Stochastic resonance* (SR). How can nerve systems benefit from such noise enhanced coherence? This question arises frequently, because it is difficult to understand how an actual system can make use of the improved coherence. Here we demonstrate that SR can improve the detection performance for aperiodic signals in a parallel neural network. A two-layer network is constructed to simulate the mechanosensory system in the crayfish tail fan. The first layer contains 1000 neurons which mimic the hair cells. The second layer contains two neurons which evaluate the output from the first layer and generate responses to detected inputs. Uncorrelated noise is added to each cell of the system. For subthreshold signals, the noise-response function shows a peak at an optimal noise level. The probability of signal detection improves in the presence of noise. Also, the time precision of the spike response to the inputs is improved. For supra-threshold signals, adding noise to the system only degrades the performance of the system, that is, the detection probability and the time precision show only a monotonous decline. In conclusion, a system containing elements which process inputs in parallel can improve its performance by adjusting the internal noise level to an optimum.

Research supported by the Office of Naval Research

428.7

STARTLE RESPONSE NEURAL SYSTEM AND ITS CHOLINERGIC ACTIVATION BY CEREBRAL INTERNEURONS IN THE MOLLUSC, *CLIONE LIMACINA*. T.P. Norekian* and R.A. Satterlie. Dept. Zoology, Arizona State Univ., Tempe, AZ 85287 & Friday Harbor Labs, Friday Harbor, WA 98250.

The holoplanktonic pteropod mollusc *Clione limacina* exhibits an active avoidance behavior, or escape swimming, which includes an initial, high-speed startle response that is followed by a period of fast swimming. The startle response is controlled by a pair of large pedal motoneurons with very low membrane potentials and extremely high thresholds for spike generation, whose activity is independent of the normal swim pattern generator. Each Startle motoneuron directly innervates ipsilateral swim musculature in the wings: one motoneuron induces contraction of dorsal swim musculature and dorsal flexion of the wings (d-phase), and the other motoneuron produces ventral flexion of the wings (v-phase). Two interneurons have been identified in the cerebral ganglia (Cr-St), which produce strong activation of the ipsilateral d-phase Startle motoneurons. Each spike in a Cr-St neuron produces a fast, high amplitude (up to 50 mV) EPSP in Startle motoneurons. This 1 spike:1 EPSP ratio and the stable short synaptic latencies (2 ms) persist in high Mg^{++}/high Ca^{++} sea water suggesting monosynaptic connections. Synaptic transmission between Cr-St interneurons and Startle motoneurons appears to be cholinergic. 10 μM atropine reversibly blocks EPSPs in Startle neurons. Hexamethonium and d-tubocurarine partially block EPSPs, although much higher concentrations are required. Exogenous acetylcholine (1 μM) produces a dramatic depolarization of Startle motoneurons in high Mg^{++} sea water. Cr-St interneurons and Startle motoneurons are also electrically coupled, however the coupling is weak. Stimuli which are known to initiate escape responses in intact animals, such as tactile stimulation of the tail, produce excitatory inputs to Startle motoneurons and Cr-St interneurons. This study was supported by NIH grant NS-27951 and NSF grant IBN-9319927.

428.8

REFLEX ACTIVATION OF PNEUMOSTOME MOTOR NERVES IN THE PULMONATE SNAIL, *Helix aspersa*. E.M. Adams*, W.J. Benedict and D.M. Shope, Dept. of Biology, Univ. of Scranton, Scranton, PA 18510-4625.

Helix aspersa possesses a single lung in which gas exchange takes place. The lung opening is regulated by muscles innervated by neurons exiting the visceral and right parietal ganglia in three nerves: the anal (AN), medial right parietal (RPNm), and lateral right parietal (RPNl) nerves. This study assessed the reflex activation of these nerves. An isolated ganglion-pneumostome preparation was used to study (1) the rate of increase (dV/dt) of nerve activity and (2) the stimulus-response latency in each nerve in response to electrical stimuli applied individually to AN, RPNm, RPNl, or intestinal nerve (IN). Whole nerve recordings were obtained from electrodes placed on the nerves. Subsequent micro-electrode mapping indicated that many cells having axons travelling in the RPN were located in the right parietal ganglion at the root of the nerve. Our results indicate that while reflex activation of RPN was possible, IN stimulation produced the greatest increase in activity and shortest latency in each of the three pneumostome nerves. Among the pneumostome nerves, RPNl activity was increased more than in either RPNm (-50%) or AN (-69%). Stimulus-response latency was also shortest between IN and RPNl and longer in both RPNm and AN. An inverse relationship was noted between latency period and magnitude of nerve response. (Supported by a grant from HHMIG.)

428.9

SYNAPTIC MECHANISMS ASSOCIATED WITH REFLEX ACTIVATION OF MOTONEURONS IN HERMIT CRAB ABDOMINAL NERVOUS SYSTEM. William Chapple*,Dept. Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269-4156.

Two prominent features of the reflex activation of ventral superficial muscle motoneurons in the fourth segment of the hermit crab abdomen are its complex phasic response and its adaptation. Intracellular recording from several of these motoneurons indicates that the mechanisms for these features are probably synaptic. The identity of the motoneurons was confirmed by antidromic stimulation of the motor root, and by a 1:1 association of motoneuron spikes with extracellular potentials in the motor root and EJPs in the muscles, both during tonic firing and during activation of the motoneuron by depolarizing current. Electrical stimulation of mechanoreceptor afferents in the first ganglionic root produces a complex synaptic potential consisting of a short latency (15 ms) EPSP followed by a longer latency (50 ms) IPSP; both are abolished by 10^{-3} M curare. Both components increase with stimulus intensity and habituate at frequencies greater than 1 Hz. A third component composed of a longer latency (200 ms) low frequency train of EPSPs was not abolished by curare.

Supported by a grant from the University of Connecticut Research Foundation

428.10

INVERTEBRATE ELECTROSENSATION: PASSIVE ELECTRORECEPTION MEDIATES PREY DETECTION IN THE CRAYFISH. M.J. Weissburg, S.J. Levett, P. Steullet, D.H. Edwards, and C.D. Derby*, Dept. of Biology, Georgia State Univ., Atlanta, GA, 30303.

The ability to detect weak electric fields is a sensory capability previously known only in vertebrates (*e.g.* sharks and rays, bony fish, urodeles, and monotremes). Here, we report the first observations of responses to weak electric fields in an invertebrate, the crayfish *Procambarus clarkii*. Behavioral observations indicate that crayfish can detect dipole fields that are similar in intensity to fields produced by small freshwater animals. Field strengths as low as 100 μV/cm elicit local search and intense digging and grabbing behaviors at the electrodes. These behaviors are not seen in the absence of the field. The percentage of animals grabbing or digging increases with field strength to reach a level of about 80% at field strengths near 300 μV/cm. Animals are more sensitive to low frequency (1-4 Hz) than high frequency (10 Hz) stimulation. Observations indicate that the first two pairs of walking legs must be in close proximity to the electrodes for a response to be evoked. Initial physiological evidence suggests the presence of electrosensitive neurons in these appendages. Data from single unit extracellular recordings show that the firing rates of some neurons can be modulated when stimulated by dipole fields of 100-400 μV/cm. These responses show graded increases or decreases in firing rate with increasing field strength, and are sensitive to field polarity. We suggest that electrosensitivity in crayfish functions in a similar context to passive electroreception in other animals, namely as a way to locate cryptic prey.

Supported by NSF IBN-95 14409

428.11

ELECTRICAL ACTIVITY EVOKED AT THE CRAYFISH BRAIN IN RESPONSE TO VISUAL STIMULATION WITH MOVING BARS. [1]Oscar H. Hernández, [2]Jesús Serrato, [3]Jesús Hernández-Falcón, and [4]Fidel Ramón*. [1]Ctro. Inv. Enf. Tropicales, Campeche, Camp., [2]Depto. Fisiol. Biof. & Neurosci. CINVESTAV del IPN, Depto. Fisiol., Fac. Med., UNAM, and [4]Div. Posgrado, Fac. Med., UNAM, Mexico.

We are studying characteristics of the electrical activity evoked at the brain of crayfish chronically implanted with extracellular electrodes. Animals were placed in a chamber with the eyes above the water level to avoid the air-water interface. Stimuli were black and white bars of various widths, angles and speeds, moving linearly across clear or dark backgrounds. In agreement with previous results by Wiersma and Yamaguchi (1967), short trains of spikes were recorded in response to leading and trailing edges of black bars wider than about 4° at a distance of 30 cm. Some combinations of bar characteristics and speed elicited delayed electrical activity and animal movement. Electrical activity was also recorded in response to moving black squares as small as 2 cm^2. White bars against a clear background do not produce responses. Results suggest that under these worst-case conditions, crayfish brain activity can be evoked by moving objects larger than about 3° at a speed of about 25°/sec. Supported by: Prog. Div. Posgrado No. 359.

428.12

A STUDY OF INTERACTIONS BETWEEN OLFACTION AND TASTE IN CENTRAL NERVOUS SYSTEM OF A SLUG. A. Yamada*, T. Kimura, A. Iwama, Y. Suzuki[1], S. Kawahara[1], Y. Kirino[1] and T. Sekiguchi. Tsukuba Research Center, SANYO Electric Co.,Ltd., Tsukuba, Ibaraki, 305 JAPAN, [1]Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo, 113 JAPAN.

To understand the mechanisms of learning and memory which results from integration of olfaction (conditioned stimulus) and taste (unconditioned stimulus), the investigation of interactions between these two senses is important. The procerebral (PC) lobe, an olfactory center of the terrestrial slug *Limax marginatus*, exhibits intrinsic oscillations in its field potential. Olfactory input from the nose modulates the frequency or the amplitude of the oscillations. On the other hand, gustatory input from the lip generates bouts of feeding motor program (FMP) in the buccal nerves. In this study, we made morphological and physiological investigation into interactions between these two senses.

In morphological study (Co++ backfill through three lip nerves), we observed dense stain on the mesocerebrum and metacerebrum, but no stain on the PC. On the other hand, when we applied solution of quinidine sulfate to the lips in cerebral-buccal-lips preparation, the frequency of the PC oscillation increased. Even if we applied taste solution to one lip, there was an increase of frequency in both ipsilareral and contralateral PC. The modulation occurred in the preparation with and without buccal ganglia, and started at the same time as the FMP. Electrical stimuli to a buccal nerve which triggered FMP generated no modulation of the PC frequency. These results suggest that there are parallel pathways which share a common initiator, and that the integration of olfactory and gustatory information occurs within the cerebral ganglion.

The present study was supported by Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

428.13

TEMPORAL RESOLUTION OF GENERAL ODOR PULSES BY OLFACTORY SENSORY NEURONS IN AMERICAN COCKROACHES. W.C. Lemon* and W.M. Getz. ESPM - Div. of Insect Biology, Univ. of California, Berkeley, CA 94720.

Behavioral and physiological evidence indicates that insect pheromone receptor cells respond to pulses of pheromone concentration as they occur down wind from a point source, but the abilities of insect sensory neurons sensitive to general food odors to respond to pulses of odor are unknown. Temporal response characteristics of olfactory sensory neurons of female American cockroaches *Periplaneta americana* in response to general odors were measured using series of short odor pulses (1.2 - 50 Hz). Odor pulses were delivered to olfactory sensilla in a moving airstream controlled by electromagnetic valves. The responses of sensory neurons were recorded with a tungsten electrode placed at the base of the sensillum. The temporal responses of sensory neurons mirrored the temporal changes in stimulus concentration, which was estimated by replacing the odorant with oil smoke and measuring the concentration of smoke passing through a light beam. Spike frequency varied with odorant concentration with surprisingly fine temporal resolution. Cockroach olfactory sensory neurons were able to reliably follow 40 Hz pulses of a pure odorant (1-hexanol) and a complex odor blend (coconut). Lower concentrations of odorants elicited responses with lower peak spike frequencies that still retained the temporal resolution of the stimulus pulses. Thus, responses of olfactory sensory neurons can reflect fine structures of non-uniform distributions of general odorants in a turbulent odor plume and not only the average concentration. Supported by UC Experimental Station.

428.14

MEASUREMENT OF NEURAL ACTIVITIES IN AN ANTENNAL LOBE OF A MOTH BRAIN BY HIGH-SPEED, VOLTAGE-SENSITIVE DYE IMAGING. K. Okada*[1], R. Kanzaki[2], and K. Kawachi[1, 3]. 1)Kawachi Millibioflight Project, ERATO, JRDC, Tokyo 153, Japan. 2)Inst. of Biol. Sci., University of Tsukuba, Tsukuba, 305, Japan. 3)Res. Cent. for Adv. Sci. and Techno., University of Tokyo, Tokyo 153, Japan

We have improved an optical recording technique with high spatio-temporal resolution in order to investigate neural activities of an in vivo insect brain. We succeeded in acquiring neural activities of an antennal lobe neurons of a sweet potato hawk moth, Agrius convolvuli, elicited by an electrical antennal stimulation under a stereoscopic microscope condition. When the synaptic transmission was not blocked, hyperpolarization followed by depolarization were consistently recorded in the antennal lobe. While synaptic transmission was blocked by replacing the Ca^{2+} with Mg^{2+} or application of the picrotoxin, the hyperpolarization was reduced and was recovered by washing. No effect was observed in the depolarization. This suggests that the hyperpolarization is to be IPSPs of the antennal lobe neurons and the IPSPs may be caused through GABA-sensitive chloride channels (GABA-A like channels) of the antennal lobe neurons. Identical results were consistently observed by a repeat experiment, indicating that our optical recording system is available to investigate the neural systems of an in vivo insect brain.

428.15

SENSORY INTEGRATION IN THE LEECH AP CELL. C. E. Osborn* and B. Zipser. Depts. of Anat. and Physiol., Michigan State University, East Lansing, MI 48824

The AP cell of the leech is commonly used as a paradigm for studying the control of axon development in the leech nervous system, but the function of the AP cell is not known. Earlier work showed that the AP cell receives direct input from central sensory neurons, the touch (T), pressure (P) and nociceptive (N) cells. We have now found evidence for other mechanosensory input. Using a standard body wall preparation, we recorded intracellularly from the AP cell while producing small amplitude surface waves. These waves did not excite the central mechanoreceptors (T, P, or N cells) and presumably acted via their effect on sensillar wave detectors. The greatest effect on the firing rate of an AP cell was for waves delivered to the contralateral body wall of its own segment, or to the next anterior or posterior segment, but ipsilateral stimuli within its own segment was also effective. This input may be direct: using confocal microscopy we found that the AP processes bilaterally colocalize in the synaptic neuropil of the CNS ganglia with those sensory afferents that express CE1. Several lines of evidence suggest that this carbohydrate markers identifies mechanoreceptors. These findings, together with our earlier EM findings of colocalization between processes of the AP cell and the optic tract, suggests that the AP cell integrates a wide range of sensory information. NIH NS25117

428.16

SEROTONIN AND OCTOPAMINE MIXTURE EFFECTS ON THE SWIM MOTOR PATTERN IN THE LEECH, *HIRUDO MEDICINALIS*. L.S. Gilchrist* and K.A. Mesce. Graduate Program in Neuroscience (L.G. and K.M.) and Department of Entomology (K.M.), University of Minnesota, St. Paul, MN 55108.

Through triple intracellular recordings, we have found that stimulation of either touch (T) or pressure (P) mechanosensory neurons activates both serotonergic cell 21 and the dorsolateral octopamine-immunoreactive (DLO) neuron. Based on these observations, we decided to investigate the action of mixtures of these two biogenic amines on leech nerve cords.

Mixture effects of octopamine and serotonin on the swim motor pattern were monitored by recording swim bursts extracellularly in the dorsal posterior (DP) nerve. Whole nerve cords of *Hirudo medicinalis*, including both the head and tail brains, were used in our studies. A mixture of 50μM octopamine and 50μM serotonin was bath applied to the nerve cord. Additional experiments were performed with either 25μM or 100μM octopamine and serotonin mixtures. For comparative purposes, nerve cords were subjected to either 50μM octopamine or 50μM serotonin.

As compared to octopamine or serotonin alone, we found that bath application of the 50μM or 100μM octopamine and serotonin mixtures resulted in a greater reduction in overall DP nerve activity. While both serotonin and octopamine have been reported to increase swimming activity in nerve cord preparations (Hashemzadeh-Gargari and Friesen, '89), we found that washout of the serotonin and octopamine mixture was the most reliable way to induce long-term expression of the swim motor pattern. Supported by NSF Award 9419216 to K.A.M.

428.17

MOTONEURON ACTIVITY IN FREELY SWIMMING MEDICINAL LEECHES J.A. Murray*, R.J.A. Wilson, W.B. Kristan Jr.. Dept. Biology, 0357 University of California, San Diego, La Jolla, CA 92093-0357

We have developed a method of recording activity in nerve roots in intact behaving leeches with a fine silver wire extracellular electrode. We have used this technique to record extracellularly from the dorsal posterior nerve (DP). During swimming, we recorded bursts consisting of regularly-spaced spikes of constant amplitude. In semi-intact swimming preparations, similar bursts in the DP nerve are produced by an identified dorsal motoneuron, Cell 3, a dorsal excitor (DE) motoneuron involved in generating a contraction of the dorsal longitudinal muscles during each swimming cycle. Cell 3 recorded in freely swimming animals fired at a significantly higher rate (~90 Hz) than in semi-intact, restrained preparations (~50 Hz, Kristan et al. 1974; J Comp Physiol 94:155). Hence, recordings from semi-intact preparations, which compromise internal hydrostatic pressure, may differ quantitatively from natural firing patterns.

The bursts of Cell 3 impulses occurred when ventral muscles in that body segment were still partially contracted and well before the dorsal longitudinal muscles reached maximal contraction. This delay between the bursts of the Cell 3 units and dorsal contraction suggests that sensory feedback from the dorsal stretch receptors does not influence the duration or amplitude of the DE bursts within the same cycle of the swimming pattern. Other sensory feedback may, however, affect swimming in different ways. For instance, leeches reaching the end of the observation tank sometimes continued to undulate. During this "stationary swimming", swimming period (time/cycle) and burst duration increased significantly while the average firing rate decreased.

Although still preliminary, these recordings of the swimming motor program in intact leeches have indicated possible modes of sensory feedback that were not foreseen from experiments on semi-intact preparations.

These experiments were supported by an NIH Fellowship (MH10705) to JAM and an NSF Research Grant (IBN11432) to WBK.

428.18

DIFFERENTIAL RESPONSE OF RETZIUS CELL TO WARMING OF THE SKIN IN TWO SPECIES OF LEECHES. G. Maximov & A.L. Kleinhaus*. Dept.of Cell Biology & Anatomy, New York Medical College, Valhalla, N.Y. 10595.

Recently, we showed that the activity of the serotonergic Retzius cells increases during the appetitive phase of feeding in *Hirudo medicinalis*, but in contrast to previous claims, decreases at the onset of ingestion (J. Exp.Biol., 1996). The mechanism underlying this reduction is not understood at this time. Earlier work suggested that raising the temperature of the dorsal lip, but not the body wall, is an appropriate stimulus for the initiation of feeding. We now show that raising the temperature of the lip or skin on any part of the body including the tail from 23° - 35 °C significantly increases the firing frequency of the Retzius cells of *Hirudo* both in the subesophageal and segmental ganglia. These results suggest that heat detected by any part of the body may alert the animal to a warm-blooded food source, assuming heat perception represents an appropriate appetitive stimulus.

Previous findings showed that heating the lip for less than two minutes caused a rapid transient increase in the firing frequency of the Retzius cell which remained elevated above baseline for at least two minutes after the stimulus was turned off. To ascertain the response of the R cell to a thermal stimulus that is a closer facsimile to the heat stimulus during a feeding episode we warmed the lip or body wall for a prolonged period. We found that the Retzius cell did indeed respond transiently but returned to baseline within five minutes even if the stimulus was maintained. These findings are in line with our observations that the activity of the Retzius cell slows down at the beginning of ingestion.

In a comparative study we have found that the Retzius cell of *Macrobdella* does not respond to heat applied to either the lip or body wall. This is an interesting finding since both *Macrobdella* and *Hirudo* will feed on warm blooded animals. Supported by NIH grant # 5R01NS18055-10 to ALK.

428.19

QUANTIFICATION OF THE MOTOR PATTERN DURING INGESTION IN THE LEECH, *HIRUDO MEDICINALIS*. R. J. A. Wilson* & A. L. Kleinhaus. Dept. of Cell Biology & Anatomy, New York Medical College, Valhalla, N.Y.10595.

Several aspects of the ingestive behaviour of the blood-sucking leech, *Hirudo medicinalis*, have been described. After attaching to a host, the leech extends its jaws, bites through the skin, displays a feeding specific pattern of midbody contractions (peristalsis) and becomes less reponsive to noxious stimuli. Although the neural basis of several behaviors in the leech has been studied, the cellular basis of feeding remains largely unknown.

We have recently developed a semi-intact preparation that allows recordings to be made from exposed ganglia in caudal segments whilst the rostral portion of the animal attaches to, bites through and feeds from a blood-filled sausage. Preliminary data, obtained from this preparation by recording extracellularly from nerve roots, suggests that the motor pattern which accompanies ingestion (peristalsis), persists in the denervated segments (Wilson et al. 1995, 1996). Here we present a quantitative analysis of peristalsis-associated activity recorded using EMG's from the muscles in the body wall of intact feeding leeches. The motor activity during ingestion is variable, only weakly rhythmic and can be divided into four classes: (a) rostral to caudal waves; (b) caudal to rostral waves; (c) discrete contractions of individual segments; and (d) dorsal-ventral undulations that resemble swimming and only occur when the animal is under water. Although the dorsal-ventral undulations resemble swimming, the rostral-caudal and caudal-rostral waves have an intersegmental travel time more in par with the intersegmental delays that occur during crawling. The same muscle groups appear to be active during both the peristaltic waves and the discrete contractions. To determine the effects of blood flowing through the gut we performed similar experiments on animals in which the gut had been cannulated in a rostral segment. Supported by NIH grant # 5R01NS18055-10 to ALK.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: INVERTEBRATE
SENSORY AND MOTOR SYSTEMS III

429.1

G-PROTEIN SPECIFIC ION CHANNEL MODULATION IN A CILIATE M.J. Marino, T.G. Sherman and D.C. Wood*. Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

The ciliate protozoan *Stentor* contracts in response to mechanical stimuli. These stimuli open mechanoreceptor channels producing a depolarizing receptor potential. Suprathreshold receptor potentials trigger action potentials and resultant contractions. GTP-γ-S, GDP-β-S, and cholera toxin alter the responsiveness of *Stentor* to mechanical stimuli by modulating mechanoreceptor channels or by changing the action potential threshold suggesting the involvement of G-proteins. Additionally, β-endorphin depresses mechanoreceptor channel function through a pertussis toxin-sensitive pathway. In search of these modulatory G-proteins, we cloned partial cDNAs for 4 unique *Stentor* G-protein subunits which exhibit 24-57% identity with vertebrate G-proteins. Functionally important amino acids in the vertebrate G-proteins were conserved in *Stentor* G-proteins. Intracellular injection of antisense oligonucleotides for specific subunits was used to determine which of these G-protein subunits modulate *Stentor* ion channels and thus control its behavior. Antisense oligonucleotide directed against a cloned G_s-like subunit sequence and one of the three G_i-like sequences produced significant enhancements in mechanical stimulus sensitivity, whereas antisense against the other two G_i-like sequences produced no observed behavioral effects. Antisense against one of the G_i-like subunits blocked the inhibitory action of β-endorphin, whereas antisense against the other subunits did not. Therefore, specialized G-protein subunits appear to have evolved early to produce modulation of specific ion channels and alteration of overt behaviors.
(Private foundation support)

429.2

MEMBRANE ION CURRENTS AND FUNCTIONAL PROPERTIES OF SPIKING AND NONSPIKING *APLYSIA* BUCCAL MUSCLES. Scott, M.L.*, V. Brezina, and K.R. Weiss. Dept. Physiol. & Biophys., Mt. Sinai Sch. Med., New York, NY 10029.

Molluscan muscles display different functional properties, which may optimize them for their specific roles in behavior. We have been studying a pair of antagonist muscles in the *Aplysia* feeding system, the accessory radula closer (ARC) and the radula opener (I7 - I10) muscles. The ARC is a nonspiking muscle, with maintained contractions directly graded by synaptic depolarization. This may provide sustained power necessary to grip food while it is pulled into the mouth. In contrast, the opener muscles spike when depolarized by synaptic input or stretch. This may enable the openers to contract rapidly at the proper time to release food into the esophagus.

To see if these differences reflect different membrane properties, we compared ion currents and voltage behavior expressed in enzymatically isolated fibers from the two muscles. Both muscles expressed similar currents, with variable amplitudes, including several K^+ currents (inward rectifier, A-type, delayed rectifier, and Ca^{2+}-activated) and an L-type Ca^{2+} current. Furthermore, unexpectedly, isolated fibers from both muscles displayed a similar continuum of voltage behavior, from completely inexcitable to fully spiking. Excitability was increased or decreased by different physiological modulators. Our hypothesis is that the currents are so balanced that small changes in their amplitude or key properties can lead to qualitatively different voltage behavior. We have identified three differences in currents that are likely to predispose the opener muscles to spike: their A-type K^+ current activates at more depolarized voltages than in the ARC, their inhibitory ACh-activated Cl^- current is much smaller, and they express a modulator-activated inward current. To test our hypothesis and examine the roles of specific currents in voltage behavior, we have developed a Hodgkin-Huxley type model of the muscle fibers. We are also evaluating how the ion currents, spiking, and stretch contribute to the different contractions of the two muscles. Supported by NIMH and the Whitehall Foundation.

429.3

ABERRANT LARVAL LOCOMOTION IN *DROSOPHILA* Na^+ AND K^+ CHANNEL MUTANTS REVEALED BY COMPUTER-ASSISTED DYNAMIC IMAGE ANALYSIS SYSTEM. J.W. Wang*, A.W. Sylwester, D.R. Soll and C.-F. Wu. Dept of Biol. Sci., Univ. of Iowa, Iowa City, IA 52242

Genetic dissection has provided insights into molecular mechanisms of the nervous system controlling behavior in *Drosophila*. There have been fewer attempts to exploit information with regard to larval behavior compared to the well established adult behavior, partly due to technological difficulties. We employed a computer-assisted motion analysis system useful for characterizing the peristaltic locomotion of *Drosophila* third-instar wandering larvae, and investigated the importance of ion channels to different quantifiable parameters in behavior repertoires. The gene *Hyperkinetic (Hk)* encodes a K channel beta subunit, which modulates IA channels in larval muscles; and *paralytic* (*para*) encodes a Na channel alpha subunit expressed in the nervous system. Using the Dynamic Image Analysis System (DIAS) initially developed for motion analysis of ameboid cells, we have analyzed videotaped behaviors of *Drosophila* larvae crawling on agar slabs. Results demonstrate that mutant larvae exhibited marked locomotion abnormalities. *Hk* and *para* mutant larvae turned, respectively, less and more frequently than wild-type larvae. In addition, both *Hk* and *para* larvae crawled at slower velocities than wild-type larvae, apparently due to a lower efficiency in conversion of muscular contraction to distance translocation. Our results demonstrate that DIAS is a powerful tool to reveal subtle difference in animal behavior to quantify mutational effect on the interplay of discrete behavioral components. Supported by NIH grants to D.R.S. and C.-F.W.

429.4

AMILORIDE-SENSITIVE AND INSENSITIVE RECEPTOR SITES IN INSECT TASTE CHEMORECEPTORS. A. Liscia, A. Bigiani*, R. Majone, P. Muroni, P. Solari, I. Tomassini Barbarossa and R. Crnjar. Dip. Biologia Sperimentale, Sez. Fisiologia Generale, Università di Cagliari, Viale Poetto 1, I-09126 Cagliari, Italy; (*) Dip. Scienze Biomediche, Sez. Fisiologia, Via Campi 287, I-41100 Modena. Italy.

In vertebrates salt and sugar reception mechanisms are known to involve both amiloride-sensitive and insensitive (AIC) cation conductance components. In insects instead, no information is yet available on amiloride effects on membrane receptor sites. We thus studied whether salt and sugar chemoreception is mediated by amiloride-sensitive cation conductance mechanisms in the blowfly *Protophormia terraenovae*. Spike discharges obtained by means of the tip-recording technique were processed by spike waveform analysis in order to identify the various firing sensory cells. The results of our experiments show that: a) spike firing frequency of the "salt" cell in response to NaCl, sucrose and fructose is not influenced by the addition of amiloride (100 and 500 μM); b) addition of amiloride does not influence the spike firing frequency of the "sugar" receptor cell in response to sucrose, but depresses the response to fructose; c) amiloride 100 μM only lowers the response of the "water" cell to sucrose and fructose, whereas at 500 μM it depresses the responses to all stimuli; d) pure amiloride, both at 100 and 500 μM, generally evokes a weak response from the "salt" cell. Our results point to the absence of amiloride-sensitive receptor sites in the "salt" cell. Instead, the "sugar" cell seems to bear an amiloride-sensitive furanose site. However, since the fructose response is not completely abolished, an amiloride-insensitive component (AIC) may also be present. As for the "water" cell, the inhibitory effect of amiloride seems to support the idea that this cell shows properties of a typical receptor cell, in contrast with the hypothesis of a simple osmometer mechanism.

Funded by Italian Board of University (M.U.R.S.T.; 40% and 60% Funds).

429.5

CHEMOSENSORY ADAPTATION AND DESENSITIZATION BY RECEPTOR DOWN-REGULATION IN PARAMECIUM. M.Kim, H. Kuruvilla, T. Hennessey and C. Fourtner.* Dept. of Biol. Sci., S.U.N.Y.A.B., Buffalo, N.Y. 14260.

Like sensory neurons, Paramecium integrate sensory information as somatic membrane potential changes. In general, chemoattractants cause sustained hyperpolarizations while chemorepellents, such as GTP and lysozyme (at micromolar concentrations), produce transient depolarizing somatic receptor potentials that trigger Ca^{++}-based action potentials and consequent bouts of backward swimming. Forward swimming returns after about 10 min. in either repellent due to chemosensory adaptation, an important process that is not well described in eukaryotic cells. Using a combination of behavioral bioassays, intracellular electrophysiology and in vivo chemoreceptor binding assays (with Scatchard analysis), we have shown that chemosensory adaptation is due to a gradual loss of surface receptors. The chemoreceptor potential amplitude also decreases along the same time course. These responses are specific for each chemorepellent. Lysozyme adapted cells (0.01uM for 10 min.) lose their behavioral and electrophysiological responses to lysozyme as well as their surface ^{3}H lysozyme binding sites but their responses to GTP (and ^{32}P GTP binding) are unaffected. Similarly, GTP adapted cells (10uM for 14 min.) respond normally to 0.01uM lysozyme and bind ^{3}H lysozyme but they lose their GTP responses and ^{32}P GTP binding sites. All of these changes seen are reversible within 10 min. after removal of the repellent. Cells adapted to either repellent still respond fully to other stimuli such as 40mM K+, 10mM Na+, 8mM Ba++ and the chemoattractant, acetate (5mM). We propose that lysozyme and GTP have separate high affinity surface receptors on the body membrane that are specifically down-regulated during chemosensory adaptation. Both receptors may activate the same somatic, receptor-operated Ca++ conductance to generate their respective chemoreceptor potentials, suggesting a common sensory transduction pathway with at least two different receptors. Supported by NSF grant MCB9410756 to T.M.H.

429.6

TRANSDUCTION CHANNELS OF A CL^- RECEPTOR NEURON IN THE LEECH, *HIRUDO MEDICINALIS*. A. Wenning[1]*, C. F. J. Erxleben[1] and R. L. Calabrese[2]. [1]Fakultät für Biologie, Uiversität Konstanz, 78434 Konstanz, Germany; [2]Biology Department, Emory University, 1510 Clifton Rd., Atlanta GA 30322.

Following a blood meal, the Cl^- concentration of leech blood triples. These changes are monitored by the nephridial nerve cell (NNC), a peripheral neuron which innervates the leech nephridium. At normally, low extracellular Cl^- concentrations $[Cl^-]_o$, the NNC is spontaneously active. Its membrane potential (V_m = -37 mV) is governed by E_{Cl}, due to a high resting Cl^- conductance. With increase in $[Cl^-]_o$, the NNC hyperpolarizes by 23 mV) towards E_K due to a decrease in Cl^- conductance (Wenning & Calabrese 1991). Here we report on the poperties of the postulated Cl^- channels and the mechanism by which they are regulated.

Cell attached patches were made on the soma of the NNC. At the NNC's resting potential, we recorded inward currents through a 48 (±8; s.d.) pS channel with either high or low $[Cl^-]$ in the recording pipette. Single-channel current amplitudes increased upon hyperpolarization and decreased with depolarization. The reversal potential was near the resting potential. The channel open probability was independent of the membrane potential. Changing the bath solution from high to low $[Cl^-]$ led to an increase of channel openings and channels closed upon superfusion with high $[Cl^-]$, regardless of the Cl^- concentration in the pipette. Since the open probability of the channels under the pipette is altered by changes in $[Cl^-]_{bath}$, we conclude the involvement of an intracellular signalling pathway rather than direct interaction of extracellular Cl^- with the channel.

Supported by DFG We 745/4-3 to AW and NS24072 to RLC.

429.7

ELECTROPHYSIOLOGICAL AND ULTRASTRUCTURAL OBSERVATIONS ON THE FIRST-ORDER GIANT NEURON OF THE GIANT FIBER SYSTEM OF THE SQUID. L.D. Pozzo-Miller*[1], J.E. Moreira[1], and R. Llinás[2]. Marine Biological Laboratory, Woods Hole MA, 1 Laboratory of Neurobiology, NINDS, NIH, Bethesda MD, and 2 Department of Physiology & Neuroscience, NYU Medical Center, New York NY.

The giant fiber system controlling the mantle contraction responsible for water/jet propulsion in the squid consists of two sets of three giant neurons organized in tandem. The first- and second-order cells are located in the head, and the third-order, that originate the "giant axon", in the stellate ganglion. The axons of the first-order neurons decussate in the midline forming a chiasma, where complete axoplasmic fusion occurs. This chiasma allows integration across the midline into a single symmetrical connectivity. After the bridge, first-order axons branch and establish mixed synapses with several second-order axons. The largest of these neurons form the presynaptic element at the giant synapse in the stellate ganglion. The first-order neurons are multipolar exhibiting dendritic branches that arise from somatic level, resembling vertebrate neurons, and in particular, the Mauthner cell of fish. The dendrites and soma of these cells receive massive synaptic input from the eye, statocyst, most of the skin surface propioception, and the supraesophageal ganglion. In order to define the integrative properties of such enormous synaptic load, especially given its incredible diversity, intracellular recordings and electron microscopic observations were implemented on first-order neurons in an isolated squid head preparation. Resting membrane potential was 42±1mV, and membrane input resistance was 12±3MΩ (n=6). Spontaneous bursts of action potentials, and spikes evoked by extracellular stimulation of afferent regions of the magnocellular ganglion were sensitive to bath applied TTX and intracellular QX-314. The amplitude of the action potentials (20-40mV), the delay for the intracellular blockade of spikes by QX-314 (10-20 minutes from impalement), and the morphological observation of abundant synaptic input to dendrites and soma, suggest that the site of initiation of action potentials is remotely located from the recording site at the soma. It seems likely that subthreshold postsynaptic potentials, the sunning logic of which we have just begun to decipher, are integrated at dendritic and somatic levels, and propagate electrotonically down to the site of Na^+-dependent spike generation, possibly at the cytoplasmic bridge where the two first-order neurons fuse. The characterization of the electroresponsive properties of the first-order neurons provides a description of the single cell integrative properties to the wide variety of sensory inputs which controls the complex rapid behavior of the squid.

Research supported by The Grass Foundation. The Lakian Foundation. and the Marine Biological Laboratory.

429.8

THE EFFICIENCY OF INFORMATION TRANSFER FROM TRANSDUCTION TO ACTION POTENTIAL ENCODING IN MECHANORECEPTOR NEURONS. Mikko Juusola* and Andrew S. French Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada.

Little is known about how effectively single sensory neurons transmit stimulus information from transduction to action potential encoding. We examined the coding efficiency of primary mechanoreceptor neurons in a spider slit-sense organ using repeated sequences of pseudorandomly modulated displacement stimuli, and found that the information content of the analog receptor current was converted into trains of discrete action potentials with ~85% efficiency. This high efficiency resulted from low-pass filtering of the signal by the mechanoreceptor membrane as the receptor current was transformed into receptor potential. The removal of high-frequency noise boosted the signal-to-noise ratio at high-frequencies of the transduction signal more than 10-fold, before action potentials were encoded with about 30% efficiency. These data suggest that during dynamic stimulus modulation, a low intrinsic noise level during spike generation allows sensory neurons to accurately and reliably transform the sensory input into spike sequences.

429.9

INDIRECT CONNECTIONS BETWEEN CERCAL SENSORY NEURONS AND GIANT INTERNEURONS OF THE FIRST INSTAR COCKROACH. E.S. Hill* and J.M. Blagburn Dept. of Biology, Univ. of Puerto Rico and Institute of Neurobiology, UPR Medical Sciences Campus. 201 Blvd. del Valle, San Juan, PR 00901

The cercal sensory system of the first instar cockroach (*Periplaneta americana*) has two filiform hairs on each cercus, termed lateral (L) and medial (M). Each hair is associated with an individual sensory neuron. These neurons make connections with giant interneurons (GIs) in the terminal abdominal ganglion. The L sensory neuron is known to make direct connections with GIs 3, 4 and 6, and M with GIs 1, 2, 4 and 5. We were interested in determining whether indirect (polysynaptic) connections are present between sensory neurons and GIs. The appropriate sensory neuron was stimulated by mechanically pushing the filiform hair. The other hair on the same cercus was immobilized with Vaseline and the other cercal nerve was crushed. Simultaneous intracellular recordings were made from a GI known to have a monosynaptic connection from that sensory neuron, and from the GI under test. Criteria for determining the existence of an indirect connection were: (1) lack of correspondence between spontaneous EPSPs in the reference GI and those in the test GI, and (2) a delay between the onset of the evoked responses in the two GIs. Physiological evidence suggests a strong indirect, excitatory connection between the L sensory neuron and the contralateral GI1. An indirect, inhibitory connection was found between the M sensory neuron and the ipsilateral GI6. GI7 was found to receive indirect input from the contralateral L. A burst of EPSPs was observed in GI7 coincident with cessation of activity in the sensory neuron, suggesting a possible tri-synaptic pathway with an inhibitory and an excitatory interneuron. These experiments have shown that filiform hair sensory input can reach post-synaptic GIs via polysynaptic excitatory and inhibitory pathways. These pathways do not simply fine tune the receptive fields of the GIs, but may make an important contribution to their directional sensitivity.

Supported by NIH grant NS07464, and an NSF EPSCoR grant.

429.10

INTRACELLULAR CA^{2+} DYNAMICS ON DENDRITIC PROCESSING OF THE WIND-SENSITIVE INTERNEURONS IN THE CRICKET. H. Ogawa*[1], Y. Baba[2], K. Okada[3], K. Oka[4]. 1), 3) Kawachi millibioflight project, ERATO, JRDC, Tokyo 153, Japan. 2) Neuro-Cybern. Lab., Res. Inst. for Electronic Science, Hokkaido Univ.,Sapporo 060, Japan. 4)Dept. of System Design Engin., Fac. of Sci. and Tech.,Keio Univ., Yokohama 223, Japan.

Some kinds of wind-sensitive interneurons, which contain the ascending interneurons such as giant interneurons, and the spiking and nonspiking local interneurons, have been identified in the terminal abdominal ganglion of the cricket (Mendenhall and Murphy, 1974; Baba et al.,1991, 1995). These neurons integrate the cercal afferents and convey information about air currents to motor systems driving the various behavior such as avoidance response. To visualize chemical computation in dendritic processing, we examined intracellular Ca^{2+} change in these cercal interneurons in the cricket, with a flucrescent imaging technique using confocal laser-scanning microscopy and a fluorescent calcium indicator, 'Calcium Green 1'. Depolarization caused by current injection or electrical stimulation of cercal sensory nerves could induce fast Ca^{2+} increase on dendritic site of these interneurons. This result provides an evidence of the voltage-dependent Ca^{2+} channels located on dendritic site of these interneurons. The amplitude and latency of Ca^{2+} increase by ipsilateral cercal afferents stimulation were different from those of contralateral one. The spatio-temporal differences between Ca^{2+} responses to ipsi- and contralateral cercal afferents stimulation suggest the importance of chemical computation in dendritic integration of synaptic inputs coding wind signals.

429.11

Neural mapping of multiple functional parameters in the cricket cercal sensory system. Sussan Paydar*, Caitlin C. Doan, Gwen A. Jacobs. Dept. of Molecular and Cell Biology, Univ. of California, Berkeley, CA 94720.

The objective of this study is to determine how different functional parameters are organized within the neural map of the cricket cercal system. Mechanoreceptor hairs in this system are sensitive to the direction and dynamics of the air currents in the animal's environment. Directional tuning and the dynamic sensitivities to air currents are determined by the orientation of the hair socket on the cerci and the hair length respectively. Previous studies have shown that afferents associated with long hairs (1000μ-2000μ) constitute a single functional class. They are most sensitive to low frequency air currents and form a continuous map of wind direction in the terminal ganglion. In this study we examined the anatomical and functional characteristics of another class of afferents that are associated with medium length hairs (500μ-700μ), which respond maximally to high frequency air currents. We assessed their morphological characteristics and studied the spatial relationships of their terminal arbors with respect to each other and to the long-hair afferents with corresponding directional sensitivities. Afferents were filled with cobalt chloride, anatomically reconstructed, scaled, and aligned to a common set of highly reproducible fiducial marks. Preliminary data suggest that (1) arbors of the medium-hair afferents are significantly smaller than their corresponding long-hair afferents with the same directionality; (2) medium-hair afferents project mostly to the same directional target areas as their long-hair afferent counterparts (however some of their arbors extend to regions that are not occupied by long-hair afferents); and (3) medium-hair afferents appear to form a continuous map of wind direction similar to that observed for the long-hair afferents. The maps of direction formed by long-hair and medium-hair afferents are spatially segregated in some regions. These findings suggest that the representation of dynamic sensitivity may also be mapped in this system. This work was supported by NIH grant R29NS29847 to G.A.J.

429.12

SENSORY AND MOTOR CONTROL OF CERCAL DISPLACEMENT IN THE CRICKET. K.A. Killian* and J.P. Bollins. Dept. of Zoology and Center for Neuroscience, Miami University, Oxford, OH 45056.

Organisms are exposed to a barrage of sensory information which must be integrated by the central nervous system in order to produce behaviorally relevant motor output. We have begun to characterize a simple reflex system in the cricket *Acheta domesticus* with which to investigate this multisensory integration at the cellular level.

Wind puffs directed across the pair of cerci located on the terminal abdominal segment of orthopteran insects produces synchronous displacement of both cerci toward the midline. This cercal movement is produced by the contraction of muscles located at each cercal joint. Cobalt chloride backfills of a cercal motor nerve stained five efferent neuronal somata within the terminal abdominal ganglion (TAG). We have begun to anatomically and physiologically characterize one neuron, an excitatory cercal motor neuron, in detail. This neuron's soma lies in the 9th segment of the TAG and its dendrites are located in the dorsal neuropil and cercal glomerulus. Depolarizing current injected into the neuron's soma or dorsal dendrite produces medial displacement of the ipsilateral cercus with displacement amplitude determined by the number of action potentials elicited from the neuron. Motor neuron activity is evoked by several sensory stimuli including wind puffs on the cerci, manual displacement of the cercus and tactile stimulation of the ovipositor. The effectiveness each sensory input has in generating a motor response is under investigation. (Supported by Dept. of Zoology, Miami University).

429.13

PROCESSING OF MOVEMENT AND POSITION DEPENDENT FEEDBACK IN THE CRAYFISH BASAL LIMB. P. Skorupski* Dept. Physiol., Univ. Bristol, Bristol BS8 1TD, UK.

Movement of the basal joint of the crayfish leg evokes both positive and negative feedback reflexes in the motor neurons that control that joint. Positive feedback reflexes, termed assistance reflexes, occur when leg promotor motor neurons (PMNs) are excited by a promotion signal, and remotor motor neurons by a remotion signal. In each case the proprioceptive inputs generating assistance reflexes are restricted to a subgroup of the motor pool. In a parallel system of negative feedback, separate motor subgroups are excited by inputs that generate resistance reflexes. During fictive locomotion both subgroups of motor neurons within a pool fire in phase, but the recruitment order within a burst is always assistance group followed by resistance group (Skorupski et al, J. Neurophysiol. 67, 648, 1992).

Feedback corresponding to static limb position generates negative, but not positive feedback reflexes. This organization of movement dependent positive feedback and position dependent negative feedback may constitute a peripheral oscillator that can entrain the central oscillator (Elson et al, J. Neurophysiol. 67, 530, 1992). For PMNs, yet another subgroup is preferentially excited by position dependent feedback. The degree of static joint remotion is sensed by a nonspiking afferent termed the S fibre. At least two PMNs receive direct electrical input from the S fibre, but little or no excitatory input from the dynamically sensitive T fibre, which senses the angular velocity of joint remotion. In addition the S fibre makes weaker excitatory connections to other PMNs, and it inhibits, via a di- or polysynaptic pathway, low threshold PMNs of the assistance group. This inhibitory pathway may serve to prevent unwanted activation of the peripheral oscillator during maintenance of stable joint position.

Supported by the Biotechnology and Biological Sciences Research Council (UK).

429.14

SOMATOSENSORY INFORMATION PROCESSING IN THE LEECH LOCAL BEND NETWORK: STIMULUS ENCODING AND BEHAVIOR. J.E. Lewis* and W.B. Kristan Jr., Biology Department., University of California, San Diego, 9500 Gilman Dr., La Jolla, CA, 92093-0357.

Local bending in the leech, *Hirudo medicinalis*, is a withdrawal reflex elicited by a mechanical stimulus to the tubular body wall. Bending away from a stimulus results from the contraction of longitudinal muscles in the vicinity of the stimulus and relaxation of muscles on the side opposite to the stimulus. Video analyses showed that behavioral output was centered within 10% (about 2mm) of the stimulus location. To address how this level of accuracy is achieved, we have made estimates on the accuracy of stimulus representation by the mechanosensory P neurons. After quantifying P cell firing statistics and spatial tuning properties, we used previously described stimulus reconstruction methods and found that the accuracy of P cell representation of stimulus location matched that of the behavior itself. This suggests that, either no information is lost through the remaining network layers (interneurons and motorneurons), or more likely, there are additional important features of stimulus encoding. This illustrates the importance of behavioral context in these types of analyses, and ideally, of monitoring sensory input and behavioral output simultaneously. We have extended our behavioral analyses with electromyographic (EMG) measurements at many body wall locations in semi-intact preparations. We can now measure the behavior along with the activity of *all* of the activated P cells on a trial-by-trial basis, and thus test more rigorously different models of stimulus encoding and information processing.

Supported by NRSA Predoctoral Fellowship MH10677 (JEL) and NIMH Research Grant MH43396 (WBK).

429.15

MULTIPLE SENSORY INPUTS ONTO A SET OF SEGMENTALLY HOMOLOGOUS INTERNEURONS IN THE LOCUST. K. R. Prier* and G. S. Boyan, Zool. Inst. der Uni Basel, Rheinsprung 9, 4051 Basel, Switzerland. (Present Addresses: KRP: Inst. of Neurosci., U Oregon, Eugene, OR 97403; GSB: Zool. Inst. der Uni München, D-80333 München, Germany.)

The segmental nature of the insect nervous system offers a unique opportunity to study the effects of the specialization of peripheral structures on central processing neurons in the CNS. In the locust, the pleural chordotonal organ (plCO) system is a segmentally repeating sensory system arrayed along the body wall of the animal. This system has specialized to respond to three distinct sensory modalities. In the abdominal segments, the plCOs have remained in their basic form and respond mainly to respiratory vibrations. In the first abdominal segment, they have specialized to detect air-borne sound as tympanal organs (TOs). In the second and third thoracic segments, they have specialized to detect wing vibrations during flight as wing-hinge chordotonal organs (wCOs).

The input from these three different sensory modalities is processed by a large number of identified interneurons, including many identified neurons derived from neuroblast 7-4 in each segment. This suggests an important role for the progeny of neuroblast 7-4 in the processing of chordotonal information. Among these are neurons 714, 531, and 529, which are three segmentally homologous interneurons in the second thoracic, third thoracic, and first abdominal neuromeres, respectively. Electrophysiological experiments show that neurons 714 and 529 respond to all three of these sensory modalities, while neuron 531 has specialized to receive exclusively tympanal input. The data from these three interneurons give insight into how the central processing elements of the basic plCO system have adapted to the segmental specialization of the sensory system.

This work was funded by a NSF Graduate Research Fellowship and a grant from the Swiss Nationalfonds.

429.16

ANATOMY AND PHYSIOLOGY OF AUDITORY RECEPTORS IN THE AUSTRALIAN FIELD CRICKET *TELEOGRYLLUS OCEANICUS*

K. Imaizumi* and G. S. Pollack Department of Biology, McGill University, Montreal, Quebec, Canada, H3A 1B1

The cricket's tympanal organ consists of ca. 70 receptor cells, which respond to frequencies from 3 up to 100 kHz. Although auditory behavior and neural processing by interneurons have been well studied, little is yet known about the anatomy and physiology of cricket auditory receptors. We are using single unit recording and staining techniques to address this question.

Single receptors have clear best frequencies, but also have additional sensitivity peaks at other frequencies. The several sensitivity peaks correspond well to multiple sensitivity peaks seen in the whole tympanal nerve with extracellular recording. Based on their best frequencies, most receptors fall into one of two broad types, low frequency receptors (4-5.5 kHz) and ultrasound receptors. These frequency ranges are known to be important for intraspecific communication and escape behavior, respectively.

Receptors can be roughly classified into two groups based on the anatomy of their axon terminals. Some receptors end near the midline in a densely packed arbor. Other receptors have more lateral arbors with widely spread endings, which extend further posteriorly than those of the first type. The first morphological type includes both low and high frequency (ultrasound) receptors. The second type appears to include only low frequency receptors.

Auditory receptors are, thus a diverse population, both anatomically and physiologically.

Supported by NSERC Canada

429.17

SIGNAL PROCESSING BY A MECHANOSENSORY ARRAY: DYNAMICS OF CRICKET AIR MOVEMENT RECEPTORS.

Leslie C. Osborne and John P. Miller* University of California, Berkeley, CA 94702

The cricket's cercal sensory organs consist of an array of frequency-tuned, directional mechanoreceptors that detect, filter, and encode local air movements. Unlike the mammalian cochlea, where efferent neural feedback can fine-tune the responsiveness of the auditory transducers, mechanical filtering of stimulus information in the cercal system is accomplished by physical design alone. A hair's mass and the viscoelastic properties of its socket affect the resonant properties of the mechanosensor. A hair's length with respect to the boundary layer and its location on the cercus affect its flow environment and therefore its response properties. Mechanical filters were calculated from high-speed video recordings of hair responses to calibrated noise and tone burst stimuli (n>36). Comparing the mechanical filters of the hairs with physiological recordings of sensory afferent responses shows that the proportion of hair response bandwidth that is reliably encoded into spikes varies with receptor length. The mean frequency of the coding range of long hair afferents coincides with the mean frequency of the long hairs' coherent mechanical response. In contrast, the mean frequency of the coding bandwidth of shorter hair afferents is significantly greater than that of the short hairs' mechanical response. Thus, the overlap of the mechanical tuning curves for long, medium and short hairs is greater than the overlap in frequency sensitivity of the innervating afferents. If stimulus frequency information is extracted by comparison of activity in different length classes of the mechanosensors, these features may maximize the bandwidth over which the cricket can reliably distinguish stimulus frequency in the presence of noise, given the mechanical constraints on its receptors. (NIH grant to J.P.M.)

CORTEX: CONNECTIVITY

430.1

INTRACORTICAL CONNECTIONS BETWEEN MOTOR CORTICAL ZONES CONTROLLING ANTAGONIST MUSCLES.

L. Bertrand, C. Capaday*, H. Devanne and B.A. Lavoie. Centre de Recherche en Neurobiologie, Université Laval, Québec, (Qc) Canada, G1J 1Z4

Experiments were done on 8 cats lightly anesthetized with nembutal to determine whether motor cortical zones controlling antagonist muscles are synaptically interconnected. Motor cortical zones controlling wrist flexors, or extensors, were identified by microstimulation and intramuscular EMG recordings (stimulation: 11 pulses at 333 HZ, currents 10-40 μA). The position of each zone of interest was marked by a small ink spot on the surface of the cortex and on a scaled drawing of the cortical surface (cruciate region). Following the identification of wrist flexor and extensor zones the anterograde tracer biocytin was injected into one, or two, wrist extensor zones at three depths (400, 800 and 1500 μm) from the cortical surface. A small injection of HRP – producing a dark brown spot of approximately 500 μm – was made in layer II/III of one or more wrist flexor zones. Similar HRP injections were made in the deep layers of wrist extensors zones that were not labeled by biocytin. The HRP injections served to mark the position of potential targets of biocytin labeled terminals. In all animals biocytin labeled terminals (blue) were found in cortical zones controlling the same muscle(s) and also in zones controlling the antagonists. There was no apparent difference in the number of terminals in synergistic or antagonistic cortical zones. Most terminals were found in the upper cortical layers (II-III), but some were also present in the lower cortical layers (V-VI). We conclude that there exist intracortical circuits linking motor cortical zones controlling antagonist motoneuron pools. Elucidating the nature and function of these circuits will be a challenging endeavour. (Supported by MRC of Canada)

430.2

ANATOMICAL CONNECTIVITY OF ELECTROPHYSIOLOGICALLY-IDENTIFIED MOVEMENT REPRESENTATIONS IN THE MI HAND AREA OF SQUIRREL MONKEYS.

E.J. Plautz*, G.W. Milliken, & R.J. Nudo. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77030.

Intracortical microstimulation (ICMS) techniques were used to generate bilateral maps of the primary motor cortex (MI) hand area in adult squirrel monkeys. Microelectrode penetrations were made at 250 μm interpenetration distances until the total extent of the distal forelimb region was explored. In a separate surgical procedure, a bi-directional tracer (biotinylated dextran amine, BDA) was injected iontophoretically into a region of locally uniform movement representation (e.g. a contiguous cluster of wrist extension sites). Regions were selected based on the results of the original mapping procedure and were confirmed by limited ICMS sampling immediately prior to tracer injection. To permit transport of the tracer, monkeys were sacrificed after a 2-week survival period. The cortex was flattened and cut tangentially at 40 μm thickness. BDA label was visualized using the ABC method followed by reaction in DAB. Sections were examined for the location of the injection site, anterogradely-labeled axons and synaptic terminals, and retrogradely-labeled neuronal somata.

Results were evaluated for both *extrinsic* projections between MI and other cortical areas as well as for *intrinsic* projections within MI. Injected sites were found to be reciprocally connected with several *extrinsic* cortical fields, including the (presumed) arcuate premotor area, SMA, and SI. *Intrinsic* terminal fields and retrogradely-labeled cells were primarily confined to zones of distal movement representations (i.e. digit and wrist regions), with relatively little labeling in more proximal zones (i.e. elbow and shoulder regions). These results extend previous findings on intrinsic connectivity within primate MI by demonstrating a greater degree of specificity in connections between functional modules.

Supported by NIH MH10963 (EJP), NS 09366 (GWM), and NS 30853 (RJN). This work was done during the tenure of an Established Investigatorship from the American Heart Association.

430.3

DIRECT CORTICO-MOTONEURONAL (CM) CONNECTIONS IN THE SQUIRREL MONKEY (*Saimiri sciureus*)

R.N. Lemon, M.A. Maier*, E. Olivier, S.N. Baker and T. Morris+ Sobell Dept. of Neurophysiology, Institute of Neurology, Queen Square, London WC1N 3BG and +SmithKline Beecham Pharmaceuticals, Harlow, Essex CM19 5AW UK

Are direct CM connections essential for relatively independent finger movements? In the squirrel monkey, which has rather poorly developed hand skill, anatomical evidence suggests that there are few CM projections from motor cortex to intrinsic hand muscle motor nuclei (Bortoff & Strick, 1993 *J. Neurosci.* **13**, 5105-5118). We have made an electrophysiological study of the corticospinal (CS) system in this species.

In 5 adult squirrel monkeys under chloralose anaesthesia, CS fibres were excited by stimulation of the pyramidal tract (PT). The fastest CS axons conducted at 47.3 to 50.7 $m.s^{-1}$. We recorded intracellularly from 80 motoneurones identified from the radial, median or ulnar nerve. The response to a single PT stimulus was usually a small EPSP with a short segmental delay (0.7-1.1 ms), confirming its monosynaptic origin. These EPSPs were weak (mean only 0.9 mV) and had slow rise times (1.2-2.5 ms); they may derive from CM inputs to distal dendrites, possibly located outside lamina IX. Many motoneurones showed only weak responses to single stimuli but multiple shocks were followed by a large (>2 mV) EPSP with a disynaptic segmental delay (>1.4 ms).

In squirrel monkeys under ketamine sedation, we found that either single PT stimuli or magnetic stimulation (figure of-eight coil) of the motor cortex produced short-latency EMG responses in thenar muscles. The mean (± SD) latency was 9.1 ± 0.2 ms, a value comparable with that in the much larger macaque, which may reflect the slow CS fibres, weak monosynaptic pathway, and need for temporal summation of oligosynaptic effects in *Saimiri*. We suggest that the larger, faster-rising CM monosynaptic EPSPs of the macaque and other dexterous primates are of critical importance for the performance of fine finger movements.

Supported by SmithKLine Beecham Pharmaceuticals

430.4

INVESTIGATION INTO A POSSIBLE C3-C4 PROPRIOSPINAL TRANSMISSION OF CORTICOSPINAL EXCITATION TO FOREARM MOTONEURONS IN THE MACAQUE MONKEY.

M.A. Maier[1], M. Illert[2], P.A. Kirkwood[1]*, J. Nielsen[2] and R.N. Lemon[1], [1]Sobell Dept. of Neurophysiology, Institute of Neurology, London WC1N 3BG, UK, [2]Dept. of Physiology, University of Kiel, D-24098, Germany

In the cat, corticospinal excitation of forelimb motoneurons (MNs) is disynaptically mediated via C3-C4 propriospinal neurons, in addition to segmental effects. Such a relay has been suggested to exist in man, though no direct investigations have yet been performed in primates.

In five monkeys (*m. fascicularis*) anaesthesia was induced with ketamine and surgery carried out under isoflurane. We performed intracellular recordings from identified forearm MNs in C5-C7 (chloralose anaesthesia with neuromuscular paralysis, pneumothorax and artificial ventilation). The medullary pyramid (PT) and the lateral reticular nucleus (LRN) were stimulated. A total of 113 MNs were recorded, 46 before and 67 after a C5 dorsolateral funiculus lesion. With this lesion we attempted to abolish the PT inflow to the forelimb segments but to spare more ventral propriospinal axons.

With intact spinal cord 70% of the MNs showed clear monosynaptic EPSPs upon PT stimulation, few had disynaptic EPSPs. Most common were monosynaptic EPSPs followed by disynaptic IPSPs. LRN stimulation evoked a variety of effects: monosynaptic excitation was the most common. After C5 lesion 42% of the MNs showed small monosynaptic EPSPs from the PT and only 20% had possible disynaptic EPSPs. Half of these late EPSPs were evoked by a single stimulus, did not show temporal facilitation and may have been monosynaptic from surviving slow PT fibres. LRN effects were virtually unchanged. Under these experimental conditions there is little evidence for a C3-C4 propriospinal mediation of corticospinal inputs to cervical MNs in the macaque monkey. *Supported by The Wellcome Trust*

430.5

SPINAL CORD TERMINATIONS OF THE MEDIAL WALL MOTOR AREAS. R.P. Dum* and P.L. Strick. Research Service, VAMC and Depts. Neurosurgery & Physiology, SUNY-HSC@Syracuse, NY 13210.

In macaques, substantial corticospinal projections originate from the supplementary motor area (SMA) and the two caudal cingulate motor areas which lie on the dorsal and ventral banks of the cingulate sulcus (CMAd and CMAv). We used anterograde transport of WGA-HRP to examine the pattern of spinal terminations of efferents from these motor areas. For comparison, we also examined the pattern of termination of efferents from the primary motor cortex (M1). All of the cortical areas examined terminated in the "intermediate zone" of the cervical spinal cord (laminae V-VIII) *and* in the ventral horn (lamina IX). Terminations from M1 were the densest, those from the CMAd and CMAv were least dense, and those from the SMA were intermediate. The terminations in lamina IX from all the cortical areas were densest dorsolaterally in regions where motoneurons which innervate distal forelimb muscles are located. Thus, the SMA, CMAd, and CMAv appear to be like M1 in having direct connections to spinal motoneurons, particularly those innervating muscles of the fingers and wrist. Within the intermediate zone, terminations from the SMA and M1 were densest at 3 sites: 1) dorsolaterally in laminae V-VII; 2) dorso-medially in lamina VI, at the base of the dorsal horn; and 3) ventromedially in laminae VII/VIII. In contrast, efferents from the CMAd terminated most densely at the dorsolateral site, whereas those from the CMAv were concentrated in the dorsomedial site. Thus, the CMAd and CMAv may influence different aspects of segmental motor control by connecting with separate sets of last order interneurons. These results suggest that the medial wall motor areas have the potential to generate and control movement through connections that are independent of M1. Furthermore, the corticospinal projections from the medial wall may provide an anatomical substrate for the recovery of motor function which follows damage to M1 or its output pathways. Support: VA Rehab. R&D and USPHS 24328 (PLS).

430.6

PARIETAL INPUTS TO DORSAL VS. VENTRAL PREMOTOR AREAS IN THE MACAQUE MONKEY: A MULTIPLE ANATOMICAL TRACING STUDY. J. Tanné*(1), D. Boussaoud (1), N. Boyer-Zeller (1), V. Moret (2) and E.M. Rouiller (2). (1) INSERM U94, 69500 Bron (France); (2) Univ. Fribourg, CH-1700 Fribourg (Suisse).

The dorsal (PMd) and ventral (PMv) premotor areas are both involved in visually guided limb movements. Recently, we *(Tanné et al., Neuroreport, 7: 267-272, 1995)* described direct visual inputs to PMd from medial and lateral parietal cortex, and from the parieto-occipital sulcus. These findings together with a substantial body of psychophysical and neurophysiological evidence support the view that PMd and PMv might be part of two separate visuomotor channels: a reaching channel involving PMd, and a grasping channel involving PMv. The present study was aimed at directly comparing the distribution of parietal cells projecting to PMd vs. those projecting to PMv, by means of injections of multiple retrograde tracers within the two areas in the same monkeys.

We found that many parietal areas project only or mostly to PMd. These include the parieto-occipital area (PO), areas 7m and 7a, the caudal part of the medial intraparietal area (MIP), the medial dorsal parietal area (MDP), and the ventral and lateral intraparietal areas (VIP and LIP, respectively). Projections directed to PMv, but not to PMd, arise from the anterior portion of the posterior bank of the intraparietal sulcus (AIP), the adjacent fundus and part of the medial bank. Parietal regions projecting to both PMd and PMv are the rostral part of area MIP, and areas 7b and 5 on the convexity of the inferior and superior parietal lobules, respectively. Within MIP and 7b, labelled cells were largely segregated: PMv-projecting cells are located rostrally, PMd-projecting cells are located caudally, with a small region of overlap in between. The findings show that PMd and PMv receive projections from largely separate regions of parietal cortex, and support the distinction between reaching vs. grasping visuomotor pathways. Supported by INSERM (Paris) and the Swiss National Science Foundation.

430.7

ORGANIZATION OF FACE REPRESENTATION IN THE CINGULATE GYRUS OF THE RHESUS MONKEY. R.J. Morecraft, C.M. Schroeder and J. Keifer*. Department of Anatomy and Structural Biology, University of South Dakota School of Medicine, Vermillion, SD 57069

Areas 24c (or M3) and 23c of the cingulate gyrus represent a unique part of the cerebral cortex because both areas receive direct input from prefrontal and limbic cortices and both send projections to the spinal cord, red nucleus, basal ganglia and isocortical motor areas. Although a face representation has been identified in area 24c, it not known if a face representation resides within area 23c. Neuroanatomical and electrophysiological methodologies were used in 4 rhesus monkeys to investigate the organization of face representation in the cingulate gyrus and examine the possibility that cingulate face areas give rise to descending projections which target brainstem centers regulating face movements. Fluorescent tracer injections placed into electrophysiologically-defined subsectors of the primary (M1) and supplementary (M2) motor cortices demonstrated that the rostral part of area 24c and area 23c project to the face representations of M1 and M2. Injections of biotinylated dextran amine involving the rostral part of area 24c and area 23c demonstrated a direct projection from both regions to the facial nucleus (FN). The area 24c projection ended primarily in the dorsolateral part of the FN and the area 23c projection in the lateral part. Our data demonstrate that areas 24c and 23c each contain a face representation directly affecting cortical as well as subcortical centers controlling the face. The observation of face representation in the rostral part of areas 24c (M3) and 23c (M4) suggest the formation of two complete simisculi in cingulate cortex. Our results also indicate that face representation may assist in defining the rostral border of areas 24c and 23c. The projection from M3 to the dorsolateral part of the FN suggest preservation of upper facial movement following middle cerebral artery damage may be due in part, to sparing of the corticofacial projection from the cingulate motor cortex. (Support: USDSM Faculty Development Award; Howard Hughes Medical Institute Award and NSF OSR-9452894) (NeuroReport, 7:1996)

430.8

TOPOGRAPHY OF PROJECTION TO THE MOTOR CORTEX FROM FUNCTIONALLY IDENTIFIED CELL GROUPS IN CEREBELLAR NUCLEUS INTERPOSITUS ANTERIOR IN THE CAT. H. Jörntell* and C.-F. Ekerot. Dept of Physiology and Neuroscience, Lund University, Lund, Sweden.

The projections to the motor cortex from different cell groups (sites) in the forelimb area of nucleus interpositus anterior (NIA) were investigated in barbiturate anaesthetized cats. NIA-sites were identified by their cutaneous climbing fibre receptive fields (cf-rfs) and microstimulated with relatively large microelectrodes. Motor cortical surface and depth responses were recorded with small surface and microelectrodes, respectively.

The cortical responses were mainly located in the lateral part of the anterior sigmoid gyrus and displayed a fine topographical organization. The response topography for a given site was coupled to the exact location of its cf-rf. Powerful cortical projections were found from sites with cf-rfs located ventrally and radially on the paw and forearm, especially if the sensitivity centre of the cf-rf was on the first digit. The response amplitudes for sites with other types of cf-rfs were progressively smaller the more the cf-rf involved the dorsum of the paw or the ulnar forearm. For functional comparisons, the cortical projections from face and hindlimb areas of NIA and from the posterior interposed and dentate nuclei were also investigated.

Importantly, the relations between the topographical patterns for different NIA sites were constant despite that the relations to anatomical landmarks on the cortical surface occasionally could deviate from the usual pattern. This is in contrast to the large interindividual variations in the detailed topography of motor output that have been reported by several authors.

This work was supported by the Swedish Medical Research Council (project no. 8291), the Medical Faculty at the University of Lund, Greta and Johan Kocks Stiftelser, Thorsten and Elsa Segerfalks Stiftelse.

430.9

CORTICOCORTICAL AND THALAMIC CONNECTIONS OF MEDIAL AGRANULAR CORTEX (AGm) IN PRAIRIE VOLES (*Microtus Ochrogaster*). R.L. Reep* and B. Kirkpatrick, Dept. of Physiol. Sci., Univ. of Florida, Gainesville, FL 32610; and Dept. of Psychiatry, Univ. of Maryland School of Medicine, Baltimore, MD 21228.

Fluorescent axonal tracers were used to investigate the connections of AGm in prairie voles. As in rats, the rostral and caudal portions of AGm have different but partially overlapping patterns of connections.

Caudal AGm has extensive cortical connections with orbital cortex, cingulate and retrosplenial cortex, caudal Par1, Par2, posterior parietal cortex and visual areas. Rostral AGm has similar connections with areas Par1 and Par2, orbital connections limited to area VLO, no connections with posterior parietal or retrosplenial cortex, and greatly reduced connections with visual areas. Rostral AGm receives input from area AGl, whereas caudal AGm does not. The axons linking rostral and caudal AGm with other cortical areas travel in the deep gray matter of layer VI rather than in the white matter.

Thalamic labeling after caudal AGm injections was prominent in nuclei AM, VL, MDl, CL, Po and LPm. Labeling in nucleus VM and Rh formed a halo around the periphery of nucleus Sm. Rostral AGm has a similar pattern, but lacks input from LPm.

Supported by the University of Florida College of Veterinary Medicine and the Maryland Psychiatric Research Center.

430.10

THALAMOCORTICAL AFFERENTS TO LAYER I OF RAT SENSORY-MOTOR CORTEX, B.D. Mitchell*, B. Clancy and L.J. Cauller, GR 41, Cognition and Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083.

Layer I of the neocortex is a dense synaptic zone consisting of horizontal axons running in all directions (Cauller *et al*, 1996) making synapses with supragranular neurons and the distal apical dendrites of the layer V corticobulbar pyramidal neurons (R.M. Brown *et al., ibid*). In addition to the "backward" corticocortical and widespread layer VII projections (Clancy and Cauller, *ibid*), thalamic inputs also terminate in layer I of the rat although the horizontal extent of these thalamic projections has not been fully determined. We have studied the origin and extent of the thalamocortical projections to layer I of primary sensory/motor areas of rat neocortex. Thalamic origins of layer I inputs were identified by retrograde tracers, diamidino yellow and Fast Blue, applied directly to the pial surface (~1 mm^2; 10 min. application) with penetration no deeper than layer II verified by the absence of labeled cells in layer IV which are consistently labeled by layer II injections. Applications in the forepaw area labeled cells scattered throughout VPl and Po thalamus in addition to a dense cluster of cells in VM. More anterior applications in lateral or medial agranular frontal cortex labeled many more cells in VL thalamus than found in VPl following application to the forepaw area. In addition, a dense cluster of cells in VM was also found following the frontal applications indicating VM projects widely to layer I across sensory/motor cortex. Injections of dextranamine (10kMW) into VM anterogradely labeled fibers extending to layer I with numerous horizontal branches. These anatomical findings are consistent with electrophysiological results (K. Paul *et al., ibid*) showing VM stimulation excites superficial cortical layers. Supported by a grant from the Whitehall Foundation.

430.11

CONNECTIVITY AND HIERARCHY IN THE OPOSSUM ISOCORTEX

S. MARTINICH, M.N.PONTES, E. VOLCHAN*. Lab. of Neurobiology II, IBCCF°, Federal University of Rio de Janeiro, RJ 21949-900. Brasil.

The analysis of tangential patterns of connections obtained after injections of anterograde and/or retrograde tracers (Fast Blue, Diamidino Yellow, HRP, WGA-HRP) into different cortical fields of *Didelphis marsupialis* have allowed us to state the following rules: 1) A primary area of one particular sensory modality connects unilaterally with primary and *unimodal associative* areas from other modalities. 2) The density of intrahemispheric connections between two areas increases with spatial proximity. 3) A primary area has multi-order contralateral inputs but their contralateral outputs are strictly homotopic. 4) Frontal (F), parietomarginal (PM) and insular (INS) cortices receive bilateral inputs from primary and associative (*uni or multimodal*) areas. 5) The connectivity of F, PM and INS is greatest with multimodal associative or "transitional" regions, decreases with unimodal associative areas and is lowest with primary areas. 6) The topography of connections is progressively coarser along an *ascendent* sequence of primary, unimodal and multimodal associative areas. 7) *Descendent* connections scatter more than *ascendent* ones. 8) Specific connections can emerge from areal modular subdivisions. These principles conform to a topological-functional organization which minimizes connection distances and hierarchizes information processing in the opossum isocortex.

Financial Support: CNPq, FENORTE, FINEP.

BASAL GANGLIA: FUNCTION III

431.1

IMPAIRMENT OF REACHING AFTER MUSCIMOL INACTIVATION OF GLOBUS PALLIDUS PARS INTERNA IN THE MONKEY W.T. Thach*, A.J. Bastian and J.W. Mink. Depts. of Neurology, Anatomy & Neurobiology and Program in Physical Therapy, Washington. Univ. School of Med., St. Louis, MO 63110.

The purpose of this study was to investigate the kinematics of a reach and grasp in the monkey before and after reversible inactivation of the globus pallidus pars interna (GPi).

A rhesus monkey sat in a modified primate chair and reached in a para-sagittal plane to grasp a 0.5 cm cube of apple that was presented at shoulder height and in front of the monkey. Markers were applied to arm overlying the shoulder, elbow, wrist, and tip of the index finger. Movements were videotaped at 60 fields/s, digitized with a Peak Performance video-based motion analysis system, and analyzed using custom-written software. In each session the monkey performed 12 - 15 reaches before and 12- 15 reaches after injection of 0.5 µl of muscimol (1 µg/ µl) into GPi. The boundaries of GPi had been mapped previously with single unit recording during a trained wrist movement. Reaching was impaired after injection into 9 of 10 sites, each 1 mm apart. The impairment was qualitatively similar across sites, but the largest deficits were seen after injection into the more ventral and medial sites.

After all 9 GPi inactivation experiments that produced a deficit, reaching was slow and the monkey tended to over-reach the target in the vertical plane and under-reach in the horizontal plane. Separate kinematic analysis of movement at the different joints indicated that velocity of elbow extension was slowed by up to 50% whereas elbow flexion was nearly normal. In this task, extension of the elbow is aided by gravity and by interaction torques due to shoulder flexion and is thus accomplished by controlled relaxation of the elbow flexors. The inability to relax the elbow flexors is consistent with previous findings that GPi inactivation causes a tonic flexor bias and impairment of 'turning off' previously active muscles compared to 'turning on' active muscles. These results support the hypothesis that the basal ganglia act to inhibit competing motor mechanisms.

(Supported by NIH grants K08 NS01808 (JWM) and R01 NS12777 (WTT) and by the McDonnell Center for Higher Brain Function)

431.2

NEOSTRIATAL NEURONAL ACTIVITY BECOMES BETTER RELATED TO MOVEMENTS WHEN CONDITIONS BECOME UNPREDICTABLE. R.J. Nelson*, M.A. Lebedev, I. Opris and J.M. Denton. Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, 875 Monroe Avenue, Memphis, TN 38163.

Recent evidence suggests that neostriatal (NS) neuronal activity may become better related to movements and sensory cues under novel behavioral conditions. We tested whether these improvements could occur after single trial changes in predictability of the linkage between cues, movements and rewards. Monkeys made vibratory or visually-cued ballistic wrist flexions and extensions after holding a steady position for 0.5-2.0s against a light load. They were rewarded 75% of the time, pseudorandomly, for correct performance. Activity during groups of trials followed by rewards (Regular trials) was compared to that during trials following ones in which rewards were withheld (After trials). Animals were cared for according to NIH guidelines.

To date, 93 confirmed and 156 presumed NS neurons have been studied because their firing patterns changed before movement or after sensory cues. Reaction times (RTs), activity onsets relative to cue (R1) or movement (R2) onsets were calculated. Changes in position, velocity, acceleration and neuronal activity were compared to yield coefficients of determination (cD). Times at which the best cDs occurred (cT) relative to movements and rise times (R2-cT) were computed . Comparing After with Regular trials, RTs and R2s were shorter (38ms and 51ms - vibratory trials, 14ms and 15 ms visual trials, respectively). For vibratory but not visually-cued trials, R1s were shorter (~13ms). CTs were not different for either go-cue modality. The cDs were significantly larger in vibratory but not visually-cued trials, indicating a better correlation of neuronal activity with movement parameters. Rise times were significantly shorter for both vibratory (~40ms) and visually-cued (~17ms) trials.

These observations suggest that NS neuronal activity becomes better correlated with movements during unpredictable conditions, but does so differently dependent upon the type of sensory stimuli present. Thus, NS neurons may be gated during predictable behavioral conditions. This improved correlation parallels improvement in several task-related kinematic parameters. Supported by **NIH Gr NS 26473**.

431.3

THE NIGROSTRIATAL DOPAMINE SYSTEM INFLUENCES ACTIVITY OF TONICALLY ACTIVE STRIATAL NEURONS THROUGH D2-CLASS DOPAMINE RECEPTORS. K. Watanabe, N. Matsumoto, A.M. Graybiel and M. Kimura*. Fac. of Health and Sport Sci. Osaka Univ. Toyonaka Osaka, 560 JAPAN, and Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge MA 02139 U.S.A.

Tonically active neurons (TANs) of the monkey striatum fire at low rates, appear not to fire in relation to movement, but show responses to conditioned sensory stimuli in behaving monkeys who learn a sensorimotor conditioning task. The acquired response has been shown to occur in 50-70% of TANs recorded in the caudate-putamen. Intrastriatal infusion of the toxin, MPTP, diminishes this population response to ca. pre-conditioning levels of cue responsiveness (ca. 15-20%; Aosaki et al., 1994). This result suggests that intrastriatal dopamine is required for the conditioned responses of the TANs. In the present study, our goal was to identify the dopamine receptor subtypes through which the conditioned responsiveness of TANs is influenced. One monkey (*Macaca fuscata*) was conditioned with a click sound that was paired to the delivery of a liquid reward. TAN units were recorded in the putamen and caudate nucleus. Before conditioning, 14 % of TANs responded to the clicks. After 2 to 3 weeks of conditioning, more than 60% of TANs became responsive to the clicks. Dopamine receptor antagonists were locally applied through the cannula containing the tungsten wire recording electrode while the activities of TANs were recorded following behavioral conditioning. Infusion of the D2-class antagonist, (-) sulpiride (1 µl, 20 µg/µl, n=2), blocked or sharply reduced the responses of TANs to the reward-predictive clicks. However, neither of two D1-class antagonists, SCH23390 (10 µg/µl, n=4) and cis-flupenthixol (30 µg/µl, n=2), detectably modulated the responses of TANs to the clicks. These results suggest that the nigrostriatal dopamine system can influence the expression of conditioned responses of TANs in the primate striatum through D2-class dopamine receptors. Supported by Japanese Ministry of Education grant #07408035 and NIH Javits Award R01 NS25529.

431.4

Functional MRI Activation of Multiple Basal Ganglia-Thalamocortical Sites During Self-Generated Sequences of Arm Movements *V. Menon*, J. Johnson, E. V. Sullivan, K. O. Lim, G. H. Glover and A. Pfefferbaum*

Departments of Psychiatry and Behavioral Sciences and Radiology, Stanford University School of Medicine and VA Palo Alto Health Care System, CA 94305.

A number of cortico-subcortical loops may underlie planning, control, and execution of internally generated movements. In this study, brain areas involved in self-generated sequences of arm movements were investigated using fMRI. Four right-handed subjects performed an experiment consisting of 12 alternating 40s epochs of rest and a motor task. During the motor task, subjects first generated 3 numbers between 1 and 4 and then made arm movements to corresponding labeled positions on a keypad. Subjects repeated this procedure with a new sequence till they were instructed to stop. Functional images were acquired on a conventional 1.5T scanner using a T2* weighted gradient echo spiral pulse sequence with a temporal resolution of 4s. Twelve axial slices (overlap across subjects: $-8mm < z < 46mm$ in Talairach coordinates) were acquired with slice thickness of 6mm and in-plane resolution of 2.8mm. Statistical Parametric Mapping was used to analyse the data. Results reported here are for $Z > 5$ ($p < 0.001$, corrected for multiple comparisons). Multisubject averaging revealed significant activations of left caudate, left putamen, bilateral pulvinar (left > right), left ventral anterior-ventral lateral and right lateral-posterior nuclei of the thalamus, bilateral dorsolateral prefrontal cortex (BA 9/46, right > left), bilateral pre-motor cortex (BA 6), bilateral anterior cingulate cortex (BA 24), left insula, bilateral superior and inferior parietal lobules (BA 7 and BA 40/39), bilateral temporo-occipital cortex (BA 19/37) and anterior vermis. These results indicate that multiple basal ganglia-thalamocortical sites are engaged during self-generated sequences of arm movements. The pattern of activation is consistent with previous electrophysiological and histochemical findings of multiple basal ganglia-thalamocortical loops. *Supported by The Sinclair Fund, NIH (MH30854, AA05965, AA10723) and the Department of Veterans Affairs.*

431.5

NEURONAL ACTIVITY IN THE PUTAMEN AND CLAUSTRUM DURING PERFORMANCE OF MULTIPLE MOVEMENTS. K.Shima, E. Hoshi and J.Tanji*, Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai, 980, Japan

The purpose of the present study was to examine how neuronal activity in the putamen and claustrum is involved in performance of multiple movements. Two monkeys (*Macaca fuscata*) were trained to perform three different movements (push, pull or turn a manipulandum) sequentially in a temporal order. Individual movements were initiated from a neutral position, and each movement was triggered by a tone signal. Initially, a visual signal indicated what movement should be performed, and subsequently the same sequential movements were performed in the absence of the visual signal. In both putamen and claustrum, over 70% of task-related neurons were active in relation to execution of movements, and remaining neurons were active during waiting periods before go signals. In the putamen, a vast majority (over 80%) of movement-related neurons were active in relation to execution of a particular movement among the three movements, while only 6 % were active in relation to all three movements. By contrast, a large population(73%) of claustral movement-related neurons exhibited relation to all three movements. In a group of putamen neurons, magnitudes of activity changes during execution of movements were influenced by differences in the order of movements to be performed. Such activity was not common in the claustrum. These striking differences suggest that the putamen and claustrum are differentially involved in sequential execution of multiple movements.

431.6

ENSEMBLE RECORDINGS FROM STRIATUM OF FREELY-BEHAVING RATS M. Jog C. Connolly V. Hillegaart M. Wilson and A.M. Graybiel* Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139

To study the functional organization of the striatum, we have developed an electrophysiological recording system with 4-6 mobile tetrodes implanted in the striatum of freely behaving rats.

Tetrodes were implanted surgically into the dorsolateral striatum of 17 rats. Each tetrode was then advanced independently from the brain surface over a period of 3-5 days, and recordings were made for up to 12 weeks. Locations of tetrodes were histologically confirmed. Each spike was registered as an event on all four channels of a tetrode. Units were identified and analyzed using an in-house cluster analysis package developed for this purpose (Jog, Connolly, Hillegaart, Graybiel in prep.). A 3-D spike event plot generated clusters in parameter space. Location, frequency and phase of firing of each cluster was computed for each tetrode.

We have analyzed 7 cell pairs from 3 animals to date. Selected pairs were from 3 tetrodes in rat 1, and 1 tetrode each from rats 2 & 3. Two pairs showed a low firing rate of 0.5Hz and stayed in phase, with calculated distances between units in the pairs of 100 μm and 22 μm. One in-phase pair fired at 4Hz with interunit distance of 39 μm. Two other pairs were out of phase with their partners at lower rates (0.5 Hz and 0.1 Hz), but were in phase at 2 Hz with interunit separations of 200 and 212 μm, respectively. A final pair showed firing out of phase at 0.5Hz but abrupt phase locking at 3Hz. This appeared behaviorally related to increased motor activity or changed sensory input prompted by turning off the room lights. Inter-unit distance was 88 μm.

Results from these experiments suggest that correlated striatal activity between unit pairs can occur at low and high frequencies. Those units that are out of phase at lower frequencies can become in-phase at higher frequencies. This phenomenon occurred in units that were both close and at a distance. The one instance of abrupt change in phase and frequency with lights off suggests that phase relationships may be correlated with behavior. Supported by the McDonnell-Pew Foundation, the Medical Research Council of Canada, and NIH Javits Award R01 NS25529.

431.7

TIME-FREQUENCY ENCODING OF VARIATIONS IN REACTION-TIME PERFORMANCE BY NEURONAL ENSEMBLES IN THE RODENT STRIATUM AND PREMOTOR CORTEX Mark G. Laubach* and Donald J. Woodward, Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157

The goal of this study was to examine whether variations in the firing patterns of neurons in the rodent striatum and premotor cortex are predictive of an animal's performance of a tone-cued, variable-delay, simple reaction-time task. A multi-site single-unit recording method was used to record from 10 to 30 neurons simultaneously. Spikes were collected in peri-event histograms (1 msec bins, 400 msec prior to the operant response) for trials with the fastest and slowest reaction-times, defined by the quartiles of the reaction-time distribution. 77 of 360 (~20%) striatal neurons and 36 of 185 (~20%) neurons in the medial agranular cortex recorded in 6 rats exhibited phasic modulations in spike output prior to the operant response (preparatory activity). A quantitative analysis of 50 neurons with wavelet discriminant analysis (Buckheit and Donoho 1995) found that variations in the firing patterns of 24 (~50%) of the neurons were predictive of variations in reaction-time performance at >20-50% over chance levels of prediction. Variations in reaction-time performance were associated with variations in the rate and temporal structure of bursts of spikes immediately prior to the operant response and in oscillatory patterns of spikes during the foreperiod. Spectral analysis found that the oscillatory firing patterns had power at 5-7 (n=6), 8-12 (n=12) and 15-25 (n=10) Hz. When groups of 4 to 8 simultaneously recorded neurons with preparatory activity were combined for discriminant analysis, there was a modest improvement in classification (~10%) over that of the best neuron in the group. Cross-correlation analysis found that spikes from neurons with oscillations were synchronized during the epoch of oscillation (JPSTH, 4 msec bins). These data suggest that information related to reaction-time performance is encoded in the firing patterns of neurons in the striatum and cortex, is related to the 10-20 Hz "mu" rhythm and is largely redundant across the neuronal population. *Supported by AA05396 to MGL and DA02338 to DJW.*

431.8

FORCED OR VOLUNTARY LOCOMOTOR ACTIVITY ELICITS FOS-LIKE IMMUNOREACTIVITY IN THE RAT STRIATUM. **W.M. Struthers* and D. Wirtshafter. Department of Psychology, University of Illinois at Chicago, Chicago, IL 60612.**

The striatum is known to be involved in production of locomotor activity. Additionally, Fos-Like Immunoreactivity (FLI) has been used as a marker for trans-synaptic activation of neurons in the nervous system. Given the striatum's involvement in locomotor activity, two experiments were conducted in an attempt to elicit FLI in the striatum behaviorally. In the first experiment, males were placed in a running wheel and forced to run intermittently for one hour. Results showed that forced running induced FLI in the striatum.

In an additional experiment, estrogen and progesterone pretreatment of ovariectomized females produced a significant increase in voluntary running wheel activity. Striatal FLI was observed in these females, although to a lesser degree than that seen in forced running animals. There was an absence of FLI in the striatum of vehicle injected controls and also in steroid pretreated females who were not allowed access to the running wheel. These results suggest that Fos is produced in the striatum as a result of increased locomotor activity (Supported by NS33992).

431.9

EXTRACTION OF NEOSTRIATAL FEATURES FROM DEOXYGLUCOSE AUTORADIOGRAMS. S.M. Feldman*†, J.R. Cavanaugh†, L.M.Goldbloom‡ and L.L. Brown‡, †Center for Neural Science, New York University, New York, NY 10003 and ‡Department of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

We used feature detection procedures in conjunction with deoxyglucose autoradiography to reveal a combinatorial map of somatosensory representation in rat neostriatum (Brown, PNAS, 1992). However, because the changes in metabolic activity that accompany tactile stimulation are subtle, it is often difficult to define the extent of a detected feature. We here describe a computer-assisted technique for detecting peaks of metabolic activity in 2DG autoradiograms that allows for improved definition of the edges of detected features, which can better be related to the neostriatal substrate.

Tactile stimulation was applied to awake rats after *iv* injection of radiolabelled deoxyglucose. Image analysis was carried out on digitized autoradiograms of coronal brain sections, with optical density coded as grey level. To detect features with high contrast edges, we used an iterative thresholding procedure to identify features that exceeded an empirically established preset minimum area (the equivalent of a circle with dia=425μm; this was the size of the largest features observed). The algorithm we used automatically reduced the preset minimum area if the detected feature grew too quickly during iterative thresholding. Features with lower contrast edges grow more quickly than do features with higher contrast edges as the thresholding "window" includes increasingly lower gray levels. Higher contrast features do not grow as quickly, and are selectively extracted by the procedure. In this way we were able reliably to define and delimit the individual peaks of high optical density that resulted from the tactile stimulation. Supported by USPHS Grants NS21356 and MH19524.

431.10

THE STRIATAL REPRESENTATION OF VIBRISSAE: CORRELATION OF ANATOMIC AND FUNCTIONAL STUDIES IN THE RAT. L.M. Goldbloom*, D.M. Smith and L.L. Brown. Depts. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

In primary somatosensory cortex, the body map is segregated into receptive fields where leg, arm and face are represented in non-overlapping regions and functionally related neurons are in the same vicinity. In contrast, metabolic mapping studies suggest that the striatum is organized in such a manner that the map of the body is in multiple disconnected regions where overlap between leg, arm and face may occur. To examine the underlying anatomy, an anterograde tracer was injected into electrophysiologically defined areas of somatosensory cortex. To uncover the functional neural activity, autoradiography using (^{14}C)deoxyglucose was employed during tactile stimulation.

We found that somatosensory cortex projections from layers III, Va and Vb of the vibrissae area to the striatum had a distinct pattern that differed from the forearm and hindlimb projections. The dorsolateral striatum contained the major projection field while there were smaller projection fields medially. These projections extended in the antero-posterior direction from about 2.5 mm behind bregma up to 2.0 in front of bregma. The functional neural activity displayed discrete metabolic peaks in areas that corresponded to the anatomical projections, but the metabolic functional map was not as extensive as the corticostriate projections. We conclude that the body map is distributed in the striatum to allow extensive integration beyond sensorimotor function as opposed to the more segregated functions seen in primary somatosensory cortex. Supported by NIH NS21356

431.11

SOMATOSENSORY STIMULATION IN AWAKE RAT INDUCES PATCHY DOMAINS OF METABOLIC ACTIVITY IN THE STRIATAL MATRIX COMPARTMENT. L.L. Brown*, L.M. Goldbloom, D.M. Smith, S.M. Feldman, A.M. Graybiel. Dept. of Neurology, Albert Einstein College of Medicine, Bronx NY 10461; Center for Neural Science, NYU, NY, NY 10003; Dept. of Brain & Cognitive Sciences, MIT, Cambridge MA 02139.

The striatum has a large sensorimotor region in which cells fire in relation to movement with a rough foot-up, head-down topography. This striatal region receives strong inputs from somatosensory and motor cortex. In the monkey, inputs representing particular body parts are organized in distributed arrays that in cross section appear as patches in the matrix ("matrisomes"). In the rat, neural metabolism peaks related to somatosensory stimulation also form patchy, distributed arrays. We used the [^{14}C]deoxyglucose mapping technique to study functional activity in the striatum in unanesthetized, restrained rats given somatosensory (light touch) stimulation for 45 min on the hindlimb or vibrissae. Sites of peak metabolic activity were identified by computer-assisted methods and striosome and matrix compartments were identified by ^{3}H naloxone binding. Peak metabolic activity appeared in the dorsolateral caudoputamen in distributed sets of patches ca 150-300μm wide and separated by 150-300 μm. Nearly all of the activity was in the matrix: there was only 1-5% overlap of metabolic peaks with striosomes. Some patches of metabolic activity abutted striosomes but others were separated from them. Almost every metabolic patch was clearly centered in the matrix. These findings demonstrate that the matrix compartment of the sensorimotor striatum is organized into patchy functional domains that can be activated by natural stimulation of localized body parts. These domains may correspond to matrisomes. Supported by NIH NS 21356 and NIH Javits Award NS 25529.

431.12

SIMULTANEOUS INTRACELLULAR RECORDING OF SPONTANEOUS ACTIVITY IN PAIRS OF STRIATAL SPINY NEURONS *IN VIVO*. E. A. Stern*[1], D. Jaeger[2], and C. J. Wilson[1], [1]Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN., [2]Division of Biology 216-76, Caltech, Pasadena, CA.

Intracellular recordings were made from pairs of striatal spiny neurons in rats anesthetized with urethane. These neurons exhibited spontaneous, subthreshold membrane potential shifts, resulting in a two-state behavior of the membrane potential. The depolarized (Up) state of the neurons has previously been shown to result from convergent synaptic activity interacting with nonlinear membrane properties. We analyzed the correlation of membrane potentials among pairs of striatal neurons on three time scales: slow spontaneous subthreshold membrane potential shifts, high-frequency membrane potential fluctuations in the Up state, and spike activity. Correlated activity was measured with crosscorrelation, cross-spectral density, and time-dependent correlation analysis. Some pairs of neurons showed highly correlated slow spontaneous membrane potential shifts, while in others, the membrane potentials fluctuated independently. Few significant correlations of fluctuations within Up states or spike activity among neurons were found. The strength of the correlated activity decreased with distance between the cells. Previous studies have shown that the spontaneous membrane potential fluctuations of corticostriatal and striatal neurons have periodic and stochastic components. Within correlated pairs of striatal neurons, both the periodic and stochastic components were correlated. This shows that the stochastic as well as the periodic components of the corticostriatal input are preserved through convergence of the corticostriatal connectivity. NIH NS20473 and Sloan Foundation

431.13

ACTION POTENTIAL THRESHOLD OF MEDIUM SPINY NEURONS IN THE RAT NEOSTRIATUM IN VIVO. J. R. Wickens and C. J. Wilson* Dept Anatomy and Neurobiology, Univ. Tennessee, Memphis, TN 38163.

Previous work has shown that dopamine, acetylcholine and possibly other neuromodulators act in part to alter the threshold for action potential generation in striatal neurons, or the sensitivity of the threshold to the recent history of membrane potential changes. We measured the threshold for action potentials evoked in identified spiny neurons in urethane-anesthetized rats under three different conditions: (1) electrical stimulation of the contralateral cerebral cortex, (2) brief directly-applied current pulses, (3) spontaneous action potentials occuring during spontaneous episodes of depolarization (Up state). Threshold was defined as the point along the action potential trajectory at which the rate of depolarization exceeded 4 mV per msec. All neurons in the sample exhibited spontaneous membrane potential shifts, but not all fired action potentials while in the Up state. In both spontaneously firing neurons and silent ones, the threshold for action potentials evoked by current pulses was significantly higher than for those evoked by cortically-evoked PSPs. There was no significant threshold difference between spontaneously firing and silent spiny neurons. There was also no significant difference in thresholds of spontaneous action potentials occuring at the onset of an Up state and those occurring after a prolonged period of depolarization. Application of more intense current pulses adjusted to reproduce the EPSP rate of rise produced firing at correspondingly lower thresholds. Most of these observations were reproduced by rapid voltage-sensitive inactivation of fast sodium and potassium channels in a compartmental model of the spiny projection neuron. NIH NS20743 & H.R.C. (NZ)

431.14

HALOPERIDOL AND CLOZAPINE INDUCE FOS-LIKE IMMUNOREACTIVITY IN THE PRIMATE BASAL GANGLIA. D. Wirtshafter*. Dept. Psychol., Univ. Ill. at Chicago, Chicago, Il 60607-7137.

Several authors have shown in rodents that the dopamine antagonist haloperidol induces Fos expression in the striatum and nucleus accumbens and we have found that this drug also induces Fos expression in the entopeduncular nucleus and substantia nigra. In contrast to haloperidol, the atypical neuroleptic clozapine appears to induce staining more effectively in the accumbens than in the striatum

In the current studies we examined the patterns of Fos expression induced in the monkey by haloperidol and clozapine. Eight *macaque fasciculata* were injected with either haloperidol (0.125, 0.5 or 2.5 mg/kg) or its vehicle three hours prior to sacrifice. Almost no Fos like immunoreactivity (FLI) was seen in the basal ganglia of animals injected with vehicle. Haloperidol induced a dose dependent increase in FLI in the striatum, accumbens, internal pallidal segment and substantia nigra. Nigral staining was most pronounced in the medial pars reticulata, but a few lightly stained neurons could also be detected in the pars compacta. Pronounced FLI was also seen in the lateral septum and in the bed nucleus of the stria terminalis. Only a few labeled cells could be seen in the external pallidal segment. These results are similar to those obtained in rat and are compatible with theories of basal ganglia organization which propose that blockade of D2 receptors excites striatopallidal cells resulting in disinhibition of nigral and entopeduncular neurons. In contrast, clozapine (25 mg/kg) induced only a small amount of staining in the striatum whereas much heavier labeling was seen in the accumbens. Pronounced staining was seen in the septum, bed nucleus of the stria terminalis and the islets of Calleja, but only a few labeled cells were seen in the substantia nigra or globus pallidus. This pattern may be related to the low instance of extrapyramidal side effects produced by clozapine.

Supported, in part, by NS33992

431.15

NEUROLEPTIC-INDUCED ORAL DYSKINESIAS IN RATS TREATED WITH OLANZAPINE, SERTINDOLE AND HALOPERIDOL. K. Sakai* X.-M. Gao and C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland, School of Medicine, Baltimore, MD 21228.

Chronic treatment of schizophrenia with typical neuroleptics is associated with tardive diskinesia (TD) in humans, a condition often modeled by vacuous chewing movements (VCMs) in rats. The atypical antipsychotic drug, clozapine has no (or a low) incident of either TD in humans or VCMs in rats. But, because of its side effects, it has been important to develop new atypical neuroleptics. Sertindole (Abbott and Lundbeck) and olanzapine (Lilly) are new antipsychotic drugs, both of which have very low motor side effects in humans. We quantified VCMs after administration of sertindole (0.5 mg/kg/day or 2.0 mg/kg/day), and olanzapine (0.5 mg/kg/day or 2.0 mg/kg/day) in comparison with haloperidol (1.5 mg/kg/day) and water controls for six months. VCM rates are 3.3±0.5 and 5.1±2.7 VCM/5 min. respectively for the two doses of sertindole, 4.6±1.3 and 3.9±0.9 VCM/5 min. respectively for the two doses of olanzapine and 10.0±2.5 VCM/5 min. for haloperidol compared to 2.0±0.5 VCM/5 min. for water alone (mean±S.E). Only with haloperidol was the VCM rate higher than with water. None of the groups treated with either sertindole or olanzapine showed VCMs higher than the water group. VCMs of the 0.5 mg/kg/day of sertindole group and 2.0 mg/kg/day of olanzapine group were significantly lower than that of the group treated with haloperidol. Plasma drug concentrations measured in trunk blood collected at sacrifice were 39.4±4.8 and 218.9±13.4 ng/ml respectively for the two doses of sertindole, 1.5±0.5 and 9.3±2.3 ng/ml respectively for the two doses of olanzapine and 13.0±3.2 ng/ml for haloperidol. Thus, two new antipsychotic drugs, which clinically show low motor side effects, have a lower incident of VCM in rats. Research supported by NIMH (MH 37073).

431.16

THE EFFECTS OF ANTIPSYCHOTIC DRUGS ON LIMBIC SYSTEM OUTPUT TO THE MEDIODORSAL THALAMUS: DEPENDENCE ON AN INTACT PREFRONTAL CORTEX. A. Lavin* and A.A. Grace *Depts. Neuroscience and Psychiatry, CNUP, Univ. Of Pittsburgh, Pittsburgh, PA, 15260.*

Numerous studies into the pathophysiology of schizophrenia have pointed to the importance of the relationship between cortical and dopaminergic afferents to the n. accumbens in this disorder. However, comparatively little is known about how the n. accumbens ultimately affects the thalamocortical system via its projections to the output neurons in the ventral pallidum (VP). In this study, we examined the impact of classical and atypical antipsychotic drugs on the VP output to the thalamic mediodorsal nucleus (MD). Using *in vivo* intracellular recordings, we examined the effects of acute administration of the classical antipsychotic haloperidol (HAL) and the atypical antipsychotic drug clozapine (CLOZ) on VP and MD cell physiology. In intact rats, both HAL and CLOZ produced a decrease in the spontaneous firing rate of VP cells (62% and 92%) while producing a parallel increase in MD cell firing rate (by 145% and 59%, respectively). In rats that had received an ibotenic acid lesion of the prefrontal cortex (PFCtx) 1 month prior the recordings, MD cells exhibited a significantly higher spontaneous firing rate. However, the MD cells also showed a significantly smaller change in firing frequency in response to HAL. The results of this study suggest that the ability of antipsychotic drugs to activate the VP-MD system depends on an intact prefrontal cortex. Furthermore, given the hypofrontality reported to be present in schizophrenics, this may provide an explanation for the greater tolerance of these patients to the acute sedative actions of antipsychotic drugs. This work was funded by NIH grant # MH455156.

431.17

CHARACTERIZATION OF D3 DOPAMINE RECEPTOR FUNCTION IN D3 KNOCKOUT MICE. A. Carta, R. Rius*, S. Fuchs, D. Accili, and C.R. Gerfen. Lab of Neurophysiology, NIMH; Lab of Developmental Endocrinology Branch, NICHD, Bethesda, MD 20892; Weizmann Institute, Rehovot, ISRAEL.

The dopamine D3 receptor subtype, a member of the D2 class of dopamine receptor subtypes, has been implicated in "limbic" functions, largely on the basis of two lines of evidence. First, the D3 receptor displays a restricted expression in ventral forebrain structures, including "limbic" cortices, the lateral septum, ventral striatum and islands of Calleja. Second, the D3 receptor displays a high affinity for "atypical" neuroleptics, D2 receptor antagonists with limited clinical motoric related side effects. Such circumstantial evidence is limited as there are many other receptors which share such anatomical distribution and pharmacologic profiles. To study the contribution of the D3 receptor to the effects of atypical neuroleptics, we have examined mice that lack a functional D3 dopamine receptor. The present study examined the induction patterns of immediate early genes in the forebrain of wild type and D3 deficient mice treated with the atypical neuroleptic clozapine using quantitative in situ hybridization localization of the mRNAs encoding c-fos and zif268. In the wild type mouse forebrain, clozapine treatment (15 and 30 mg/kg) results in immediate early gene induction in the cingulate and piriform cortex, lateral septal area, islands of Calleja and striatum. The pattern of induction in homozygote mice deficient in the D3 receptor was similar except for a reduction of clozapine-mediated immediate early gene induction in the striatum. These results suggest that although the pattern of immediate early gene induction by clozapine matches the distribution of the D3 dopamine receptor in many areas, in most areas, with the exception of the striatum, such induction is not attributable to blockade of this dopamine receptor subtype. (supported by NIMH)

431.18

EFFECTS OF DOPAMINE AGONISTS AND ANTAGONISTS ON OPTICAL RESPONSES RECORDED IN RAT FRONTAL CORTEX SLICES AFTER STIMULATION OF THE SUBCORTICAL WHITE MATTER. H. Kita*, K. Oda, M. Tanifuji, and K. Murase. Univ. Tennessee, Dept. Anat. and Neurobiol., Memphis, TN 38163 & Fukui Univ., Dept. Inf. Sci., Bunkyo, Fukui 910, Japan.

Effects of dopamine agonists and antagonists on the optical responses of the frontal cortex slices to stimulation of the subcortical white matter were examined using a voltage sensitive dye and a high speed image sensor. Repetitive white matter stimulation of the slices treated with bicuculline evoked optical responses propagating to the overlaying cortex. The responses often lasted for more than 400 msec. Bath application of NMDA antagonists, CPP and APV, resulted in a large reduction in the optical responses. Bath application of D1 agonist, c-APB, consistently decreased the intensity of the early part (i.e., during repetitive stimulation) of the response but increased the intensity and the duration of the later part of the response. D1 antagonist, SCH 23390, also increased overall intensity and duration of the response. The effects of c-APB and SCH 23390 were additive. Bath application of D2 agonist, quinpirole, reduced the response. Application of D2 agonist, eticlopride, did not cause significant change in the response but it effectively blocked the quinpirole effect. c-APB and quinpirole effects were difficult to reverse even when washing the slices for an extended period. However, the decrease of the response by quinpirole was restored or reversed by application of c-APB. Similarly, the increase of the response by c-APB was reversed by quinpirole. This study suggests that dopamine may modulate NMDA receptor mediated responses in the cortex through both D1 and D2 receptors. Supported by NIH, USA and MESC, Japan.

431.19

AN ELECTRON MICROSCOPIC ANALYSIS OF NEOSTRIATAL DOPAMINE FIBERS. J. Q. Ren* and H. Kita Dept. Anatomy and Neurobiol, Coll. of Med., Univ. of Tennessee, Memphis, TN 38163.

An electron microscopic analysis of rat neostriatal tyrosine hydroxylase (TH) immunoreactive fibers was performed to reveal details of morphological features of dopaminergic fibers. TH-immunohistochemistry of the neostriatum revealed darkly stained patch areas and surrounding less darkly stained matrix areas. Blocks of tissues obtained from the patches and matrices areas were cut into thin serial sections and examined under the electron microscope and photographed. The morphological features of TH-immunoreactive fibers (TH-fibers) were analyzed with the aid of computers. The average diameters of the TH-fibers in the patch and matrix were 0.241 and 0.268 μm, respectively. The volume of the neuropil occupied by TH-fibers was 6.7 % in patches and 4.0 % in matrices. The average length of TH-fibers contained in an unit volume of the neuropil was estimated to be 0.94 and 0.42 $\mu m/\mu m^3$ in patch and matrix areas, respectively. Thirty two axons which were oriented roughly perpendicular to the plain of thin sections were selected and the synapses formed by these axons were counted. Each fiber formed synapses with both dendritic shafts and spines. The average number of synapses per unit length of TH-fibers was 0.5/μm. This and the length of the fibers in the blocks gave the density of synapses to be $0.47/\mu m^3$ in the patch and $0.21/\mu m^3$ in the matrix.

Supported by NS-25783 and NS-26473.

431.20

INTERACTIONS BETWEEN AMYGDALA AND PREFRONTAL CORTICAL AFFERENTS TO THE NUCLEUS ACCUMBENS AND THEIR MODULATION BY DOPAMINE RECEPTOR ACTIVATION

H.Moore, and A.A. Grace, Depts. of Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh PA 15260.*

The basolateral amygdala (BLA), hippocampus and prefrontal cortex (PFC) provide excitatory inputs to the nucleus accumbens (NAC). Recently, O'Donnell & Grace (1995) demonstrated that the hippocampal input is necessary for the depolarized phase of the bistable membrane potential of NAC neurons. Moreover, it is only during this hippocampally-mediated depolarized state that PFC stimulation can evoke spikes in NAC cells. Given the strong input from the BLA to the NAC shell, we propose that the BLA inputs to the NAC might play a similar permissive role in PFC-NAC throughput. This was tested using extracellular single-unit recordings of NAC cells to characterize their responses to single- and paired-pulse stimulation of the PFC and to trains of stimuli in the BLA. We then tested whether a conditioning train of BLA stimuli would affect the probability of a PFC-evoked spike. Over 80% of the cells showed short-latency spikes in response to PFC stimulation but not to BLA stimulation, while a small proportion fired at longer latencies to stimulation of either site. In the PFC-responsive cells, a conditioning train of BLA stimuli, which in itself did not evoke spikes, increased the probability of a spike evoked by threshold-intensity PFC stimulation. Systemic administration of apomorphine, while decreasing the effectiveness of PFC stimulation in small set of these cells, more reliably decreased the BLA facilitation of PFC stimulation in the majority of cells. We conclude that the inputs from the BLA may shift a subset of NAC cells into a state in which they are more receptive to PFC inputs, and, furthermore, that BLA facilitation of PFC inputs is preferentially modulated by dopamine. *Supported by USPHS MH45156 and an NRSA Fellowship to H.M.*

BASAL GANGLIA: DOPAMINE

432.1

ACTIVATION OF D_2 DOPAMINE RECEPTORS REDUCES Ca^{2+} CURRENTS IN RAT NEOSTRIATAL CHOLINERGIC INTERNEURONS Zhen Yan*, Wen-Jie Song and D. James Surmeier Dept. of Anatomy & Neurobiology, School of Medicine, University of Tennessee, Memphis, TN 38163.

The D_2 dopamine receptor mediated modulation of Ca^{2+} channels in neostriatal cholinergic interneurons was studied by combined whole-cell voltage-clamp recording and single cell RT-PCR. Cholinergic interneurons were identified by the presence of choline acetyltransferase mRNA. Of the 17 interneurons examined with RT-PCR, nearly all (~90%) co-expressed D_2 (short and long isoforms) and D_{1b} dopamine receptor mRNAs, whereas D_{1a} receptor mRNA was found in only a small subset (~20%) of the sample.

D_2-class agonists reversibly reduced N-type Ca^{2+} currents, whereas D_1-class agonists had little or no effect on these currents. This reduction was blocked by the D_2 antagonist sulpiride. The D_2 modulation was almost eliminated by application of N-ethylmaleimide at concentrations previously shown to selectively inactivate $G_{i/o}$-class G proteins or by dialysis of GDPβS. The D_2 modulation had rapid onset kinetics and was unaffected by application of cAMP analogs. Furthermore, the modulation was not attenuated by strong, depolarizing pre-pulses.

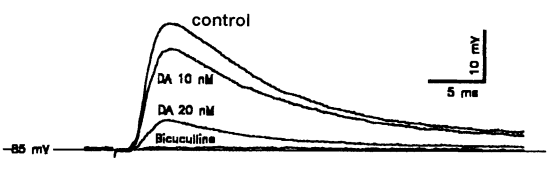
Although the modulations produced by D_2 dopamine receptors and M2 muscarinic receptors were occlusive, activation of protein kinase C disrupted muscarinic modulation of Ca^{2+} currents, but not the dopaminergic modulation. Taken together, our results suggest that activation of D_2 dopamine receptors in cholinergic interneurons reduces N-type Ca^{2+} currents through a $G_{i/o}$ class G-protein. Although M2 and D_2 signaling pathways both utilize $G_{i/o}$ proteins that target N-type Ca^{2+} channels, the D_2 pathway appears to rely upon elements that are not regulated by protein kinase C.

This work was supported by USPHS grants NS 26473 and NS 34696.

432.2

PRESYNAPTIC DOPAMINE MODULATION OF GABA-MEDIATED INTRASTRIATALLY EVOKED DEPOLARIZING POSTSYNAPTIC POTENTIALS . A. Delgado, A. Sierra, R. Valdiosera and J. Aceves.* Department of Physiology. CINVESTAV-IPN. 07300 México, D.F., México.

To study the role of dopamine in intrastriatal synaptic transmission, we used intracellular recording techniques in rat brain slices. Here we examined the role of D2 receptors. The experiments were done in the presence of CNQX and AP5 to eliminate the glutamatergic component of the evoked postsynaptic potential. In 30% of the recorded (n=33) neurons, dopamine (100 nM) reversibly depressed (by 60%) electrically evoked, picrotoxin-sensitive postsynaptic potentials, without changing the electrical properties of the impaled neurons. The effect was 1) antagonized by sulpiride (100 nM), 2) mimicked by quinpirole (20 nM), and 3) dose-dependent (see figure below). The IC50 was 13 nM. These results indicate the presence of presynaptic D2 receptors modulating GABA-mediated transmission within the striatum. It remains to be determined the location of the receptors, whether on collaterals of neighboring spiny projection neurons or on local circuit cells.

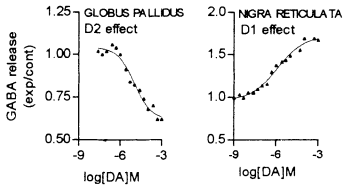


Supported by CONACYT grant 1245-N9203.

432.3

DIFFERENTIAL MODULATION OF GABA RELEASE BY D2 AND D1 RECEPTORS IN THE BASAL GANGLIA OF THE RAT. B. Floran., F. Paz-Bermudez., A. Sierra., J. Hernandez*., and J. Aceves. Department of Physiology, Biophysics and Neurosciences. CINVESTAV-IPN. 07300 México, D.F.

GABA projection neurons appear to have preferentially either D1 or D2 receptors. Here, we have explored whether the same segregation exists on their nerve endings at the target nuclei.



The effect of DA or its agonists (quinpirole or SKF 38393) on [^{3}H] GABA release was studied in K^+-depolarized slices from globus pallidus (Gpall), caudate-putamen (CP), substantia nigra pars reticulata (SNr) and entopeduncular nucleus (EPN). D2 effects were studied blocking D1 receptors with SCH 23390 and D1 effects blocking D2 receptors with l-sulpiride. Quinpirole and dopamine inhibited GABA release in Gpall and CP, but had no effect in SNr and EPN. SKF and dopamine markedly increase the release in SNr, EPN, CP and only slighly in Gpall. Dose-response relationship (see fig.) showed similar values (1.3-4.6 μM) beetwen the EC_{50}'s for D1 receptors in all the studied nuclei, but for D-2 receptors IC_{50} was three orders of magnitude higher in Gpall than in CP (Gpall= 8.14 μM ; CP=28.8 nM).
These observations suggest a differential modulation of GABA release by D1 and D2 receptors, and a differential location on striatal projections. They also suggest heterogenities beetwen D2 receptors.

432.4

DOPAMINE EXCITES RAT NEOSTRIATAL LARGE ASPINY NEURONS IN VITRO. Toshihiko Aosaki* and Yasuo Kawaguchi Lab. Neural Circuits, Inst. Phys. & Chem. Res. (RIKEN), Nagoya, Aichi 456, Japan.

Accumulating evidence suggests that dopamine (DA) inhibits release of acetylcholine (ACh) from cholinergic large aspiny neurons of the neostriatum via activation of D2 DA receptor. In order to test this ACh-DA balance hypothesis directly, we made whole-cell patch clamp recordings from identified large aspiny neurons in a brain slice preparation to study the actions of dopaminergic agents on them. Unexpectedly, bath-applied DA (1-100 μM) evoked either depolarization or an inward current in 13 out of 21 cells tested. The remaining cells showed no response (7/21) or an outward current (1/21). Quinpirole (1-10 μM), D2-like DA receptor agonist, evoked an inward current in 7 cells, an outward current in 3 cells and no responses in 6 cells out of 16 cells tested. SKF 38393 (1-10 μM), D1-like DA receptor agonist, evoked an inward current in 8, an outward current in 1 and no response in 5 out of 14 cells examined. The excitatory effect of DA was mostly accompanied by a decrease in input resistance. These results suggest that the neostriatal large aspiny neurons are quite heterogeneous in terms of dopamine actions and that, contrary to the hypothesis, DA excites rather than inhibits them. We suspect that, although there are no immunohistochemial studies on D3 receptor distribution in the neurons so far, the observed inward current by quinpirole might be due to activation of D3 DA receptors, because D2 receptor is reported to be coupled to K-channels, D4 receptor mRNA expression is very low in the dorsal neostriatum, and quinpirole cannnot differentiate D2 from D3 receptors. The observed actions of SKF 38393 might contain a component of D5 DA receptor activation, because, although in situ hybridization study (Le Moine et al., 1991) showed that 30% of the neurons contained D1 receptor mRNA, recent immunohistochemical study (Bergson et al. 1995) showed a mismatch that the neurons were labeled only by D5 receptor antibodies, not by D1. Supported by Frontier Research Program.

432.5

CORRELATION OF ELECTROPHYSIOLOGICAL AND BEHAVIORAL OUTPUT OF THE RAT BASAL GANGLIA AFTER INTRASTRIATAL INFUSION OF DOPAMINE (DA) AGONISTS. L.P. Martin*, D. Ozsirin, A. Zelenchuk, J.R. Stellar and B.L. Waszczak. Depts. of Pharmaceutical Sciences and Psychology, Northeastern University, Boston, MA 02115.

Current basal ganglia functional models predict that DA acts on striatal efferents to inhibit output from the substantia nigra pars reticulata and internal pallidal segment (SNr/GPi). This inhibition should, in turn, disinhibit the thalamus to facilitate movement. We are testing this model in intact (unlesioned) rats by attempting to correlate changes in single unit activity of SNr neurons with motor (i.e. behavioral) effects observed in the 20-30 min after striatal infusions of DA agonists given at identical doses, infusion volumes and stereotaxic coordinates. For behavioral studies, we have fabricated a remote, computer-operated infusion pump which allows automatic infusion of drugs through a previously-implanted striatal cannula, avoiding behavioral activation due to handling. Rats are filmed to allow later assessment of stereotypies and circling behavior, and photobeam-breaks give an index of locomotor activity. In parallel SNr recording studies, rats are acutely implanted with striatal cannulae, and SNr cell firing is monitored before and 20-30 min after infusions. While preliminary, results are contrary to predictions of the model in that large (1 μl) unilateral infusions of d-amphetamine (AMPH) into the rostral dorsomedial striatum elicit only sporadic and modest changes in basal ganglia output in either assay. For instance, in anesthetized rats both 1 and 20 μg AMPH infusions (n=4-5 each) caused a few brief (<1 to 3 min) inhibitions (<20% of baseline) as well as some brief to sustained (>5 min) increases in firing (>20% of baseline) in about half of SNr cells tested. The remainder showed no changes. Anesthesia appeared not to be a factor since similar, minor fluctuations were seen in awake, locally-anesthetized rats. Future studies will attempt to elicit (and correlate) behavioral and electrophysiological output using other DA agonists, striatal injection sites, and bilateral infusions. (Support by NS 23541)

432.6

DOPAMINE MODULATES MEMBRANE EXCITABILITY OF STRIATAL SPINY NEURONS VIA DARPP-32 SIGNAL TRANSDUCTION PATHWAY S-P. Onn*[1], A.A. Fienberg[2], V.A. Pieribone[2], P. Greengard[2], and A.A.Grace[1]. *Depts of Neurosci. and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260[1] & Lab. Molec. & Cell. Neurosci. Rockefeller University, New York, NY 10021[2]*

DARPP-32, dopamine- and cAMP-regulated phosphoprotein, is anatomically associated with striatal neurons that contain D1 receptors. However, the role of DARPP-32 in mediating DA actions on striatal neurons is not clear. In this study, we examined the effects of D1 and D2 agonists on the membrane physiology of identified striatal neurons recorded in slices obtained from wildtype vs DARPP-32-knockout mice. Bath application of SKF 38393 (5-10 uM) alone resulted in a 193% increase in the amplitude of current injection required to trigger spiking in spiny neurons. In contrast, SKF38393 failed to cause this reduction in the excitability of spiny neurons recorded from mutant mouse brain slices. Furthermore, subsequent bath application of D2 agonists quinpirole (5-10 uM) to D1-treated slices resulted in a membrane depolarization and a decrease in threshold currents to nearly the pre-drug (i.e., before D1) level in identified spiny neurons recorded from striatal slices from wildtype but not knockout mice. This net excitation in wildtype mice was opposite to the inhibitory actions typically observed when D1 and D2 agonists were co-applied. Thus, the temporal sequence of D1 and D2 receptor stimulation is critical for determining the net effect on cell excitability. Such an interaction is not present in mouse striatal neurons in which DARPP-32 signal transduction has been disrupted. Support by USPHS MH45156, MH42217 and MH40899(to P.G.) and APDA (to A.A.F.)

432.7

THE D_2 AGONIST QUINPIROLE ATTENUATES STRIATAL *c-fos* EXPRESSION INDUCED BY SEROTONERGIC, OPIATE AND MUSCARINIC AGONISTS. D.F.Cook* & D.Wirtshafter. Dept. Psychology, University of Illinois at Chicago, IL 60607-7143.

Pretreatment with the dopamine D2 agonist quinpirole has been shown to markedly potentiate striatal fos-like immunoreactivity (FLI) induced by the administration of D1 agonists. Additionally, it has been demonstrated that FLI is elicited following injections of several serotonin (5-HT) agonists with different profiles of receptor subtype selectivity. In order to investigate whether D2 activation would potentiate 5-HT agonist-induced striatal FLI, the current study examined quinpirole pretreatment on FLI induced by several 5-HT agonists. The systemic administration of fenfluramine, TFMPP, RU-24969 and DOI produced significant increases in striatal FLI which were significantly reduced by quinpirole pretreatment; these results are in contrast to the potentiation observed when quinpirole is combined with D1 agonists.

Furthermore, quinpirole pretreatment significantly reduced striatal FLI induced by the muscarinic agonist pilocarpine and the opiate agonist morphine. Collectively, these data suggest that FLI induced in this region by 5-HT, pilocarpine and morphine may be mediated through a dopaminergic mechanism. One interpretation of these results would suggest that quinpirole activates D2-like autoreceptors which reduce striatal dopamine release that is necessary for 5-HT, opiate and muscarinic induction of FLI. (Supported by NS33992.)

432.8

BLOCKADE OF DOPAMINE RECEPTORS AMPLIFIES CORTICAL ACTIVATION ON INDUCTION OF IMMEDIATE-EARLY GENES IN THE STRIATUM. S. Berretta*, Z. Sachs and A. M. Graybiel. M.I.T. Dept. of Brain and Cognitive Sciences, Cambridge, MA 02139

Dopamine is thought to induce long-term changes in the efficacy of corticostriatal linkages. Dopamine D2-class receptor antagonists have been found to enhance the excitatory effects of cortical fibers on striatal neurons (Brown and Arbuthnott, 1983) and to reverse the decrease in excitability induced by amphetamine (Garcia-Munoz et al., 1991). To test whether D2-blockade amplifies cortically-induced immediate-early gene (IEG) expression in the striatum, we combined, in awake freely-moving rats, stimulation of the prefrontal cortex by epidural local application of low concentrations (0.03 mM) of the GABA-A antagonist picrotoxin (Berretta et al, 1994) with low doses of haloperidol (0.05 mg/kg; i.p.), primarily a D2-class antagonist at the chosen dose (Sumiyoshi et al., 1994). We monitored striatal Fos- and JunB-like IEG proteins by immunohistochemistry; positive nuclei were counted with a quantitative computer-assisted method. At the doses chosen, neither haloperidol nor picrotoxin induced more than slight IEG expression in the striatum (haloperidol: n=6, Fos 2.7 ± 3.0 nuclei/striatal section, JunB 12.0 ± 21.4; picrotoxin: n=6, Fos = 0.3 ± 0.8; JunB 9.1 ± 15.7). Co-administration of the subthreshold doses of picrotoxin and haloperidol produced a strong synergistic effect (n=9; Fos= 68.1 ± 79.6 SD; one-way ANOVA: p=0.0002; JunB= 133.5 ± 160.2, one-way ANOVA: p=0.005). Fos and JunB were induced in a distribution pattern similar to that induced by higher doses (0.3 mM) of picrotoxin alone. These results suggest that cellular activation and protein regulation in postsynaptic neurons induced by corticostriatal fibers is reduced by D2-class dopamine receptor activation. Our results also suggest one possible mechanism of action for antipsychotic drugs: they could correct the hypofrontality found in schizophrenia by amplifying corticostriatal signals, possibly through increased release of glutamate. Funded by the Stanley Foundation and NIH Javits Award R01 NS25529.

432.9

DOPAMINE RECEPTOR AGONISTS REGULATE LEVELS OF THE SEROTONIN 5-HT_{2A} RECEPTOR AND ITS mRNA IN A SUBPOPULATION OF RAT STRIATAL NEURONS. J.-J. Soghomonian[1]*, F. Radja[2], T. Reader[2] and N. Laprade[1]., [1]Centre de Recherche en Neurobiologie, Fac. Med., Univ. Laval, Quebec (QC.) G1K 7P41 and [2]Department of Physiology, Univ. of Montreal, CP6128, Succ.A, Montreal (QC.).

The effects of dopamine receptor agonists on the levels of the striatal serotonin 5-HT_{2A} receptor and its mRNA were investigated in rats lesioned with 6-hydroxydopamine (6-OHDA) as neonates. The mRNA encoding for the 5-HT_{2A} receptor was detected by *in situ* hybridization histochemistry and the binding to 5-HT_{2A} receptors was revealed with [^{125}I]DOI. In adult control unlesioned rats, labeling with the 5-HT_{2A} cRNA probe and with [^{125}I]DOI was concentrated in medial sectors of the striatum. In 6-OHDA-lesioned rats, labeling with the 5-HT_{2A} cRNA probe or with [^{125}I]DOI was increased in the striatum, particularly in its lateral subdivisions. These increases were abolished after chronic systemic administration of the dopamine receptor agonists apomorphine or SKF-38393. The mRNA levels encoding for the 5-HT_{2A} receptor were further measured in individual striatal neurons after double-labeling of sections with a 5-HT_{2A} and a preproenkephalin (PPE) cRNA probe. In control unlesioned rats, 5-HT_{2A} mRNA labeling was distributed in PPE-labeled as well as in PPE-unlabeled striatal neurons. In 6-OHDA-lesioned rats, increased 5-HT_{2A} mRNA labeling was found only in PPE-unlabeled neurons and it was abolished following apomorphine or SKF-38393 administration. These results demonstrate that agonists of dopamine receptors inhibit the expression of 5-HT_{2A} receptors in a subpopulation of presumed striato-nigral neurons. We propose that this regulation plays an important role in the control of motor activity by dopamine and serotonin in the basal ganglia. (Supported by the Parkinson Foundation of Canada, NSERC-0155607, FRSQ and CRM).

432.10

EXPRESSION OF C-FOS mRNA IN STRIATUM OF RATS DEPLETED OF DOPAMINE AS NEONATES. M.J. Sandstrom[2]*, J.P. Bruno[2] and J.-J. Soghomonian[1]. [1]Centre de Recherche en Neurobiologie, Fac. Med., Univ. Laval, Quebec (QC.) G1K 7P4 and [2]Neuroscience program, Ohio State University, Colombus, OH, 43210.

Rats depleted of striatal DA as neonates (PD3) are spared the gross sensorimotor deficits seen in comparably depleted adults. Stimulation of D1 or D2 receptors is sufficient for sensorimotor behavior in adults depleted of DA as neonates whereas co-activation of D1 and D2 receptors is necessary for intact rats or those depleted of DA as adults. We now report the effects of D1-receptor stimulation on c-fos mRNA levels in striatal neurons of adult unlesioned rats and rats lesioned with 6-OHDA as neonates. A [^{35}S]-labeled c-fos cRNA probe was detected at regional and cellular level after *in situ* hybridization histochemistry. Following acute administration of the agonist SKF-38393 (12.5mg/kg; i.p.), a potent increase in c-fos mRNA levels was observed in lesioned but not in unlesioned rats. By comparison, the mRNA levels encoding for the two GAD isoforms (GAD65 and GAD67) were also higher in lesioned rats injected with SKF-38393 but this difference was attributed to the lesion alone rather than the SKF-38393 challenge. On emulsion radioautographs, c-fos mRNA was distributed in neurons throughout the striatal surface of lesioned rats. Quantification at the cellular level in a dorso-lateral sector demonstrated that c-fos mRNA was expressed by an average of 79% of all striatal neurons such as visualized with an eosin-hematoxylin stain. These results suggest that in rats depleted of DA as neonates SKF-38393 induces c-fos in a majority of striatal projection neurons including the striato-nigral and -pallidal neurons. Double-labeling studies are currently underway to demonstrate the phenotype of striatal neurons showing c-fos induction. We also anticipate comparing these results with those seen in animals depleted of DA as adults (Supported by the Parkinson Foundation of Canada, NSERC-0155607 (JJS) and the Whitehall Foundation (JB)).

432.11

GAD67 and GAD65 mRNA levels are regulated in different striatal neurons following neonatal 6-hydroxydopamine (6-OHDA) lesions and treatment with dopamine receptor agonists. N. Laprade * and J.-J. Soghomonian. Centre de Recherche en Neurobiologie, Hôpital de l'Enfant-Jésus, Québec, Qué., Canada, G1J 1Z4.

We analyzed the levels of mRNAs encoding for the two isoforms of glutamate decarboxylase (GAD65 and GAD67) in striatal neuronal subpopulations following neonatal dopamine depletions with 6-OHDA and treatment with dopamine receptor agonists. mRNA levels encoding for the GAD65 or the GAD67 isoforms were measured in distinct subpopulations of striatal neurons after double *in situ* hybridization histochemistry using ^{35}S-labeled GAD65 or GAD67 and digoxigenin-labeled PPE cRNA probes (to identify striatopallidal neurons). Following neonatal 6-OHDA lesion, adult rats exhibited increased GAD65 mRNA levels in striatonigral and increased GAD67 mRNA levels in striatopallidal neurons. Chronic treatment with the dopamine D1 receptor agonist SKF-38393 increased GAD65 and GAD67 mRNA levels in striatonigral neurons only. The D1 dopamine receptor antagonist SCH-23390 reversed the effect of SKF-38393 on GAD65 mRNA levels but only partially reversed the increase in GAD67 mRNA levels. Chronic treatment with apomorphine increased GAD67 mRNA levels in both striatopallidal and striatonigral neurons whereas it increased GAD65 mRNA levels in striatonigral neurons only. These results confirm that the mRNAs encoding for the two GAD isoforms are differentially regulated in striatal neuronal subpopulations. In addition, the data suggest that the regulation of the two GADs isoforms in GABAergic striatonigral neurons is dependent on their pattern of activation (Supported by the Parkinson Foundation of Canada and NSERC 0155607).

CEREBELLUM: PHYSIOLOGY, MODELS

433.1

CEREBELLUM INVOLVEMENT IN TIMING OF MOTOR RESPONSES M.A. Weiler*, D. Storzbach, W. Spaulding, J. Sunderland. Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD 21228.

We sought to determine brain areas involved in timed motor responses, hypothesizing frontal lobe involvement in prediction of regular intervals. We studied eleven right handed normal males using positron emission tomography and O^{15} labeled water to measure blood flow during performance of a simple reaction time task. Subjects were scanned during a finger-lift task during a baseline slow finger-lift and two conditions of rapid finger-lift; one with regular prepatory intervals and another with irregular prepatory intervals, all presented visually by a computer and repeated three times. Scan data was realigned, normalized, smoothed and analyzed using Statistical Parametric Mapping (SPM) software. Contrary to our hypothesis the cerebellum showed increases compared to the baseline and strong negative correlations to reaction time performance. There was also some indication of increases compared to the irregular interval condition. Frontal activation was more pronounced in the baseline and irregular condition. These results support cerebellar involvement in timing and "motor-set." Support: NIMH R03MH49433.

433.2

EVIDENCE FOR DENTATE NUCLEAR INVOLVEMENT IN MOTOR PREPARATION. M.S. Milak*, V. Bracha, J.R. Bloedel. Barrow Neurological Institute, Phoenix, Arizona 85013.

The purpose of this investigation is to study the role of the dentate nucleus in the planning and execution of goal-directed movements. In this experiment an instructed delay paradigm was employed to determine whether this nucleus was essential for retaining information regarding target location for a specified period before the movement was initiated. Specifically, the cats were trained to stabilize their posture, watch for a light indicating the position of one of two targets, retain the information regarding the location of the target for 1 - 1.5 sec after the target light was turned off, and respond to a go signal by reaching for and contacting the correct target ("Light-off" task). If the correct target were contacted, a food reward was provided. To evaluate the animals' performance in this task, the number of correct responses in the "Light-off" paradigm was contrasted with the same measurement in a paradigm in which the light indicating the correct target remained on throughout the trial ("Light-on" task). Cats with chronically implanted guide tubes over the cerebellar nuclei were tested using both paradigms in the same session before and after the temporary inactivation (800ng muscimol/1µl saline) of the dentate nucleus ipsilateral to the performing limb. We found that the animals' performance on the "Light-off" task fell to chance level during inactivation, whereas the performance on the "Light-on" task did not change significantly. In addition, there was a significant increase in the number of spontaneous responses in both tasks after the muscimol injection. These data suggest that the capacity to remember the location of the target for a brief period and/or to incorporate the specified target into the motor plan is impaired when the dentate is inactivated. The findings support the general view that this nucleus plays a role in the processing related to motor preparation. Supported by NIH grants NS30013 and NS21958.

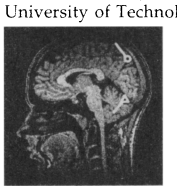
433.3

DYNAMICS OF SACCADE BURST NEURONS IN THE BASAL INTERSTITIAL NUCLEUS IN THE MONKEY CEREBELLUM.
R. Kawagoe, Y. Takikawa, N. Miyashita*, and O. Hikosaka.
Dept. of Physiology, Juntendo Univ. Sch. of Med., Tokyo 113, Japan.

In order to study the saccade controlling mechanism of the cerebellum, we recorded saccade-related neurons in the dentate nucleus and peri-dentate regions in a monkey. A half of these neurons (32/69) exhibited omni-directional burst activities preceding the onset of the saccade. They were located in the area just ventral to the dentate nucleus, which corresponded to the region reported by Langer (1985) as the basal interstitial nucleus. A prominent feature of these neurons was the relationship of the duration of their burst activities and the duration of saccades. Thus, the burst onset and the burst peak preceded the saccade onset by 16.3±0.4 ms and 7.6±1.0 ms respectively, while the burst end preceded the saccade end by 32.5±1.8 ms (in visually guided saccades). These time relationships were kept nearly constant for different saccade amplitudes, directions, and different types of saccade (i.e. visually guided, memory-guided, and spontaneous saccade). Consequently, the burst duration was linearly related to the saccade duration regardless of different dynamics of saccade. These results suggest that neurons in the basal interstitial nucleus may carry the information to determine the saccade duration.

433.4

NONINVASIVE DETECTION OF HUMAN CEREBELLAR ACTIVITY ASSOCIATED WITH VISUALLY-GUIDED SACCADES.
V. Jousmäki*, M. Hämäläinen, and R. Hari. Brain Research Unit, Low Temperature Laboratory, Helsinki University of Technology, FIN–02150 Espoo, Finland.



Visually-guided saccades, necessitating precise control of eye movements, are known to involve cerebellar activation. We recorded magnetoencephalographic (MEG) signals associated with visually-guided horizontal saccades (extent 21°) from 8 healthy adults. MEG signals, averaged on the basis of horizontal electro-oculogram, showed prominent eye-ball generated deflections close to the orbits, coinciding with the electro-oculogram, and posterior signals peaking 145–195 ms after the saccade onset. Source modelling suggested temporally overlapping activation (*i*) in the posterior parietal cortex close to midline and (*ii*) in the cerebellum. Decrease of illumination delayed the peak activation in both areas by 15–20 ms and dampened the parietal source more than the cerebellar one (62% vs. 35%, respectively). Our results show, for the first time, that signals from the human cerebellum can be recorded noninvasively by means of MEG. The observed cerebellar signals, following the saccades, may reflect reafferent input.
Supported by the Academy of Finland and by the EC's HCM Programme through Large-Scale Facility BIRCH at LTL, HUT.

433.5

HEAD MOVEMENT CONTROL DEMONSTRATED IN THE C2 ZONE OF THE FLOCCULUS.
C.I. De Zeeuw*, S.K.E. Koekkoek, M. Arts, J. V.D. Burg, and J. Voogd. Department of Anatomy, Erasmus University Rotterdam, The Netherlands.

The rabbit flocculus consists of five zones (1,2,3,4, and C2). Microstimulation in zones 1 to 4 can evoke short latency eye movements that rotate predominantly about the vertical axis or a horizontal axis perpendicular to the ipsilateral anterior semicircular canal (135° axis). In the present study, we investigated what type of movements can be evoked by stimulation in zone C2. Dutch Belted rabbits were prepared for electrical stimulation in the flocculus, and search coils were implanted on their head pedestal and left eye. The stimulation sites were marked by lesions and evaluated in acetylcholinesterase stained sections. In all animals in which the C2 zone of the flocculus was stimulated, short latency head movements could be evoked. The largest response component of these movements was usually a *clockwise* rotation about the ipsilateral 135° axis in the horizontal plane, but rotations into other directions and other movements, such as translations, also occurred. The amplitude varied from 0.5 to 7 degrees and the peak speed occurred 30-80 msec after movement onset and reached a maximum value of 54 deg/sec. The latencies varied from 15 to 60 msec; the average latency of the five stimulations with the shortest latencies was 19 msec. The smallest threshold was 4 μA (average 16 μA). In some cases the stimulations in zone C2 evoked simultaneous head and eye movements. The largest response component of the eye movements was usually also a rotation about the ipsilateral 135° axis, but in the opposite, i.e. *counter clockwise*, direction. When we stimulated zone 3 for control, we were unable to evoke short latency head movements. The present study is the first to show that the C2 zone is involved in head movements. The observation that electrical stimulation in zone C2 cannot only produce a head movement, but also a concomitant eye movement in the opposite direction, raises the possibility that the C2 zone is involved in the control of gaze stabilization during head movements.

433.6

SIMPLE SPIKE ACTIVITY MODULATED BY VESTIBULARLY-EVOKED CLIMBING FIBER RESPONSES IN THE RABBIT CEREBELLAR NODULUS.
H. Fushiki*[1,2] and N.H. Barmack[1]. [1]R.S. Dow Neurol. Sciences Inst., Portland, OR 97209, [2]Dept. of Otolaryng., Toyama Med. & Pharm. Univ., Toyama 930-01, Japan.

In the rabbit uvula-nodulus, vestibular information is mapped onto parasagittal zones by climbing fibers (CFs). Vestibular stimulation in the plane of the ipsilateral posterior and contralateral anterior semicircular canals modulates climbing fiber responses (CFRs) in a zone restricted to the most medial 1.5 mm. Lateral to this zone lies a zone of Purkinje cells whose CFRs are modulated optimally by vestibular stimulation in the plane of the ipsilateral anterior and contralateral posterior semicircular canals. Inserted between these two semicircular canal-related CF zones is a zone of Purkinje cells whose CFRs are excited by posterior-anterior optokinetic stimulation of the ipsilateral eye. Within each of these zones, simple spikes (SSs) are modulated reciprocally with CFRs. In rabbits anesthetized with chloralose-urethan, we have used vestibular stimulation to evoke CFRs within particular zones and we have determined the effect of these evoked CFRs on SSs within the same and adjacent zones. CFRs and SSs were recorded during roll vestibular stimulation in the CF "optimal plane" and "null plane".

In all Purkinje cells, SSs were modulated reciprocally during rotation in the CF "optimal plane". In approximately 30% of the recorded cells, the modulation of SSs led the modulation of CFRs from the same Purkinje cell. The modulation of SSs was always reduced during rotation in the CF "null plane". We conclude that: 1) The influence of CFRs in one CF zone within the uvula-nodulus is restricted to SSs within the same zone, and 2) Modulation of a CFR is not necessary for modulation of the SS in the same Purkinje cell. Rather, this reciprocal modulation might be attributed to CF-driven Golgi cells whose axons are distributed orthogonally with respect to the sagittal CF zones. 3) The vestibular and optokinetic CF zones in the nodulus provide a map of visual-vestibular space that may be used to regulate movement. *Supported by NEI EY04778.*

433.7

SIMPLE-SPIKE ACTIVITY OF FLOCCULAR PURKINJE CELLS RESPONDING TO VERTICAL PURSUIT AND PITCH ROTATION IN ALERT MONKEYS.
K. Fukushima*, J. Fukushima, H. Tsunekawa and C.R.S. Kaneko. Dept. of Physiol., Hokkaido Univ. Sch. Med., Sapporo, 060 Japan and Dep. of Physiol. & Biophysics Regional Primate Res. Ctr., Univ. of Washington, Seattle, WA 98195

Recently, we suggested that the vertical vestibulo-ocular reflex (VOR) neural integrator in cats requires a transfloccular inhibitory pathway (i.e., contralateral posterior canal inputs that inhibit anterior canal related VOR interneurons; Fukushima & Kaneko, 1995). To test this scheme in primates, we recorded simple-spike activity of Purkinje (P) cells in the flocculus and ventral parafloccului of Japanese macaques that were trained to track a smoothly moving spot during whole-body rotation. The preferred direction of the majority of vertical pursuit P cells was downward. Down pursuit P cells were excited by up pitch, whereas up pursuit P cells respond to down pitch during fixation of an earth-stationary spot. About half of the related P cells showed phase reversal during VOR suppression compared to their responses during VOR enhancement, similar to the behavior of GVP cells. The remaining half were different; their response amplitudes decreased during VOR suppression and increased during VOR enhancement while their phase response was consistent in the 3 conditions (i.e., within 90°) suggesting that they receive eye velocity-related responses. Response phase of some of these cells reversed during vertical rotation in the roll plane, consistent with responses induced by vertical canal inputs. We are now analyzing these discharge properties quantitatively. Supported in parts by JMESC grants (07680886, 07252201, 07671043), Marna Cosmetics, and PHS grants EY06558 (NEI) and RR00166(NIH).

433.8

RESPONSES OF SQUIRREL MONKEY VERTICAL ZONE FLOCCULUS PURKINJE CELLS DURING ADAPTATION OF THE VESTIBULO OCULAR REFLEX.
R. Arikan and S. M. Highstein*. Dept. of Otolaryngology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Vertical zone floccular Purkinje cells (P-cells) are hypothesized to have a role both in the control and adaptation of the vertical vestibulo ocular reflex (vVOR). P-cells were recorded in four alert naive and vVOR adapted squirrel monkeys using standard techniques during VOR in the light (VORl), dark (VORd), visual following, enhanced and suppressed VOR (VORe and VORs) at 0.5 Hz. VOR gains in naive monkeys averaged 0.9, and P-cells were modulated when the eyes moved down. 25% were modulated during gaze velocity, and 75% during eye velocity only. Mean naive head velocity sensitivity of gaze velocity cells was 0.76 sp/deg/sec with mean phase lag of 30 deg. Of 8 gaze velocity cells recorded from high gain animals (mean gain 1.1) mean head velocity sensitivity decreased to 0.69 sp/deg/sec with mean phase lag of 76 deg. Of 12 cells recorded from low gain animals (mean gain 0.7) mean head velocity sensitivity increased to 1.20 sp/deg/sec with mean phase lag of 4 deg. While gaze velocity P-cells did not modulate during VORd in naive animals, when the gain was high, cells responded in phase with head velocity with a phase lag of 180 deg; when VOR gain was low, cells responded out of phase with head velocity sensitivity and phase lag of 0 deg on the average. These findings demonstrate that head velocity sensitivity and phase relationships of P-cells change during adaptation of the VOR and support the hypothesis that these cells play a role in adaptation or motor learning of the vVOR. Supported by NIH grant EY 05433.

433.9

EFFECTS OF NUCLEUS PREPOSITUS HYPOGLOSSI LESIONS ON THE VISUAL CLIMBING FIBER ACTIVITY IN THE RABBIT'S FLOCCULUS. M. P. Arts, C. I. De Zeeuw and J. I. Simpson*, Dept. Physiology and Neuroscience, NYU School of Medicine, New York, NY 10016.

Like other parts of the inferior olive, the caudal dorsal cap, which is the source of the visually modulated vertical axis climbing fibers of the flocculus, has one predominant excitatory input and one predominant inhibitory input. The excitatory input comes from the ipsilateral nucleus of the optic tract and the dorsal terminal nucleus. The inhibitory (GABAergic) input comes from the contralateral nucleus prepositus hypoglossi (NPH) (De Zeeuw et al. 1993, 1994; Barmack et al. 1993). Little is known about the function of the NPH input. In an attempt to change this situation, the NPH was aspirated unilaterally in 9 rabbits (anesthetized with ketamine, xylazine and acepromazine) to determine if the spontaneous complex spike activity of Purkinje cells in the ipsilateral flocculus changed and/or if the depth of modulation to visual stimulation changed. Spontaneous activity was recorded in the dark. Visual stimulation consisted of 15 cycles of whole-field movement for 5 sec each in the excitatory and inhibitory directions. Prior to lesion, the average spontaneous activity in the dark was 1.08±.31 spikes/sec (mean±s.d.; n=19). After lesioning from 60% to more than 95% of the part of the NPH that projects to the contralateral dorsal cap, the spontaneous activity was not significantly different (1.16±.35 spikes/sec, n=20; t-test). Modulation depth was characterized by the ratio of the firing rates during excitation and inhibition. This ratio was not significantly different after lesioning (median 6.98 before, n=32; 8.10 after, n=27; Mann-Whitney test). The absence of a change in either the spontaneous activity or the modulation depth is puzzling. A next step is to block the GABAergic input directly in the dorsal cap by microinjection of picrotoxin. (NS-13742).

433.10

INFERIOR OLIVE SENSITIVITY IS REDUCED BY INCREASED CEREBELLAR OUTPUT. K. M. Horn*, P. L. E. van Kan, T. J. H. Ruigrok and A. R. Gibson. Division of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

Interpositus (IP), the output nucleus of intermediate cerebellum, has a strong GABAergic projection to the rostral dorsal accessory olive (rDAO). Many nucleo-olivary fibers terminate close to gap junctions between olivary neurons, and the nucleo-olivary pathway may mediate coupling between neurons. Another possible function of the nucleo-olivary pathway may be to modulate olivary sensitivity, since several studies indicate that sensitivity changes during movement. If the IP-rDAO projection is largely concerned with the regulation of olivary coupling, increased IP activity might reduce the size of rDAO receptive fields, since a given neuron would be less coupled to the activity of neighboring neurons. If the IP-rDAO projection is mainly involved in regulating sensitivity of rDAO neurons, increased IP activity should reduce the responses of rDAO neurons to somatosensory stimuli.

Two cats were anesthetized with sodium pentobarbital. Cutaneous receptive fields of rDAO neurons were mapped, and stimulating electrodes were implanted at several locations across the receptive fields. Interpositus neurons were excited by either cooling the overlying cerebellar cortex or by injection of bicuculline into the IP. Cooling cerebellar cortex led to a 40% reduction of the evoked rDAO activity across all stimulation sites; no change in receptive field size was apparent. Bicuculline injections eliminated all activity in rDAO. The predominant effect of increased activity in interpositus is inhibition of rDAO, more refined testing would be required to reveal the action of inhibitory synapses near gap junctions.

Supported by NIH grant NS30013.

433.11

NON-CLOCK-LIKE DISCHARGE OF CELLS IN THE DEEP CEREBELLAR NUCLEI OF THE AWAKE BEHAVING MONKEY . J.G. Keating* and W.T. Thach. Dept. of Anatomy, Washington Univ. Sch. Med. St. Louis, MO 63110.

We have recorded from deep nuclear cells in two Rhesus monkeys during the performance of a targeted wrist flexion/extension task.

Previously we showed that no oscillatory discharge can be found in complex spike recordings from Purkinje cells when monkeys performed non-cyclic wrist movements (Keating and Thach, J Neurophys 73 (4) 1329-40, 1995). There we questioned the physiological relevance of quasi-periodic inferior olive discharge seen in reduced and drugged preparations. Using the same analysis method we have examined deep nuclear cell discharge for periodicity (frequencies up to 100Hz). Interspike intervals for all spikes recorded for a unit were placed in 1ms bins. Autocorrelation analysis was done. The autocorrelograms were then Fourier transformed to reveal the spectral frequency of the units discharge. Periodicity was seen in the 2Hz range for some of the 89 units analyzed. No periodicity was found near 10Hz. To determine the nature of the peri-2Hz periodicity, we examined cell discharge during the "move" phase of the task, and during the "hold" phase of the task. The peri-2Hz signal was prominent during the move phase, and less so during the "hold" phase. In addition, spectral analysis of wrist position during the "move" phase revealed a prominent component also at 2Hz. As was seen for complex spikes, units with large numbers of spikes gave flatter more even histograms than those with fewer, arguing against the existence of any underlying periodic tendency.

The failure to observe clock-like timing in awake behaving animals, in either complex spike or deep nuclear cell discharge argues against the inferior olive acting as a "motor clock". (supported by NIH grant NS12777)

433.12

OPTICAL MEASUREMENTS OF CEREBELLAR RESPONSES TO SURFACE STIMULATION IN AN ISOLATED IN-VITRO CEREBELLUM. D. Cohen, I. Segev and Y. Yarom.* Department of Neurobiology, Life Sciences Institute, The Hebrew University, Jerusalem, Israel.

Cerebellar responses to surface stimulation were analyzed in an isolated guinea pig cerebellum, which was maintained at room temperature and perfused, via the vertebral arteries, with physiological solution. The voltage sensitive dye RH-414 was applied extracellularly for 1 hour (0.05mg/ml). Optical signals were recorded from 128 photodiodes organized in a 12x12 array. Using a 10x power objective, each element of the array detects light from a surface of about 200µmx200µm. The signals were amplified, sampled and digitized, with 12-bit accuracy. Using two A/D boards (AT-MIO-64F-5) a time resolution of about 300 µsec per channel was obtained.

Surface (parallel fiber) stimulation generates a wave of changes in fluorescence (of up to 0.2%) that propagates along the cerebellar folium at a constant velocity of 0.2m/s. This wave of activity is characterized by a fast initial positive peak followed by a prolonged tail. Both components were partially blocked by CNQX (10^{-5}M). The positive wave of activity is confined, by what seems to be a lateral inhibition, to a 400µm beam. The inhibition is recorded as a negative signal located laterally to the active beam. A train of stimuli delivered to the parallel fibers activates on-beam inhibition which is expressed as a slow, negative signal that follows the positive response. Neither the lateral inhibition nor the width of the activated beam are affected by the train of stimuli. The on-beam inhibition, which is non-linearly related to the number of stimuli in the train, was reversibly blocked by bicuculin (10^{-5}M).

We conclude that: 1. The optical signals recorded from the isolated cerebellum reflect the electrical activity of the cerebellar cortex. 2. This optical system enables the detection of excitatory and inhibitory synaptic potentials from layers as deep as that of the Purkinje cells (the target site of the basket cell lateral inhibition). 3. These results reconfirm the concept that both the lateral inhibition, as well as the on-beam inhibition, shape the spatio-temporal organization of the cerebellar cortex activity. Supported by HFSF and the ONR.

433.13

CEREBELLAR UNITS WITH SIMPLE AND COMPLEX SPIKE ACTIVITY HAVE DIFFERENT RESPONSES TO CLICK BEFORE AS WELL AS AFTER CONDITIONING WITH CLICK AS A CS. C. Woody*, A. Nahvi, G. Palermo, J. Wan and E. Gruen. UCLA Med. Ctr., Los Angeles, CA 90024

Mean onsets and profiles of increased activity to click were compared before and after conditioning in neurons with complex spikes [Type I - initial spike followed 1-3 ms by multiple spikes; Type II - followed < 1 ms (in absolute refractory period); Type III - followed < 3 ms by spikes in baseline noise] and only simple spikes (S). Recordings were from flocculus and lateral and medial ansiform areas of cats. Type I & III cells are thought to be Purkinje cells with complex spikes representing climbing fiber responses. Type II cells are thought to be coupled Purkinje/Golgi II cells (Nahvi et al., Ribak et al. Exp. Neurol. 1980a,b). S cells were other cerebellar neurons plus some Purkinje cells not identified due to the low frequency of complex spike activity.

Before conditioning, baseline activity differed among the four groups (two tail T tests), and was greatest in Type I cells. Type II cells had the greatest mean response to click (activity relative to baseline) with a peak 18Z above baseline noise. S cells had the second largest response (10Z above baseline). Activity of Type II cells best reflected temporal properties of the click.

After conditioning, baseline activity decreased significantly in each of three groups that were compared (Type I & II cells and S cells). Differences in response to click were found in each cerebellar region before as well as after conditioning. In S cells the response to click increased 8Z and that to hiss 17Z above baseline mean after conditioning. In cells with Type II complex spikes, the response to click did not change significantly after conditioning (<1Z difference), but the response to hiss increased (7Z). The findings demonstrate differences in acoustic response by cell type as well as cerebellar region and behavioral state. (Supported by HD 05958.)

433.14

INFLUENCE OF THE BÖTZINGER COMPLEX (BÖtC) ON THE APNEA PRODUCED BY CAPSAICIN. Z. Zhang, F. Xu, L.Y. Lee and D.T. Frazier*. Dept. of Physiology, Univ. of KY, Lexington, KY40536

Intravenous capsaicin elicits pulmonary chemoreflexes (apnea, rapid shallow breathing, bradycardia, and hypotension) presumably through the stimulation of pulmonary C-fibers. The present study was designed to evaluate the influence of the BÖtC on the apneic component of the above reflex responses. Experiments were performed on 7 anesthetized, paralyzed and ventilated cats using the phrenic neurogram (PN) as the index of the respiratory motor output. Capsaicin (3.3-10µg/kg) was administered via left femoral vein to produce a pronounced apnea. After bilateral BÖtC lesions, the same dosage of capsaicin failed to produce a significant apnea, i.e., the expiratory time (T_E) was not statistically different from the baseline T_E. The baseline T_E following the lesions was somewhat shorter than in the intact but not significantly different. In 6 other animals, the apneic response elicited by capsaicin injections were not affected by bilateral lesions of pontine respiratory group (PRG). The baseline T_E was prolonged but not significantly. These results suggest that an intact BÖtC is important for the expression of the apneic component of the reflexes elicited by capsaicin while the PRG is not. (Supported by HL40369)

433.15

DYNAMICS OF A DETAILED MODEL OF THE GRANULAR LAYER OF THE CEREBELLUM. R. Maex, B.P. Vos* and E. De Schutter. Lab. voor Theoretische Neurobiologie, UIA, B-2610 Antwerpen, Belgium.

In order to provide a detailed Purkinje cell model (De Schutter E. & Bower J. 1994 J.Neurophysiol. 71, 401) with a more realistic pattern of parallel fiber synaptic inputs, we initiated simulations of the granular layer of the rat cerebellar cortex. The parameters of the one-compartmental model neurons were adapted from Gabbiani et al. (1994 J.Neurophysiol. 72, 999) for granule cells and chosen to reproduce known current-clamp data for Golgi cells. A circuit of tens of Golgi cells and thousands of granule cells positioned on an array along a folium was then activated by random mossy fiber input. The dynamics of this network depends principally on the distribution of the excitatory synapses on Golgi cells. A highly dominant parallel fiber excitation of Golgi cells forces all neurons in the network, over a length of several millimeters, to fire synchronized and at regular time intervals. This oscillatory behavior gradually breaks down when the strength of parallel fiber synapses is decreased and/or the strength of mossy fiber synapses on Golgi cells is increased. The average granule cell firing rate decreases strongly if Golgi cell axonal arbors overlap, but only when the Golgi cells are not synchronized, i.e. when their mossy fiber inputs dominate. Our results suggest that the Golgi cell ⇔ granule cell feedback loop is able to generate a very rhythmic pattern of parallel fiber activity. Moreover, if granule cells receive inhibition from a pair of Golgi cells, a dynamical switching between the Golgi cells' dominant sources of excitation would be a powerful mechanism for gain control.

Grants from HFSPO, NFWO (Belgium) and UA (Antwerp).

433.16

A QUALITATIVE MODEL OF CEREBELLAR SACCADIC CONTROL. J.L. Krichmar*, L. Hunter and J.L. Olds., Krasnow Institute for Advanced Study at George Mason University, Fairfax, VA.

Evidence suggests that the oculomotor vermis (OV) region of the cerebellar cortex is necessary for the execution and maintenance of accurate saccades. We present a unique computational model, based on current knowledge of the anatomy and physiology of the OV, that tests probable strategies for saccadic processing. The model employs the Qualitative Reasoning Neuron (QRN) methodology to create a highly detailed model of the Purkinje cells (PC), stellate cells and basket cells that are found within the cerebellar cortex. The technique is extremely efficient because no quantitative values need to be calculated. However, accurately detailed and predictive properties emerge from QRN simulations. QRN demonstrates that a PC population code can accurately encode the direction of a saccade. QRN demonstrates that a state machine is adequate to store and execute a saccadic motor program in the OV; we were unable to accurately recreate empirical observations using an alternative feedback controller approach. The firing patterns of QRN, during intact and lesion experiments, qualitatively match in detail recordings in the OV during saccades. QRN simulates adaptation from inaccuracies in gradual and rapid adaptation paradigms. The adaptation is achieved with an error signal carried by the parallel fiber input. The QRN model demonstrates adaptation is possible when climbing fiber (CF) is periodic and contains no error signal. Instead, CF input may dictate when an evaluation of the previous movement should occur. QRN suggests plausible explanations for the dysmetria seen in cerebellar lesion, excessive tremor, and Wallenberg's syndrome patients. This research illustrates the ability of QRN to efficiently simulate complex behaviors without sacrificing important details.

This research was supported by the Krasnow Institute for Advanced Study.

433.17

A MODEL OF ADAPTATION IN THE CEREBELLUM FOR LEARNING THE MODULATION OF THE VESTIBULO-OCULAR REFLEX (VOR).

Olivier J.M.D. Coenen* and T.J. Sejnowski. UC San Diego, Howard Hughes Medical Institute, CNL, The Salk Institute, PO Box 85800, San Diego, CA 92186-5800.

The gain of the VOR in monkeys is modulated by many inputs including eye position, vergence angle and otolith signals. These inputs insure stabilization of retinal images during head movements regardless of gaze direction, head rotation axis and translation. We have shown that a dynamical model which combines these inputs can match the VOR dynamics observed in monkeys (Coenen & Sejnowski, NIPS 8, MIT Press). Based on a cerebellar theory that we have developed, we have extended this dynamical model to explain anticipatory gain changes of the VOR with vergence movements (Snyder & King; Vision Res. 32:3,1992). In the model, the cerebellum predicts the sensory feedback which is used to improve performance. Adaptation in the cerebellum and the cerebellar nuclei is mediated by the inferior olive. We demonstrate the importance of synchronous firing of climbing fibers for appropriate adaptation when the synergy of multiple Purkinje cells is necessary for good performance.

The modulation of the VOR with eye position, vergence and otolith inputs is a highly nonlinear function which can be learned with our model. Computer simulations of the model indicate how the cerebellum may selectively combine input signals to adaptively reduce VOR retinal slip. This is accomplished by constructing predictive representations of neural signals which can modulate the gain of the VOR in anticipation of new behavioral conditions.

Supported by a McDonnell-Pew Foundation Fellowship and The Howard Hughes Medical Institute.

VESTIBULAR SYSTEM: BEHAVIORAL STUDIES

434.1

NON-LINEAR HABITUATION OF THE GOLDFISH VESTIBULO-OCULAR REFLEX. E. R. Dow and T. J. Anastasio*. University of Illinois, Beckman Institute, 405 N. Mathews Ave., Urbana, IL 61801.

Experiments in mammalian species show that the vestibulo-ocular reflex (VOR) habituates following prolonged low-frequency stimulation. Using linear system descriptors, the VOR can modeled as a high-pass filter. Habituation is a form of time-variance in the VOR. In mammals, habituation can be characterized by an increase in low-frequency phase lead and a commensurable decrease in gain that can be modeled by a decrease in the VOR time constant.

In habituating the goldfish VOR, we also found an increase in phase lead that could be explained by an decrease in the VOR time constant. However, we found an extreme low-frequency gain reduction that was incommensurable with the linear system description of phase. This suggests a non-linear component to habituation of gain in the goldfish VOR. Analysis of VOR time series data during habituation show several different time-variant and non-linear phenomena including: either a gradual or an abrupt bilateral or unilateral decrease in gain, complete unilateral rectification, DC shift which can result in spontaneous nystagmus, magnitude-dependent response only to large accelerations (dead-zone non-linearity), or some combination of these.

Habituation can be eliminated permanently by cerebellectomy or transiently by injecting lidocaine onto the Purkinje cells of the vestibulo-cerebellum. A nonlinear, recurrent neural network model can simulate the full spectrum of these phenomena simply by modifying the Purkinje cell inhibition of the vestibular nuclei.

This work was supported by NIH grant MH50577.

434.2

EFFECT OF SPACEFLIGHT ON VESTIBULO-OCULAR REFLEXES (VORS) DURING ANGULAR HEAD MOTION. D.L.Tomko* and J.O.Clifford. Gravitational Research Branch, Life Sciences Division, NASA Ames Research Center, Moffett Field, CA 94035-1000

VORs stabilize the eyes when the head moves. During Earth-horizontal (E-H) pitch or roll rotations, canal and otolith stimuli occur coincidentally. In Earth-vertical (E-V) pitch or roll rotations, only canal signals occur. In cats and squirrel monkeys, pitch/roll VOR gains during E-H motion have been shown to be closer to one (more compensatory) than during E-V motion, implying that otolith modulation plays a role in producing angular VORs (AVORs). The present experiments replicated this study in rhesus monkeys, and examined how spaceflight affected AVOR gain. During yaw, pitch and roll (0.5 - 1.0 Hz, 40-50°/s pk) motion, 3-d eye movements were recorded in 4 Rhesus monkeys using scleral search coils. Mean E-H and E-V pitch VOR gains were 0.85 and 0.71, respectively. Torsional VOR gains during E-H and E-V roll were 0.47 and 0.39. Gains were thus more compensatory during E-H pitch or roll, when otolith modulation was added to canal stimuli. Two of the 4 monkeys flew for 11 days on the Soviet COSMOS 2229 Biosatellite, Bion 10 in 1992. E-H pitch VOR gains measured immediately (within 48 hrs) post-flight were attenuated, with gain values similar to those during pre-flight E-V head pitch. Torsional VOR gains in response to head roll were reduced in one flight subject and were similar to pre-flight values in the second. Horizontal yaw VOR gains in the flight subjects were similar pre- and post-flight. Results of both components of this study support the notion that signals of angular motion by semicircular canals interact with otolith modulated signals to produce compensatory AVORs during E-H pitch.

Support-NASA Space Medicine Tasks 199-16-12-23 & 106-30-04-40

434.3

EFFECT OF HEAD POSITION ON TORSIONAL AND HORIZONTAL VESTIBULAR OCULAR REFLEX (VOR) ADAPTATION. D. Solomon*, D. Straumann, D. S. Zee. Dept. Neurology, Johns Hopkins Hosp, Baltimore, MD 21287

We studied short-term torsional (T) and horizontal (H) VOR adaptation. Five normal subjects underwent sinusoidal vertical axis whole-body rotation for 30 minutes (.3 Hz, pk-vel 37 deg/sec) viewing a chair fixed radial pattern (x0 viewing). The head was pitched up or down 35°. Eye movements were kept near zero orbital elevation and recorded in complete darkness with a dual search coil. VOR gain was (mean peak eye velocity over 10 cycles)/(peak chair velocity). Static ocular counter-roll gain following adaptation of the angular TVOR was unchanged.

Mean control TVOR gain was 0.43 (.31 to .64), decreasing 15% following head up and 28% following head down training. Horizontal gains followed a different pattern: mean control HVOR gain was .85 (.67 to .95), decreasing 37% following head up and 25% following head down training. Eye angular velocity (ω) axes were calculated relative to a head fixed coordinate system. The orientation of ω in the x-z plane was determined by the ratio of horizontal to torsional peak velocity. ω tilted on average 19.6° (13.5° to 26.2°) from the head's yaw axis (stimulus axis=35°) prior to adaptation, consistent with the observed gain anisotropy. Following x0 viewing, the mean tilt was 25.4° (18.9° to 32.2°) with head up and 19.8° (15.6° to 27.3°) with head down training. Following post-adaptation testing, VOR was measured with subjects imagining a chair fixed target in darkness. With the head up, TVOR gain decreased 39% and ω tilted 46.6°. With the head down, TVOR gain decreased 67% and ω tilted 25.2°. HVOR gain decreased 80% with head up and 73% with head down.

These results suggest that torsional and horizontal VOR gain adaptation, even when elicited together, are subject to different influences (differential contributions of the horizontal and vertical semicircular canals, neck afferents or graviceptive cues) depending upon head orientation.

NIH Grants P60-DC00979 and 1 F32 EY06577-01, Schweiz. Stift. f. med.-biol. Stip.

434.4

ROLL- AND PITCH-TILT PERCEPTION AND EYE MOVEMENT DURING LOW-FREQUENCY LINEAR ACCELERATION. S.H. Seidman*, L. Telford, and G.D. Paige, Dept. of Neurology and Ctr for Visual Science, Univ. of Rochester, Rochester, NY 14642

The otolith organs transduce linear acceleration, and respond to both translation and tilt stimuli. This ambiguity seems to be largely resolved using a frequency-parsing scheme, by which high-frequency signals convey translation, and low-frequency, tilt. To quantify the dynamic properties of low-frequency linear acceleration processing, we asked human subjects to report their perception of tilt during centripetal acceleration profiles generated by constant-velocity centrifugation (127°/sec) while the radius of centrifugation was dynamically modulated at 0.005, 0.01, or 0.025Hz (±50cm). Centripetal forces were aligned with the naso-occipital or inter-aural axes, producing resultant force profiles consistent with ±14° of pitch- or roll-tilt, respectively. Subjects reported tilt perception by maintaining a laser spot on the horizon. This spot was located straight-ahead during pitch-tilt trials or displaced 20° horizontally during roll-tilt trials. Perception during roll-tilt was also assessed by aligning a projected light bar with the perceived vertical. In different trials, eye movements were monitored in darkness while subjects attempted to maintain gaze on the perceived horizon.

Subjects perceived tilt, and not translation, during all trials. Perceptual responses for all horizon-matching tasks (pitch and roll) were similar. Gains averaged ~0.55 with a phase lag of ~20° at 0.005 Hz, decreasing to ~0.35 with a phase lag of ~30° at 0.025Hz. The overall dynamics of the bar-alignment task were essentially the same as for the horizon-matching task, with similar phase lags and an attenuation with increasing frequency, but with mean gains roughly twice as large. Eye movement responses mimicked corresponding perception responses, and were compensatory for tilt. These findings are consistent with low-pass dynamics of tilt processing from otolithic signals. (Supp. by NIH grants AG06442, RR09283, EY01319)

434.5

EFFECTS OF MOTOR LEARNING ON THE RESPONSE OF THE VESTIBULO-OCULAR REFLEX TO CURRENT PULSES AND HIGH-FREQUENCY ROTATION IN CATS. D. M. Broussard*, J. A. Hong, J. K. Bhatia and A. R. Butt. Playfair Neuroscience Unit, Univ. of Toronto, Toronto, Ontario M5T 2S8, Canada.

Motor learning modifies the response of the cat's horizontal angular VOR in a frequency-dependent fashion. We induced motor learning by unilateral vestibular damage (injury-induced learning) and by fitting cats with telescopic lenses (optically-induced learning). After a unilateral labyrinthectomy, the gain of the VOR at 5-10 Hz recovered to near-normal values, but a large dynamic asymmetry remained. There was no residual asymmetry at 0.5-2 Hz. Plugging the horizontal canal did not eliminate the canal's contribution to the VOR at high frequencies; after a bilateral plug, the VOR at 10 Hz had a near-normal gain, although plugging introduced a significant phase lead. Nevertheless, learning induced by a unilateral plug failed to restore symmetry in the VOR gain at 5-8 Hz. In general, the VOR's response to rotation at 0.05-2 Hz showed larger changes during optically-induced learning, and better recovery of symmetry during injury-induced learning, than the response at 5-10 Hz. We activated vestibular primary afferents synchronously with a 0.1-ms biphasic current pulse applied to the labyrinth; this stimulus evoked a biphasic twitch of eye velocity. During optically-induced learning, the learning- related change in the evoked response was small and most of it appeared after the first peak in eye velocity. However, during injury-induced learning, large changes appeared in the amplitude and latency of the response to a current pulse. Some of the changes were transient, persisting only for a few hours or days after surgery. (Supported by MRC:MT-12254)

434.6

AUTOMATED 3D ANALYSIS OF FREE-FALL TEST ON HEMI-LABYRINTH-PLUGGED RATS. L. Simon*, Z. Papp, I. Rényi+, P. Csillag+ Dept. of Anatomy, Semmelweis University Medical School and +Section of Image Analysis, KFKI-Research Inst. for Measurement and Computing Technique, Budapest, H-1450 Hungary

Plugging of the labyrinth serves as an earth-based model of motor control and orientation disorders of vestibular origin in weightlessness. After the unilateral surgical treatment under Nembutal anaesthesia (35 mg/kg.,i.p.) the essential motor asymmetry of animal results in side twirls (saltos) at free-fall test (1,5 m unconditioned fall from a cage onto a soft net, preventing any injury of the animals). The reduction of the number and of completeness of side turns indicates the ongoing sensorimotor adaptation. Stereo pairs of pictures of free fall were recorded by two CCD cameras in 20 mS sequence, and digitally stored in PC.

Three steps of analysis were performed as follows:
i, false-colored pairs were used for anaglyph observations on the test scenes. ii, computerized stereogrammetric measurements were manually made for identifiable points of the body scheme.
iii, Automatic stereo evaluation was attempted through motion field analysis of picture pairs. Surface shells of the animal's view given by a pair of cameras should than be completed either by a more complete (4 to 6 cameras) recording system, or by using a background mirror for the backside shell, in a merging procedure. A model of animal and the sequence of free fall should than be elaborated for abstract parameters of free fall.

434.7

Asymmetries in the horizontal VOR after unilateral plugging of the three semicircular canals in the squirrel monkey. D.D. Backous,* D. Lasker, L.B. Minor, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21203

Eye movements in response to 3000 deg/s^2 impulses of angular acceleration delivered with the horizontal canals aligned in the yaw plane have been recorded (search coil) in darkness in two squirrel monkeys before and after unilateral canal plugging. Prior to plugging, horizontal vestibulo-ocular responses began after a latency of 7.9 ± .4 ms (mean ± s.d.) with eye velocity rising at an acceleration gain of .81 ± .09 until the first fast phase that occurred at 83 ± 26 ms. Velocity gain for 150 deg/s plateau head velocity was .74 ± .13.

Within one week after unilateral canal plugging, ipsilesional acceleration gain decreased to .46 ± .10 with the first fast phase occurring 123 ± 27 ms into the response. Contralesional acceleration gain and timing of the first fast phase were unchanged after plugging. Ipsilesional velocity gain decreased to .42 ± .01 whereas contralesional velocity gain measured .56 ± .09. A further decrease in ipsilesional velocity gain was noted for velocity steps up to 500 deg/sec. Responses to .05-8 Hz sinusoidal rotations showed decreased gain after plugging with cut-off in eye velocity occurring for ipsilesional half-cycles at rotations reaching 500 deg/s maximum velocity. These directional asymmetries noted after unilateral canal plugging have been modeled through a closed-loop system with inhibitory commissural inputs and varying rotational sensitivities of secondary vestibular neurons.(Supported by NIH DC02390 and by the American Otological Society)

434.8

ADAPTATION OF VESTIBULO-OCULAR REFLEX AFTER SELECTIVE SEMICIRCULAR CANAL PLUGGING D. E. Angelaki*, B. J. M. Hess, and J.-I. Suzuki. Neurology Dept., Univ. Hospital Zürich, 8091 Zürich, Switzerland and Univ. of Mississippi Medical Center, Surgery (Otolaryngology) Dept., Jackson, MS 39216, USA

The three-dimensional spatial and temporal vestibulo-ocular reflex (VOR) properties were investigated in five rhesus monkeys, two with the lateral semicircular canals plugged, two with the right anterior/left posterior canals plugged and one with all but the left posterior canal plugged. Three-dimensional VOR was systematically tested in these animals at different head positions) i.e., upright, supine, prone, ear-down and intermediate positions) during transient displacements and sinusoidal oscillations about an earth-vertical axis. Acutely after plugging, VOR gain was minimal during rotation in the plugged canal planes. Over time, however, VOR gains increased in a frequency-specific manner: At high frequencies, VOR gains reached values up to ≈ 0.3-0.5 (1.1 Hz) after approximately three to five months. In contrast, there was no significant change in VOR gain at frequencies below approximately 0.1 Hz. These gain changes were also accompanied by a spatio-temporal reorganization of slow phase velocity, whereby the adapted responses were characterized by non-cosine tuned properties such that the spatial specificity of the elicited eye movement was restored at the expense of temporal response properties. These results suggest a complex spatio-temporal modification of three-dimensional vestibulo-ocular connectivity within the oculomotor system.

Supported by grants from NIH (EY10851), NASA (NAGW-4377), the Air Force Office of Scientific Research (F49620) and the Swiss National Science Foundation (#31-32484.91).

434.9

THREE-DIMENSIONAL EYE MOVEMENT DEFICITS AFTER REVERSIBLE LESIONS OF THE INTERSTITIAL NUCLEUS OF CAJAL (IC) OF THE ALERT MONKEY. C. Helmchen, L. Fuhry, H. Rambold, U Büttner*. Dept. of Neurology, University of Munich, 81377 Munich, Germany

The mesencephalic interstitial nucleus of Cajal (iC) is thought to be the neural integrator for vertical and torsional eye movements. We performed reversible lesions in iC of the alert monkey (Macaca mulatta) to study the following questions: 1) Do iC lesions cause upbeating or downbeating spontaneous nystagmus ? 2) Is torsional nystagmus direction-specific and due to a vestibular imbalance and/or a deficit of the neural integrator ? 3) Are there different time constants of the torsional exponential decay as has been shown for the vertical exponential decay after iC lesions suggesting different time constants and hence a parallel processing of neural integration (Crawford and Vilis 1993) ? 4) How do iC lesions affect the torsional vestibulo-ocular reflex (VOR) ? 5) How do iC lesions affect vertical saccade amplitude and saccade velocities ?

Rhesus monkeys were trained to sit in a primate chair with the head immobilized. Spontaneous eye movements were recorded and monkeys were a) brought in different static roll and pitch positions and b) passively rotated sinusoidally in the pitch and roll plane. iC was localized by single unit recordings according to their typical neuronal discharge.

iC lesions (Muscimol) consistently showed a downbeating spontaneous nystagmus with downward gaze evoked nystagmus. During the late course of the lesion there could be an additional upbeating gaze evoked nystagmus but we never exclusively found an upbeat nystagmus. Listing's plane was shifted to the contralesional side (up to 20 deg). There was always an ipsilesionally beating torsional nystagmus, a strong asymmetry of the torsional VOR with diminished or lost torsional quick phases to the contralesional side. Time constants of exponential drift ranged from 100 -700 ms. Vertical oculomotor range became significantly smaller but saccade amplitude/velocity relationship remained normal. Horizontal saccades were not affected. [Supported by the Deutsche Forschungsgemeinschaft].

434.10

OTOLITH INPUT TO THE CENTRAL VELOCITY STORAGE IN HUMANS. T. Haslwanter*, M. Fetter, S. Mayr, J. Heimberger. Dept. of Neurology, Univ. Hospital, D-72076 Tübingen, Germany.

The central velocity storage in humans can be charged not only through input from the semicircular canals - the primary source of information about the velocity of the head in space - but also through optokinetic or otolith stimulation. Recent experiments which tested the response of nystagmus immediately after constant velocity rotation to repositioning of the body in space have shown that there are fundamental differences of the velocity storage in humans and monkeys: while in monkeys the axis of eye rotation stays approximately aligned with the earth vertical axis, in humans it is aligned with the bodyfixed yaw-axis. To investigate these differences between humans and monkeys in more detail, we have tested the 3-dimensional eye-movement response of 11 humans to off-vertical-axis rotation (OVAR) at 100°/s, with the axis of rotation tilted by 15° or 30° with respect to the earth vertical axis. During OVAR, the velocity storage gets charged through dynamic otolith input, and produces in monkeys a unidirectional eye-velocity which is modulated with the orientation of the body in space. Our results show that in humans the steady state average eye-velocity is close to zero, but still shows a significant modulation with the body position. This experiment emphasizes the differences of the 3-dimensional velocity storage organization in humans and monkeys. *Supported by DFG SFB 307 - A2*

434.11

3D OPTOKINETIC RESPONSES IN HUMANS ARE NOT INFLUENCED BY THE GRAVITO-INERTIAL FORCE VECTOR. M. Fetter*, G. Pfaff, J. Heimberger, T. Haslwanter. Dept. of Neurology, University of Tübingen, 72076 Tübingen, Germany.

When optokinetic nystagmus is elicited in monkeys by movement of the visual surround in their yaw plane, and the animals are in a tilted position in the roll or pitch plane, inappropriate (cross coupled) vertical or torsional eye movement components appear both during optokinetic nystagmus (OKN) and afternystagmus (OKAN). This has been explained by models assuming a „gravity centered" 3D velocity storage in monkeys. To test if similar cross coupled components can also be observed in humans, we studied in 10 normals the effect of head position on 3D optokinetic responses. Subjects were seated in a 3D rotator. Optokinetic responses were elicited by a planetarium-like optokinetic stimulator (40°/s for 40 s). Movement of the left eye was monitored using a 3D magnetic search coil during and after stimulation. Subjects were positioned at different roll angles (0-90° right ear down) with the optokinetic stimulation axis varying in the roll plane relative to the subject and at different pitch angles (0-90° nose up) with the stimulation axis varying in the pitch plane. Apart from the well-know fact that torsional optokinetic responses are weak in humans, OKN and OKAN showed identical eye rotation axes for identical optokinetic stimulation axes relativ to the head, independent of the head position relativ to gravity. Unlike in monkeys, no cross coupled components other than those due to smaller torsional than vertical or horizontal OKN gains could be detected. These findings support the notion of a fundamentally different organization of 3D velocity storage in humans and monkeys. *Supported by DFG SFB 307-A2*

434.12

THREE-DIMENSIONAL ANALYSIS OF NORMAL HUMAN CALORIC NYSTAGMUS S. T. Aw[1], M. Fetter[2], T. Haslwanter[2], J. Heimberger[2], G. M. Halmagyi[1]*. Eye and Ear Research Unit, Royal Prince Alfred Hospital, Sydney, NSW 2050 Australia[1], Department of Neurology, University of Tuebingen, D-72076 Germany [2].

The aim of this study was to investigate whether some response components of the caloric nystagmus can be attributed to a stimulation of the vertical semicircular canals. Each caloric irrigation was maintained for 3 minutes during which time the subject's head was positioned in the multi-axis rotator in one of four positions; such that either the lateral, anterior or posterior canals was horizontal, or the lateral canal was vertical. Following irrigation of the right external auditory canal with warm and cold air at 44°C and 27°C respectively, three dimensional eye movement recordings, using a dual search coil technique, showed horizontal, vertical and torsional nystagmus in 5 normal subjects. The results of the analysis were expressed as quaternions. We showed that the predominant response was from the lateral canal. The contribution of vertical canals to the caloric response was evidenced by the appropriately changing vertical component of the nystagmus when either the anterior or posterior canal stimulation was minimised by being positioned horizontally. We also irrigated 4 subjects, each continuously with warm air while the head was pitched at different angles. The lateral canal was initially pitched 45° backwards from the earth vertical position in each subject and then pitched forwards in nine 45° steps through a range of 270°, at 3 min intervals. We showed that the horizontal component of the nystagmus approximated a cosine function that varied with head pitch angle and confirmed that thermovective mechanism played an important role in caloric nystagmus. The vertical and torsional components were in the inverse direction of the horizontal component.

Supported by NH&MRC, Department of Neurology Trustees RPAH, Australia and Duetsche Forschungsgemeinschaft.

434.13

THE USE OF A VIRTUAL REALITY DISPLAY FOR VESTIBULAR AND OCULOMOTOR RESEARCH. P. D. Kramer*, D. Roberts, M. Shelhamer, and D. S. Zee. Johns Hopkins University, School of Medicine, Baltimore, MD 21287.

Testing of the vestibular system requires a vestibular stimulus (motion) and/or a visual stimulus. We have developed a versatile, relatively low cost ($13,000), stereoscopic visual display stimulus, using "virtual reality" (VR) technology. The display has a field of view of at least 110° vertically and horizontally, and is under computer control. We tested 2 subjects for smooth pursuit and stare optokinetic nystagmus (OKN) and after-nystagmus (OKAN); 4 subjects for short-term adaptation of the angular vestibulo-ocular reflex (AVOR); and 2 subjects for short-term adaptation of the linear VOR (LVOR). All tests were performed with a conventional display (OKN drum or laser target) and with the stereoscopic VR display.

OKN slow phase velocity averaged 16% greater with the VR display. Both displays produced OKAN; the conventional OKN drum produced OKAN of greater amplitude and longer duration. Smooth pursuit gains were almost identical with both displays.

Using the VR device and a rotating chair in a 1 hr visual-vestibular conflict paradigm, we were able to induce adaptive changes in AVOR phase (lead and lag) and gain (increase and decrease) that ranged from 50% to 100% of those induced with identical paradigms using an OKN drum as the visual display. Adaptive changes in LVOR phase were also induced on a linear sled, averaging ~16° after 20 min. There was no conventional visual stimulus for comparison in this case; we have no visual stimulus capable of producing a moving visual scene with a phase that is adjustable relative to sled motion.

While VR has some limitations, such as aliasing at high stimulus velocities and difficulty accommodating subjects who wear spectacles, it can provide a versatile visual stimulus for both the clinic and laboratory.

Supported by NIH Grants DC00111-01, DC00979-06, and the Whitaker Foundation.

434.14

A NEW METHOD FOR MEASURING PODOKINETIC AFTER-EFFECTS WITHOUT VESTIBULAR STIMULATION. K. Weber, WA Fletcher, G. Melvill Jones, E Block. Dept. of Clinical Neurosciences, University of Calgary, Calgary, Canada T2N 4N1.

In previous communications (Gordon et al, EBR 102:540-545, 1994; Weber et al, Soc Neurosci Abstr 21:2085, 1995) we described a robust non-visual "podokinetic" somatosensory/motor system thought to control spatial orientation during locomotion through trunk rotation relative to the space-stationary stance foot. However, after adaptive remodelling of the system by prolonged "walking-in-place" on a rotating treadmill, subjects could no longer walk straight without vision. Instead they all described markedly curved pathways (podokinetic after-effect) without any sense of turning, despite incurring body angular velocities an order of magnitude above vestibular sensory threshold. Similarly, following adaptation by "stepping-in-place" over the axis of the turntable, subjects turned relative to space without perception of turning. We now ask whether such UNPERCEIVED vestibular stimulation did nevertheless influence the subconscious podokinetic after-effect? Subjects were equipped with an electromagnetic search coil which senses angular POSITION and servo-drives the turntable at a proportionate angular VELOCITY in the OPPOSITE direction. After adaptation subjects stepped-in-place over the axis of the turntable, using the search coil to servo-control turntable velocity until they sensed it was NOT turning. The turntable tachometer then registered both magnitude and direction of the after-effect (UNPERCEIVED foot rotation-re-trunk) without any relevant vestibular stimulation. Initial results from 3 subjects and 3 conditions showed no obvious difference from the corresponding previous responses in which actual rotation relative to space occurred. Consequently, we conclude that the original expression of podokinetic after-effects described previously was not significantly influenced by the associated vestibular stimulation.

Funding: T. Rosza Foundation; MSI Foundation.

434.15

RESULTS OF VESTIBULAR REHABILITATION AFTER ACOUSTIC NEUROMA RESECTION. H Cohen* and K T. Kimball. Depts. of Otorhinolaryngology and Medicine, Baylor Coll. Med., Houston, TX 77030

Immediately following resection of an acoustic neuroma or vestibular nerve section patients have decreased vestibular-ocular reflex (VOR) gain, decreased postural responses, vertigo, disorientation and decreased independence in daily life skills. Although patients recover some physiological responses rapidly they remain impaired for long periods of time. Exercise treatments are often used in the attempt to facilitate that improvement, although little evidence supports the use of these treatments.

In this study pre-operative subjects with acoustic neuromas were pre-tested on caloric tests, sinusoidal tests of the horizontal VOR, computerized dynamic posturography, and qualitative measures of vertigo, gait, and independence in daily life tasks. For the 4 to 5 inpatient days after surgery subjects were treated twice daily at bedside in attention or exercise groups and then retested at discharge and at monthly intervals for three months. Subjects improved significantly on measures of vertigo and functional skills during the course of the week, and improved on physiological measures during the chronic recovery period. Exercise facilitated functional recovery.

Supported by NIDCD R03 DC01732-02 and the Clayton Foundation for Research.

REFLEX FUNCTION: ANIMAL STUDIES

435.1

DYSFUNCTIONAL CONTROL OF THE FLUCTUATIONS OF THE H REFLEX IN THE NEUROLOGICAL MUTANT *taiep* RAT. J.R. Eguibar[1*], J.E. Salceda-Ruanova[1], M. Roncangliolo[2] and E. Manjarrez[1]. [1]Institute of Physiology, B. Universidad Autónoma de Puebla, Puebla, Pue., México. [2]Dept. of Physiology, Fac. of Sciences, Universidad de Valparaiso, Chile.

Presynaptic inhibition is associated with the control of the afferent information flow into the spinal cord. Electrical stimulation of group I flexor afferents diminish the fluctuations (variance) of the monosynaptic reflexes (Rudomin and Dutton, 1969). *Taiep* rat is a neurological mutant that progressively presents tremor, ataxia, immobility episodes, epilepsy and paralysis (Holmgren et al., 1989). Comparison of the fluctuations of the H reflexes in normal rats and in *taiep* rats, allowed us to evaluate the effects produced by electrical stimulation of pathways that mediate presynaptic inhibition. We studied 9 control rats and 4 *taiep* rats (2 months age), under urethane or ketamine anesthesia. Stimulation was delivered through the sciatic or medial gastrocnemius (MG) nerves and the H reflexes were recorded by means of platinum electrodes inserted in the interdigital muscles of the hindlimb, or in the MG muscle, respectively.

Our experiments showed that conditioning stimulus of common peroneous nerve decreased the amplitude (A) and variance (S) of the H reflexes in normal rats: A=31.1% of control value, with S from 0.127 to 0.011; but not in the *taiep* rats A=81.7% of control value, with S from 0.005 to 0.003. This effect was maximal at 20-25 ms and had an average duration of 180 ms. The present results suggest that in young *taiep* rats the pathways associated with a reduction of the variability of H reflexes are dysfunctional. It seems possible that these alterations may be due, at least in part, to a failure in the presynaptic inhibition mechanisms in the spinal cord of *taiep* rat. *Partly supported by CONACyT 3710 (JRE), and SNI, México. JESR was fellow from CONACyT.*

435.2

INCREASED STRETCH REFLEX EXCITABILITY CAUSED BY SPASTICITY FOLLOWING SPINAL CORD INJURY IS PREDOMINANTLY CAUSED BY THE REFLEXIVE ATTRIBUTES OF A MONOSYNAPTIC REFLEX.

Browd, C.R., Thompson, F.J., Carvalho, P.M., Semple-Rowland, S.L.* Dept. of Neuroscience, Univ. Of Florida Brain Institute, Gainesville, FL 23610-0244.

Spasticity has been clinically defined as a velocity dependent increase in the lengthening tension of the affected muscles. Increases in reflex (Thilmann, 1991) and non-reflex-mediated (Sinkjaer, 1993) stiffness have been documented in spastic subjects. The threshold of monosynaptic reflexes in the stretch reflex pathway were significantly reduced (Thompson, 1993) subsequent to midthoracic spinal contusion injury. This experiment tested the respective contributions of reflexive compared to non-reflexive properties, to the exaggerated velocity dependent resistance to muscle lengthening in the spastic rat. This was determined by analysis of velocity dependent lengthening resistance using a repeated measures paradigm. An anesthetic was used to isolate the non-reflexive response from the reflexive component of the stretch. We have seen under general anesthesia of a normal rodent, only passive qualities are present. There was a marked attenuation of RMS of the EMG by 87.6% at a velocity of 306 deg/sec. The stiffness of the triceps surae muscle was also attenuated by 46.8% at the same velocity. The tentative conclusion would therefore be that the reflex mediated stiffness of the dynamic stretch reflex provides the predominant contribution to velocity dependent increase in ankle torque. (Supported by: R01 NS 33333-01A1, the Brain and Spinal Cord Injury Rehabilitation Trust Fund, and the Spinal Cord Research Foundation-PVA-1226.01)

435.3

HINDLIMB MOVEMENTS OF ADULT RATS WITH TRANSECTED SPINAL CORDS. J.G. Broton*, X.M. Xu, M.B. Bunge, S. Lutton, T. Cuthbert, and B. Calancie, The Miami Project to Cure Paralysis, Depts. Neurol. Surg. and Cell Biology and Anatomy, University of Miami, Miami FL

In studies of therapeutic interventions after adult rat spinal cord injury, restoration of hindlimb movements has often been equated with recovery of voluntary function. The present investigation describes the reflex hindlimb movements of rats with transected spinal cords. Movements were observed while rats were suspended over a moving treadmill.

Adult female rats underwent spinal cord transections at T8-T10. Rats were videotaped 1, 2 and 4 weeks post-transection (post-t) with the treadmill surface moving at 0.05, 0.10 or 0.15 m/sec, for a minimum of 2.5 minutes. The videotape was replayed, and the times during which specific hindlimb movements (or their absence) were seen (i.e. dragging, scratching, kicking, flexion, extension, or stepping movements - unilateral or bilateral) were noted. Times were scored as percent of the total time analyzed for that rat. When 20s of continuous bilateral stepping movements (BSMs) were seen, the coordinates of ink spots on the left forelimb and hindlimb were determined and used to calculate position auto- and crosscorrelations for that time period.

Five of the seven tested rats displayed non-weight-supported BSMs at times ranging from 20-46% of the total time analyzed. BSMs were first evident 2 wks post-t. Average autocorrelation coefficients for the hindlimb were 0.34± 0.14 at 0.05m/s and 0.40±0.14 at 0.10m/s. Forelimb autocorrelations were 0.25± 0.05 at 0.05m/s and 0.43±.18 at 0.10m/s. The average hindlimb cross-correlations with the forelimb were 0.22±0.07 at 0.05m/s and 0.11±0.07 at 0.10m/s, much lower than control data from able-bodied rats (0.71±0.12 at 0.10m/s).

These results indicate that movements that may easily be confused with voluntary movements - BSMs - can be generated by the isolated adult rat spinal cord. The use of correlation analysis may be helpful in the determination and quantification of voluntary hindlimb function after spinal cord injury in rats.

Supported by The Miami Project to Cure Paralysis.

435.4

STRETCH AND H REFLEXES IN SOLEUS MUSCLE OF HIGH DECEREBRATE CATS DURING TONIC AND PHASIC CONTRACTIONS. De Serres, S.J.*, Misiaszek, J.E., Stein, R.B. and Pearson, K.G., Department of Physiology, University of Alberta, Edmonton, Canada, T6G 2H7.

Opposite relationships exist between reflex force and background muscle activity when stretching the triceps surae muscles during tonic and phasic contractions. While the reflex force decreases when the background activity increases in a tonically contracting muscle, the reflex force increases when the background force is increased by phasic activation (locomotion). This difference could arise from events at two sites: 1) altered fusimotor drive at the muscle spindle, or 2) altered transmission through the reflex arc. In the present study, electrically evoked soleus H reflexes were compared to mechanical stretch reflexes of the same muscle during both types of contraction in high decerebrate cats. H reflexes arise from activation of the primary afferents proximal to the muscle spindles and thus are not influenced by fusimotor drive. Electrical stimuli or mechanical stretches were applied during periods of either tonic contractions or spontaneous locomotion entrained by a treadmill. The muscle was held isometric during both types of contraction. During locomotion the H reflex modulated strongly as its peak-to-peak amplitude increased with increasing background activity. During tonic contractions the amplitude of the H reflex also increased with increasing background activity. In general, H reflexes obtained during tonic contractions were greater than reflexes obtained during locomotion. The stretch responses behaved in a similar fashion to previous studies. That is, the reflex force and EMG increased with increasing background activity during locomotion, but decreased with increasing background activity during tonic contractions. The lack of a difference, between the tonic and phasic conditions, in the relationship of the H reflex with background activity suggests that the difference described for the stretch reflex arises from a variation in fusimotor drive between the two types of contraction. Supported by MRC (Canada) and AHFMR (Alberta).

435.5

EFFECTS OF STIMULATION OF EXTENSOR MUSCLE AFFERENTS DURING WALKING IN ADULT RATS. K. Fouad and K.G. Pearson*. Dept. of Physiology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Little is known about the influence of muscle proprioceptors on the walking pattern in the rat; the only information comes from studies in neonates. In P4-6 animals stimulation of extensor afferents during extension causes either no change or a truncation of extensor activity, depending on the intensity of the stimulation. Low or high intensity stimulation during flexion can truncate or prolong flexor activity respectively (O. Kiehn personal communication).

To address this issue in adult rats, we stimulated extensor nerves in the hindlimbs of decerebrate animals walking on a wheel. Bouts of walking either occurred spontaneously or were evoked by electrical stimulation of the mesencephalic locomotor region (MLR). An ankle extensor nerve (either Lg-Sol or Mg) was cut close to the muscle and the proximal end tied into a stimulation cuff, which was anchored to the tibial nerve. To monitor the stimulus intensity, a recording cuff was placed around the sciatic nerve. Stimulating an extensor nerve (300-500 ms duration, 50 Hz, 2-5 x T) during the early extensor phase of the step-cycle resulted in prolongation of the extensor burst and a delay of the flexor burst. This effect was weaker, but qualitatively similar to the effect of extensor nerve stimulation in the cat. Stimulation at the end of the flexor burst resulted in a delay of extension, a phenomenon that does not occur in the cat. These effects were larger during spontaneous walking compared to those during MLR evoked walking. Stimulating during other phases of the step cycle did not influence the timing of locomotor activity. No differences were found in stimulating Mg or Lg-Sol afferents. Because these observations differ from those in neonatal rats, we conclude that a major change occurs in the integration of afferent input to the walking pattern generator during late postnatal development.

This work was supported by a grant of the DFG to KF and by the MRC to KP.

435.6

SYNAPTIC EFFECTS OF TRICEPS SURAE (TS) GROUPS I AND II AFFERENT FIBERS IN TS SCRATCHING MOTOR PROGRAM. S. H. Dueñas*. Lab. de Neurofisiología, Departamento de Neurociencias, CUCS, Universidad de Guadalajara, Guadalajara, Jalisco, México.

The present study was performed to analize the synaptic actions of TS groups I and II afferent fibers in TS motoneurons during fictive scratching in thalamic cats (n=12). TS group Ia and Ib afferent fibers were activated by TS tendon vibration (180-200Hz and 25-35 Hz, respectively), and group II afferent fibers by medial gastrocnemious (MG) nerve electrical stimulation (2-3 x T). TS Ia afferent fibers did not evoke fictive scratching episodes but a small increase (10%) in scartching cycle duration ocurred. TS burst amplitude increased as TS homonymous and heteronynimous monosynaptic reflexe amplitude before (10-30 ms) and during scratching (extensor) phase increased. TS Ib afferent fibers evoked episodes of fictive scratching but scratching cycle duration changes were not observed (n=4). In the period elapsing between Ib stimulation and the appearence of scratching episodes, forerunner exitatory posynaptic potentials (EPSPs) occurred in MG motoneurons. TS group II afferent fibers reduces tibialis anterior scartching postural (aimung) period duration, produces MG tonic motoneuron activity at the onset of scratching episodes and prolonged (2-3 times) the MG cyclic scratching phase duration with a consequent flexor phase reduction. Summing effects of group I and II afferent fibers in extensor Ia rhytmically activated interneurons and in TS motoneurons could prolong extensor and reduce flexor scratching cycle phases during TS motor program.

Supported by U de G. Acuerdo 0206. Prog.02. D.I.

435.7

A MODERN APPRAISAL OF DENNY-BROWN'S CONCEPTIONS OF GRASPING AND AVOIDING. J.A. Vilensky* and S. Gilman. Dept. of Anatomy, Indiana Univ. Sch. of Med., Ft. Wayne, IN 46805 and Dept. of Neurology, Univ. of Michigan Sch. of Med., Ann Arbor, MI 48109.

"Grasping" and "avoiding" responses are two positive clinical signs that Denny-Brown observed in his patients after localized brain lesions, and that he was able to reproduce in monkeys by cerebral ablations. Recent investigations of these responses in patients using imaging techniques have tended to support Denny-Brown's association of grasping with frontal lobe lesions and avoiding with parietal lobe lesions. Here we illustrate the types of grasping (traction response, grasp reflex, instinctive grasp reaction) and avoiding (avoiding response, levitation) phenomena that Denny-Brown described and how each may be elicited using sketches, photographs and a videotape.

We also illustrate how grasping and avoiding responses fit into Denny-Brown's view on nervous system integration. He believed that the CNS was organized such that reactions to sensory stimuli have "positive" and "negative" elements. Positive reactions (e.g., grasping) are those that reach out into the environment and negative reactions (e.g., avoiding) retreat from the environment. Based on this perspective, Denny-Brown explained how, for example, the chorea of Huntington's disease results from released but conflicting positive and negative cortical automatisms.

The continued verification of Denny-Brown's conclusions on the localization of grasping and avoiding phenomena suggests that his views on nervous system integration should be reexamined in the light of contemporary data, e.g., the success of pallidotomy for relieving some of the disorders of Parkinson's disease.

Supported by PHS NS33782.

435.8

A DESCRIPTION OF THE LICK-CHEW RESPONSE IN THE RABBIT. K. Asher, N. R. Myslinski* and J. Buxbaum. Department of OCBS, School of Dentistry, University of Maryland, Baltimore, MD 21201.

The lick-chew response (LCR) is a complex oral-facial reflex seen in the rabbit after tooth pulp stimulation. It has been used to study the pharmacology of trigeminal pain systems, but has never been described in detail. This paper attempts to characterize this reflex in order to make it a more useful experimental technique. Six adult naive New Zealand white rabbits (1.5 - 3.0 kg) were prepared with 0.1 mm cavities in their maxillary incisors bilaterally half way between the gingival margin and incisal edge of the tooth. Electrical stimuli were applied to the tooth pulp using a ramp stimulator and the voltage that evoked the LCR was recorded. Monopolar EMG recordings were made of the masseter and digastric muscles. The LCR occurred in 2 phases: the bruxing phase and the licking phase. Time and distance measurements were made of multiple aspects of each phase. The bruxing phase averaged 4.26 sec and the licking phase averaged 6.1 sec. The average number of licks was 15. In order to determine the location of the critical circuits of this response the skulls of 2 additional anesthetized rabbits were opened and serial cross sections of the brains were performed. The sections began near the anterior commissure and proceeded caudally while testing the LCR after each section. The LCR remained intact after sections up to and including the pons-midbrain junction. (Supported in part by NIH Grant RR10162-01.)

435.9

HABITUATION OF THE EYEBLINK AND OF FULL-BODY STARTLE: A COMPARISON OF TWO REFLEX RESPONSES. A. S. Powers*[1] and J. Cranney[2]. [1]Department of Psychology, St. John's University, Jamaica, NY 11439, and [2]School of Psychology, University of New South Wales, Sydney, New South Wales 2052, Australia.

In rats, the acoustic startle response is typically measured as a full-body response, while in humans, it is measured as an eyeblink. The present study investigated whether both measures would show the same patterns when examined in a single species. We used the same procedures in two experiments in rats, one examining the full-body startle and the other the eyeblink. In one experiment we implanted electrodes in 10 rats for recording electromyograms (EMG's) from the orbicularis oculi muscle, which closes the eye. Then blink responses to a loud (105 dB) white-noise stimulus were measured in one 80-trial session. This stimulus was presented every 10 sec for 40 trials. Ten seconds after the fortieth trial, the rats received a single 0.5 s, 0.5 mA footshock. Within 72 s of receiving the footshock, another sequence of 40 acoustic stimuli began. Freezing, a measure of fear, was recorded throughout the session. In the other experiment, a separate group of 10 rats was run on the same sequence of stimuli, but their full-body response was measured in a startle chamber. Freezing was also recorded in this group. The results showed that EMG, startle, and freezing in both experiments showed an initial sensitization, followed by habituation during the first 40 trials, then another sensitization caused by the footshock. Freezing was more marked in the EMG experiment, and the degree of sensitization to the shock was less. The results indicate that eyeblink and full-body startle follow similar patterns and suggest that the full-body startle in rats is probably measuring the same processes as the eyeblink in humans.

Supported by a travel grant to ASP from the Australian Psychological Society.

435.10

A SIGNAL DETECTION THEORY ANALYSIS OF THE EFFECTS OF QUINPIROLE ON ACOUSTIC STARTLE IN THE RAT. D. S. Leitner[1]*, E. M. Girten[1] and D. P. Carmody[2]. [1]Psychology Dept., St. Joseph's Univ., Philadelphia, PA 19131, and [2]Psychology Dept., St. Peter's College, Jersey City, NJ 07306

If a weak sensory event (a prestimulus) is presented prior to the onset of a startle-eliciting stimulus (SES), the amplitude of the subsequently elicited startle reflex will be reduced. Currently no consensus concerning the best way to quantify this phenomenon exists. The present study used analyses based on signal detection theory (SDT) to quantify the ability of gaps, brief silent periods in otherwise continuous 60 dB broad-band noise, to reduce the amplitude of the rat's acoustic startle reflex. The SDT analyses were compared to analyses of amplitude, and difference and percentage scores. Also examined were the effects of the dopamine D_2/D_3 receptor agonist quinpirole on startle amplitude and the inhibition produced by gaps. Dopamine D_2 receptor agonists have been shown to interfere with the ability of prestimuli to modulate startle amplitude.

Twenty-four rats were exposed to four stimulus configurations representing the factorial combination of two gaps (4- and 50-ms-long) presented at two interstimulus intervals (20 and 50 ms) in addition to control trials of the SES alone. These five types of trials were presented 25 times each in block-random sequence. Each subject was tested once following an injection of saline (1 ml/kg) and once following an injection of quinpirole hydrocholoride (5 mg/kg).

Analyses based on amplitude showed that quinpirole decreased startle amplitude, and its effects on amplitude reduction produced by gaps varied with stimulus configuration. Because of the simultaneous drug-induced decrease in startle amplitude and the ability of the gaps to reduce startle amplitude, these data were not readily interpretable. Analyses based on difference scores showed that quinpirole reduced the ability of a gap to inhibit startle. Analyses based on percent and SDT showed a similar pattern in that quinpirole enhanced the ability of a gap to inhbit startle.

435.11

IMIQUIMOD ELICITS EMESIS IN FERRETS THAT IS MEDIATED BY AREA POSTREMA (AP) BUT DOES NOT DIRECTLY ACTIVATE NEURONS IN THE AP OF BRAIN STEM SLICES. N. L. Strominger[1]*, R. J. Brady[2], D. O. Carpenter[2] and G. Gullikson[3]. [1]Albany Medical College, Albany, NY 12208, [2]Wadsworth Labs, School of Public Health, Albany, NY 12201 and [3]3M Pharmaceuticals, St. Paul, MN 55144.

The immunomodulator, imiquimod, has been shown to have antiviral and antitumor properties in animal models. It has also been reported to alter cytokine levels in both animals and humans. However, imiquimod appears to be emetic. Subcutaneous administration of ≥10 mg/kg imiquimod to ferrets elicits emesis with latencies as short as 2'; 12 and 15 mg/kg were optimal doses. Emetic responsiveness was eliminated by complete ablation of the AP, but was unaffected by bilateral supradiaphragmatic section of the vagus nerve. This indicates that the emesis is produced by an activation of the chemical trigger zone, the AP.

Brain stem slices (400 μm), prepared from deeply anesthetized ferrets, were preincubated in oxygenated Krebs-Ringer and then mounted in a submerged slice recording chamber. Extracellular recordings of spontaneous and ionophoretically-evoked activity of AP neurons were obtained for up to 8 hours while the effect of bath applied imiquimod was determined. Under control conditions neurons showed a low frequency spontaneous discharge. Introduction of imiquimod (concentration range 10^{-7} to 5×10^{-6} M) had no effect on neuronal firing. With ionophoresis of glutamate from an independent micropipette, a brief excitatory response was obtained. The bath application of imiquimod also did not significantly alter this neuronal response. Imiquimod at concentrations of up to 50 μM does not significantly alter the activity of AP neurons. We conclude that imiquimod does not directly excite area postrema neurons. It is likely that imiquimod causes synthesis and release of some emetic substance. Supported by 3M Pharmaceuticals.

MUSCLE

436.1

IMPAIRED VELOCITY-SENSITIVE RESPONSE TO STRETCH IN PLANTARFLEXORS AFTER SPINAL CORD LESION. A. Lamontagne, F. Malouin, C.L. Richards*, F. Dumas and D. Tardif. Physiotherapy Dept., Faculty of Medicine, Laval University, Quebec City, Canada, GIK 7P4

The primary aim of this study was to compare the effect of stretch velocity on the non-reflex resistive torque (RT) of the plantarflexors in subjects (n=6) with a complete chronic (1-3 yrs) spinal cord injury (SCI) to normal (n=12) subjects (CTL). Series of 5 passive ankle movements, ranging from -35° to 5° of dorsiflexion (DF), were randomly imposed at 5°/s, 10°/s, 20°/s, 40°/s, 60°/s, 120°/s, 180°/s using a Kin-Com dynamometer. Surface electrodes detected unwanted activity during the tests. RT of the last 4 trials of each series recorded at -10° and 0° of DF was averaged. The effect of velocity on RT values was determined by an analysis of variance (ANOVA) followed by post-hoc procedures and net RT increments (RT at each velocity minus RT at 5°/s) were compared between the SCI and CTL groups using t-tests. Results first show that RT recorded at 5°/s for the SCI group was similar to that of the CTL group, which contrasts with increased muscle stiffness seen in spastic disorders of cerebral origin (cerebral palsy, stroke). Secondly, a significant increase in RT was found at a higher velocity ($p<0.01$) in the SCI group (60°/s) compared to the CTL group (40°/s). Finally, larger ($p<0.001$) net increments of RT were calculated at 180°/s for the CTL group than for the SCI group whose RT plateaued at 60°/s. These results show that plantarflexors in subjects with a chronic and complete SCI have no increase in muscle stiffness but demonstrate an impaired velocity-sensitive behavior, especially at high velocity. This impaired behavior likely reflects changes of muscle tensile properties that may have taken place because of disuse and of the alteration of descending neural input. (Supported by MRC Canada)

436.2

MUSCLE FATIGUE IN INDIVIDUALS WITH HEALED BURNS, S.W.Chau, D.M.M. St-Pierre*, M.Choinière and D.R. Garrel. School of Physical and Occupational Therapy, McGill University, and Centre des grands brûlés, Hôtel Dieu de Montréal, Québec H3G1Y5, Canada.

The purpose of the study was to compare the effects of a submaximal intermittent isometric fatigue protocol of the quadriceps in individuals with healed burns (BURNS) versus healthy controls (CONTROLS). Seven subjects (40±11.9 y) with second and third degree burns (mean 44.2 ± 12.3% total body surface area, range of 35.0 to 67.3%) were evaluated at 53.4 ± 15.1 months (range 31 to 74) post-trauma. They were matched to five CONTROLS (43.2±12.9 y) of similar fitness level. The fatigue protocol consisted of two sets of 50% maximal voluntary contractions (MVC) held for 10s, with 3s ramps followed by a 4s rest. The inability to maintain the target level during the 10s hold marked the end of a set and 10 mins were allowed between each. All subjects performed contractions while seated with hips and knees flexed at 90°. Voluntary and evoked contractile properties were evaluated pre, post and during the two 10 min recovery periods. Preliminary data revealed that BURNS performed significantly fewer total contractions than CONTROLS (9.6±2.4 vs 13.0±2.3 respectively, p=0.04). The first set of fatiguing contractions produced similar changes in MVC, activation as measured by the interpolated twitch technique, M-wave and twitch contractile properties, suggesting that the mechanisms underlying fatigue were similar in both groups. The second set of fatiguing contractions produced greater decreases in MVC in the BURNS (39.6 versus 32.9%, respectively, p=0.05,). However, BURNS also had a greater degree of inactivation (15.0 versus 5.9%, respectively, p=0.008). These findings suggest that the greater fatigue in the BURNS was associated with a decrease in central drive. Funded by FRSQ-FPQGB and NATO.

436.3

M-WAVE DEPRESSION AND POTENTIATION FOLLOWING ECCENTRIC MUSCLE CONTRACTION. A.B. Leger, P. Bawa and T.E. Milner*. School of Kinesiology, Simon Fraser University, Burnaby, B.C. V5A 1S6.

Previous studies have shown a decrease in amplitude and an increase in duration of the M-wave during fatiguing concentric muscle contraction. This study examined the changes in the M-wave immediately after (post) and 24 hours following a series of maximal eccentric contractions. Nine male subjects performed an exercise regime of 100 maximal eccentric contractions with the FDI muscle in abduction-adduction. A maximal M-wave was elicited by supramaximal stimulation of the ulnar nerve proximal to the wrist joint and recorded with surface EMG electrodes placed over the muscle belly. Accurate repeated placement was ensured by marking the skin.

Peak to peak amplitude and peak to peak duration decreased significantly from the pre- to post-test (-23.6% and -11.8%, respectively; $P<0.05$). This decline in M-wave amplitude may have been the result of neuromuscular propagation failure which has been reported to occur during fatiguing concentric contractions. The M-wave was tested again 24 hours later. Peak to peak amplitude was found to have increased by 47.7% over the pre-test values ($P<0.01$). No significant differences were found in peak to peak duration or delay from stimulus to M-wave onset. Since maximal voluntary force was significantly lower than pre-test (-19%, $P<0.001$), it is unlikely that the M-wave potentiation was due to increased motor unit recruitment. Repeated high intensity eccentric contractions have been reported to cause damage to muscle fibers. We suggest that the M-wave potentiation was due to changes in membrane properties produced either directly or indirectly by the injury to muscle fibers. These findings may be useful in determining which fibers are most susceptible to injury during eccentric exercise.

Supported by a grant from the Natural Sciences and Engineering Research Council of Canada

436.4

RE-EVALUATION OF MUSCLE WISDOM USING PHYSIOLOGICAL STIMULATION RATES. A.J. Fuglevand*. John B. Pierce Laboratory, 290 Congress Ave., New Haven, CT 06519.

Motor unit discharge rates decline by about 50% over 60 s of a maximum voluntary contraction (MVC) from an initial rate of ~30 Hz (Bigland-Ritchie et al. *J Physiol* 1983). It has been suggested that the decline in discharge rate functions to optimize force output by maintaining a match between activation rate and the changing mechanical properties of motor units and by protecting against conduction failure associated with prolonged high discharge rates (Bigland-Ritchie et al. 1983). This hypothesis, known as muscle wisdom, was based in part on studies in which muscle force was shown to decline more rapidly when stimulation was maintained at a high rate than when stimulus rate was reduced over time (Jones et al. *Exp Neurol* 1979). The stimulus rates used by Jones et al., however, were higher than that normally encountered during MVCs. The purpose of this study was to compare force loss under constant and declining-stimulus rate conditions using rates similar to those that occur during voluntary effort. Isometric force and surface EMG responses were recorded from human adductor pollicis in response to supramaximal stimuli delivered to ulnar nerve. Four fatigue tasks were performed on separate days: 1) stimulation at 30 Hz for 60 s, 2) stimulation decreased from 30 to 15 Hz over 60 s, 3) intermittent stimulation at 30 Hz, 330 ms, 1/s, for total of 1800 stimuli, 4) sustained MVC for 60 s. Immediately following each fatigue task, 2.5 s trains were delivered at 30 and 15 Hz. Force loss was greatest for the MVC condition (~60%) and similar across the three stimulation conditions (~40%). In all cases, change of stimulus rate from 30 to 15 Hz at the end of the fatigue task caused force to decrease (17±12%). These findings imply that diminution of discharge rate may not function to preserve force during fatigue. Supported by NIH grant AR 42893.

436.5

INCOMPLETE MUSCLE ACTIVATION AFTER TRAINING.
T.Hortobágyi*, K.Scott, N.Lambert†, J.Hill†, and C.Webb†.
Biomechanics Lab and †Dept. of Physical Therapy, East Carolina University, Greenville, NC 27858.

One aspect of neural adaptation to exercise is an increase in muscle activation allowing for greater strength following training. Because eccentric contractions and electromyostimulation (EMS) tend to preferentially recruit large motor units, we postulated that training with voluntary eccentric or with EMS-evoked eccentric contractions should result in complete muscle activation. Eighteen women (age 18-40 y) were randomly assigned to a voluntary (VOL), an EMS, or a control group. VOL and EMS groups trained the quadriceps muscle at the same but increasing force levels for 6 wks, 4/wks using voluntary eccentric or EMS-evoked eccentric contractions. The Table shows the results (mean ±SD in N, * $p < .05$). Because EMS-evoked forces exceeded maximal

Training	Test mode	Pre		Post		Δ	%Δ
VOL	VOL	456	71	617	158	161	35*
	EMS	238	125	310	128	72	30*
	Ratio (%)	57	34	51	17	-6	
EMS	VOL	505	97	605	108	100	20*
	EMS	277	86	615	85	338	122*
	Ratio (%)	58	13	120	40	62	

voluntary forces after training in the EMS group (120% for the ratio), voluntary muscle activation was not complete after training. Even if one becomes trained, an inhibitory mechanism may protect muscles and joints from excessive forces during eccentric contractions.

Supported in part by NICHD-30422.

436.6

PRELIMINARY ANATOMIC EVIDENCE OF PARTITIONING IN THE MUSCLES OF THE ANTERIOR COMPARTMENT OF THE HUMAN LEG, B.W. Bakkum*, K. Russell, T. Adamcryck and M. Keyes. The National College of Chiropractic, Lombard, IL 60148.

Many mammalian muscles appear to be partitioned into neuromuscular segments. Each segment of a muscle is a subvolume of fibers that is innervated by an individual muscle nerve branch and contains motor unit territories with a unique array of physiological characteristics. The muscles of the anterior compartment of the human leg have not been investigated for anatomic evidence of these neuromuscular segments. From each of 10 embalmed adult cadavers, one leg (4 - right, 6 - left) was dissected to expose the muscles of the anterior compartment and the branches of the deep fibular nerve. Perimysium consistently partitioned the tibialis anterior (TA) muscle into three segments: lateral longitudinal, medial longitudinal and oblique. The extensor digitorum longus (EDL) and extensor hallucis longus (EHL) muscles each had two segments: longitudinal and oblique. The fibularis tertius muscle had a muscle belly distinct from EDL and a separate nerve branch in 40% of the legs. Muscle nerve branches to each of the longitudinal segments of TA were found to arise consistently, with one exception, from the recurrent genicular nerve. A branch to the oblique segment of TA was found in 70% of the legs. Branches to both segments of EDL were consistently located. Branches to the longitudinal and oblique segments of EHL were found in 80% and 90% of the legs, respectively. These results show that there is gross anatomic evidence of three neuromuscular segments in TA and two segments each in EDL and EHL. Supported by Natl. Coll. Chiro.

436.7

OLDER ADULTS EXHIBIT A REDUCED ABILITY TO MAXIMALLY ACTIVATE THEIR ELBOW FLEXOR MUSCLES. G.H. Yue*, S.J. De Serres and R.M. Enoka. Department of Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH, 44195.

Reorganization of the sensorimotor system due to aging results in a reduction in the maximal muscle force that can be voluntarily produced by older adults. Although a decrease in muscle mass contributes significantly to the decline in muscle force, little is known about whether the neural command for a maximal voluntary contraction (MVC) is adequate to achieve full muscle activation. The purpose of this study was to determine the ability of elderly subjects to maximally activate their biceps brachii muscle using the twitch interpolation technique. This method consisted of delivering a supramaximal electrical stimulus to the muscle while an MVC was sustained. Two groups of volunteers (young adults, <35 yrs; older adults, >65 yrs) performed isometric MVCs with the elbow flexor muscles of the left arm. Each maximal contraction lasted for about 3 seconds during which a short train of supramaximal stimuli (10 pulses at 100 Hz, 0.1-0.3 ms in duration) was applied to the biceps brachii muscle. All the elderly subjects demonstrated an inability to fully activate their biceps brachii muscle. In contrast, 38% of the young subjects were able to maximally activate their muscle. The remaining young subjects showed a comparable deficit in voluntary activation to the elderly subjects. These findings suggest that an inability to achieve a full muscle activation is among the physiological changes seen in the aging sensorimotor system.

Supported by a NIH grant (AG 09000-04) to RME.

436.8

EFFECTS OF DIFFERENT MECHANICAL ACTIVATION PATTERNS ON THE FUNCTIONAL PROPERTIES OF INACTIVE SOLEUS MUSCLES. V. H. Zhong, R. R. Roy*, J. A. Hodgson, E. J. Grossman and V.R. Edgerton. Brain Research Institute and Dept. Physiological Science, UCLA, LA, CA 90095.

Inactivity was produced in the soleus muscle of adult, female cats via spinal isolation (SI), i.e., complete cord transections at T12-13 and L7-S1 and bilateral deafferentation between the transection sites. After 1 week of recovery, mechanical activity was imposed via maximum stimulation of the soleus nerve at 40 Hz for 300 ms, once/sec for 30 min/day, 5 days/week during either the lengthening (SI-L) or shortening (SI-S) phase of a simulated step cycle or isometrically (SI-I). Tendon force measurements verified maximum muscle stimulation. After 4 months, the mass, maximum tetanic tension (Po), tension produced at 20 Hz (P20), isometric contraction time (CT), maximum velocity of shortening (Vmax) and fatigue index (FI) for each experimental group expressed as % of control were:

Group (n)	mass (g)	Po (g)	P20 (g)	CT (msec)	Vmax (mm/sec)	FI
(a) SI (13)	$33\pm2^{b,c,d}$	$17\pm1^{b,c,d}$	$12\pm1^{b,c,d}$	56±6	179 ± 12^{d}	93±2
(b) SI-S (12)	55 ± 4^{a}	$33\pm3^{a,d}$	$25\pm3^{a,d}$	58±3	158 ± 13^{d}	99±5
(c) SI-L (11)	53 ± 3^{a}	46 ± 4^{a}	32 ± 4^{a}	61±4	194 ± 10^{d}	102±2
(d) SI-I (13)	63 ± 3^{a}	$47\pm3^{a,b}$	$41\pm3^{a,b}$	54±3	$115\pm6^{a,b,c}$	89±3

(Letters indicate a significant difference from that group at $P < 0.05$. All values except for the Vmax for SI-I and all FI were different from normal control)

Thus inactivity resulted in a dramatic loss of force generating potential and a shift towards faster velocities of shortening, whereas the fatigue properties were unaffected. All mechanical interventions ameliorated the loss in mass and force potential, whereas only the isometric regime had a significant effect on Vmax. These data suggest that short bouts of high-load, isometric contractions were, in general, the most efficacious in maintaining the normal functional properties of inactive soleus muscles. Supported by NIH Grant NS16333.

436.9

SYNAPTIC PLASTICITY IN A POPULATION OF NEUROMUSCULAR JUNCTIONS. S. A. Jaboori and R. S. Manalis*. Department of Biology, Indiana University-Purdue University Fort Wayne, IN 46805.

In order to study synaptic plasticity in populations of neuromuscular junctions, a paired-pulse stimulus protocol was used to indirectly stimulate pharmacologically blocked frog cutaneous pectoris nerve-muscle preparations. Blockade was achieved either presynaptically with high Mg^{++}(3.0 mM) - low Ca^{++}(0.3 mM) Ringer's or postsynaptically with curare (2 μM) in normal Ringer's. Synaptic plasticity, or, more specifically, facilitation, was used to describe the increase in the TEST response relative to that of the CONDITIONING response. The amplitude of the test response divided by that of the conditioning response yielded a T/C ratio, which was our measure of facilitation. In either case, the T/C ratio was maximal at short interpulse intervals (5-30 msec) whereas it decreased toward unity as the interval approached 1000 msec. Comparison of contractile and EPP ratios illustrates: 1) the time courses of facilitation are very similar and 2) contractile ratios are considerably greater than are EPP ratios. For contractile ratios, some members of the motor unit population were close to threshold upon the arrival of the conditioning stimulus, and some of them were recruited to contract when the test stimulus arrived. Changes in synaptic plasticity occurring within a population of nerve-muscle synapses is an example of integrative physiology.

Supported by Indiana University-Purdue University Fort Wayne.

436.10

NEUROMUSCULAR COMPARTMENTS OF THE MASSETER MUSCLE PRODUCE UNIQUE FORCE TRAJECTORIES. A.W. English* and C.G. Widmer. Dept. Anat. Cell Biol., Emory Univ., Atlanta GA 30322 and Dept. Oral Surgery, Univ. Fla., Gainesville FL 32610

The rabbit masseter contains at least 25 neuromuscular compartments. To determine the mechanical effect of activating different compartments, they were activated using visually guided intramuscular micro-stimulation. Reaction forces were measured using a multiaxis force moment transducer mounted via two pins attached to small drill holes in the sides of the mandible. Evoked EMG activity was recorded from multiple sites to document the very localized nature of the contractions. Each compartment produced a jaw closing force of different magnitudes, but each also produced significant ipsilateral (working) or contralateral (balancing) forces relative to the side of stimulation and also moved the mandible rostral (protrusion) or caudal (retrusion). The relative contributions of these three components determines the resultant trajectory of force. Each compartment produced a relatively unique trajectory of force. In the coronal plane, the range of trajectories associated with different compartments is greater than 90°. Our results are consistent with our previous hypothesis that each neuromuscular compartment exerts a distinct mechanical effect on the skeletal system. We propose that the neuromuscular compartments of the rabbit masseter form elements by which the nervous system interacts with the musculoskeletal system to bring about precise control of movements of the mandible. Supported by DE11536 (AWE), HD 32571 (AWE), DE00333 (CGW), and DE10130 (CGW) from the USPHS.

436.11

VELOCITY SENSITIVITY OF SHORT-RANGE STIFFNESS AND YIELD OF HETEROGENEOUS MUSCLES IN THE CAT. C.M.J.I. Huyghues-Despointes*, T.A. Abelew and T.R Nichols. Department of Physiology, Emory university, Atlanta, GA 30322, USA.

The responses of intact cat soleus (SOL) to ramp stretches resemble the responses obtained from the type I single muscles fibers of which this homogeneous muscle is composed. The intrinsic mechanical properties of heterogeneous muscles, such as flexor hallicus longus (FHL), are expected to also reflect the properties of its active muscle fibers and their underlying cellular mechanisms. Preliminary data show that some type II muscle fibers exhibit velocity-dependent short-range stiffness (SRS) and yielding (Malamud et al., *J. Neurophysiol.*, in press). However, the velocity sensitivity could be masked by the filtering properties of the in-series tendon. Since the mechanical properties of a heterogeneous muscle have not been explored, we hypothesized that any velocity sensitivity of short-range stiffness (SRS) and yield expressed by single muscle fibers should also be apparent in the intact muscle. To test this hypothesis we stretched cat FHL and SOL at different velocities and different lengths of in-series tendon in anesthetized or decerebrate cats. Muscles were electrically or reflexly activated. Regardless of the length of the tendon, the heterogeneous FHL muscle expressed a distinct yield which is not characteristic of most type II single fibers. As expected, the SRS of both hetero-and homogeneous muscles was consistently higher when the muscle tendon was shortened. We found no velocity sensitivity of SRS in either muscle. Our results confirm the linearity of the response during the short-range period, further validating the assumption that prior to yield the muscle-tendon unit behaves much like a spring. However, following the yield point, the response becomes highly non-linear.

(Supported by NS 20855 and HD 32571)

436.12

DEHYDRATION ATTENUATES FLEXOR MUSCLE CONTRACTILITY IN ACUTE LEAD-TREATED MICE. M.A. Fahim*, F. El-Sabban, F.S. D'souza and S. Singh. Dept. of Physiology, Faculty of Medicine, U.A.E. University, P.O. Box 17666, Al Ain, United Arab Emirates.

Effect of water deprivation for 24 h on skeletal muscle contractility of lead-treated mice was investigated. Exposure to lead was made by i.p. injections for 24 h of lead acetate dissolved in a 5% glucose solution. Three doses of lead were used: low, 0.1; intermediate, 0.5 and high, 1.0 mg/kg. Comparative analyses of *in situ* muscle isometric contractile characteristics were studied in urethane-anesthetized (2 mg/g, i.p.) control and lead-exposed in both hydrated and dehydrated male mice. In hydrated mice, acute lead treatment did not affect muscle contraction characteristics. However, water deprivation for 24 hr. brought about reduction of twitch tension by a factor of 4, compared to hydrated control animals. In dehydrated animals, control muscle twitch tension reached 3.64 ± 0.14g. Under dehydration condition, lead treatment did not affect muscle contractile speed, but significantly reduced the twitch tension in all three lead-treated groups. The recorded twitch tension values were: 1.68 ± 0.27, 1.74 ± 0.06 and 1.83 ± 0.01g for low, intermediate and high dose, respectively. There was a marked elevation in the tetanic tension in all lead-treated animals, which indicated changes in the contractile apparatus function. These results revealed that acute lead treatment had no effect, but concurrent dehydration significantly ($P < 0.01$) affected contractile strength of the skeletal muscle.

436.13

EXPRESSION OF AN ALTERNATIVELY SPLICED ISOFORM OF NITRIC OXIDE SYNTHASE IN DIFFERENTIATED SKELETAL MUSCLE.

F. Silvagno, H. Xia and D.S. Bredt*. Dept. of Physiology, Univ. of California at San Francisco Sch. of Med., San Francisco, CA 94143

In skeletal muscle the calcium-dependent, neuronal form of nitric oxide synthase (nNOS), is membrane bound and strictly associated with the dystrophin glycoprotein complex. The nNOS mRNA in muscle differs from the typical neuronal form because of a 102-base pair cassette inserted by alternative splicing between exons 16 and 17. A polyclonal antibody specific for a peptide encoded by the alternative splice has been developed. Western blotting indicates that this novel muscle isoform, nNOSμ, occurs only in skeletal and cardiac muscles. nNOSμ expression appears only after the fusion of myotubes in primary culture. The catalytic activity of nNOSμ, expressed in transfected COS cells, is similar to that of the neuronal isoform. A peptide from the alternatively spliced cassette is phosphorylated *in vitro* by a calmodulin-dependent kinase (CamK), and this may modify the catalytic properties of the enzyme. Interestingly, among the substrates for CamK in muscle are dystrophin and the sarcoplasmic reticulum calcium channels, suggesting a simultaneous regulation of NOS and these proteins by phosphorylation.

SOURCES OF SUPPORT: NIH, MDA, Council for Tobacco Research.

436.14

INCREASED CHLORIDE CONDUCTANCE IN CULTURED TRISOMY 16 MOUSE TONGUE MUSCLE SUGGEST A MECHANISM FOR THE HYPOTONIA IN DOWN SYNDROME S. Peng*, S. Rapoport, R. Pearce, and Z. Galdzicki Lab of Neuroscience, NIA, NIH, Bethesda, MD 20892

Trisomy 16 (Ts16) mouse is a genetic animal model of Down syndrome (DS; human trisomy 21, Ts21). DS patients develop hypotonia in striated muscle, including tongue. A whole-cell patch-clamp method was used to study membrane properties of isolated mouse embryonic Ts16 and diploid control tongue muscle cells in primary culture. Recordings were obtained using physiological solutions from colcemid induced myoballs. Membrane resistance (R_m) and capacitance (C_m) were measured from a holding potential of -60 mV. Mean Ts16 R_m was 369 MΩ (SEM, ±31; n=26), whereas control R_m was 505 MΩ (SEM, ±38; n=33). Thus the mean Ts16 R_m was significantly reduced (27%) as compared to control. However, we did not detect a significant difference in C_m between the two groups of cells. In addition, we applied depolarizing voltage steps to elicit total membrane current. The mean normalized maximum outward conductance ($G_{max\,out}/C_m$) was 491 pS/pF (SEM, ±58; n=25) and 840 pS/pF (SEM, ±78; n=33) for Ts16 and control, respectively. This 42% reduction in $G_{max\,out}/C_m$ was significant. However, no significant difference in inward current or conductance was found. Direct measurements of Cl^- current showed that the mean of $G_{max\,Cl}/C_m$ was 103 pS/pF (SEM, ±10 n= 25) and 68 pS/pF (SEM, ±6.6 n=25) for Ts16 and control cells, respectively. Alterations in membrane excitability as a result of this increased Cl^- conductance could contribute to the hypotonia in DS.

436.15

SPHINGOSYLPHOSPHORYLCHOLINE-INDUCED CALCIUM MOBILIZATION AND CELLULAR PROLIFERATION IN PORCINE VASCULAR SMOOTH MUSCLE CELLS. M.-Y. Ho*, T.-Y. Chin and S.-H. Chueh Biochemistry Dept. National Defense Medical Center, Taipei, Taiwan.

Sphingolipid metabolites, including sphingosine, sphingosine-1-phosphate (S-1-P) and sphingosylphosphorylcholine (SPC) have recently been implicated as lipid mediators in signal transduction pathways. We have investigated the SPC-induced cell proliferation and calcium mobilization in porcine aortic vascular smooth muscle cells (VSMC). Cytosolic $[Ca^{2+}]_i$ was monitored by fluorescence indicator, fura-2. As previously reported, unchallenged VSMC were found to fall into one of two functionally distinct groups: those with stable $[Ca^{2+}]_i$ and those with spontaneous oscillating $[Ca^{2+}]_i$ with various on-and-off times that might reflect the intrinsic properties within each and every VSMC. Application of SPC elicited a fast rise in $[Ca^{2+}]_i$ in a dose-dependent manner with apparent EC_{50} at 0.5 μM and 1 μM in the presence and absence of extracellular Ca^{2+}, respectively. SPC-induced $[Ca^{2+}]_i$ elevation was unaffected by ryanodine or ER CaATPase blocker, thapsigargin (TG); yet was diminished by application of 2 mM caffeine or preincubation with 50 ng/ml PTX. SPC was also found to stimulate quiescent cell growth of VSMC and syngeristically enhanced PDGF-induced cell proliferation by 1.8 folds. The data indicates that SPC is a potent mitogen and mobilizes Ca^{2+} from caffeine-sensitive Ca^{2+} store via a PTX-sensitive G protein pathway in porcine VSMC.

436.16

IMMUNOCYTOCHEMICAL AND ELECTROPHYSIOLOGICAL CHARACTERIZATION OF CONDITIONALLY IMMORTAL HUMAN SKELETAL MUSCLE CELLS, I. Deschênes, J. Puymirat and M. Chahine*. Laval Hosp. Research Center and CHUL, Laval Univ. Fac. Med., Sainte-Foy, Quebec, Canada.

Primary human muscle cells culture is a good experimental tool but has a lot of limitations. The use of human muscle immortalized cell lines is a good alternative.

In this study, we immortalized human skeletal muscle cells using the human vimentin promotor controlling the expression of a SV40 thermosensitive T antigen. Human myoblasts were obtained from a postmortem biopsy and purified by limiting dilution. Isolated cells were transfected with a plasmid vector encoding the SV40 T antigen. We characterized those immortalized cells by immunocytochemistry and electrophysiology. Normal immortalized cell lines proliferate in dividing medium at 34°C and differentiate into myotubes in differentiating medium by increasing the temperature to 39°C. Myoblasts expressed vimentin and desmin, and myotubes expressed several markers of differentiation (dystrophin, carbonic anhydrase II and fast myosin heavy chains). The immortalized cells were also characterized electrophysiologicaly using the patch-clamp technique. Sodium currents were recorded from both myoblasts and myotubes (myoballs). On myoballs, 100nM (tetrodotoxin) TTX induced a reduction of 90.6% of the amplitude of the current. On myoblasts, at 1μM of TTX, we observed a reduction of only 20.7% of the amplitude of the current. Those results show that these sodium currents are comparable to what was reported in primary skeletal muscle cells where two types of sodium channels coexist: insensitive to TTX in early development and sensitive in adult form. Those data suggest again that immortalized human skeletal muscle cells do differentiate. We also recorded from those cells a sustained potassium current sensitive to tetraethylamonium (TEA). The immortalization of skeletal muscle cells from human deseased muscles was also succesfull. Our results suggest that immortalized cells may represent a strong model to study the molecular mechanisms of muscle dystrophy.

Supported by the Association Française contre les Myopathies.

436.17

DEGENERATION AND RE-INNERVATION OF SUBSTANCE P-LIKE IMMUNOREACTIVE NERVES TO THE CARDIAC MUSCLE OF THE MONKEY (MACACA FASCICULARIS) AFTER BILATERAL VAGOTOMY. S.S.W. TAY and W.C. WONG*. Department of Anatomy, National University of Singapore, Singapore 119260.

Bilateral vagotomy has a drastic effect on substance P-like immunoreactive (SP-IR) nerves innervating the cardiac muscle. At 1 week postoperation, fragments of SP-IR nerve fibres were observed in the interstitial spaces between cardiac muscle fibres. A few macrophages were present in the vicinity of the degenerating SP-IR debris. At 1-3 months postoperation, most of the SP-IR nerve fibres had disappeared. However, the muscle fibres appeared to be normal At 6 months postoperation, there was re-innervation of the cardiac muscle fibres by SP-IR nerve fibres. Fine varicose and non-varicose nerve fibres were observed to traverse towards the cardiac muscle fibres. A few scattered macrophages could still be observed. By 12 months postoperation, numerous fine varicose and non-varicose nerve fibres were seen in the interstitial spaces between individual or groups of cardiac muscle fibres. These fine nerve fibres formed reticulated networks, similar to those seen in the sham-operated control animals. It is concluded that there is re-innervation of the cardiac muscle fibres by SP-IR nerve fibres in long term bilaterally vagotomized monkeys. The origin of these SP-IR nerve fibres remains unknown.

436.18

SPECTRIN-BASED MEMBRANE SKELETON OF SKELETAL MUSCLE: EFFECT OF DENERVATION. M. Williams, N. Porter, D. Zhou and R.J. Bloch*, Departments of Physiology and Neurology, University of Maryland School of Medicine, Baltimore, MD 21201

We have used isoform-specific antibodies and confocal microscopy to learn how spectrins and ankyrins are organized at the sarcolemma and intracellular membranes of skeletal muscle. The spectrins are present at the sarcolemma of fast twitch fibers in a rectilinear lattice arranged over the Z and M lines of superficial myofibrils, and in longitudinally oriented lines. No spectrins are detected in the spaces between these lines. Slow twitch fibers, by contrast, have a less well-defined lattice in which it is harder to identify spectrin-free regions. Denervation of fast muscle alters the spectrin lattice to a pattern resembling that of slow fibers. Denervation of slow muscle has no obvious effect on its spectrin organization. The levels of expression of the spectrin proteins do not change significantly following denervation. Notably, ß-fodrin, which is absent from adult muscle fibers but present in embryonic muscle, is not up-regulated by denervation.

Denervation also affects expression of ankyrin. An alternatively spliced product of the ankyrin 1 gene is normally expressed at higher levels in fast twitch than in slow twitch fibers. After denervation, this splice form of ankyrin decreases in fast twitch fibers and becomes almost undetectable in slow fibers.

Supported by the NIH and the Muscular Dystrophy Association.

436.19

INNERVATION-INDEPENDENT TRANSCRIPTIONAL REGULATION OF THE ACETYLCHOLINESTERASE GENE IN ADULT SKELETAL MUSCLE FIBERS. C. Boudreau-Larivière*, R.Y.Y. Chan, F.A. Mankal and B.J. Jasmin, Department of Physiology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5.

mRNAs encoding acetylcholinesterase (AChE) are highly concentrated within the postsynaptic sarcoplasm of adult skeletal muscle fibers where their expression is markedly influenced by nerve-evoked electrical activity. To determine whether transcriptional regulatory mechanisms account for the localization and activity-dependent regulation of AChE transcripts, we examined the pattern of expression of the LacZ reporter gene driven by the functional rat AChE promoter (FRAP) along muscle fibers. Direct injection of FRAP-LacZ gene constructs into diaphragm and tibialis anterior (TA) muscles resulted in intense and widespread transgene expression in both muscles 7 to 42 days later. In diaphragm muscle, LacZ expression was observed near and away from the phrenic nerve region indicating that FRAP is capable of directing expression in both synaptic and extrasynaptic compartments. Analysis of cryostat sections further showed that expression of the transgene was confined to muscle fibers and confirmed similar transcriptional activity in synaptic and extrasynaptic regions. Deletion of a 600 bp fragment outside of the basal promoter region of FRAP abolished its transcriptional activity. Subsequent experiments revealed that the 600 bp fragment does not possess promoter activity. Similar results were obtained in vitro by transfecting these various constructs into C2 cells and monitoring histochemically and biochemically LacZ expression. In separate experiments, we examined by nuclear run-on assays transcriptional activity of the AChE gene in denervated muscles and showed that it remained essentially unchanged in comparison to controls despite a marked reduction in the levels of both 2.4 and 3.2 kb AChE transcripts. Taken together, these results suggest that transcription of the AChE gene in adult muscle fibers is largely innervation-independent. Furthermore, these findings are coherent with the notion that the AChE basal promoter requires an enhancer element for transcriptional activity in skeletal muscle fibers in vivo.

Supported by a grant from the Medical Research Council of Canada (MRC) to BJJ. BJJ is a Scholar of the MRC.

436.20

EFFECT OF PERINATAL DENERVATION ON THE STRUCTURE OF CHICKEN LEG MUSCLE SPINDLES. Alfred Maier*. Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294.

Leg muscles of chickens were denervated by cutting the sciatic nerve 1-6 days after hatching. Muscles were examined for regenerating spindles during periods of recovery ranging from 1-8 weeks. There was variability in how muscles responded to denervation, presenting mild to severe deficits. Inflammatory infiltrate and conspicuous degeneratve changes were recognized as early as 6 days after nerve section. The greatest number of degenerating spindles was observed 1-2 weeks after cutting the nerve. The fewest spindles were encountered 2-3 weeks after denervation when immunostaining for neurofilament was negative or weakest. By 5-8 weeks of regeneration the equatorial region was still more affected than the polar region. The volume of the periaxial space was greatly reduced, intrafusal fiber outlines were diffuse, and the perifibril sheath presented fetal characteristics. At polar regions there was some atrophy of slow and fast fibers, but myosin heavy chain profiles were not greatly altered, and the proportions of slow to fast intrafusal fibers were not significantly different from those in the intact contralateral muscles. In developing mammalian spindles sensory innervation is necessary for slow-tonic MHC to be expressed (Kucera and Walro, Histochemistry 90:151-160, 1988). Intrafusal fibers containing slow-tonic myosin heavy chain were present in the chicken spindles that had regenerated, suggesting that afferents reinnervated the spindles and induced slow-tonic myosin heavy chains; however, the general anatomy at the equator continued to show abnormal features, despite its presumed innervation by afferents. This supports the alternate view that afferent innnervation of chicken spindles is not the only formative factor. This argument is especially valid when considering that chicken extrafusal fibers have no sensory innervation, but also express slow-tonic myosin heavy chains.

436.21

A THYROID HORMONE-DEPENDENT MECHANISM DIRECTS FIBER TYPE-SPECIFIC EXPRESSION OF FAST SKELETAL MUSCLE TROPONIN I IN TRANSGENIC MICE H. L. Bradshaw* and K. E. M. Hastings, Molecular Genetics Laboratory, Montreal Neurological Institute, McGill University, Montreal, P.Q. Canada H3A 2B4

We are using transgenic mice to study the developmental regulation of fiber type-specific contractile protein isoforms in skeletal muscle. We have studied the gene expression pattern in transgenic mice that carry an *E.coli* beta-galactosidase reporter construct (LacZ1) driven by promoter/enhancer elements of the quail troponin I gene (TnI) of fast muscle. TnILacZ1 is expressed only in skeletal muscle, and is further restricted to fast, not slow muscle fibers. During the first two weeks of postnatal life, TnILacZ1 progresses from low level, equivalent expression in all fast fibers to a marked differential expression pattern that is correlated with the appearance of the three distinct adult fast fiber subtypes IIB, IIX, and IIA. The result is a striking range of TnILacZ1 expression, ie. IIB > IIX > IIA. This differential expression is unexpected given that the endogenous TnIfast gene shows equal expression in all three fast subtypes in the adult mouse. The results suggest that the adult levels of muscle gene expression are achieved by a two-step mechanism, ie. an initial gene activation in all fast fibers at or before birth followed by an augmentation of transcription to adult levels. In perinatal animals rendered hypothyroid by propythiouracil treatment, TnILacZ1 expression remains at low, equal levels for up to 21 days. This result indicates that the augmentation of TnILacZ1 gene expression in maturing IIB and IIX fibers involves a thyroid hormone-dependent mechanism. Thus, the TnILacZ1 transgene has provided novel insight into muscle gene regulatory mechanisms. Our present efforts are focused on further characterization of this thyroid-dependent mechanism. (Medical Research Council of Canada).

437.1

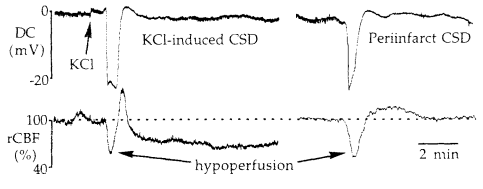
IN VIVO MONITORING OF CORTICAL NADH USING LASER-INDUCED FLUORESCENCE SPECTROSCOPY IN ANAESTHETISED RATS. A.REX*, K.SCHMALZIGAUG, F.FINK[1] and H.FINK Institute of Pharmacology and Toxicology, Humboldt University, 10098 Berlin,[1]FHTW, Warschauer Platz 6, 10245 Berlin, FRG.
The nicotinamide adenine dinucleotide (NADH) is an important coenzyme for energy transfer in the mitochondrial respiratory chain and a parameter of cellular metabolism. NADH can be detected by fluorescence and various fluorometric methods have been developed. In this study a commercial laser fluorescence detector [LLA GmbH, Berlin] was used to induce and measure NADH fluorescence on the cortical surface. The aim of the study was to assess the suitability of the laser induced fluorescence for *in vivo* and on line measurement of NADH in neuroscience.
Preliminary tests using the vasodilator nitro-glycerine showed that an increased blood flow in the brain increased not only NADH fluorescence but also the scattered light of the excitation pulse. Including a compensation factor for the scattered light the NADH fluorescence was corrected to exclude occurring hemodynamic artefacts. The effects of potassium cyanide and cortical spreading depressions (CSD) on cortical NADH fluorescence were determined. Potassium cyanide inhibits the mitochondrial respiratory chain at the cytochrome oxydase. Local administration of 1µmol potassium cyanide near the optic fibre resulted in a transient increase of NADH fluorescence. A single CSD is characterised by EEG disturbances accompanied by an initial excitation and then depression of cortical neurones. The occurrence and the time course of the CSD were determined by parallel registration of the cortical direct current slow potential. Single CSD induced by local application (1cm from the fibre) of 1.5µmol potassium chloride decreased cortical NADH fluorescence followed by a 5-10 minutes increase. These changes in NADH levels correspond with the excitation and inhibition of neuronal metabolism, respectively.
In summary, the measurement of NADH fluorescence using the laser technique allows the determination of changes in oxidative phosphorylation with high regional selectivity and time resolution. This work is supported by the BMBF (Fkz. Fosped), the DFG (INK 21/A1-1) and the LLA GmbH Berlin.

437.2

DIASCHISIS-INDUCED METABOLIC IMPAIRMENT IS ASSOCIATED WITH REDUCTION IN NADPH-DIAPHORASE HISTOCHEMISTRY
M.G. Leggio*, L. Mandolesi, A. Graziano, L.G. Grammaldo, L. Petrosini, and M. Molinari [0]. Dept. of Psychology, University of Rome "La Sapienza" and Inst. of Psychology, CNR, Rome; [0] Inst. of Neurology, Catholic University, Rome, Italy.
Diaschisis is currently used as a "cover" name to indicate different phenomena of transitory postlesional functional impairment whose neurobiological substrates are at best ill-understood. A very common clinical evidence, that has deserved by clinicians the term of diaschisis, is the crossed modification of cerebral blood flow rate (CBFr) observed in cerebral cortex or cerebellum after focal cerebellar or cortical lesions, respectively. The neurobiological correlates of these events have not yet been elucitaded. The present study was aimed to investigate the neocortical neurochemical changes induced by cerebellar lesions. Under barbiturate anesthesia, adult rats underwent hemicerebellectomy by suction and were perfused one month after surgery. The metabolic activity in the cerebral cortex was evaluated by means of cytochromoxidase (CO) quantitative histochemistry, while nitric oxide (NO) expressing neuronal population was evidenced by NADPH-diaphorase (NADPH-d) histochemistry. Densitometric analysis of the CO reaction indicates that hemicerebellectomy produces oxidative neuronal activity depression in contralateral prefrontal and parietal cortices. In the same areas a significant reduction was observed in the number of NADPH-d positive neurons, as well as in the intensity of the NADPH-d reaction. Further analysis on CO and NADPH-d expression in frontal, temporal and occipital cortices are at present under processing. The present data demonstrate that unilateral cerebellar lesions in rat induce depression of the activity in functional related cortical areas. In light of the proposed role of NO as mediator in local CBRFr changes, the reported association between NADPH-d and CO reduction suggests NO neuronal system as a candidate in affecting postlesional CBRFr changes. Funding: CNR & MURST grants to LP & MM.

437.3

A PRONOUNCED HYPOPERFUSION PRECEDES THE HYPEREMIA DURING KCl-INDUCED AND PERI-INFARCT CORTICAL SPREADING DEPRESSIONS IN MICE. M.A. Moskowitz, C. Ayata, M. Fujii, V. Limmroth*. Stroke and Neurovascular Res., Massachusetts General Hospital, Charlestown MA 02129.
We investigated the regional cerebral blood flow (rCBF) response to KCl-induced and spontaneous peri-infarct spreading depressions (CSD) in mice. rCBF was measured by laser Doppler-flowmetry and was coupled to DC potential recordings from the same cortical area by glass micropipettes. KCl-induced CSD was accompanied by an early and pronounced hyperperfusion (51±21% of baseline; ±SD; n=5) simultaneous with the DC shift, after which rCBF increased to a peak (134±23%), followed by a prolonged hypoperfusion (67±4%). This prolonged hypoperfusion gradually and completely recovered within 40 min. Intraluminal filament occlusion of middle cerebral artery caused spontaneous peri-infarct CSDs (~9/hr; n=5) that spread in the nonischemic cortex. rCBF changes induced by peri-infarct CSDs were similar to those induced by KCl including an initial hypoperfusion preceding the hyperemia. Our findings showed that rCBF response to CSD in mice is different from other species, including rats, in that it has a pronounced hypoperfusion that was highly reproducible. Therefore, in addition to the metabolic load imposed by CSD-induced ionic imbalances, the active vasoconstriction during CSD may result in a larger mismatch between the rCBF and the energy demand in ischemic penumbra.
Supported by NS10828.

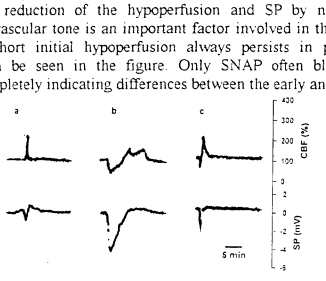


437.4

ENDOTHELIN (ET) 2 OVEREXPRESSING RATS ARE RESISTANT TO THE ELICITATION OF CORTICAL SPREADING DEPRESSION (CSD) WHEREAS THEY PRODUCE NORMAL PERIINFARCT DEPOLARISATIONS (PID). Reuter, U., Arnold, G., Wiegand, F., Lindauer, U., Liefeldt, L.[1], Paul, M.[1], Dirnagl, U.* Dept. of Neurology, Humboldt University, Berlin, and [1]Dept. of Pharmacology, Free University, Berlin, Germany
ETs are potent vasoconstictor polypeptides, capable of blocking gap junction coupling in astrocytes (*Giaume, Eur J Neurosci, 4:877:1992*). Because CSD involves a cerebrovascular response (hyper- followed by hypoperfusion) and their propagation is thought to involve astrocytic gap junctions *(Nedergaard, Science 263:1768; 1994)* we investigated the characteristics of CSD in normotensive transgenic (tg) rats overexpressing human ET-2 in the brain. In addition, we characterized PIDs in ET-2 transgenic rats. PID is a CSD like phenomenon occuring after focal cerebral ischemia linked to ischemic tissue damage. CSD was induced by KCl application on the dura mater over the frontal cortex in pentobarbital anesthetized rats. Two calomel DC-electrodes were positioned on the dura mater fronto-parietally (together with a laser Doppler flowprobe, LDF) and parieto-occipitally. In the wildtype animals (n=5) typical CSDs were elicited by KCl application (neg. DC potential, CBF-hyperemia). In contrast, in ET-2 tg rats (n=5) no CSDs (DC or CBF) could be elicited. Even when cortical pin prick was used as a stimulus, CSD could only be elicited in 2 of 5 tg rats, in which it did not propagate to the distant DC-electrode. In contrast, after induction of focal cerebral ischemia by distal middle cerebral artery occlusion combined with bilateral common carotid artery occlusion in halothane/N_2O anesthetized rats, both wild type (n=4) and tg rats (n=3) showed spontaneous PIDs which propagated from one DC electrode parieto-frontally (over the penumbra) to one located parieto-occipitally (no LDF measurement). In summary, ET-2 tg rats are resistant to the elicitation of CSD, whereas they produce normal PIDs. We speculate that ET mediated blockade of gap junctions prevents CSD propagation and that CSD and PID propagate by different mechanisms.

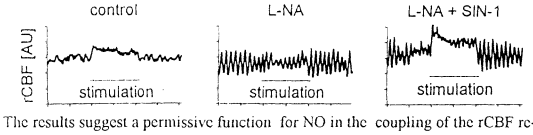
437.5

THE SPREADING DEPRESSION RELATED ISCHEMIC EVENT IS PARTIALLY ABOLISHED BY VASODILATORS. J.P. Dreier*, K. Körner, A. Görner, T. Back, U. Lindauer, A. Villringer, U. Dirnagl. Dept. of Neurology, Humboldt University, Berlin, Germany
We have recently reported the induction of a spreading depression (SD) related ischemic event (spreading ischemia, SI) by elevated cerebrospinal K^+ (20 mM) and NOS inhibition (1 mM L-NA) in a rat cranial window preparation. During SI the cerebral blood flow (CBF) sometimes declined to 10 % of the baseline and occasionally remained below 30 % for more than 20 min. Brain topical superfusion of the NO-donor SNAP did not block the SD but reduced the hypoperfusion. In this study we tested the effect of adenosine, papaverine, 8-bromo-cGMP, or nimodipine. We found that all vasodilators did not block the SD but reduced the hypoperfusion. Amplitude and duration of the SD related surface potential (SP) were reduced as well (fig.: CBF (upper trace) and SP (lower trace), a = 0 min: control, b = 180 min: superfusion of L-NA plus elevated K^+, c = 240 min: L-NA plus elevated K^+ plus nimodipine. Note reduction of the hypoperfusion and SP by nimodipine). We conclude that the vascular tone is an important factor involved in the mechanism of SI. However, a short initial hypoperfusion always persists in presence of the vasodilator as can be seen in the figure. Only SNAP often blocks the initial hypoperfusion completely indicating differences between the early and later phases of SI.



437.6

NITRIC OXIDE (NO) IS A PERMISSIVE FACTOR IN THE REGIONAL CEREBRAL BLOOD FLOW (RCBF) RESPONSE TO WHISKER DEFLECTION IN THE RAT. T.Wolf, J.Schulz*, O.Peters, C.Busch, F.Wiegand, U.Dirnagl U.Lindauer. Dept. of Neurology, Humboldt University, Berlin, Germany.
Nitric oxide (NO) is involved in the coupling of the rCBF response to neuronal activation (for review see Iadecola, *Trends Neurosci* 16:206-214, 1993). Recently several studies have challenged the role of NO as a mediator, and proposed a modulatory function. It has been shown that the vasodilation to hypercapnia (Iadecola et al., *Am. J.Physiol* 267:R228-R235) and extracellular potassium (Dreier et al, *J.Cereb.Blood Flow Metab.*15:914-919), when attenuated by NO synthase (NOS) inhibition, can be restored by NO donors. We investigated the role of NO in the coupling of rCBF to somatosensory stimulation. In 5 α-chloralose anesthetized Wistar rats, equipped with a closed cranial window (dura mater removed) over the whisker barrel cortex, rCBF responses to mystacial vibrissae deflection (2-3x/sec, 60 sec stimulation period) were measured with laser-Doppler flowmetry (LDF). After attenuation of the CBF response due to topical NOS inhibition (L-NA, 10^{-3}M), the NO donor SNAP ($5x10^{-5}$M in 10^{-3}M L-NA) or SIN-1 (10^{-5}M in 10^{-3}M L-NA) was superfused over the cortical surface. CBF responses (% of resting flow) were significantly reduced during NOS inhibition from 17.7±2.5 % to 7.1±2.8 % and increased again during SNAP application to 12.9±1.2 %. An example with SIN-1 is shown in the figure.

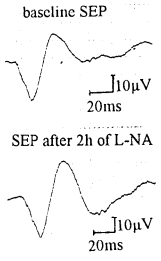


The results suggest a permissive function for NO in the coupling of the rCBF response to somatosensory stimulation in the rat. (Supported by the DFG).

437.7

NITRIC OXIDE SYNTHASE (NOS) INHIBITION DOES NOT AFFECT SOMATOSENSORY POTENTIALS (SEPS) EVOKED BY WHISKER DEFLECTION IN THE RAT. U.Lindauer*, J.Schulze, D.Megow, U.Dirnagl. Dept. of Neurology, Humboldt University, 10098 Berlin, Germany.

Nitric oxide (NO) is involved in cerebrovascular regulation and neurotransmission. Ngai et al. (*Am.J.Physiol.*269:H1803-H1810, 1995) described a suppression of SEPs evoked by electrical sciatic nerve stimulation due to NOS inhibition in the rat. They concluded that NOS-inhibition reduces cerebrovascular responses by suppression of neuronal activity. We investigated the influence of NOS inhibition on neuronal activity during whisker deflection. In 6 rats a closed cranial window (dura mater removed) was implanted over the right whisker barrel cortex with a silver ball-tip electrode positioned over the pial surface. After switching anaesthesia from halothane/N_2O to α-chloralose-urethane, single contralateral whisker hairs (2-3, same row) were continuously deflected (3 Hz). 100 consecutive SEPs were averaged, using a Nihon Kohden Neuropack Monitor. NOS inhibition was induced by brain topical superfusion of 10^{-3} M L-Nitroarginine (L-NA) for 2 hours and SEPs were recorded 0.5, 1, and 2h after start of L-NA application. During NOS inhibition (NOS activity was inhibited by 85±9 %, measured by citrulline conversion HPLC assay) SEPs did not change, neither in amplitude (57±25 μV at baseline, 59±27 μV at 0.5h, 56±28 μV at 1h, 57±28 μV at 2h of NOS inhibition), nor in latency. A typical example is given in the figure. We conclude that inhibition of NOS in the cerebral cortex, which significantly reduces regional cerebral blood flow responses, does not change neuronal activity due to whisker deflection. (Supported by the DFG)



437.8

NEURONAL NITRIC OXIDE IS CRITICALLY INVOLVED IN CEREBRAL CAPILLARY FLOW RESPONSE TO HYPOXIA. A. G. Hudetz*, B. Biswal, J. D. Wood, J. P. Kampine. Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226.

Nitric oxide (NO) has been suggested to play a role in cerebral hyperemic response to hypoxia. Whether NO synthase (NOS) containing neurons may selectively regulate blood flow in cerebral capillaries under normoxic and hypoxic conditions has not been elucidated. We determined the effect of selective neuronal NOS inhibitor 7-nitroindazole (7-NI) on cerebrocortical capillary flow velocity and its response to moderate hypoxia. Capillary circulation of the right parietal cortex was studied using epifluorescence video-microscopy through a closed, perfused cranial window in pentobarbital-anesthetized, artificially ventilated (30% O_2 in N_2), adult male Sprague-Dawley rats. The velocity fluorescently labeled red blood cells (V_{FRBC}) in individual capillaries was measured from 1-minute long video recordings using frame-by-frame analysis. Hypoxia was achieved by lowering the inspired O_2 concentration to 15% for 5 minutes; the arterial pressure was maintained with methoxamine. A separate group of rats was treated with 7-NI, 20 mg/kg ip., and the response to hypoxia was tested 45 minutes later. 7-NI decreased V_{FRBC} from 0.72±0.06 to 0.64±0.05 mm/s (-11%, p<0.001, N=7). Repeated injections of the NO donor MAHMA-NONOate (10^{-4}M) under the cranial window produced transient, 2-3-fold increases in V_{FRBC}. In the control group, V_{FRBC} increased during hypoxia from 0.64±0.06 to 0.85±0.09 mm/s (+34±7%, p<0.001, N=7). In the 7-NI treated group, V_{FRBC} failed to increase in hypoxia (0.64±0.05 to 0.58±0.04 mm/s, -7±3%, p<0.05, N=7). These results suggest that neuronal NOS is involved in the maintenance of red blood cell flow in cerebral capillaries and that NO from neuronal NOS plays a critical role in the cerebral capillary flow response to moderate hypoxia.

Supported by AHA grant GIA-95009340 and NSF grant BES-9411631.

437.9

INHIBITION OF NITRIC OXIDE SYNTHASE ATTENUATES BLOOD FLOW INCREASE EVOKED BY CLIMBING FIBER ACTIVATION IN CEREBELLAR CORTEX. N. Akgören*, I. Rubin and M. Lauritzen. Dept. of Med. Physiol.and Dep.of Biochem., Univ. of Copenhagen; Dept. of Clin. Neurophysiol. Glostrup Hospital, Denmark.

In this study, we examined the laminar profile of CBF changes after stimulation of climbing fibers (CFs) by harmaline injection and after stimulation of parallel fibers (PFs) by local electrical stimulation. Inhibition of nitric oxide (NO) synthase (NOS) by N^G-nitro-L-arginine (L-NNA) attenuates the PF-evoked CBF rise. Harmaline increases cGMP levels of cerebellar cortex that may be related to NOS activation. Therefore we investigated the effect of NOS inhibition on the CBF rise evoked by harmaline stimulation in halothane-anesthetized and artificially ventilated rats. CBF was monitored by laser-Doppler flowmetry using a combination probe holding four combinations of wavelength and fiber separation to record CBF in four different depths in cerebellar cortex. NOS activity was blocked by topical application (1mM) and/or local injection of 300 nl (20 mM) of L-NNA.

Harmaline (40mg/kg i.p) induced a uniform CBF increase by 99-136 % in all layers of cerebellar cortex, whereas stimulation of PFs increased CBF by 48 ± 8 % mainly in the upper layers. The latency to onset of harmaline induced CBF increase was 15-20 min and the evoked CBF increase was maximal for 0.5-1 h and then gradually declined to baseline values in the following 1-3 h. Local application of TTX (20 μm) abolished both PF- and CF-evoked CBF rises. NOS activity increased by 20 % after harmaline injection (p>0.05). L-NNA inhibited NOS activity by 90 % (p<0.05) and attenuated harmaline-evoked CBF increase by 60-70 % in the upper layers gradually declining with depth. PF-evoked CBF increase was attenuated by 40-60 %. These findings suggest that different input systems (CFs and PFs) ending on the same target cell share similar mechanisms in coupling of neuronal activity to CBF.

437.10

NITRIC OXIDE SYNTHASE (NOS) INHIBITION SUGGESTS THERE ARE REGIONAL DIFFERENCES IN THE ROLE OF NITRIC OXIDE (NO) IN CBF AUTOREGULATION IN NEWBORN PIGLETS. J. T. Berkenbaugh, Jr., J. J. Palacino, A.-L. Sirén* and J. T. O'Neill. Uniformed Services Univ. of Health Sciences, Bethesda, MD 20814.

We examined the effect of NOS inhibition with N^{ω}-nitro-L-arginine methyl ester (L-NAME, 50 mg/kg) on total brain and regional blood flows (mL/min/100g) measured by microsphere technique in 12 piglets (<14 d) at baseline and at elevated MAP obtained by aortic occlusion and/or norepinephrine infusion. Cerebral perfusion pressure (CPP) was increased from baseline to 111±2 mmHg in Control (CON) group (n=6), and to 110±3 mmHg in L-NAME (n=6). Cortical gray blood flow increased (65±8 to 119±20) in CON but not in L-NAME (52±4 to 47±2). Blood flow (BF) to cerebrum increased in CON (52±11 to 74±15) but not in L-NAME (43±4 to 37±4). Brainstem BF remained constant in CON, but medulla-pons BF decreased in L-NAME (52±5 to 38±3) as CPP was increased. Oxygen uptake of the cerebrum was not different between groups. These results indicate that NOS inhibition attenuates increased BF to the cerebrum and cortical gray matter, and may contribute to a decrease in brainstem BF, when CPP is increased in newborn piglets. NO production may contribute to forced vasodilation and breakthrough of autoregulation in the cerebrum and cortical gray matter, and NO may also contribute to the maintenance of brainstem BF by counteracting myogenic constriction when CPP is increased.

(Funded by DCI, Walter Reed Army Medical Center, Washington, DC)

437.11

NITRO-L-ARGININE-SENSITIVE REGIONAL CBF AUGMENTATION DURING HYPERCAPNIA IN MICE WITH TYPE III NITRIC OXIDE SYNTHASE GENE DELETION J.Ma*, W.Meng, C.Ayata, P.L.Huang, M.C.Fishman, M.A.Moskowitz Stroke and Neurovascular Regulation Lab & Cardiovascular Research Center, MGH, Harvard Medical School, Charlestown, MA, 02129

We investigated the regional cerebral blood flow (rCBF) changes during hypercapnia (5%CO_2) and the effects of L-NA in SV-129 (Wt, n=12) and type III NOS deficient mice (n=14) under controlled physiological conditions and urethane anesthesia. We measured rCBF by laser-Doppler flowmetry over a closed cranial window. Resting rCBF decreased by ~25% at 60min after topical L-NA (1 mM) superfusion in wild-type mice only. Hypercapnia augmented rCBF approximately 50% in both wild-type and type III NOS mutant mice. L-NA superfusion (1mM, 60min) inhibited this increase by approximately 60% in both strains. Our results suggest that: (1)NO partially mediates hypercapnic rCBF increase in both wild type and type III NOS mutant mice, (2) type I NOS, but not type III NOS, mediates the rCBF response to hypercapnia in both wild type and type III NOS mutant mice, (3) NO generated by type III NOS contributes to resting rCBF during normocapnia in the wild type mouse.

This study is supported by the Massachusetts General Hospital Interdepartmental Stroke Program Project (NS10828) (MAM), NS33335 (PLH), by Bristol-Myers Squibb unrestricted award in Neuroscience (MAM) and by a sponsored research agreement with Bristol-Myers Squibb (MCF).

437.12

OBLIGATORY ROLE OF NITRIC OXIDE IN GLUTAMATE-MEDIATED VASODILATION IN CEREBELLAR CORTEX G. Yang* and C. Iadecola, Dept. of Neurology, Univ. of MN, Minneapolis, MN 55455.

Electrical stimulation of the cerebellar parallel fibers (PF) increases cerebellar blood flow (BFcrb), a response mediated by glutamate (J. Neurophysiol 75:940, 1996). Nitric oxide synthase (NOS) inhibitors attenuate the vasodilation suggesting that NO is involved in the vascular response (AJP, 270: R914, 1996). In this study we investigated whether administration of NO donors could counteract the attenuation by NOS inhibitors of the vasodilation produced by PF stimulation. In anesthetized rats, the cerebellar cortex was exposed and the site was superfused with Ringer. PF were stimulated with microelectrodes (100 μA, 30 Hz) and BFcrb recorded by a laser-Doppler probe. During Ringer superfusion, PF stimulation increased BFcrb by 55±5% and hypercapnia by 75±5% (n=5). Superfusion with L-NA (1 mM) attenuated the response to PF stimulation (-47±5%) and hypercapnia (-46±7%). After L-NA, superfusion with the NO donors SIN-1 (100 μM; n=5) or SNAP (5 μM; n=5) re-established resting BFcrb (p>0.05 from before L-NA) and reversed the L-NA-induced attenuation of the response to hypercapnia (p>0.05 from before L-NA). However, NO donors did not reverse the attenuation of the response to PF stimulation (before SIN-1:-47±5%; after SIN-1:-57±7%; p>0.05). The NO-independent vasodilator papaverine (100 μM; n=5) did not re-establish the response to either hypercapnia or PF stimulation. Topical L-NA inhibited NOS activity, measured by the citrulline assay, by 98±1% (p<0.05). Thus, in PF stimulation, unlike in hypercapnia, NO donors cannot counteract the effects of NOS inhibition on the vasodilation. The data suggest that while in hypercapnia basal levels of NO are needed to facilitate vasodilation initiated by other mechanisms, in the vasodilation produced by PF stimulation glutamate-induced NOS activation is required to produce NO which in turn mediates the vasodilation. Thus, NO plays an obligatory role in the glutamate-induced vasodilation produced by PF stimulation.

437.13

HOMOCYSTEINE IMPAIRS NITRIC OXIDE-RELATED CEREBROVASODILATION BY SUPEROXIDE-DEPENDENT MECHANISMS. F. Zhang*, A. Slungaard[1], G. Vercellotti[1] and C. Iadecola, Dept. of Neurology and Medicine[1], Univ. of MN, Minneapolis, MN 55455.

Elevated plasma levels of homocysteine (HC) may be a risk factor for ischemic cerebrovascular diseases. However, little is known about the cerebrovascular effects of HC. HC may impair cerebrovascular function by producing activated oxygen species. We studied whether HC alters the reactivity of the cerebral circulation and, if so, whether this effect depends on O_2^- generation. In halothane-anesthetized rats the parietal cortex was exposed and superfused with Ringer. Cerebrocortical blood flow (CBF) was monitored by a laser-Doppler probe. With Ringer superfusion, CBF increased with hypercapnia (+134±7%), with topical application of acetylcholine (ACh; 10 μM; +35±3%), of the NO donor SNAP (500 μM; +66±6%) or papaverine (PAP; 1 mM; +100±6%)(n=5). Superfusion with HC (1 mM) or Cu^{2+} (40 μM) alone did not perturb resting CBF nor responses to hypercapnia, ACh, SNAP or PAP (p>0.05; n=5). However, superfusion of HC+Cu^{2+} reduced resting CBF (-18±4%) and attenuated (p<0.05) responses to hypercapnia (-43±9%), ACh (-71±4%) or SNAP (-42±4%), but not PAP. The cerebrovascular effects of HC+Cu^{2+} were prevented by co-administration of superoxide dismutase (SOD; 1000U/ml; n=5). SOD alone did not affect resting CBF or CBF reactivity (n=5). The observation that HC+Cu^{2+} attenuates the response to hypercapnia, ACh and SNAP, but not to the NO-independent vasodilator PAP, suggests that HC+Cu^{2+} selectively impairs NO-related cerebrovascular responses. The fact that SOD prevents such impairment, indicates that the effect of HC is O_2^--dependent. The data support the conclusion that O_2^- generated by the reaction of HC with Cu^{2+} inhibits NO-related cerebrovascular responses by scavenging NO, perhaps, through peroxynitrite formation. O_2^--mediated scavenging of NO might be one of the mechanisms by which hyperhomocysteinemia predisposes to cerebrovascular diseases.

437.14

STIMULATION OF ENDOTHELIAL P2u PURINOCEPTORS DILATES THE RAT MIDDLE CEREBRAL ARTERY (MCA) THROUGH A MECHANISM INVOLVING NITRIC OXIDE (NO) AND A PATHWAY SENSITIVE TO TIN PROTOPORPHYRIN-9. **J.P You, T.D. Johnson, W.F. Childres, W. Durante, and R.M. Bryan, Jr.* Dept. of Anesthesiology, Baylor College of Medicine, Houston, TX 77030**

We previously reported that stimulation of P2u receptors by UTP dilated the rat MCA through an unknown mechanism. The purpose of this study was to investigate this mechanism. Rat MCAs were isolated, cannulated at each end with a micropipet, and pressurized to 85 mm Hg. The MCAs were immersed in a bath (37° C) and luminally perfused. Resting outside diameter of the MCAs in one study was 213 ± 6 μm (n=11). The MCAs showed large concentration dependent dilations when UTP was applied luminally. The maximum dilation was 30% and the EC_{50} (concentration producing half the maximal response) was 4.2 X 10^{-7} ± 1.1 X 10^{-7} M (n=13). The dilations elicited by UTP were completely blocked by removal of the endothelium (n=9). L-NAME (10^{-5} M), an inhibitor of NO formation, did not alter the maximum response to UTP (n=9) but did shift the EC_{50} to the right tenfold. The L-NAME insensitive component of UTP dilation was not altered by 10^{-5} M indomethacin (n=6) or 5 X 10^{-5} M aspirin (n=5). This indicates that cyclo-oxygenase metabolites such as prostacyclin were not involved in the dilation. The L-NAME insensitive component of the dilation was blocked by the heme oxygenase inhibitor, tin protoporphyrin-9 (SnPP-9) at concentrations of 10 μM (n=5). Heme oxygenase is the enzyme responsible for the synthesis of CO. We conclude that the dilation mediated by the stimulation of P2u receptors on endothelium of the rat MCA involves NO and a pathway sensitive to SnPP-9. (PHS grant PO1 NS 27616)

437.15

L-NAME INDUCED REDUCTION OF CEREBRAL BLOOD VOLUME CAN BE RESTORED BY OPIATE RECEPTOR BLOCKADE WITH NALTREXONE. K. Komjati*, J. Bencze, M. H-Velkei and P. Sandor, Experimental Research Dept.- 2nd Inst. of Physiology, Semmelweis Univ. of Medicine, Budapest, H-1082 Hungary

Involvement of L-arginine-nitric oxide system and the endogenous opioid peptide system in the regulation of regional (hemispheric) cerebral blood volume (CBV) and local hypothalamic blood flow (HBF) was investigated in Urethane anesthetized, ventilated, normoxic, normocapnic, male Spraque-Dowley rats (n=8). CBV and HBF was measured simultaneously, CBV by Tomita's photoelectric method in our modification (Sándor et al Life Sci 1986) and HBF by Aukland's H_2-gas clearance method. Selective blockade of the NO-synthase enzyme by 30 mg/kg i.v. administered L-Name (Nω-nitro-L-arginine methyl ester) resulted in a significant reduction of both hemispheric CBV (from 5.25±0.72 vol% to 4.13±0.51 vol%, p≤0.01) and local HBF (from 0.89±0.09 ml/g/min to 0.60±0.07 ml/g/min, p≤0.05), while systemic arterial pressure increased and heart rate decreased significantly. General opiate receptor blockade by 2 mg/kg i.v. administered Naltrexone caused no change in the HBF, MAP and HR values of the NOS-blocked animals. CBV, however, returned to it's steady state control value (5.4±0.72 vol%) at the same time. These findings suggest 1.) a major role of the L-arginine-nitric oxide system in the regulation of both hemispheric CBV and local HBF of the rat, and 2.) a possible involvement of opioid peptides and/or opiate receptors in the NOS-blockade induced reduction of the cerebral blood volume.
(Supported by OTKA-3/8-T-017790 Grant)

437.16

HALOTHANE INHIBITS THE PRESSOR BUT NOT THE CEREBROVASCULAR EFFECT OF THE NITRIC OXIDE SYNTHASE (NOS) INHIBITOR 7-NITROINDAZOLE (7NI) IN RATS. Y.S. Zagvazdin*, G. Sancesario, M.E.C. Fitzgerald and A. Reiner, Dept .of Anatomy & Neurobiology Univ. of Tennessee and Dept. of Biology, Christian Brothers Univ., Memphis, TN, and Dept. of Neurology Univ. di Roma "Tor Vergata", Rome, Italy

Inhibition of endothelial NOS reduces cerebral blood flow (CBF) and leads to a profound systemic hypertension in rats. Decrease in CBF after inhibition of NOS by 7NI, a relatively specific inhibitor of the neuronal isoform of NOS, is associated with a moderate pressor effect. The pressor effect of L-arginine analogs or 7NI is blunted by using halothane for maintenance or even for induction of anesthesia, presumably by interference with endothelial NO production (Dawson et al., 1993; Zagvazdin et al., 1996, European. J. Pharmacol.). The goal of this study was to examine whether the cerebrovasoconstrictor effect of 7NI is abolished by halothane. Sprague-Dawley rats were anesthetized using urethane either with or without induction by halothane or using halothane (2%) only. CBF, measured by Laser Doppler flowmetry, decreased after 7NI injection (50 mg/kg i.p.) in all groups by 20-28 %. Increasing the dose of 7NI (75 mg/kg i.p.) did not result in a greater reduction in CBF. In contrast, mean arterial blood pressure after 7NI was increased significantly only if halothane was not used. Thus, the systemic pressor and cerebrovascular effect of 7NI is mediated by a different mechanism. The inability of the halothane to abolish the decrease in CBF after 7NI suggests a contribution of neurally derived NO to the maintanance of cerebrovascular tone.
Supported by EY-05298 (A.R.), AG-10538 (M.E.C.F.) & "Altri Interventi" (G.S.)

437.17

DISTURBANCE OF CEREBRAL BLOOD FLOW AUTOREGULATION FOLLOWING SUBARACHNOID HEMORRHAGE IS ATTRIBUTABLE TO ISCHEMIA IN RAT: A PET STUDY. S. Yamamoto[1]*, S. Nishizawa[1], H. Tsukada[2] and K. Uemura[1]. [1]Dept. of Neurosurgery, Hamamatsu University School of Medicine, [2]Central Research Lab., Hamamamatsu Photonics K.K. , Hamamatsu, 431-31, Japan

The effects of subarachnoid hemorrhage (SAH) on cerebral blood flow (CBF) response to induced hypertension were studied during chronic vasospasm in anesthetized Sprague-Dawley rats. SAH was induced by intracisternal injection of autologous blood (SAH group) or saline (control group). This method produces angiographical vasospasm of major arteries 48h later in the SAH group (Yamamoto et al., *Soc. Neurosci. Abstr.* 20:1761, 1994). During this period, CBF was measured in each side of parietal and occipital sections using positron emission tomography (PET) with or without induced hypertension in the SAH group (n=5) and the control group (n=6). Mean arterial blood pressure (MABP) was increased from 94±8 to 140±1 mmHg by the injection of phenylephrine. Autoregulatory Index (AI) expressed as delta CBF (%) per 10-mmHg increase in MABP was employed to analyze CBF response. Intracranial pressure (ICP) was measured in different animals 48h after SAH was induced.

SAH did not affect ICP 48h later (control 9.2±0.30 vs. SAH 10.0±0.47 mmHg, n=5). However, SAH significantly reduced (p<0.0001) basal CBF (ml/100g/min) by 26.2% (control 60.0±1.9 n=24, SAH 44.3±4.5 n=20). A territorial CBF that decreased by 50% or more over controls was used to define ischemia and was identified in five out of 20 regions (25%) in the SAH group. AI (%/10-mmHg) was 13.5±2.4 in the control group (n=24). In the SAH group AI decreased (p<0.05) to 4.5±2.5 in the non-ischemic areas (n=15), while in the ischemic areas (n=5) AI increased (p<0.05) to 25.2±4.1.

During chronic vasospasm following SAH, the spastic artery by itself is resistant to hypertension. Marked increase in CBF in case of hypertension is attributable to ischemia following SAH.

(Supported by the Research Grant of Hamamatsu University)

438.1

MAPPING PATHOLOGY TO METABOLISM: COREGISTRATION OF STAINED WHOLE BRAIN SECTIONS TO PET. M.S. Mega, S.S. Chen, A. Tiwari, T.J. Karaca, L.L. Thompson*, H.V. Vinters, P.M. Thompson, A.W. Toga. Lab of Neuro Imaging, UCLA School of Medicine, Los Angeles, CA 90024.

Objective. Demonstrate a methodology using a 3D cryomacrotome reference for the coregistration of PET and stained whole brain sections.

Background. Localizing histologic data to functional imaging requires a common spatial reference. Whole brain slices, obtained from cryomacrotome sectioning, can be localized to a high resolution 3D digital reference reconstructed from the sectioned block-face. This model serves as a warping reference for functional imaging and histologic data. Using this methodology we evaluated the contribution of neurofibrillary tangles (NFTs) to the metabolic abnormalities seen on FDG-PET in a patient with Alzheimer's disease.

Methods. PET was obtained 8 hours before death and the brain was frozen 12 hours after death. Cryomacrotome images were obtained every 500µ in the coronal plane. Every 1 cm a 250µ whole brain slice was collected and stained with the Gallyas method to reveal NFT density. Digital images of these stained sections were elastically warped onto the coregistered PET-cryo dataset to evaluate associations between NFT density and regional hypometabolism.

Results. Hypometabolism was generalized but greatest in the posterior temporoparietal and frontal cortex, NFT density was maximal in the limbic and paralimbic cortex. Microscopic verification of NFT staining was not possible due to freeze artifact.

Conclusion. Cryomacrotome reconstruction serves as a reference space for warping pathological and functional data allowing an evaluation of the cellular basis for metabolic defects in humans. (This project was supported by a VA Neuroscience Fellowship, an NIA Alzheimer's Disease Center grant P30, AG10123, and a Human Brain Project grant NIMH/NIDA:P20MH/DA52176.)

438.2

3D PROBABILISTIC ATLAS AND AVERAGE SURFACE REPRESENTATION OF THE ALZHEIMER'S BRAIN, WITH LOCAL VARIABILITY AND PROBABILITY MAPS OF VENTRICLES AND DEEP CORTEX. Thompson PM, Mega MS, Moussai J, Zohoori S, Xu LQ, Goldkorn A, Khan AA, Coryell J, Small G*, Cummings J and Toga AW, Laboratory of Neuro Imaging, Dept. Neurology and Division of Brain Mapping, UCLA School of Medicine, Los Angeles CA 90095.

A high-resolution probabilistic atlas of the Alzheimer's brain was constructed from a reference archive of MRI scans of patients with clinically determined Alzheimer's disease. MRI volumes, acquired from 10 subjects (5 males, mean age: 72 yrs.) as datasets of 170 contiguous T_1-weighted 1-mm thick slices, were digitally transformed into Talairach stereotaxic space. Outlines of 29 different structures were directly identified at high magnification on sagittally-resampled sections. Connected systems of parametric meshes were used to model major lobar, ventricular and cytoarchitectural boundaries in 3 dimensions. These included complex internal trajectories of the parieto-occipital, calcarine, cingulate, marginal and callosal sulci and Sylvian fissure in both hemispheres. The ventricular system was partitioned into a system of 14 connected surface elements whose junctions reflected cytoarchitectonic boundaries of the adjacent tissue. A family of surface maps was constructed, encoding statistical properties of local anatomical variation across the surfaces of individual structures. A complete system of probability density functions was computed, yielding confidence limits on surface variation within the Talairach stereotaxic grid. Local variability maps revealed striking directional trends in the patterns of anatomic variation. Confidence limits on surface variation increased dramatically towards the posterior Sylvian and anterior cingulate cortex. These probabilistic atlasing techniques provide a basis for the generation of anatomical templates and expert diagnostic systems which retain information on inter-subject and inter-group variations in brain architecture.

Grant Support: (PT:) Howard Hughes Medical Institute, United States Information Agency, US-UK Fulbright Commission; (AWT:) Human Brain Project (NIMH/NIDA:P20MH/DA52176), NSF (BIR9322434), NLM (LM/MH05639) and NCRR (RR05956).

438.3

CORTICAL FLAT MAPS OF THE VISIBLE MAN LINKED TO THE TALAIRACH STEREOTAXIC ATLAS

H.A. Drury* and D.C. Van Essen, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO, USA 63110.

We have generated computerized reconstructions of the surface of cortical layer 4 for both cerebral hemispheres of the Visible Man (Visible Human Project, National Library of Medicine). The surface areas of the left and right hemispheres were 757 cm^2 and 795 cm^2, respectively. We then generated 2-D cortical flat maps for each hemisphere using our automated flattening algorithm. The geography of cortical convolutions can be readily visualized on the flat maps using displays of mean curvature.

Comparison of the cortical geography of the left hemisphere to that of the right hemisphere shows strong similarities in their overall size and shape and close correspondence between most major sulci. However, there are conspicuous differences in the pattern of many minor sulci. Although the cortical geography of the flat maps of the human cortex is clearly more complex than those of the macaque, the overall configuration of the maps share important similarities across species.

We transformed the volume of the Visible Man and associated cortical surface data to the Talairach coordinate system. A surface-based coordinate system was also established on the flat map of each hemisphere (with each origin chosen as the ventral tip of the central sulci). Because the 3-D representation has been transformed to Talairach coordinate space, each point on the flat map can be assigned a unique 2-D surface-based coordinate and a corresponding 3-D Talairach coordinate. Representation of activity foci from PET and fMRI (reported using Talairach coordinates) can readily be modeled using an appropriate volume distribution, allowing visualization of which gyri and sulci of the Visible Man are within a specified distance of any given Talairach coordinate. Linkage between these first flat maps of the entire human cerebral cortex and the Talairach atlas will help in incorporating data from the many approaches available for studying cerebral organization and function. Supported by NIH Human Brain Project MH52158 and NIH EYO2091.

438.4

CHARACTERISATION AND REMOVAL OF RESPIRATORY, CARDIAC AND VASOMOTOR OSCILLATIONS IN DYNAMIC BRAIN IMAGES. [1]P. P. Mitra, [1]B. Pesaran, [1]S. Ogawa*, [1,2]D. Kleinfeld, [3]K.Ugurbil. [1]Bell Laboratories, Lucent Technologies, Murray Hill, NJ 07974, [2] Physics Dept., UCSD, La Jolla CA 92093, [3]CMRR, University of Minnesota, Minneapolis MN 55455.

Different techniques to infer the in vivo dynamics of electrical activity in brain, including fMRI, wide-field optical imaging of intrinsic signals and with voltage-sensitive dyes, are corrupted by the presence of physiological fluctuations. Using data from fMRI studies of humans and a rat model, as well as optical imaging studies of the rat barrel cortex, the characteristics of these fluctuations were investigated to develop optimal post-processing strategies.

The main sources of fluctuations are respiration, cardiac oscillations, and vasomotor activity. Each source has defined frequencies and is spatially coherent Conventional schemes, such as gating of data acquisition based on the heart rate, are found to be inadequate due to variability in the oscillations. We are able to extract these oscillations using a combination of singular value decomposition (SVD) (principal components analysis) and multiple window spectral techniques. The removal of signals that are solely correlated with a physiological mode can be done on the basis of the imaging data alone; however, auxiliary measurements of the relevant physiology, e.g. the EKG, assist in the process.

In both fMRI and optical imaging, respiration causes global changes characterised by a spatial structure resembling a derivative of the image, corresponding to motion. Cardiac oscillations in fMRI have a more complex spatial structure, presumably originating in hemodynamic effects. In optical images where resolution is higher, the dilation of the vessels is also observed. The vasmotor oscillations also show a complex spatial structure. In addition, in the rat studies we observed singular events associated with the blood vessels that could not be ascribed to any of the above sources. (Supported by Bell Labs and NIH grant RR08079).

438.5

FMRI BLOOD OXYGENATION LEVEL DEPENDENT CONTRAST (BOLD) CHANGES REFLECT CHANGES IN BLOOD FLOW. F.M. Lalonde, A.W. Song, J.V. Haxby and A. Martin*. National Institute of Mental Health, NIH, Bethesda, MD, 20892.

An inversion recovery (IR) MRI pulse sequence sensitive to regional changes in blood flow was used to investigate the relationship between blood flow and blood oxygenation. Four right-handed female subjects (24 to 29 years of age) viewed line drawings of common objects and noise patterns. Each stimulus was presented for 180 msec; one per 2 seconds. Subjects were instructed to silently name the objects and stare at the patterns. Alternating lists of 10 objects and 10 noise patterns were presented. This on-off sequence was repeated three times during each run. Two subjects viewed each object only once. The other two subjects viewed the same objects in a different order during the second on-off sequence of each run. Each session had between 6 to 8 runs. One, 10 mm sagittal slice, centered 30 mm from the left lateral surface, was obtained from a GE Signa 1.5T scanner using a gradient echo EPI sequence (TR = 3000 msec, TE = 40 msec, FOV = 24 cm, 64X64 matrix). Protons within the slice were saturated using a 180 degree inversion pulse (TI = 1000 msec) prior to data collection. Changes in signal intensity were therefore primarily due to unsaturated protons flowing into the slice. The results from this study agree with previous findings obtained using the BOLD contrast effect. Namely that the silent naming of objects was associated with increased signal intensity in the inferior temporal gyrus and decreased signal intensity in the perisylvian region, relative to viewing noise patterns. Moreover, the more subtle BOLD signal intensity changes observed when objects are shown a second time (Martin et al., Neurosci. Abstr., 1995) were also detected with the IR sequence. These results support the notion that BOLD contrast signal intensity changes are tightly coupled to changes in blood flow. Funded by the NIMH, DIRP.

438.6

NEGATIVE FUNCTIONAL MRI SIGNALS AND MODELS OF BLOOD FLOW AND OXYGEN METABOLISM J.G. Ojemann†, R. C. McKinstry§, T. E. Conturo§, A. Z. Snyder†, E. Akbudak§, R.L.Bucknert, R.L. Grubb Jr.†*, and M.E. Raichle†§ Departments of †-Neurology and Neurological Surgery and §-Radiology, Washington University School of Medicine, St. Louis, MO.

The signal in functional magnetic resonance imaging (fMRI) arises from increased blood oxygenation due to uncoupling of regional cerebral blood flow (rCBF) and oxygen metabolism (rCMRO2) during functional activation. In the resting state, however, rCBF and rCMRO2 are tightly correlated. Under barbiturate anesthesia, parallel decreases in CBF and CMRO2 have been observed. If this relationship holds for regional changes during decreased functional activity, then uncoupling of rCMRO2 and rCBF would not occur and no signal would be seen by fMRI. To test this hypothesis, we examined two paradigms known to induce rCBF decreases and increases from a resting state. Subjects underwent whole brain echo-planar imaging at 1.5T (Siemens Vision, contiguous 8mm slices, TR=2s, TE=50ms) during runs of multiple, alternating blocks of control and task: one group (n=4) with vibratory stimulation of the left hand (control = eyes closed) and the other (n=6), word stem completion task (control = visual fixation). Task minus control images were created after realignment and normalization across time. In the vibratory study, reliable signal increases were seen in right primary sensory cortex and reliable decreases in right primary motor cortex. In the stem-completion study, strong increases were seen in visual cortex and left prefrontal cortex along with robust signal decreases in medial parietal cortex. Our results imply that the resting correlation between rCBF and rCMRO2 does not apply bidirectionally to focal activity modulation. But negative fMRI signals do not distinguish between neuronal activity decreases due to "task" performance vs. increases in the "control" state. In the former case, negative fMRI signals suggest that rCMRO2 does not couple with rCBF decreases. Further understanding the relationship of rCBF and rCMRO2 will require defining a resting brain state relative to which uncoupling may occur.

(Supported by NIH grant NS 06833 and the Charles A. Dana foundation)

438.7

STIMULUS INDUCED FOCAL DECREASE IN DEOXY-HAEMOGLOBIN CONCENTRATION RESOLVES DURING SUSTAINED VISUAL STIMULATION.

H Heekeren, H Obrig, R Wenzel, J Ruben, C Hirth, U Dirnagl, A Villringer*
Dept Of Neurology, Charité, Humboldt-Univ., Berlin, Germany

Near-infrared spectroscopy (NIRS) monitors the time course of the haemodynamic response to functional neuronal activation. In previous studies we demonstrated a decrease in deoxygenated haemoglobin [deoxy-Hb] in response to motor and visual stimulation[1,2]. The [deoxy-Hb] response develops with a 5-6 s latency and is almost stable during the stimulation period (up to 30s). In the present study we examined whether the [deoxy-Hb] response is also stable over a longer stimulation period. We continuously measured oxygenation changes in 6 subjects during 5 min of visual stimulation followed by 3 min of rest (8 cycles per subject) using a NIRO-500-monitor (Hamamatsu, Japan), interoptode distance 3.5 cm, sampling time 1 s, optodes placed over the right occipital cortex. The stimulus consisted of a coloured dodecahedron. The results confirmed the [deoxy-Hb] decrease during the first 30 s. However a gradual increase in [deoxy-Hb] occurred, almost reaching the baseline level after 4 min of stimulation. There is a pronounced post-stimulus overshoot lasting about 1 min. According to the current theory of neurovascular coupling the decrease in [deoxy-Hb] results from an increase in rCBF not mirrored by an equally large increase in oxygen consumption[3]. The results may therefore indicate changes in the degree of this „focal uncoupling" between rCBF and oxygen consumption over the course of longer stimulation periods, although habituation of neuronal activation can not be excluded. These findings are in line with recent functional MRI studies using a similar experimental protocol showing a vanishing BOLD-contrast[4,5].

(1) Obrig, et al., J Appl Physiol (1996), in press; (2) Wenzel, et al., J Biomed Opt (1996), in press; (3) Fox and Raichle, Proc Natl Acad Sci 83, 1140-1144 (1986); (4) Hathout, et al., J Magn Reson Imaging 4, 537-543(1994); (5) Frahm J et al., MRM 35, 143-148 (1996)

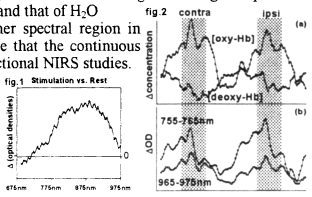
Fig.: Concentration changes of deoxy-Hb [arbitrary units] during 5 min of visual stimulation (n=6).

438.8

CONTINUOUS SPECTRUM NIRS APPROACH CAN QUANTIFY OXYGENATION CHANGES IN RESPONSE TO FUNCTIONAL STIMULATION IN HUMANS.

H.Obrig*, J. Ruben, C.Hirth, R. Wenzel, U. Dirnagl and A. Villringer
Dept. of Neurology, Charité, Humboldt-Univ., Berlin, F.R.Germany

With a commercial NIRS monitor (NIRO-500) we have previously shown changes in cerebral oxygenation in response to motor and visual stimuli[1,2]. Whereas commercial NIRS monitors use discrete single wavelengths to calculate concentration changes in oxy and deoxy haemoglobin a new approach illuminates the tissue with a continuous NIR spectrum, thus enhancing spectral information[3]. To test the feasibility of this approach for functional activation studies we examined 8 ss. performing a motor task for 20s contra- and ipsilaterally to probe positioning over the left motor region (40s of rest). Ten cycles of each condition were performed in an interleaved fashion. The NIRS system was composed of a halogen lamp (400-1100nm), a spectrograph spectrally resolving the collected light and a CCD camera with a 567 point (= 0.5nm spectral) resolution. Spectra were collected every second and averaged across all subjects with respect to movement onset for both conditions. Fig.1 showing the difference in optical densities at all wavelengths between the resting and the stimulation period, demonstrates that the optical properties of the tissue change over the whole spectral range from 675 to 975nm. Fig.2a shows the changes in [oxy-Hb] and [deoxy-Hb] when applying the algorithm of Cope et al.[4] confirming the lateralized hyperoxygenation previously described. Fig.2b plots the integral over the OD changes covering the spectral absorption peak of deoxy-Hb at 760nm and that of H_2O at 970nm indicating changes in either spectral region in response to the stimulus.We conclude that the continuous spectrum approach is feasible for functional NIRS studies. Assuming a constant, known concentration of H2O the [deoxy-Hb] can be quantified using water as a reference chromophore according to the theory of Matcher [3].



1.Obrig, Adv Med Biol. 1995, 2. Heekeren, abstr.this volume, 3. Matcher, PhysMed Biol 1994.4.Cope MedBiolEngComput 1988.

438.9

CEREBRAL OXYGENATION CHANGES DURING PERFORMANCE OF DIFFERENT MOTOR TASKS C. Hirth[1], H. Obrig[1], K. Villringer[2], A. Thiel[3], R. Wenzel[1], W. Oertel*[4], W. Mühlnickel[5], H. Flohr[5], U. Dirnagl[1], A. Villringer[1] Dep. of Neurology Humboldt-University (HU) Berlin[1], Dep. of Radiology Free University (FU) Berlin[2], Dep. of Med. Comp. Sci. FU Berlin[3], Dep. of Neurology, Philipps-University Marburg[4], Dep. of Clinical Psychology, HU Berlin[5].

Near infrared spectroscopy (NIRS) can be used to measure oxygenation changes induced by functional brain activation (1) We applied multisite NIRS (NIRO 500. Hamamatsu, Japan) in combination with anatomical MRI to study localisation and spatial distribution of functional oxygenation response during performance of three different motor tasks. 5 subjects performed finger, elbow and foot movement in a fixed order. Concentration changes of oxy-Hb and deoxy-Hb were continuously measured (sample time 1s) (2). Measurements were obtained from 8-10 different positions over the left parietal cortex using C3 as a fix point. Measurement positions were localised on brain surface of 3D rendered MRI. All tasks were followed by a localised increase in [oxy-Hb] and a decrease in [deoxy-Hb]. Whereas only slight task dependent spatial differences in [oxy-Hb] were found. [deoxy-Hb] demonstrated topographical differences in localisation of maximal response. Significant changes in [deoxy-Hb] during finger movement were found in the vicinity of C3, and during elbow movement additionally in positions above C3. Foot movement elicited significant changes in positions near interhemispheric fissure. Localisation of maximal response corresponded anatomically to presumably activated brain regions. The results indicate that NIRS findings during functional activation are localised, however changes in [deoxy-Hb] seem to be more localised and more related to topographical location of activated brain regions than changes in [oxy-Hb]. We conclude that multilocal NIRS may be used for non-invasive functional brain mapping. Improved spatial resolution and simultaneous assessment of pathlength is necessary to enhance the potential of this method. *Obrig et al.* In press 1996; *Cope M, Delpy DT.* 1988.

438.10

EVIDENCE FOR VASCULAR REFRACTORY PERIODS OBSERVED WITH OPTICAL INTRINSIC SIGNAL IMAGING A.F. Cannestra* and A.W. Toga
Lab. of Neuro Imaging, Division of Brain Mapping, Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90095

Optical Imaging of intrinsic signals (OIS) and repetitive proprioceptive stimulation paradigms were employed to investigate potential vascular refractory periods and response capacities observed in rodent somatosensory cortex. Primary stimuli were presented to the animal, followed by a period of no stimulation (interstimulus interval), and a subsequent secondary stimulus. Close temporal proximity of repetitive stimuli decreased the magnitude (intensity and spatial involvement) of the secondary optical response. Stereotaxic evoked potentials were recorded and indicated no change in neuronal activity between stimulations. The interstimulus interval required to obtain an equal magnitude second optical response was defined as the refractory period. Increasing primary stimulus duration increased interstimulus intervals required to obtain equal secondary responses. The relationship between multiple stimulus durations (2s to 15s) and observed refractory periods was determined and may be represented by a quadratic function. Increasing secondary stimulus frequency increased secondary optical response during the refractory period, indicating the relative nature of the refractory periods. During long duration stimulations, a second stimulus initiated a response that only attained steady state level observed during primary stimulation, foregoing the initial overcompensation phase. Therefore, observed decreases in secondary response correlate with loss of luxury perfusion phenomena. Since current functional imaging techniques rely upon neurovascular behavior, this study suggests frequency and duration of repetitive cortical activation may dramatically affect intensity and extent of response. Funding was provided by NIMH (MH/NS52083)

438.11

MODULATION OF OPTICAL INTRINSIC SIGNAL TIME-TO-PEAK RESPONSE DURING TEMPORALLY STAGGERED COMPETITIVE STIMULATION. A.J. Blood* and A.W. Toga. Laboratory of Neuro Imaging and Division of Brain Mapping, Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90095.

Optical intrinsic signal imaging (OIS) was used to examine response characteristics of two temporally staggered somatosensory stimuli. Rodent whisker C1 and forelimb digits 3 through 5 were stimulated simultaneously or in staggered (overlapping, but not simultaneous) order. Time-to-peak and magnitude of OIS responses in the barrel region were determined for each paradigm and compared to lone whisker stimulated controls. Simultaneous stimulation consisted of a 3 second whisker deflection commencing at the same time as a 3 second forelimb vibration. During staggered stimulation, whiskers were deflected for 3 seconds at 10 Hz, and a 3 second, vibratory forelimb stimulus commenced after the first second of whisker stimulation. This paradigm was also reversed so that forelimb stimulation preceded whisker stimulation by one second. Simultaneous stimulation slightly decreased response time-to-peak and caused response magnitude to either increase or decrease relative to controls. Staggered competitive forelimb stimulation caused a delay of the peak OIS signal in barrel cortex. Peak response magnitude changed in the same direction from controls as did responses to simultaneous stimulation, but to a lesser degree. When the staggered competitive forelimb stimulus preceded whisker stimulation, OIS response time-to-peak decreased relative to controls. These results demonstrate the ability to alter temporal and magnitude characteristics of OIS responses by varying temporal order of competitive stimulus presentation. This suggests there are temporally dependent interactions between spatially and functionally distinct cortical systems.

This work was supported by NIH grant MH 52083.

438.12

CORTICAL BARREL PATTERNS AND THEIR CAPILLARIES AFTER POSTNATAL WHISKER LESIONS. J. SUI, C. M. ROVAINEN and T.A.WOOLSEY.* Departments of Neurology and Neurosurgery and of Cell Biology and Physiology, Washington University School of Medicine, St. Louis MO 63110.

Blood flow increases locally in rodent barrel cortex with stimulation of whiskers. The vascular units defined by injection of single penetrating arterioles are anatomically related to the barrels as neural groups. Our hypothesis is that the development of cerebral vessels is directed by the patterns and activities of neurons. The normal cytoarchitectonic pattern of barrels in layer IV of mouse SmI face cortex is altered by early damage to the mystacial vibrissae. Row-C or rows-BCD on one side of the face in groups of Swiss mice were cauterized. Animals lesioned on P1, 2, 3, 4, 5, 6, 7 or 8 were perfused with FITC-gelatin on P30; animals lesioned on P30 were perfused on P60. The brains were sectioned parallel to SmI layer IV at 100μm, and sections reacted for cytochrome oxidase (CO). The sections were scanned twice at a primary magnification of 20 x on a Zeiss LSM-310 confocal microscope; first with transmitted light to show the CO stain and a second time with epi-ilumination in three-dimensions for volume rendering of intravascular fluorescence. Images from the two channels were combined to demonstrate the relationship of the vessels to the CO staining patterns and images of the fluorescent marker were used to measure capillary density in different groups of barrels.

Supported by NIH grants NS 17763, NS 28781, HL 41075 and NS 07057, the McDonnell Center for Studies of Higher Brain Function, and the Spastic Paralysis Foundation of the Illinois Eastern-Iowa District of the Kiwanis International.

438.13

AN EVALUATION OF LINEAR MODEL ANALYSIS TECHNIQUES FOR PROCESSING INTRINSIC IMAGES OF REGIONAL BLOOD FLOW IN RAT CEREBRAL CORTEX AND TESTICLES

John Mayhew, Dewen Hu, Ying Zheng, Steven Askew, Yuqian Ho, Jason Berwick, Peter Coffey, Nicky Brown and Inglis Miller*
AIVRU Dept Psychology Univ Sheffield, Sheffield, S10 2TP, UK.
*Dept Neurobiol & Anat Bowman Grey Sch Med, Wake Forest Univ, Winston, Slalem, NC.

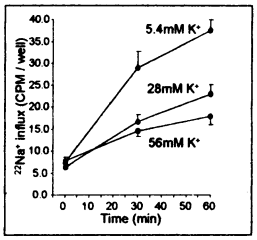
Images of the exposed cortical surface can be processed to reveal informatiot about the modulation of regional cerebral blood flow (rCBF) by neuronal activity. Images of rat sensory motor cortex and testes were processed using different analysis methodologies. The study compared the use of generalised linear models (GLM) and canonical variate analysis, against standard signal processing and de-noising methods (including principal components analysis. The GLM method is used in the analysis of fMRI data (Friston et al, NeuroImage, 1995, 2, 45-53) to identify regions of focal activity. We explore the use of this method to analyse video image data from the cortex of anaesthetised rats in which rCBF was modulated by sensory stimulation and by exsanguination. The results show statistically significant local spatio-temporal variations in rCBF in response to stimulation. Using different wavelengths of illumination, and the appropriate models of the neural haemodynamic coupling it was possible to disassociate changes in blood volume from changes in blood oxygenation. An advantage of the GLM method is that it provides an estimate of statistical reliability for the spatial structures revealed by the analysis (which of course were also revealed by the standard signal processing methods). The method is sensitive to the choice of the parameters used in the haemodynamic models (good news; it 'forces' model development). Importantly, it can be used to remove the effects of the low frequency (~0.1Hz) oscillations (the V-signal) without extensive averaging strategies commonly used. The 0.1 Hz oscillations are now known to be the principal source of widely acknowledged "vascular artefacts" present in intrinsic image data.

438.14

EFFECTS OF EXTRACELLULAR POTASSIUM ION CONCENTRATION ON SODIUM ION INFLUX INTO CULTURED RAT ASTROGLIA.

S. Takahashi[1]*, M. Shibata[2] and Y. Fukuuchi[2]
[1]Dept. of Neurology, Urawa Municipal Hospital, Urawa-shi 336, Japan and [2]Dept. of Neurology, Keio University School of Medicine, Tokyo 160, Japan.

Depolarization elicited by elevations in extracellular K^+ concentration causes rapid Na^+ influx through voltage-sensitive Na^+ channels and leads to stimulation of glucose utilization in neurons. In cultured astroglia, however, elevated extracellular K^+ does not stimulate rates of glucose utilization while direct elevation in intracellular Na^+ concentration does (Takahashi, S. *et al. Proc. Natl. Acad. Sci. USA* 92, 4616-4620). In the present study the effects of increasing extracellular K^+ concentration on Na^+ influx into cultured rat astroglia were examined. Cells were incubated with bicarbonate buffer containing 1 mM ouabain, tracer amounts of [^{22}Na]Cl, and various concentrations (5.4, 28, 56 mM) of K^+ for 0 to 60 min. Cells were digested and assayed for $^{22}Na^+$. K^+ did not facilitate but rather suppressed Na^+ influx in astroglia (Fig.). Veratridine (75 μM), which opens Na^+ channels, significantly raised rates of Na^+ influx (144%), and this elevation was completely blocked by 10 μM of tetrodotoxin, an inhibitor of voltage-sensitive Na^+ channels. These results indicate that elevated extracellular K^+ concentration does not lead to an activation of voltage-sensitive Na^+ channels in astroglia and therefore does not stimulate rates of glucose utilization.



COGNITION: FRONTAL/PREFRONTAL

439.1

A FUNCTIONAL MAGNETIC RESONANCE IMAGING (FMRI) STUDY OF VENTRAL PREFRONTAL CORTEX MEDIATION OF RESPONSE INHIBITION. B.J. Casey*, R. Trainor, J. Orendi, A. Schubert. Psychiatry Depart., WPIC, UPMC, Pittsburgh, PA 15213.

The proposed role of prefrontal cortex (PFC) in behavioral inhibition has been supported by a number of animal and human lesion studies [1, 2] These studies show that lesions to PFC, especially ventral PFC, produce deficits in performance of tasks that require inhibition of prepotent response tendencies. The current study examines differences in PFC activation during performance of a GoNoGo task in subjects with high versus low numbers of false alarms. Eighteen right-handed subjects (mean age 16 years) were scanned during task performance using echo planar fMRI. The task consisted of the subjects responding to any letter but X with 75% of the total number of trials being targets (i.e., non Xs). There were three conditions: 1) a No-go condition with nontargets (i.e. Xs) and targets intermixed; 2) a Go_S condition consisting of all targets and controlling for number of stimuli; and 3) a Go_R condition consisting of all targets and controlling for number of responses. Two groups were defined with a median-split on the number of false alarms. The results indicate that only activation in ventral PFC (i.e. orbital and inferior frontal) and anterior cingulate cortex discriminated between groups on comparisons of NoGo vs. Go conditions. The comparison of the two Go conditions did not discriminate between groups. These results lay the critical groundwork in paradigm development for examining disorders with deficits in response inhibition (e.g., ADHD) using fMRI.

1. Iversen & Mishkin (1970). *Exptl. Brain Research, 11*, 376-386.
2. Malloy et al. (1993). *Archives of Clinical Neuropsych. 8*. 185-201.

439.2

DORSOLATERAL PREFRONTAL CORTEX MODULATES VISUAL PROCESSING IN EXTRASTRIATE CORTEX. D. Swick* & R.T. Knight. Dept. of Neurology & Center for Neuroscience, UC Davis, VAMC, Martinez, CA 94553.

Dorsolateral prefrontal cortex is crucial for the control of sustained and phasic attention to environmental events. A prefrontal-thalamic inhibitory system provides a mechanism for suppressing irrelevant inputs at an early stage of sensory processing. Patients with prefrontal lesions show attention deficits, perseveration, and distractibility, in part due to a lack of inhibitory control that begins with processing in primary sensory cortices. The present experiment examined the influence of prefrontal cortex on a later stage of sensory processing. Event-related potentials (ERPs) were recorded from age-matched controls and patients with unilateral frontal lesions caused by strokes (10 left, 1 right hemisphere). Lesions were centered in areas 9 and 46, but damage extended into areas 6, 8, 10, 44, or 45 in some subjects. Subjects read centrally presented words and pronounceable nonwords and performed lexical decision or recognition memory tasks. In controls, words and nonwords both elicited a focal negative potential peaking at 165 msec and maximal at posterior temporal and occipital electrodes. The amplitude of this N170 component was greater over the left hemisphere in both tasks ($p<.01$), similar to previous studies using verbal stimuli (Neville et al., 1986; Curran et al., 1993). A related temporal-occipital N1 to lateralized nonverbal stimuli is presumably generated in extrastriate cortex (Gonzalez et al 1994; Johannes et al., 1995). N170 amplitude was reduced in frontal patients ipsilateral to damage ($p<.05$), but peak latency was unaffected. These results agree with previous findings in a visual oddball task (Knight, in press) and suggest that dorsolateral prefrontal cortex provides an ipsilateral facilitory input to neural processing in extrastriate cortex that begins within 120 msec post-stimulus.

Funded by NS21135 and PO NS17778 from the NINDS.

439.3

EVIDENCE FOR SEQUENTIAL DEVELOPMENT OF TEMPORAL THEN FRONTAL LOBE FUNCTIONS IN 4-TO-8 YEAR-OLD CHILDREN.

Monica Luciana and Charles A. Nelson*. Institute of Child Development, Univ. of Minnesota, Minneapolis, MN 55455

To investigate the emergence of cognitive functions mediated by prefrontal cortical networks, 180 normal children, ages 4 to 8 (N=30 within each age group), completed a standardized battery of computerized neuropsychological tests (the CANTAB**) to index the following functions: psychomotor speed & accuracy, sequential spatial memory, spatial working memory, planning & inhibition, set-shifting, pattern and spatial recognition, and visual paired associate learning. Performance on each task was compared across age groups matched for sex and socioeconomic status.

Results demonstrate an early emergence of abilities attributable to functioning within subcortical and temporal lobe structures (psychomotor speed/accuracy, pattern recognition, visual paired associate learning) but a more protracted development of cognitive abilities mediated by prefrontal cortex (sequential spatial memory, spatial working memory, planning, and set-shifting). The observation that performance on the prefrontal tasks has not yet approached adult values by the age of 8 is consistent with neurophysiological studies suggesting that the frontal lobe is unique among brain regions in failing to reach full maturity until early adulthood.

This work was supported by the MacArthur Foundation's Network on Development & Psychopathology. **Cambridge Neuropsychological Test Automated Battery, developed by Drs. Trevor Robbins & Barbara Sahakian. Marketed by Paul Fray, Ltd. Control Systems, Cambridge UK

439.4

ABNORMAL BASAL-GANGLIA OUTFLOW IN PARKINSON'S DISEASE IDENTIFIED WITH PET: IMPLICATIONS FOR HIGHER CORTICAL FUNCTIONS. A. M. Owen*, J. Doyon, A. Dagher and A. C. Evans. Montreal Neurological Institute, McGill University, Montreal, Quebec, H3A 2B4, Canada and Department of Psychology and Research Center in Neurobiology, Laval University, Quebec City, Quebec, G1K 7P4, Canada.

In this study we examined the effects of striatal dopamine depletion on cortical and subcortical blood flow changes during two tasks known to involve fronto-striatal circuitry. Regional cerebral blood flow was measured in six patients with moderate Parkinson's disease and in six age-matched controls while subjects performed easy and difficult versions of i) a modified Tower of London planning task; ii) a mnemonic variant of this task that required short-term retention and reproduction of problem solutions and iii) a control condition that involved identical visual stimuli and motor responses. Relative to control conditions, the planning task was associated with an increase in cerebral blood flow in the internal segment of the right globus pallidus in the age-matched controls subjects, and a decrease in the same region in the patients with Parkinson's disease. A similar inverse relationship between the task specific blood flow change observed in the control group and that observed in the Parkinson's disease patients was not found in any other subcortical or cortical area examined, including regions of the dorsolateral frontal cortex known to be involved in this task. When blood flow in the spatial working memory task was examined, a similarly specific dissociation between the two groups of subjects was observed at comparable coordinates in the right pallidum.

We conclude that striatal dopamine depletion disrupts the normal pattern of basal-ganglia outflow in Parkinson's disease and consequently, affects the expression of frontal-lobe functions by interupting normal transmission of information through frontostriatal circuitry.

This work was supported by the McDonnell-Pew Program in Cognitive Neuroscience and by the Medical Research Council (Canada) Special Project Grant SP-30.

439.5

DISTINCT PREFRONTAL ACTIVATIONS IN PROCESSING SEQUENCES OF ACTIONS AND WORDS: A fMRI STUDY AT 3T. S.Crozier[1,2], A.Sirigu[1], S.Lehéricy[1,2], P.F.Van de Moortele[2], [3]E.Guigon, B.Pillon[1], B.Dubois[1], J.Grafman[4]*, D.LeBihan[2], Y.Agid[1]. [1]INSERM U289, Hôpital de la Salpêtrière, 47, Boulevard de l'Hôpital, 75651 Paris Cedex 13, FRANCE., [2]CEA, Orsay. [3]INSERM CREARE. [4]Cognitive Neuroscience Unit, NIH, Bethesda.

Neuropsychological studies have suggested that the prefrontal cortex is important in planning sequences of actions. It has been suggested that script-like mental structures serving as vehicles for representing complex acts, are stored in the prefrontal cortex (Grafman, N.Y. Acad. of Sci., 1995). We reported that patients with prefrontal lesions are impaired at generating and sorting temporally-ordered sequences of actions (Sirigu et al., Cortex, 1995, 1996). This raises the question whether the maintenance of chronology for action sequences is subserved by specific neural substrates within the frontal lobes. This hypothesis was investigated in normal human subjects (n=5) during the processing of action or word sequences, using functional Magnetic Resonance Imaging (fMRI). Brain MRI images were acquired using Bold activation at 3 Tesla and with Echo-Planar. Subjects were shown pairs of written sentences. In condition A, they detected errors in the temporal order of *actions* depicted by the sentences (script). In condition B, they detected errors in the order of *words* within the sentences (syntax). The control condition consisted in checking for double consonants in non-words. Subtraction of the control condition from conditions A and B revealed different patterns of activation for script and syntax processing. The script task activated a large area in the dorsolateral prefrontal cortex (Brodmann areas 6 and 8) in both hemispheres. The syntax task resulted in a lesser activation of the same region but only in the left hemisphere, as well as Broca's and Wernicke's areas. These results indicate that script and syntax analysis are based on different types of sequential relations and may depend on domain-specific representations within the prefrontal cortex.

This research was supported by Fondation pour la Recherche Médicale and European Community T.M.R. Program.

439.6

INTERAREAL EEG SYNCHRONIZATION DURING SPATIOTEMPORAL REASONING IN MAN, J. Sarnthein, A. von Stein, G.L. Shaw†*, F.H. Rauscher †, P. Rappelsberger, and H. Petsche. Instit. Neurophysiol., Univ. Wien, 1090 Vienna, Austria; † CNLM, UCI, Irvine, Ca, 92717, USA.

The neuronal basis of mental activity is assumed to be related to distributed assemblies of cortical nerve cells. These neuronal cell assemblies are formed by synchronous activity of their member neurons. We measured the coherence in the signals of pairs of scalp EEG electrodes placed over different cortical areas. Synchronicity is related to coherence, which may be considered as measure for information transfer between two electrodes. Higher brain function is thought to be based on the synchronization of cell assemblies distributed within different cortical areas.

In our experiment, EEG was recorded over several minutes while (female) subjects solved spatio-temporal reasoning tasks that also required working memory. This ongoing EEG was analysed for coherence changes with respect to a resting condition. In our group of 14 subjects we found a highly significant increase of coherence between parietal and frontal cortex in the high beta frequency band (18.5 - 31.5 Hz) in both hemispheres. The EEG coherence patterns were highly reproducible over several tasks for each subject.

Using scalp EEG and the concept of distributed neuronal cell assemblies, one may thus gain insight in the cooperativity of working memory and spatial reasoning needed for complex spatio-temporal reasoning tasks.

Funding: Ralph and Leona Gerard Foundation, Seaver Institute.

439.7

SPATIAL WORKING MEMORY IN PATIENTS WITH FRONTAL LOBE LESIONS. J. V. Baldo* and A. P. Shimamura. Dept. of Psychology, University of California, Berkeley, CA 94720.

Patients with lateral prefrontal cortex lesions and elderly controls were tested on a spatial working memory task that was similar to one used in studies of prefrontal function in monkeys (Goldman-Rakic, 1987). Participants had to remember the location of a black dot presented randomly on the computer monitor. Following a delay of 0, 3, or 9 sec, participants responded by moving the mouse cursor to the remembered location. In one condition, during the delay, participants had a secondary task to perform. A series of digits was rapidly presented at central fixation, and participants had to press the mouse button every time a '0' was detected. In the control condition, the digits appeared but no response was required. Based on previous human and animal studies of prefrontal function, we hypothesized that patients would be worse overall on this working memory task and that they would be disproportionately impaired on the digit task condition and with longer delays. There was a main effect of group, such that patients were impaired overall. Numerically, this effect in the patients was exaggerated with longer delays (3 and 9 sec), compared to the baseline (0 sec). However, there was no interaction of group with task condition. Patients' and controls' performance declined comparably when they had to perform the secondary digit task. Further studies will be presented that manipulate the type of interference occurring during the delay.

This research was supported by National Institutes of Health Grants, MH48757 and NS17778, and a National Science Foundation pre-doctoral fellowship.

439.8

NEURAL ACTIVITY IN THE PRIMATE PREFRONTAL CORTEX DURING A PROBLEM-SOLVING TASK.
E. Procyk,Y.L. Tanaka and J.-P. Joseph*. INSERM U94, 16 av.du doyen Lépine, 69500 Bron, FRANCE.

It has been shown that rhesus monkeys can perform complex spatial tasks by using a trial-and-error strategy (Procyk and Joseph, in press). The animal, facing a touch-screen monitor, had to search for the touching order (chosen in a set of 6 by a computer), of 3 fixed spatial targets illuminated on the screen. After an incorrect touch, all targets were extinguished and the monkey had to start another trial from the very beginning. After a correct touch, all targets remained illuminated and the monkey could choose the following target. When the correct order was repeated 4 times, a new search was initiated. Unit activities were recorded from superior arcuate area (SA) and caudal part of sulcus principalis (SP) in 2 rhesus monkeys during execution of this task.

Preliminary data show 2 groups of activities specific to the task: 1) visual-attentional activities which are associated with the selection of the successive targets. Some of these activities show allocentric characteristics. 2) error-specific activities with spatial- and/or rank-selectivity. They reflect the encoding of an error-event that is a cue for the forthcoming choices. These preliminary data show that SA and SP are involved in attentional control on the target selection and in encoding the action feedbacks. However, putative working memory processes involved in trial-and-error strategies and in the construction and storage of the correct solution remain to be elucidated. The data suggest that these processes (memory of a movement and of its result) are quite different from those involved in simple DR-tasks (i.e. memory of a single sensory event provided by the environment) and/or are located in other structures.

439.9

Characterization of the Decision-Making Defect of Subjects With Ventromedial Frontal Lobe Damage. S. W. Anderson*, A. Bechara, D. Tranel, H. Damasio, and A.R. Damasio. Dept. Neurology, Univ. of Iowa, Iowa City, IA, 52242.

In a novel gambling task, control subjects learn to avoid the decks which yield high immediate rewards and larger future losses, and opt for choices that yield low immediate gains, but lower future losses. Subjects with ventromedial frontal (VMF) lobe damage do the opposite. Three possibilities may account for the behavior of VMF subjects: 1) Hypersensitivity to reward, so that the prospect of future (delayed) punishment is outweighed by that of immediate gain. 2) Insensitivity to punishment, so that the prospect of reward always prevails. 3) Insensitivity to future consequences, positive or negative, so that the behavior of the VMF subject is always guided by immediate prospects. To explore which possibility provides the best account, we designed a variant of the original gambling task, in which control subjects learn to prefer the decks with high immediate punishment, but higher future reward (good decks), and avoid the decks with low immediate punishment, but lower future reward (bad decks). Although a few VMF subjects (n=3) chose the good decks in the variant task, the majority (n=9) did the opposite, i.e., avoided the decks with high immediate punishment/ high delayed reward. In both the original and the variant tasks, the skin conductance responses (SCRs) of controls and VMF subjects after receiving a reward or punishment were not significantly different. The findings that most VMF subjects avoided the decks with high immediate punishment, and that they showed SCRs after receiving punishment, are inconsistent with the insensitivity to punishment explanation. The findings that most VMF subjects were not lured by the high reward in the decks with high immediate punishment, and that their SCRs after receiving a reward were not abnormally high, are inconsistent with the hypersensitivity to reward explanation. Thus, the impairment of VMF subjects on both the original and variant tasks leaves one plausible explanation, that most VMF subjects are insensitive to future consequences, whatever they may be.

Supported by NINDS PO1 NS19632, JSMF, and Centennial MRC.

439.10

Fear Conditioning After Ventromedial Frontal Lobe Damage in Humans. D. Tranel*, A. Bechara, H. Damasio, and A.R. Damasio. Div. of Cognitive Neuroscience. Dept. Neurology, Univ. of Iowa, Iowa City, IA, 52242.

The somatic marker hypothesis proposes that the ventromedial prefrontal (VMF) cortex is a critical site in a neural circuit subserving somatic state activation. We asked whether the role of the prefrontal cortex in this process is similar to that of the amygdala, i.e., to acquire simple associations between exteroceptive sensory information and interoceptive information concerning somatic states. We tested controls (n=6) and VMF subjects (n=9) in a fear conditioning experiment, using 4 different colors of monochrome slides or 4 different computer-generated tones as conditioned stimuli (CS), and a startlingly loud sound (100 db) as the unconditioned stimulus (US). Electrodermal activity (SCR) was the dependent measure of autonomic conditioning. Each experiment involved 1)a habituation phase, 2) a conditioning phase in which only one of the colors or the tones were paired with the US, and they were presented at random among the other colors or tones, 3) an extinction phase, and 4) a debriefing phase in which the subjects were asked to recall specific facts about the experiment. All control subjects conditioned and began to generate SCRs after the presentation of a slide or a tone previously paired with the US. They also recalled specific facts about the experiment. Five of the VMF subjects acquired the conditioning and recalled the facts at the same level as the controls. Four of the VMF subjects failed to show any conditioned SCRs, and were also defective in recalling the facts. Preliminary anatomical analysis reveals that the lesions in the impaired VMF subjects extended more posteriorly and probably involved the basal forebrain. The lesions in the unimpaired VMF subjects were more anterior. The findings suggest that the anterior component of the ventromedial prefrontal cortex is not necessary for the acquisition of simple associations between exteroceptive and interoceptive information.

Supported by NINDS PO1 NS19632, JSMF, and Centennial MRC.

439.11

Insensitivity to Future Consequences Following Bilateral Damage to the Human Amygdala: Contrasts With Ventromedial Frontal Lobe Lesions. A. Bechara*, G.P. Lee, R. Adolphs, D. Tranel, and A.R. Damasio. Div. of Cognitive Neuroscience, Dept. Neurology, Univ. of Iowa, Iowa City, IA, 52242.

The somatic marker hypothesis proposes that both the amygdala the ventromedial prefrontal (VMPF) cortex are parts of a neural circuit subserving decision-making. Here we hypothesize that although both structures couple exteroceptive sensory information with interoceptive information concerning somatic states, they do so at different levels, thus making different contributions to the process. Using a gambling task sensitive to the decision-making defect observed in VMPF subjects, we predicted that subjects with bilateral amygdala damage would show 1) impairment on the gambling task, 2) defective autonomic activity when given information which implies a state of reward or punishment, and 3) defective autonomic activity when pondering a choice among several options associated with various magnitudes of immediate reward and delayed punishment. We tested 3 subjects with bilateral amygdala damage, and 5 subjects with bilateral VMPF damage, during their performance of the gambling task, and while recording their electrodermal activity. All amygdala subjects were 1) impaired on the gambling task, 2) unable to generate skin conductance responses (SCRs) when given rewards or punishment (they generate normal SCRs in response to a loud startling noise), and 3) unable to generate anticipatory SCRs when choosing among various response options. By contrast, VMF subjects were similar to the amygdala subjects with respect to conditions 1) and 3), but they were normal in conditon 2). The results support the hypothesis that the amygdala and VMPF cortex make different contributions to a neural circuit subserving decision-making.

Supported by NINDS PO1 NS19632, JSMF, and Centennial MRC.

439.12

FRONTAL, PARIETAL AND CEREBELLAR NETWORKS FOR CONDITIONAL MOTOR LEARNING. M.-P. Deiber, S.P. Wise*, M. Honda, J. Grafman and M. Hallett. Med. Neurol. Br., NINDS, Lab. Neurophysiol., NIMH, Bethesda, MD 20892

Frontal cortex has been hypothesized to function in the learning and rejection of behavior guiding rules. To test this hypothesis, 6 normal, right handed subjects centrally fixated a 9° square display and dextrally moved a joystick in the direction arbitrarily instructed by: (1) a 2° visual pattern (PATTERN RULE) or (2) the pattern's location (PLACE RULE). For these MOTOR TASKS, the same 4 stimuli appeared in the same 4 places and were attended on each trial. On another day, subjects moved the joystick in a visually displayed "YES" direction if an arrow's direction agreed with each rule or in a "NO" direction if it did not (EVALUATION TASKS). Both tasks had a FIXED-response condition. Subjects improved performance over 4 consecutive scans while rCBF was measured with PET (15-O water). Variance was evaluated with principal component analysis (PCA), contrasts with SPM.

PCA revealed a first principal component corresponding to rule adherence (vs. fixed responding), which accounted for ~50% of the variance. A second component reflected a time-in-rule effect, presumably learning (~20%). Only one frontal area showed a significant increase in rCBF during learning, the caudal part of the left premotor cortex (PM, area 6). It did so during the pattern rule-evaluation task. Several frontal areas, along with the left lateral cerebellar hemisphere, showed rCBF *decreases* during learning, also during the pattern rule-evaluation task: the left ventro-orbital prefrontal (PF) cortex (area 47/12 of Petrides & Pandya) and, in the right hemisphere, the dorsal and rostral aspect of PM (PMdr) plus dorsal PF (PFd, area 9). During the place rule-motor task, a more caudoventral part of the right PM and the right dorsolateral PF (PFdl, area 46) showed decreasing rCBF during learning. A largely common posterior parietal region (area 39/40) showed decreasing rCBF during learning in all tasks. These data are consistent with the hypothesis that localized frontal areas, as well as the cerebellar and parietal networks associated with them, receive potentiated inputs (reflected by higher rCBF in the first scan compared to later ones) when routine rules need to be rejected and new ones adopted, with different frontal areas "activated" while learning different rules.

COGNITION: LANGUAGE II

440.1

AGRAPHIA WITHOUT ALEXIA: ISOLATION OF GRAPHEMIC OUTPUT IN A SPLIT-BRAIN PATIENT. **K. Baynes†, J.C. Eliassen†, and M.S. Gazzaniga‡.* †Center for Neuroscience, University of California, Davis, CA 95616; ‡Department of Psychology, Dartmouth College, Hanover, NH 03754 .**

Dejeurine first described the striking loss of reading ability in a patient who could write. Our patient VJ, a 43-year-old left-handed female, presented with agraphia following two-stage callosotomy. She continues to read for pleasure, but can perform only rudimentary writing tasks, and then not always at will. We used tachistoscopically lateralized letters, numbers, nouns, verbs, adjectives, adverbs and pictures to demonstrate the unique lateralization of language in VJ. She is able to name RVF/LH stimuli aloud, but cannot write the names. In contrast, she writes the names of LVF/RH stimuli, but cannot name them aloud. We argue that in this familially left-handed patient, phonological output is isolated in the left hemisphere whereas graphemic output is isolated in the right. This hemispheric separation of input and output for visual language suggests that the evolutionarily late skill of written language has varied cortical representation.

Supported by NIH/NIDCD grant R29 DC00811 to K.B. and NIH/NINDS grant PO1 NS 17778-14 and the McDonnell-Pew Foundation to M.S.G.

440.2

SELECTIVE SPEECH MOTOR, SYNTAX, AND COGNITIVE DEFICITS ASSOCIATED WITH BILATERAL DAMAGE TO THE HEAD OF THE CAUDATE NUCLEUS AND THE PUTAMEN. A CASE STUDY.

Emily R. Pickett, Athanassios Protopapas, Joseph Friedman†, Philip Lieberman, and James A. Anderson*. Department of Cognitive & Linguistic Sciences, Brown University, Providence RI 02912; †Department of Clinical Neurosciences, Brown University, and Department of Neurology, Roger Williams Medical Center.

Deficits in speech production, sentence comprehension, and abstract reasoning occurred in a subject having profound bilateral damage to the anterior caudate nucleus and putamen. Acoustic analyses indicated that the subject's speech was degraded due to inappropriate sequencing of articulatory gestures that involve different articulatory structures. Transitions between sounds were slow and often did not achieve target configurations. The subject had a 14% error rate comprehending distinctions in meaning conveyed by syntax in English sentences; normal controls make virtually no errors in this test. Cognitive deficits involving impaired sequencing occurred: the subject had a 70% error rate on the Odd Man Out test when making decisions within a single category. Cognitive perseveration occurred when the subject was asked to shift categories. In contrast, performance was within normal ranges in tests of lexical access and memory. The pattern of deficits provides evidence for basal ganglia involvement in the regulation of sequencing across modalities.

440.3

DETECTING ABNORMAL BRAIN ACTIVATION IN INDIVIDUAL SUBJECTS USING POSITION EMISSION TOMOGRAPHY. T. J. Grabowski*, R. J. Frank, C. K. Brown and H. Damasio. Div. Cognitive Neurosci., Dept. Neurology, Univ. of Iowa, Iowa City, IA 52242.

The application of pixelwise linear models to the analysis of position emission tomography (PET) activation images from individual subjects has not been thoroughly explored. We report the specificity and sensitivity of an outlier detection method for detecting deviations from a normative pattern of activation.

Data from a verb generation paradigm (16 subjects; 4 task-pairs) were analyzed with a standard two-way ANCOVA (Model I), two-way ANCOVA with a subject-by-task (SXT) interaction term (II), and two-way ANCOVA with an "outlier SXT" interaction term (III). Model III contrasted individual subjects with the normative group under three conditions: A. no putative outliers (control), B. simulated location outliers and C. simulated negative intensity outliers (i.e. lack of expected activation). A consistent pattern of activation occurred in individual subjects, evidenced by the absence of significant SXT interaction in Model II and the absence of outliers in target regions when individuals were contrasted with the group using Model IIIA.

When each subject was analyzed with respect to the group using Models IIIB and IIIC, simulated blood flow increase in 3 homologous regions was detected in 79% of instances (N=48) and simulated lack of activation in 6 regions of expected increase was detected in 56% of instances (N=96). Greater variance in regions with significant task effect reduced power in the latter case. Regions whose activity could be predicted to be correlated were detected jointly more powerfully than individually.

Despite the noisy nature of PET activation images, it is possible to investigate hypotheses with individual subjects when activation data from a normative group is available. This method is applicable to the undamaged hemispheres of subjects with acquired lesions.

(Supported by The Charles A. Dana Foundation and The Mathers Foundation).

440.4

ACTIVATION OF FRONTAL MOTOR AREAS DURING SILENT LIP READING: A PET ACTIVATION STUDY. S. T. Grafton*, L. Fadiga, M.A. Arbib, G. Rizzolatti. Dept. of Neurology, Univ. Southern CA School of Med., Los Angeles, CA 90033

Verbal communication relies on acoustic and visual information, with acoustic pathways normally predominating. Lip movements that are incongruous with sounds can lead to startling comprehension errors (McGurk effect). We used PET CBF imaging to trace the cortical circuitry underlying lip reading and in particular to see which speech areas were involved.

7 normal right handed subjects were scanned during: (1) control condition of object viewing; (2) observing the investigator making silent monosyllable mouth movements (e.g. ba, da, me, go...); (3) same as condition 2 with subjects silently mimicking the observed lip movements. Images were coregistered in Talairach coordinates and a threshold of $p<0.005$ was used for all categorical comparisons.

Silent lip reading vs. control activated left frontal operculum (Talairach coordinates: -39, 17, 3) in Brodmann's area B.A. 45. Mimicking vs. lip reading activated left precentral sulcus (-39,8,12), B.A. 44, bilateral precentral gyrus (-50 & +50, 5, 30), B.A. 6, and bilateral central sulcus (L>R) (-50 & +50, -12, 33), B.A. 4. The B.A. 45 activation was attenuated when mimicked movements were performed. No acoustic or "receptive" language areas were activated.

The findings suggest visual information enters the language circuit in frontal motor areas including B.A. 45, consistent with motor theories of speech.

Supported by NS01568 and Human Frontiers Science Program

440.5

EXPLAINING CATEGORY-RELATED EFFECTS IN THE RETRIEVAL OF CONCEPTUAL AND LEXICAL KNOWLEDGE: OPERATIONALIZATION AND ANALYSIS OF FACTORS. C. G. Logan*, D. Tranel, R. J. Frank, and A. R. Damasio. Div. of Cognitive Neurosci., Dept. of Neurology, University of Iowa, Iowa City, IA 52242.

Category-related effects in the retrieval of conceptual and lexical knowledge for concrete entities presented visually have been well documented in lesion studies, and more recently, with functional imaging and electrophysiological approaches. For example, brain-damaged patients may lose the ability to recognize (or to name) most animals but not tools; or the opposite pattern may obtain. Several stimulus- and subject-related factors have been proposed to underlie such effects, including homomorphy (shared shape), manipulability, characteristic motion, sensory modality of transaction, familiarity, age of acquisition, and value. Here, we report a study in which we operationalized these factors and analyzed their intercorrelations. Stimuli were slides of 215 items from the conceptual categories of animals and birds, fruits and vegetables, tools and utensils, vehicles, and musical instruments. The factors were operationalized through ratings from 218 normal control subjects or by computer analysis of the objects' digitized outlines. Principal components analysis revealed that 86% of the variability across items could be accounted for by 3 factors: Items that loaded highly on Factor 1 had high homomorphy and low ratings on characteristic motion and manipulability. Items loading highly on Factor 2 had high ratings on value, touch, and familiarity, and low age of acquisition. Items loading highly on Factor 3 had high ratings on hearing and highly distinctive sounds. In another analysis, we found that homomorphy, characteristic motion, manipulability, touch, familiarity, and value all significantly differentiated between animals and tools/utensils. These findings allow us to test the notion that the driving force behind the regionalization of neural systems related to acquisition and retrieval of conceptual and lexical knowledge results from systematic differences in physical characteristics and contextual specification of concrete entities.

Support: NINDS NS 19632, ONR N00014-91-J-1240.

440.6

PET STUDIES OF SEMANTIC ANALYSIS A. L. Roskies*, J.A. Fiez, D. A. Balota, J.G. Ojemann, M.E. Raichle, and S. E. Petersen, Washington Univ. Sch. of Med., Box 8225, St. Louis, MO 63110.

A semantic categorization task was used to examine activation related to the processing of word meaning using PET. Subjects viewed word pairs, one member of which was a category name, and indicated by keypress if the second member of the pair was an exemplar of that category. Subjects were scanned while performing this task both in an easy condition with typical category-member pairs (e.g. bird - robin), and a hard condition with atypical pairs (bird - ostrich); each word was used in both the easy and hard tasks.

Responses were slower and less accurate ($p < .01$) in the hard than the easy task. Several regions showed similar activation across tasks, including regions in the left frontal operculum and the right cerebellum. Other regions showed greater activation in the hard than the easy task ($p<.01$), including one area in right lateral cerebellum, and one at or near left BA47.

The same subjects performed two other tasks of equivalent difficulty: synonym and rhyme judgment. The left frontal operculum was again active for both tasks, while the BA47 region was more active during synonym than rhyme ($p<.07$). Previous experiments have revealed similar distinctions between a verb-generation and a stem-completion task. Both tasks activated a region in the left frontal operculum, while the verb-generation task, which had clear semantic demands, activated a more anterior frontal region near area 47.

The combined results from this and previous studies provide evidence that there are dissociable frontal regions involved in language processing, and that a region in left anterior prefrontal cortex may specifically contribute to semantic processing. *Supported by NIH grant NS06833, the Charles A. Dana Fndn., and the McDonnell Ctr. for Higher Brain Function.*

440.7

MODULATION OF N400 AND Dm EVENT-RELATED POTENTIALS BY IMAGINAL AND SEMANTIC PROCESSING OF CONCRETE AND ABSTRACT SPOKEN WORDS. W.C. West*, C.D. Hong, and P.J. Holcomb, Department of Psychology, Tufts University, Medford MA 02155.

Visually presented concrete words have been shown to elicit more negative event-related potentials beginning as early as 250 msec after stimulus onset than have abstract words (Kounios & Holcomb, 1994). The duration, amplitude, and scalp distribution of this effect was found to vary with the type of processing strategy used by subjects (West & Holcomb, 1995). In particular, semantic strategies produced large concreteness effects across the entire scalp while imaginal strategies produced smaller effects which were restricted to anterior sites. The present study replicated the methodology of West & Holcomb (1995) in the auditory modality. Two groups of 12 subjects performed a sentence verification task in which the final word of each sentence (160 trials) was either a concrete or abstract noun. For the first group, the truthfulness judgement required the use of imagery and for the second group it involved a semantic decision which was not aided by imagery. ERPs to concrete words were more negative than to abstract words between 350 and 750 msec post-stimulus onset (N400). This effect increased toward anterior sites for both groups and was larger over the right hemisphere for the semantic group. Subjects in this experiment were also tested for recall of the sentence final words after every 20 trials. Subsequently recalled concrete words elicited a more positive ERP during encoding between 500 and 900 msec post-stimulus onset than non-recalled concrete words (Dm), while recalled abstract words did not differ from non-recalled abstract words. Comparison of these results to previous ERP memory research as well as implications for the role of imagery in language processing and memory will be discussed.

440.8

BILATERAL REPRESENTATION OF COORDINATE SEMANTIC RELATIONS: EVIDENCE FROM EVENT-RELATED POTENTIALS. D. J. Wegesin.* College of Physicians and Surgeons, Columbia University, New York, NY USA.

Though language centers are situated primarily in the left hemisphere (LH), right hemispheric (RH) mediation of semantic processing has been frequently reported. We herein summarize electrophysiological evidence which supports bilateral mediation of coordinate semantic relations (i.e. shared categorical membership like apple-pear). Forty right-handed men and women completed a divided-visual field Lexical-Decision/Semantic Monitoring task. Subjects identified Pseudowords in a serially presented list of Pseudowords, Semantically-related words, and Control words. Analysis of the P2 complex - computed using the mean amplitude in the latency ranges 230 - 530 - revealed that Semantic words presented to the RVF were similar to Control words; both evoked a large P2c asymmetry - suggestive of greater LH involvement. However, when words were presented to the LVF, the asymmetry remained only for Control words. LVF Semantic words evoked more equivalent activity across the two hemispheres suggestive of bilateral processing of Semantic but not Control words. Latency analyses revealed that while both hemispheres responded differentially to Control and Semantic words, the LH did so earlier than the RH.

supported by the Eva O. Miller Fellowship at the Univ. of Minnesota

440.9

WHEN YOU LEAST EXPECT IT: CONTEXT EFFECTS AND CATEGORIZATION IN SENTENCE PROCESSING.

Kara D. Federmeier*† & Marta Kutas†°, Departments of Cognitive Science† and Neuroscience°, UCSD, La Jolla, CA 92093

Context and category membership have both been shown to influence the amplitude of the same electrophysiological component, the N400 (e.g., Kutas & Hillyard, 1984; Harbin et al., 1984). We hypothesize that this relationship arises because sentence context activates world knowledge which is stored in long term memory and organized according to perceptual and functional similarities as reflected in taxonomic categories. In this experiment we examined the extent to which category structure is used in on-line sentence processing. We recorded event related brain potentials (ERPs) as subjects read pairs of sentences for comprehension. The first sentence set up an expectation for a particular exemplar of a particular category while the second sentence ended with (1) the expected exemplar, (2) an unexpected exemplar from the expected category, or (3) an unexpected exemplar from an unexpected category (e.g., "She wanted to treat her guests to an all-American pie. She went out in the back yard and picked some (1) apples/(2) oranges/(3) carrots"). Although all unexpected items elicited an N400, it was significantly smaller (i.e. closer to the response for the expected exemplar) if the item was a member of the expected category than if it was from a different category. Moreover, we found that if the expectation for a particular item was strong the N400 to an unexpected category member was smaller than if the expectation was weak, despite the fact that its fit to the context was poorer. This not only supports our view that category structure influences sentence processing on-line but also suggests that its impact may be a function of the degree to which the category member is expected.

Funded by: Grants to M.K. from NIMH MH52893 and NICHD HD22614 and Fellowship to K.D.F. from the Howard Hughes Medical Institute.

440.10

WORD REPETITION WITHIN- AND ACROSS- VISUAL FIELDS: AN EVENT-RELATED POTENTIAL (ERP) STUDY.

M.C. Doyle and M.D. Rugg*. Wellcome Brain Research Group, School of Psychology, University of St. Andrews, St. Andrews, Fife, UK.

The present experiment investigated the sensitivity of the ERP word repetition effect to the visual field in which the words are encountered. 12 subjects performed a go/nogo lexical decision task, responding only to non-words. First and second encounters with repeating words occurred on immediately successive trials. For each visual field of presentation there were three experimental conditions. First presentation (left;right), repetition within visual field (left-left; right-right) and repetition across-visual field (left-right; right-left). ERPs (28 scalp sites) were formed for each experimental condition. Non-words were detected more quickly and more accurately when presented to the right visual field. Between 400 and 600 ms post-stimulus onset a reliable ERP repetition effect was found in each repetition condition. In the left-left and right-right conditions the repetition effect was greater over the right hemisphere than over the left, particularly at posterior sites. This pattern of results suggests that the processes reflected by the ERP repetition effect are dissociable from those responsible for lexical analysis, and are more strongly represented in the right hemisphere.

Wellcome Trust, UK.

440.11

WHAT DO FACES AND LETTER-STRINGS HAVE IN COMMON? EARLY EVENT-RELATED POTENTIAL EVIDENCE. G. Ganis*@, H.E. Schendan@, and M. Kutas. Cognitive Science, UCSD, La Jolla, CA 92093-0515. Neuropsychological and behavioral studies have noted processing differences between written words and non-linguistic visual objects. Previously we reported on the nature and time course of such processing differences from ERPs recorded to word-like (concrete nouns, pronounceable nonwords, pseudo-font strings) and object-like stimuli (line drawings of common objects and pseudo-objects) as well as icon strings, which are both word- and object- like, in three tasks. We found that word-like stimuli elicited a much larger P150 than objects or pseudo-objects. On the basis of its latency and distribution we put forth the hypothesis that this P150 may be a reflection at the scalp of the intracranial-recorded 'letter-string' N200 observed over discrete regions of the posterior fusiform gyrus (Allison et al., 1994). Intracranial 'face' N200s have been observed adjacent to intracranial 'letter-string' N200s in regions of posterior fusiform gyrus. Thus, if our hypothesis is correct, faces should elicit a scalp P150 that is very similar in distribution, amplitude, and latency to that elicited by letter-strings. To test this hypothesis, we replicated and extended out previous experiment with the addition of a face condition. Consistent with our hypothesis, results show that letter-strings and faces do indeed elicit nearly identical scalp P150s, while objects and pseudo-objects elicit much smaller P150s. The P150 to faces is probably equivalent to the 'vertex positive peak' discussed by Jeffreys (1992). Since the P150 to letter-strings is as large as that to faces, we suggest that it is not a face-specific component as has been claimed. It has been suggested that the intracranial N200s to letter-strings and faces reflect early stages of visual identification (prelexical analysis of letter-strings and face detection). If the P150, or a subcomponent of this ERP, is the reflection of the intracranial N200 at the scalp, then it can be used as an important tool to study early visual processes in intact individuals. (@These authors made equal contributions and are funded by the McDonnell-Pew Center for Cognitive Neuroscience in San Diego. *Funded by Center for Research on Language, UCSD).

440.12

ERPs AND SEX DIFFERENCES IN REGULAR AND IRREGULAR PAST TENSE VERB FORMATION. D.W. Shucard*, J.L. Shucard, G.L. Ciupak, J.J. Jaeger, A.H. Lockwood. Depts. Of Neurology, Linguistics, and Nuclear Medicine, SUNY @ Buffalo, 100 High Street (D-6), Buffalo, NY 14203. The purpose of the present study was to investigate the neural patterns of activation during the production of regular and irregular past tense forms of English verbs. Currently, there are two competing theories that implicate different brain systems or mechanisms in these functions. One theory suggests that separate systems are responsible for regular and irregular verb production, whereas the opposing theory posits that one system serves both functions.

Young adults (7 males, 7 females) performed five visually presented tasks: 1) read aloud a series of verbs, 2) read aloud a series of orthographically regular nonce verbs, 3) form the past tense of regular verbs and speak them, 4) form the past tense of irregular verbs and speak them, and 5) form the past tense of nonce verbs and speak them. Measures of cerebral blood flow with positron emission tomography (PET) and cortical event-related potentials (ERPs) were obtained simultaneously while the tasks were performed. Here, only ERP data are presented. Three peaks between 58 and 270 msecs were identified from 18 electrode sites. Several findings are noteworthy: 1) The occipital leads showed a significant condition effect ($P<.006$) indicating that the amplitude of the Peak 2 ERP component (N_1-P_2) was different across conditions, irrespective of the sex of the subject. 2) Significant sex-related ERP amplitude differences were present that were dependent on both the conditions and the scalp sites. Males, but not females, showed a significant Condition x Site interaction ($p<.009$). The highest ERP amplitude was seen at the frontal sites for the formation of past tense regular verbs and the lowest amplitude at the frontal sites for formation of past tense irregular verbs. Further, the temporal scalp sites showed a significant Sex x Condition x Hemisphere interaction ($P<.008$) indicating that there was a different pattern of hemispheric asymmetry present between males and females across conditions. The findings are related to differences in brain organization between males and females and reflect functional relationships between grammatical tasks and brain mechanisms responsible for these tasks. Supported in part by SUNY@Buffalo & Department of Veterans Affairs

440.13

UNIVERSAL GRAMMAR'S EMERGENCE FROM PROTOLANGUAGE: CORTICOCORTICAL COHERENCE COULD ENABLE BINDING AND RECURSIVE EMBEDDING. WILLIAM H. CALVIN*, University of Washington, Psychiatry, Seattle WA 98195-1800. *http://weber.u.washington.edu/~wcalvin*

A brain mechanism for recursive embedding (such as sentences within sentences: *I think I saw him leave to go home*) is considered essential for Universal Grammar (features common to all known languages except pidgins). Among the linguists' other desiderata are mechanisms for long-range dependencies, including binding of pronouns to their referents. Such binding requires longer-than-local links; recursive embedding requires structuring a hierarchy of them.

Corticocortical axon bundles are considerably worse than the incoherent fiber optic bundles where neighbors fail to remain neighbors. I have predicted (***Soc. Neurosci. Abstr.*** 1993), however, an error-correction ability that should emerge from the lattice connectivity of the superficial pyramidal neurons. Were it not well tuned, linkages would be restricted to well-practiced special cases, analogous to the mariners' signal flags – and to only a few at a time, limiting the possible associations conveyed. Embedding would be restricted to stock phrases. Properly tuned up, the error-correction circuitry could reconstitute an arbitrary spatiotemporal firing pattern in the target cortex – and thus convey novel associations, even passing them through a hierarchy of embeddings. This makes corticocortical coherence a candidate for what converted protolanguage (structureless pidgins) into Language Itself (linguists doubt that intermediate forms exist). Indeed, the transition from special-case to arbitrary code conveyance could have implemented both major innovations of Universal Grammar – embedding and long-range links. The versatile linkages described here, in the context of a darwinian process at each end to bootstrap quality, might also have helped convert the infrequently-innovating cultures of *Homo erectus* into the constantly-evolving ones of *Homo sapiens*. Funding ;-) via my two new books, ***How Brains Think*** (BasicBooks 1996) and ***The Cerebral Code*** (MIT Press 1996).

440.14

HEMISPHERIC COOPERATION DURING MOTOR PROGRAMMING AND LANGUAGE PERCEPTION.
F. Pulvermüller *, B. Mohr & W. Lutzenberger
Institute of Medical Psychology and Behav. Neurobiology, University of Tübingen, 72074 Tübingen, Germany

Execution of complex tasks involves both hemispheres. For example, sequential finger movements with only one hand are accompanied by both contralateral and ipsilateral cortical activity. To demonstrate that both hemispheres significantly contribute to lateralized processes, language processes were investigated. Word processing is speeded if not only the language-dominant left hemisphere, but, in addition, the right hemisphere is directly stimulated. Thus, in this case, the inferior hemisphere contributes to better performance. However, this does still not demonstrate that activity flow between the hemispheres is relevant for better performance. Therefore, electrocortical activity was recorded when verbal stimuli reached the left, right, and both hemispheres. Bilateral presentation led to enhancement of activity signs over left-hemispheric language areas (relative to LH stimulation), indicating that activity flow from the right hemisphere takes place. Thus, (i) both hemispheres are activated when complex tasks are being performed; (ii) both hemispheres can contribute to better task performance when they are coactivated; (iii) if coactivation leads to better task performance, activity flow between the hemispheres can be demonstrated. These data support models of interhemispheric cooperation.
Supported by the DFG (Pu 97/2, Pu 97/5, SFB 307/B1).

COGNITION: MEMORY II

441.1

DIFFERENTIAL ACTIVATION OF HUMAN CORTICAL AREAS DURING MENTAL ROTATION AND MEMORY SCANNING: A HIGH FIELD FUNCTIONAL MRI STUDY. G.A. Tagaris*, W. Richter, S-G. Kim, G. Pellizzer, P. Andersen, K. Ugurbil and A.P. Georgopoulos Brain Sciences Center, VAMC and Center for Magnetic Resonance Research, Univ. of Minnesota Medical School, Minneapolis, MN.

Six human subjects performed two variants of mental rotation and memory scanning tasks while multislice BOLD functional MR images were acquired with a 4 Tesla system. The tasks used included (a) mental rotation of a figure, (b) mental rotation of an intended movement direction, (c) memory scanning to determine the order of presentation of a sequence of lights and (d) memory scanning to generate a movement in a direction based on a serial order rule. Appropriate control tasks were used. The main results are summarized below.

(i) In both mental rotation tasks the superior parietal lobule was activated, whereas in the memory scanning tasks activation was observed predominantly in the inferior parietal lobule, (ii) all four tasks activated the precentral gyrus, (iii) cerebellum was preferentially engaged in tasks that required directed movements (tasks b and d) and (iv) all four tasks activated extrastriate visual areas.

These results reveal for the first time the differential activation of two parietal areas in mental rotation and memory scanning. They also demarcate the role of precentral motor areas and the extrastriate visual cortex as common nodes in both cognitive processes. Finally, these results demonstrate the preferential involvement of the cerebellum in cognitive operations on spatial motor representations

(Supported by NIH grants NS32919 and RR088079)

441.2

AN fMRI STUDY OF FRONTAL CORTEX AND CEREBELLUM DURING SEMANTIC AND WORKING MEMORY TASKS. J.E. Desmond*, J.D.E. Gabrieli, N. Sobel, L.A. Rabin, A.D. Wagner, C.A. Seger, and G.H. Glover. Depts. of Psychology and Radiology, Stanford University, Stanford, CA 94305.

Prior studies have shown that retrieval of semantic information activates left frontal and right cerebellar networks, and these regions are also activated during verbal working memory tasks. The present study directly compares frontal and cerebellar activation in two tasks of semantic retrieval, verb generation (VG) and levels of processing (LOP), as well as in a Sternberg working memory (WM) task. For both VG and LOP, the non-semantic control condition required deciding if a visually-presented word was in upper- or lowercase letters. The semantic task for VG required covertly generating an appropriate verb to a noun, and for LOP, covertly deciding if the word was abstract or concrete. For WM, difficult (6 letters to remember) and easy (0 letters to remember) conditions were alternated. Brain images were collected in 7 oblique axial planes rotated 25° from AC-PC line to image frontal and cerebellar structures. Functional MRI (fMRI) was obtained with a T2*-weighted gradient echo spiral pulse sequence (1.5T, TR=630 ms, TE=40 ms, flip=66°, inplane resolution=2.35 mm, thickness=6 mm). Results from 6 subjects show overlapping activation in left prefrontal areas in VG, LOP, and WM tasks, including portions of inferior and middle frontal gyri and insular cortex. However, WM produced more bilateral activation, suggesting that frontal semantic working memory networks invoked by VG and LOP may be part of a verbal working memory network. In cerebellum VG, LOP, and WM activated right HVIIB. More superiorly, VG activated right posterior HVIIA, whereas WM activated vermis and bilateral HVI (right > left). Less cerebellar activation was observed for LOP than for VG even when frontal activation was comparable, suggesting possible differences in cerebellar computation during performance of these semantic tasks. Supported by NIH (F32NS09628) and NIA (AG 12995).

441.3

RESPONSE-RELATED ERP COMPONENTS IN NEWLY LEARNED AND OVERLEARNED S-R COMPATIBILITY TASKS. C. A. Christensen* and K. J. Drake. Dept. of Psychology, Vassar College, Poughkeepsie, N.Y., 12601.

Response times (RTs) and ERP components were evaluated in two S-R compatibility tasks to evaluate whether RT prolongation makes a late response-related component (RRC) more evident. Subjects pressed either a right or left key in response to the words LEFT and RIGHT (overlearned) or CAJ and PUY (newly learned), following an instruction stimulus that indicated whether a compatible or incompatible response was required. Obvious P2, P3a and P3b peaks were observed in both the instruction-stimulus and action-stimulus ERPs, though a fourth positive peak was observed in the action-stimulus ERP only, consistent with its identification as response related. The RRC, which followed the P3b, was more evident when RTs were prolonged, on incompatible trials and early in learning the new task. RT splits of trials into the fastest and slowest halves showed that the RRC was pronounced on slow trials, though it often was less apparent or absent on fast trials because of temporal overlap with the P3b component.

441.4

WORD REPETITION EFFECTS ON EVENT RELATED POTENTIALS (ERPs) AND MEMORY IN HUMAN VOLUNTEERS BREATHING AIR OR ISOFLURANE C.Villemure, G.Plourde*, P.Fiset, D. Lorrain. Départ. Physiologie, Univ. de Montréal; Anesthesia Dept. Royal Victoria Hosp., McGill Univ., Montréal (Québec) Canada, H3A 1A1.

It is unclear whether repetition effects on ERPs reflect processes underlying implicit or explicit memory. We recorded ERPs while presenting 24 digitized spoken words repeated 10 times to 11 non-medicated controls (CTRL) and 13 volunteers (EXP) breathing 0.26% end-tidal isoflurane. Subjects responded to rare (10%) male voice targets. ERPs to female voice at Pz were averaged separately for the first, second, ninth and last presentations. Memory for words was assessed with explicit (free recall) and implicit (syllable completion) tasks. ERP repetition effects, involving a decreased P2 amplitude and an increased positivity of a late component (LC) for the repetitions, were observed in both grand averages. We measured P2 amplitude and the mean amplitude in the range 400-700 ms (CTRL) and 800-1050 ms (EXP). For CTRL (mean μV $\pm$SD; first, second, ninth and last presentations respectively) P2: 7.64$\pm$3.76, 6.81$\pm$4.72, 5.04$\pm$3.41, 4.92$\pm$4.21; LC: -1.47$\pm$3.44, -0.94$\pm$3.51, 2.79$\pm$3.55, 1.10$\pm$2.94 and for EXP P2: 1.95$\pm$2.72, 0.41$\pm$2.39, 0.88$\pm$1.85, 0.85$\pm$1.59; LC: 0.33$\pm$2.38, 0.92$\pm$1.83, 1.78$\pm$1.74, 1.62$\pm$2.24. Planned comparisons between the first and the mean of the 3 other presentations showed that, for CTRL, the amplitude differences were significant (P2: $p=0.037$; LC: $p=0.048$). They approached significance in the EXP (P2: $p=0.063$; LC: $p=0.070$). ANOVAs' results showed that both groups had implicit memory (CTRL (mean % $\pm$SD): hits=14.39$\pm$11.24; base=3.79$\pm$4.35; $p=0.029$; EXP: hits=6.41$\pm$4.99; base=3.52$\pm$4.11; $p=0.013$). T-tests indicated that only CTRL had explicit memory (CTRL: 21.97%$\pm$14.08; $p<0.01$; EXP: 1.28%$\pm$2.63; $p>0.1$). We observed evidence of ERPs repetition effects in the presence of implicit memory without free recall. We conclude that ERPs repetition effects are not linked to explicit memory measured with free recall.
Support: FCAR, FRSQ, F.E.S. of UdeM.

441.5

RETROACTIVE INTERFERENCE IN MEMORY: AN EVENT-RELATED POTENTIAL STUDY. I.Tendolkar, M.C.Doyle, M.D.Rugg . Wellcome Brain Research Group, School of Psychology, University of St Andrews, St Andrews, Fife KY169JU, UK. (SPON:European Brain and Behavior Society)

The purpose of this study was to search for an ERP correlate of retroactive interference. Word pairs were presented visually in two different study lists. At test subjects (n=16) were presented with a mixture of new items and the first members of the word pairs from list 1. They made an old/new judgement, and for items judged old, attempted to recall the item with which it had been associated in the first study list. In the interference condition, the same word was paired with different items in the two study lists (A-B; A-C). In one control condition item pairs were presented in only one list (D-E; F-G), while in another control condition same item pairs were presented in both study lists (J-K; J-K). Associates from the interference condition were recalled reliably less often than in each of the control conditions. Event-related potentials to items correctly judged old, and for which the second word was recalled, differed reliably from new items. This "old/new" effect was reliable over midline and lateral sites, and was greater in magnitude over the left than over the right hemisphere. There was no reliable difference in the size or topografy of this effect between interference and control conditions. The findings suggest that event-related potentials reflect retrieval operations yielding episode-specific information. There was no evidence for a specific electrophysiological correlate of the behavioral interference effect.

supported by Wellcome Trust, UK

441.6

EVENT RELATED POTENTIALS TO FALSE MEMORY J. Hopfinger*[1,2] E. Duezel[1,3] A.P. Yonelinas[2] E. Tulving[4] G.R. Mangun[1,2]. Center for Neuroscience[1] & Dept. of Psychology[2], University of California, Davis CA 95616; Dept. of Clinical Neurophysiology, Otto-v-Guericke University, D-39120 Magdeburg, FRG[3]; Dept. of Psychology, University of Toronto & The Rotman Research Institute of The Baycrest Center, Ontario M6A 2E1 Canada[4]

False memories were examined using a modified version of Roediger and McDermotts (1995) false recognition paradigm based on the semantic relatedness effect. Event related potentials (ERPs) were obtained during a recognition memory test for studied words, semantically related words ("lures") and nonstudied words, eliciting either remember, know or new responses. A large proportion of lures were falsely accepted. Lures and studied words elicited smaller N400 components over parietotemporal electrodes than nonstudied words, indicating semantic priming effects. Remember responses to studied words and to lures elicited more positive going ERPs than rejections and know responses. This positivity to remember responses appeared bifrontally within a time window from 600 to 1200 ms and was most prominent over right frontal electrodes. There were no qualitative differences in the ERPs elicited by remember responses to accepted studied words and lures. But more remember responses were given to accepted studied words than to lures, and this was reflected in slightly more positive going ERPs to accepted studied words as compared to accepted lures. These data indicate different ERP components to remembering and knowing and provide evidence for a physiological basis for the mental phenomenon of remembering events that did not happen. [This work was supported by NSF, NIMH, NINDS and the James S. McDonnell Foundation].

441.7

AN EVENT-RELATED BRAIN POTENTIAL CORRELATE OF CENTRAL EXECUTIVE FUNCTION: ADDITIONAL EVIDENCE. I. Kiss*, H. Pazderka-Robinson, and D. Schopflocher. Toupin Psychophysiology Lab. & University of Alberta, Edmonton, AB T5G 0B7.

In a previous series of experiments, we employed a working memory (WM) task (Morris and Jones, 1990) shown to provide access to the central executive (CE) as described in Baddeley's (1986) model of WM. Subjects viewed variable length (e.g., 2 to 5 item) series of individually presented digits followed by sets of (e.g., 2) digits. Dynamic revision (updating) of WM contents was required for subjects to correctly indicate whether sets matched the single digits immediately preceding them. We recorded ERPs to provide non-invasive, on-line access to brain electrical activity, permitting potential behavioral and electrophysiological measures associable with CE function (updating). However, the ERP data were also interpretable as reflecting non-CE processes, including activities associable with storage components of WM (i.e., rehearsal) and processes related to proactive interference. We present recent studies designed to investigate both alternate explanations. One employed visual and auditory versions of a memory updating task. A second involved specific manipulations to compare ERP correlates of rehearsal and updating and incudes several interstimulus intervals to examine proactive interference. These appear to indicate that our original interpretations of our ERP findings as reflecting CE activity remain valid. Implications for Baddeley's model and basic and applied uses for this methodology are discussed.

Baddeley, A.D. (1986). Working Memory. Oxford: Oxford University Press
Morris, N. & Jones, D.M. (1990). Br. J. Psychol, 81, 111-121.

Supported by Natural Sciences and Engineering Research Council (NSERC, Canada) and the Northern Alberta Regional Geriatric Program.

441.8

H.M. REVISITS THE TOWER OF HANOI PUZZLE. Y. Xu* and S. Corkin. Dept. of Brain and Cognitive Sciences, and the Clinical Research Center, Mass. Institute of Technology, Cambridge, MA 02139

Cohen and Corkin (1985) reported that the amnesic patient, H.M., acquired the skill for solving the Tower-of-Hanoi puzzle during 4 days of testing, ultimately solving the problem in the minimum number of moves. During their testing, a set of questions was posed to H.M. repeatedly to encourage his adoption of an effective strategy. However, when H.M. was tested again by Gabrieli et al. (1987) with a different experimenter and minimal experimenter-subject interaction (no questions posed), he failed to improve his performance in solving the puzzle. Gabrieli et al. attributed H.M.'s failure to the absence of frequent experimenter-subject interactions. The present study compared H.M.'s performance in the two conditions, administrated by a single experimenter. H.M. (age, 69; education, 12) was tested twice, first, following the Cohen and Corkin protocol, and then 4 months later, following the Gabrieli et al. protocol. On both occasions, H.M. failed to improve his performance either within or across the four days of testing. The average number of moves on each testing day was greater than twice the minimum number of moves. To our surprise, of the 8 normal control subjects (NCS) (mean age, 68.3; mean education, 12) tested under the Gabrieli et al. protocol, only 2 NCS improved their performance consistently and solved the puzzle in the minimum number of moves; the other 6 NCS performed like H.M. Among the NCS, success in solving the puzzle was not related to verbal IQ, or gender, but may have been related to prior experience in puzzle solving.

Supported by NIH grant AG06605 and the McDonnell-Pew Program in Cognitive Neuroscience.

441.9

EVIDENCE THAT NICOTINE-INDUCED COGNITIVE ENHANCEMENT DOES NOT INVOLVE ANTIOXIDANT ACTIONS. K.B. Bjugstad, B.M. Crandall, and G.W. Arendash*. Dept. of Psychology and Biology, and Institute on Aging, Univ. of South Florida, Tampa, FL 33620.

Studies involving non-neural peripheral tissues have yield conflicting results regarding an antioxidant vs. pro-oxidant potential of nicotine. Given the large number of studies that demonstrate nicotine's ability to enhance cognition in both animals and humans, the present study sought to: 1) determine if nicotine exerts antioxidant effects on brain tissue *in vitro* and *in vivo*, and 2) determine if any *in vivo* antioxidant effects of nicotine could be linked to it's ability to improve cognition. Male rats (S.D., 5 mos) were injected twice daily with nicotine (0.8 mg/kg ip.) or vehicle for 10 days. Beginning on Day 5 of injections, rats were tested for acquisition and retention in the Morris water maze. Nicotine-treated rats showed greater retention for the platform location than the saline group. Neurochemical analysis of neocortex, hippocampus, and neostriatum from behaviorally tested rats revealed no group differences in reactive oxygen species formation (DCF fluorescense) or lipid peroxidation (TBAR formation) for any brain region studied. In a follow-up *in vitro* study involving addition of nicotine (7.5-50 mM) or Desferol (7.5-50 mM) to neocortical homogenates, nicotine increased TBAR formation by 334 - 706% while Desferol decreased TBAR formation by 24 - 82% compared to saline-treated tissues. The results of these studies suggest that antioxidant actions may not be involved in nicotine's ability to improve cognition and that, to the contrary, nicotine appears to exert pro-oxidant actions on brain tissue. Supported by the USF Alzheimer's and Parkinson's Disease Research Fund.

441.10

EFFECTS OF SMOKING ON EVENT-RELATED POTENTIALS IN A MEMORY SCANNING TASK. J. A. Pineda*, C. Herrera, and A. Sandler. Cognitive Science and Neuroscience Departments, University of California, San Diego, La Jolla, CA 92093-0515.

Event-related potentials (ERPs), reaction times (RTs), and performance accuracy were recorded from smokers during the performance of a memory task under a smoking and a 12-hour deprived condition. Stimuli consisted of lists of words presented one at a time in blocks of five to a monitor located in front of the subject. Following each block, a probe word was presented. Subjects responded to the probe as either part of the previously displayed set (in-set) or not (out-of-set). In the smoking condition, the magnitude of the P300 was larger to both in-set and out-of-set probes. In contrast, latencies were different only to the in-set probes. Amplitudes exhibited a U-shaped function to in-set probes according to their position on the list. That is, words in the first and fifth position typically elicited larger potentials than those in the middle positions. These primacy and recency effects were enhanced in the smoking condition. Although latencies did not exhibit primacy and recency effects, RTs to in-set probes did show an inverted U-shaped function. Words in the first and fifth position exhibited faster RTs, with smoking causing a uniform slowing of RTs but a preservation of the inverted U-function. The P300 amplitude results support the hypothesis that smoking produces an enhanced attentional state, most likely due to the effect of nicotine on perceptual function. The P300 latency results suggest some degree of specificity in these effects. That is, memory search is specifically enhanced when an in-set but not an out-of-set probe occurs. The RT results indicate that smoking causes a slowing of motor function.

441.11

IMPLICIT MEMORY FOR NONVERBAL ASSOCIATIONS UNDER DUAL TASK CONDITIONS. G. Musen*, S.Sehgal, and W.S. Brooks Barnard College, Columbia University, New York, NY 10027.

Familiarity and unitization of items play a role in priming of nonverbal associations. When a color and an abstract shape were unitized (e.g., presented as a colored shape), evidence for associative priming was obtained. However, no evidence for priming of color-shape associations was observed when the color and abstract shape were presented separately (e.g., a shape on top of a colored frame) (Musen & O'Neill, 1994). Perhaps working memory is needed to form the association, and its capacity is reached when the stimuli are neither familiar or unitized. In contrast, when only one of these processes must be carried out during learning, priming of associations readily occurs.

If working memory resources are crucial for priming, then a secondary task should interfere with priming. The present experiments examined this issue. We used both a visual and an auditory secondary task across two different experiments. In the visual task, unitized colored shapes were presented on top of the same or different colored backgrounds as the shapes. Participants were to image the color and shape and to decide whether the background color was the same as or different from the color of the shape. There were no errors on the secondary task, and evidence for associative priming was still obtained. However, in the auditory tone-counting task, errors were made in the counting task and associative priming was not obtained. It is possible that a more difficult visual secondary task would also disrupt priming. We suggest that difficulty of both the stimuli to be learned and task demands contribute to the probability of obtaining priming. When the stimuli are neither unitized nor familiar, or the secondary task is difficult, priming is disrupted. Thus, implicit memory is affected by resource limitations. Supported by Edward J. King Memorial Fund.

441.12

BEHAVIOR AND PHYSIOLOGY OF PICTURE-WORD PROCESSING IN HUMAN WORKING MEMORY. S.L. Provencal*, J.D. Lewine, P. Amrhein, J.C. Edgar, K. White, K. Paulson, and W.W. Orrison. New Mexico Institute of Neuroimaging and University of New Mexico, Albuquerque, New Mexico.

A Sternberg serial probe recognition task was used to examine mnemonic processing for within and between stimulus domains. At the start of each test block, subjects memorized a set of target stimuli (e.g., memory load of 2, 4, or 6 items) and determined whether a subsequently presented probe stimulus was a member of the memorized target set. Three domains of stimuli were evaluated - written words, corresponding visual pictures, and corresponding spoken words. Target-probe evaluations were either intra-domain (e.g., picture-picture) or inter-domain (e.g., picture-written word). During task performance, behavioral data were collected in conjunction with high-density electroencephalographic data (62-channels) and whole-head magnetoencephalographic data (122-channels).

Preliminary results demonstrate that inter-modality processing (e.g., picture targets and spoken probes) is markedly different from intra-modality processing for both early and long latencies. On the other hand, within the visual modality, intra- and inter-domain processing (picture-picture and picture-word) engaged comparable brain mechanisms. For example, auditory probes being evaluated with respect to picture targets tended to elicit bilateral responses, whereas picture and word probes being evaluated with respect to picture targets elicited left-lateralized long-latency responses.

MEG source modeling suggests that left hemisphere auditory cortex may be active even in the evaluation of visual stimuli (e.g., picture-picture trials). The available data suggest that both modality specific and modality independent mnemonic mechanisms are engaged in the processing of visual and auditory mnemonic information.

441.13

CHARACTERIZING rCBF RESPONSE IN A PET STUDY OF GRADED WORKING MEMORY LOAD J.D. Van Horn*, J.L. Austin-Lane, B.S. Kirkby, G. Esposito, D.R. Weinberger, and K.F. Berman Unit on PET, CBDB, NIMH, Bethesda, MD 20892

Few studies have been conducted to document the neural response to increasing cognitive load. Such responses may demonstrate linear and nonlinear (e.g. quadratic, cubic, etc.) components as the demand for neural resources increases. Within the context of a working memory paradigm, we used PET to investigate the neurophysiological effects of varying the amount of remembered material and the number of manipulations performed on that material. Nine normal subjects were studied with oxygen-15 water IV bolus PET (10 mCi/scan) on a GE Advance 3-D PET scanner. Seven different cognitive conditions were repeated four times for a total of 28 rCBF measurements pseudorandomly distributed over three days. Stimuli consisted of strings of two, four, and six elements (50% numbers, 50% letters per stimulus) presented on a computer screen for 1.5 sec. For the six cognitive conditions, subjects responded after a 4 sec delay. For the control condition, subjects immediately read the string aloud. The amount of remembered material was varied across three separate conditions (2, 4, or 6 items) in which no manipulation was required; after the delay, subjects repeated the stimulus string unaltered. The number of manipulations was varied across three separate conditions in which subjects were cued with four items upon which they performed one, two, or three separate sequencing steps during the delay; after the delay, they responded with the reordered string. All scans for each subject were aligned via the method of Woods *et al.* (1992) and analyzed with SPM95 (Friston *et al.*, 1995) using polynomial contrasts to map voxels in which there was a statistically significant ($p<0.001$) linear, quadratic, or cubic rCBF response to increasing either the amount of remembered material or the number of manipulations performed on the material. Linear increases in rCBF with increasing manipulation were seen in dorsolateral prefrontal cortex (Brodmann areas 9 and 46), bilateral inferior parietal lobule and cerebellum, whereas quadratic and cubic contrasts were also present in the cerebellum. Increasing the amount of remembered material resulted in linear rCBF increases highlighted in primary motor, supplementary motor, and premotor cortices, whereas both linear and quadratic effects were present in cerebellar areas. These data provide a systematic experimental framework for characterizing the parameters of rCBF response to varying working memory load and suggest a method for measuring the "carrying capacity" of working memory systems in patient groups.

441.14

THE EFFECTS OF HIPPOCAMPAL AND NEOSTRIATAL LESIONS ON DURATION ESTIMATION, SHORT-TERM MEMORY, AND TRANSFER PERFORMANCE OF TEMPORAL EVENTS IN THE PIGEON. J.M. Dose*, P.J. Kraemer & J.F. Zolman. Depts. of Psychology and Physiology, University of Kentucky, Lexington, KY 40506.

The role of the avian hippocampus and neostriatum in duration estimation, short-term memory (STM) and transfer performance of temporal events was studied using a duration matching-to-sample (MTS) procedure. Pigeons were initially trained on two duration MTS problems in succession. Signals consisted of 2- and 10-s durations of red or white light presented from either the ceiling or front wall of a standard operant chamber. Choice stimuli consisted of red, green, yellow and blue solid colors presented on two side keys. Correct choices were followed by 2-s access to grain before a 15-s ITI commenced; incorrect choices immediately produced the 15-s ITI. Before and after receiving bilateral aspiration lesions of either the hippocampus or neostriatum, pigeons were tested with normal (nonprobe) and probe trials in duration estimation, short-term memory, and transfer performance tasks. Nonprobe trials for all test sessions consisted of red or white signals immediately followed by the appropriate choice stimuli. Probe trials in duration estimation consisted of the red or white signals being presented for intermediate signal durations (3-, 5-, 7-, or 9-s). Compared with pre-lesion performance, pigeons were more likely to respond "long" following hippocampus lesions. Probe trials in STM involved 2- or 10-s presentation of red and white signals; but, 1-, 3-, 6-, 9-, or 12-s delays were imposed between signal offset and choice onset. Pre-lesion performance showed a "choose-short" effect, but this effect was disrupted following neostriatum lesions. Transfer probe trials consisted of red or white signals followed immediately by unexpected choice stimuli. Transfer performance was not disrupted by either lesion condition. Results will be discussed in terms of changes in temporal processing following disruption of normal hippocampal and neostriatal functioning.

441.15

DUAL-TASK PERFORMANCE OF ROTARY PURSUIT AND EYEBLINK CLASSICAL CONDITIONING. J. T. Green* and D. S. Woodruff-Pak. Dept. of Psychology, Temple Univ., Phila., PA 19122.

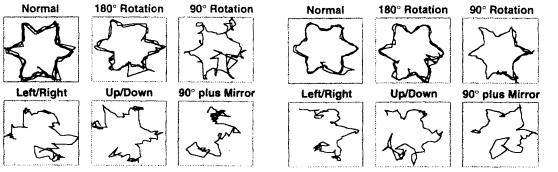
The purpose of this study was to test the hypothesis that different nondeclarative memory tasks are mediated by different brain substrates. A total of 80 young, normal adults participated in one of four conditions. Twenty subjects were simultaneously engaged in EBCC and rotary pursuit for half of an experimental session and EBCC alone for the other half. Twenty control subjects were engaged in EBCC alone for an entire session and twenty control subjects were engaged in rotary pursuit alone for an entire session. A fourth group of twenty subjects was simultaneously engaged in an explicitly unpaired paradigm and rotary pursuit for half of a session and an explicitly unpaired paradigm alone for the other half. The presumed essential brain substrate for EBCC is the cerebellum. The presumed substrate for rotary pursuit is ipsilateral motor cortex. The results revealed that performance of the rotary pursuit task caused excessive blinking during EBCC which mimicked learning by increasing eyeblinks during the CR period. When rotary pursuit was discontinued in the second half of a session, CRs declined, but to a level that remained significantly above performance of the EBCC-alone group, demonstrating that acquisition was slowed but not prevented during dual task performance. The results from simultaneous performance of the explicitly unpaired paradigm and rotary pursuit supported this interpretation because responses to the unpaired CS declined after these subjects stopped simultaneous performance of the rotary pursuit task. Subjects learned rotary pursuit in all conditions. The results provide evidence for the independence of different measures of nondeclarative memory and highlight the difference between performance and learning of EBCC. *Supported by AG09752 from NIA.*

441.16

ADAPTING TO ROTATED AND/OR INVERTED COORDINATE SYSTEMS. R.L. Savoy*, K.M. O'Craven, R. Bergida *The Rowland Institute for Science, 100 Edwin H. Land Blvd, Cambridge, MA 02142; MGH-NMR Center, Charlestown, MA; Boston University, Boston, MA*

We implemented a computer controlled generalization of the "mirror-tracing" task. Subjects controlled the movement of a cursor on a computer display to trace a fixed path. In addition to traditional mirror inversions (either left/right and up/down), we implemented 180°-rotation (which is the composition of a left/right with an up/down mirror transformation), 90°-rotation, and 90°- rotation-plus-mirror-inversion. Instructions discouraged the use of cognitive strategies and encouraged visuo-motor learning.

When both axes are reversed (180° rotation), most subjects find the task much easier than for either reversal alone. Performance in both speed and accuracy is much better than for the mirror inversion task, even shortly after the first trial begins. Performance on the 90° rotation task is particularly intriguing. Many subjects exhibit extremely poor performance for a period of time on the order of 20-120 seconds, and then suddenly perform at a level comparable to the 180° rotation task. This change is in the absence of reported cognitive changes. In contrast, when there is also a mirror inversion added to the 90° rotation, performance remains poor. (Support: The Rowland Institute for Science)



Performance for first 30 seconds *Same subject, after 3 min practice per condition*

441.17

LEARNING OF SPATIAL AND MOTOR-RESPONSE SEQUENCES IN PATIENTS WITH PARKINSON'S DISEASE. L.L. Helmuth*[1,2], U. Mayr[3], and I. Daum[1]. University of Tübingen, Germany; University of California at Berkeley, U.S.A.; University of Potsdam, Germany.

Sequence learning studies of patients with Parkinson's disease (PD) have yielded inconsistent results. Among the unresolved questions in this field are: a) whether PD patients are impaired in the acquisition of a sequence or in its execution, b) whether PD leads to comparable deficits in motor and attentional abilities, and c) whether sequence learning is related to the severity of PD.

The serial reaction time learning task used in the present study allows a separate assessment of the learning of two different types of sequence: one a stimulus-response sequence and the other a sequence of spatial positions in which stimuli appear on a screen. We compare the performance of PD patients in early and more advanced stages of the disease, and control subjects, in both types of sequence learning. Preliminary evidence suggests that early PD patients do not differ from controls in their ability to improve performance in the presence of either type of sequence, whereas more advanced PD patients may show impairments.

Supported by Deutscher Akademischer Austauschdienst (DAAD) grant A-95-04418.

441.18

EFFECTS OF FRONTAL LESIONS ON THE LEARNING OF A NEW SENSORIMOTOR MAPPING. M.J. Chouinard*, I. Rouleau and F. Richer. Lab. de Neuroscience de la Cognition, Univ. du Québec à Montréal, Montréal, Qc, Canada, H3C 3P8.

In a previous study, we have observed that frontal lobe lesions in humans result in a longer tracing time in a mirror drawing task compared to temporal patients and controls (Chouinard & Rouleau, 1994). That slowing was mainly due to more frequent episodes of non-progressive tracing suggesting that frontals have difficulty adapting to a new sensorimotor mapping. To further explore that deficit, we tested patients with frontal excisions, temporal excisions and controls (N=39) in a computerized tracing task which modified sensorimotor mappings (Cunningham,1989). The subject had to move a cursor on a graphic tablet from the center to one of 4 peripheral targets. Ten blocks of 16 trials were administered: 2 blocks with no transformation, 3 blocks with a rotation of 180° of motor space, 1 block with no transformation, and 4 blocks with a reflection of the horizontal axis of motor space. Subjects visually controlled their movements on a monitor placed in front of them and an occluding screen hid the arm from view. Frontals showed longer trace lengths in the reflection condition than the other groups but showed normal improvement with practice. Tracing speed did not increase with practice in frontals as it did for the others. The results suggest that frontal lesions affect sensorimotor control when a new mapping is involved but not sensorimotor learning.

This research was sponsored by Medical Research Council of Canada and Fonds de la Recherche en Santé du Québec.

441.19

STM & LTM FOR A SPATIAL LEARNING TASK IN BLACK CAPPED CHICKADEES AND WHITE CROWNED SPARROWS. D.W. Lee* & N.S. Clayton. Section N.P.B., UC Davis, Davis, CA, 95616.

Hippocampal involvement in the distinction between short-term memory (STM) and long-term memory (LTM) has been demonstrated repeatedly: STM is independent, but various forms of LTM depend upon the integrity of the hippocampal formation. Some species of food storing birds spend more time caching, cache more items, and leave their caches for longer periods of time during the fall than in the spring indicating that LTM may be superior during the fall. Also, the hippocampus of food storers 1) increases in volume and neurogenesis during the fall; 2) is necessary for memory formation of spatial but not visual cued tasks; and 3) is larger than non-storers. Furthermore, storing birds are better than non-storers at spatial learning tasks. If LTM for spatial learning in food storing birds is hippocampus-dependent but STM is not, storers should have superior LTM but not STM compared to non-storers. To test this hypothesis, food storing black capped chickadees *(Parus atricapillus)* and non-storing white crowned sparrows *(Zonotrichia leucophrys)* were trained to search and remove string knots on the faces of 7 visually and spatially unique "peanut feeders" to find food hidden in 1 of the feeders. They were then tested either 5 min or 24 h later. Seven new visually identical feeders were placed in the same spatial locations as those used during training; thus to find the food birds could rely on spatial but not visual cues. Data indicated that 1) both species showed memory in fewer trials when given the short rather than the long interval; 2) at both intervals, chickadees showed memory in fewer trials than sparrows; and 3) at the long interval, chickadees showed better memory for this task than sparrows. Results partially confirm the hypothesis: the food storing chickadee shows faster acquisition of both STM and LTM and superior LTM formation for this spatial learning task compared to the non-storing sparrow.

Supported by NIMH grant MH11109-02 (DWL).

441.20

PREDICTIVE CODING OF STRUCTURED FORMS THROUGH DIVERGENCE OF CORTICAL CONNECTIONS P.Grandguillaume †,Y.Burnod†, K.S.Rockland‡ SPON: European Brain and Behaviour Society †CREARE,UPMC,75252 Paris ‡Dept Neurol,U Iowa,Iowa City,IA52242

We investigate how cortical circuits along sensory pathways may handle prediction while extracting growing selectivity to structured form, accounting for the reciprocal constraints between the connectivity and the spatial and temporal form of activity. We have developed a model, continuous in time and space, of the neural mechanisms underlying this cortical function. The formalism accounts for the propagation of intrinsic and extrinsic neuronal activity within and between the cortical areas of a processing pathway. The visual pathway is taken in reference. The operation performed by the model takes place progressively during the first few hundred of milliseconds of the steady input flow.

In the model, the populations of horizontally connected neurons in each area integrate and selectively transmit synchronous spatial patterns of input activity. This activation sustained over the population plays the role of an error function. The basic operation in the model consists in progressively turning down, through backward propagation, this unspecified activation on the basis of the predicted spatial pattern transmitted to upper processing stages. The resulting spatio-temporal pattern of forward activity acts as a reinforcement for a few neurons in the upper area, while enabling the learning of specific metrical relations associating neurons of other overlapping populations. A structured form is thus broken down into a set of nested spatio-temporal forms relating locally defined temporal axes to longitudinal cortical regions. The activities of the populations of neurons representing fine components of the structured form are thus extracted from those related to coarser components while being kept embedded in them. The model also settles the related issue of how to transmit faithfully fine components parsed at lower stages through successive stages given the increasing divergence and convergence of forward connections.

The 3 stage network-model uses excitatory and local inhibitory spiking neurons and synchronization in the millisecond range. The growing divergence of forward connections parallels that of backward connections from stage n to both stages n-1 and n-2. The network is fed with arrays of roughly synchronized trains of spikes reproducing structured patterns within a matrice of random spikes. Classical receptive fields of neurons in upper stages shrink progressively, reflecting the changing spatio-temporal patterns of inputs. Unsupervised learning yields selectivity to structured patterns in populations of the upper stages. The model is discussed in relation to physiological data and to other models of interaction between the three sets of cortical connections. PG is supported by MESR.

442.1

NEURONS OF THE LATERAL NUCLEUS OF THE AMYGDALA THAT PROJECT TO THE BASAL NUCLEUS ARE INNERVATED BY CALBINDIN AND PARVALBUMIN IMMUNOREACTIVE NEURONS. W. Woodson*, and J. E. LeDoux. Center for Neural Science, New York University, 4 Washington Place, New York, N.Y. 10003

The lateral nucleus (LA) is the sensory interface of the amygdala and the origin of projections to other amygdala nuclei, such as the basal nucleus (B), that participate in the intraamygdala processing of sensory inputs. In order to determine whether LA neurons that project to the basal nucleus are innervated by different subpopulations of inhibitory cells, the retrograde tracer cholera toxin subunit B (CTb) was injected into B, and the immunocytochemical double labelling technique was employed to identify retrogradely labelled cells and calcium binding neurons in LA. Following CTb injections into B, retrogradely labelled cells were found throughout the rostro-caudal extent of LA. Morphologically distinct types of parvalbumin (PARV) and calbindin (CAL) immunoreactive (IR) neurons made presumptive axo-somatic terminals upon neighboring projection neurons in LA, as determined by confocal microscopy. Each projection cell was usually innervated by many PARV-IR and CAL-IR neurons. Terminals were made between 0.5 and 1 μm of section thickness. These results emphasize that inhibitory cells modulate the excitatory responses of LA neurons, and probably influence the flow of sensory information through intraamygdala microcircuitries. Supported by NIH-MH38774-10.

442.2

ANATOMICAL TRACING AND LESION STUDIES OF VISUAL PATHWAYS INVOLVED IN FEAR CONDITIONING MEASURED WITH FEAR POTENTIATED STARTLE. C-J SHI* & M. Davis. Dept. of Psychiatry & Psychology, Yale Univ., New Haven, CT 06508

Previous work indicated that complete removal of the visual cortex did not prevent the expression of fear potentiated startle to a visual conditioned stimulus (CS) in rats. In contrast, electrolytic or chemical lesions of rostral perirhinal cortex fully blocked the expression of conditioned fear using a visual CS. However, perirhinal lesions made before training did not block acquisition and rats could still relearn if retrained after perirhinal lesions. These results suggest that subcortical visual inputs may project directly or indirectly to the perirhinal cortex to allow visual information to access the basolateral amygdala, also critical for fear potentiated startle. This hypotheses was tested using both anatomical tracing and lesion methods.

Following iontophoretic injection of biotinylated dextran amine into different subareas of the lateral posterior nucleus of the thalamus (LP) in rats, heavy anterograde labeling was differentially present in areas Oc2L, Oc2M and caudal area Te2. Additionally, a light to moderate density of labeled fibers and terminals was consistently detected in the perirhinal cortex. This labeling concentrated in the fundus of the rhinal sulcus and extended to all layers. Electrolytic or chemical lesions after training were placed in LP, or lateral geniculate complex (LG) or both and the effects on fear conditioning were examined. Either electrolytic or chemical lesions of both LP and LG, but not lesions of LP or LG alone, blocked the expression of fear potentiated startle. However, neither electrolytic nor chemical lesions of LG/LP prevented relearning of conditioned fear trained with a visual CS. These results implicate two parallel thalamocortical pathways involved in fear conditioning with a visual CS. In addition, there must be other visual pathways to the basolateral amygdala which by-pass the visual thalamus to allow relearning to occur. [Supported by MH47840, MH00004 and AFOSR F49620-93-1-0293 DEF]

442.3

FEAR-POTENTIATED STARTLE (FPS) IN THREE INBRED MOUSE STRAINS. S. Carlson*, W.A. Falls, J.G. Turner, S.E. Webster, and J.F. Willott. Dept. of Psychol., Northern Illinois Univ., DeKalb, IL 60115.

The FPS paradigm has been widely used to study the neural basis of conditioned fear, but the unique advantages of mice as animal models have yet to be tapped in FPS research. The present study uses mice of the C57BL/6J (C57), DBA/2J (DBA), and CBA/CaJ (CBA) inbred strains in the first of a series examining FPS in mice.

Amplitude of startle was measured in 1-month-old mice in a quiet background and when a 70 dB SPL, 12 kHz tone (to be the conditioned stimulus, CS) was present. Mice were then randomly assigned to either an experimental or control group (N= 10 per group). On two consecutive days, experimental mice were presented with 10 paired tone-shock training trials (a 30-sec CS coterminating with a 0.5-sec, 0.6-mA foot shock). Control mice received unpaired tones and shocks. Startle responses were measured 1 day later with 10 CS-on and 10 CS-off trials. A ratio of startle amplitude with CS-on/CS-off was computed for each mouse for pre- and post-training sessions. These were subjected to a 3 (strain) X 2 (training condition) X 2 (pre-post test session) mixed ANOVA. FPS occurred if the CS-on/CS-off ratio was significantly larger in post- vs. pre-training sessions in experimental animals but not in controls.

None of the control groups exhibited a significant change in the CS-on/CS-off ratio for pre- vs. post-training sessions. In experimental C57 mice, the CS-on/Cs-off ratio increased significantly, by 41%, in post-training sessions, indicating the occurrence of FPS. In experimental DBA mice, the ratio increased by 146%, which was significantly greater than the increase in C57s; thus, FPS was more pronounced in DBA mice. In experimental CBA mice, the pre- post-training difference in the CS-on/CS-off ratio was only 2%, a nonsignificant effect. These findings indicate that inbred mice can be used as subjects for FPS, but that dramatic differences occur among strains with the present procedures. Elucidating the factors that underlie these differences should provide new insights into the neurobiological mechanism of FPS.

Supported by NIH grant R37 AG07554 to J.F.W.

442.4

EFFECTS OF TONE-DEPENDENT FEAR CONDITIONING ON HEART RATE IN MICE (C57BL/6J). O. Stiedl*, S. Milanovic, and J. Spiess. Department of Molecular Neuroendocrinology, Max Planck Institute for Experimental Medicine, Hermann-Rein-Str. 3, D-37075 Goettingen, Germany

Male mice were subjected to a single fear conditioning (acquisition) trial in order to investigate specific changes in heart rate (HR) and *c-FOS* immunoreactivity during and after a tone-dependent (memory) retention test. HR was measured in freely moving mice with an ECG transmitter system.

Groups of animals were subjected to different conditioning sequences during acquisition. For paired conditioning, animals were placed in a box for 3 min, then presented with a pulsed (5 Hz) tone stimulus (CS: 75 dB SPL, 10 kHz, 30 s) followed by a foot shock (US: 2 s, 0.7 mA, constant current). In addition, separation (unpairing) of CS and US by 30 s and 60 s, respectively, and exposure to tone and foot shock alone was carried out to determine whether HR effects and *c-FOS* production are specific for associative learning. Twentyfour h after training, tone-dependent retention was tested in the homecage without tone (preCS phase) followed by tone stimulation (CS phase) for 3 min each.

In all animals, HR was elevated to maximal physiological levels during the fear conditioning trial. It was impossible to differentiate, whether an animal received shock or not. However, HR was significantly increased and HR variability was significantly decreased in the CS phase compared to preCS values of tone-dependent retention in mice subjected to paired conditioning. Unpairing of CS and US by 30 s triggered a significant increase of HR and decrease in HR variability during the first but not the second and third minute of the CS phase. CS did not significantly affect HR in any other group.

Brain sections of mice are currently evaluated for *C-FOS* production with regard to the sequence and presence of CS and US during acquisition.

(Supported by the Max Planck Society and SFB 1497/C3)

442.5

EXTERNAL INHIBITION OF FEAR-POTENTIATED STARTLE: A PROCEDURE FOR INVESTIGATING THE EFFECT OF DISTRACTING STIMULI ON THE EXPRESSION OF CONDITIONED FEAR. W.A. Falls* , S. Heldt & S.E. Webster Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

Presentation of a novel stimulus in compound with a well trained conditioned stimulus results in a significant reduction in the conditioned response. Pavlov described this as external inhibition. When considered in the context of conditioned fear, external inhibition represents one way in which fear can be inhibited. With the recent interest in identifying the neural systems responsible for the inhibition of fear, it would seem important to consider the systems that may be responsible for external inhibition. The purpose of the present experiments was to investigate a procedure for producing external inhibition of conditioned fear using the fear-potentiated startle paradigm.

Rats were given 10 noise+shock pairings on each of 2 days. Testing occurred 1 day later. All rats showed conditioned fear to the noise as defined by greater startle amplitude in the presence of the noise than in its absence (i.e., fear-potentiated startle). Fear-potentiated startle to the noise was reduced on trials in which a novel light was presented in compound with the noise (i.e., external inhibition). This external inhibition cannot be attributed to the light&noise compound being perceived as a unique (i.e., configural) stimulus because rats given noise+shock training followed by light+shock training showed enhanced fear-potentiated startle on light&noise compound trials. This external inhibition also cannot be solely attributed to an inhibitory effect of the light on the startle reflex because a novel light did not reduce startle that was potentiated by an i.p. injection of strychnine. Instead, it appears that the light reduces the ability of the noise to produce fear-potentiated startle. Importantly, this reduction in fear-potentiated startle appears to be a function of the magnitude of fear-potentiated startle. Rats were given either 0, 1, 3 or 5 days of noise+shock training. Fear-potentiated startle to the noise increased as a function of training. The amount of external inhibition was related to the magnitude of fear-potentiated startle: the greater the fear-potentiated startle, the more it was reduced by the novel light.

Because a great deal is known about the neural systems involved in the expression of fear-potentiated startle, this procedure may prove useful for investigating the neural systems involved in the inhibition of fear by novel stimuli.

Supported by the Dept. of Psychology, Northern Illinois University

442.6

NMDA Antagonists Infused into the Amygdala Block Second-Order Conditioning, Measured with Fear Potentiated Startle. J.C. Gewirtz*, W.A. Falls, & M. Davis, Dept. Psychiatry, Yale University, New Haven, CT 05508, & Dept. Psychology, Northern Illinois University, DeKalb, IL 60115.

Pavlovian second-order conditioning is produced by pairing a neutral stimulus (S2) with another stimulus (S1) that previously was paired with an unconditioned stimulus (US). This was done by presenting a 3.7-s S1 coterminating with a 0.6-mA footshock, and then placing S2 in serial compound with S1. To reduce extinction of first-order conditioning, additional S1-US pairings were intermixed with S2-S1 pairings. Acquisition of second-order conditioning, assessed by fear-potentiated startle, was blocked by intra-amygdala infusion of the NMDA receptor antagonist, AP5. This suggests that NMDA receptor-dependent plasticity in the amygdala is critical for the acquisition of second-order fear conditioning.

However, AP5 could have interfered with first-order conditioning on the intermixed S1-US pairings. Hence, it is necessary to have S2-S1 and S1-US pairings on different days, so that AP5 can be infused only on S2-S1 days. To enhance second-order conditioning, which is typically weaker using this procedure, we varied the temporal arrangement between S1 and the US. Second-order conditioning was more robust with a brief S1-US interstimulus interval (0.2 s as opposed to 3.2 s), and was especially strong when fear was sustained throughout the duration of S1, achieved by varying the onset of the US during a 6.7-s S1. The fact that contiguity between S2 and the fear elicited by S1 was more critical than contiguity between S2 and the onset of S1 itself, provides support for the involvement of "stimulus-response" learning in second-order conditioning.

These procedures produce robust second-order conditioning, even when S2-S1 and S1-US pairings are presented on separate days. We are thus in a position to assess further the role of glutamate receptors in the amygdala in the acquisition of second-order conditioning. [Supported by MH 47840, MH 00004 and AFOSR F49620-93-1-0293 DEF].

442.7

THE EFFECTS OF SEPTAL LESIONS ON CONTEXTUAL SHIFTS IN CONDITIONED FEAR. P.D. Sparks* and J.E. LeDoux. Dept. of Exp. Psychology, NYU, NY, NY 10003.

Lesions of the septum increase conditioned fear (freezing) expressed in the presence of contextual, but not phasic conditioned stimuli (Sparks and LeDoux, 1995). To investigate this effect further, the effects of septal lesions were examined in a context shift paradigm. Two weeks after surgery, septal rats and sham operated controls were placed for 15 minutes in the shock chamber (chamber A, Coulborn shock chamber), without the presence of tones or shocks (day 0). For each of the following two days (days 1-2), all rats were returned to chamber A and presented with two tone-shock parings (10kHz, 74dB tone for 20 secs, co-terminating with a 0.5mA shock, of 0.5 sec duration). On days 3-4, half the septal and sham operated rats (non-shift group; NS) were placed in chamber A, and the other half (shift group; S) were placed in a new chamber (chamber B, very distinct from chamber A), and were presented with two tone alone trials daily. The time spent freezing during the tone and during a 20 sec period before the tone (freezing to the chamber cues) for days 1-4, were used as the measures of conditioning. As shown before, NS-septal rats freeze more to the chamber cues but not to the tone as compared to NS-controls. S-control rats froze less to both the tone and chamber cues as compared to the NS-controls. S-septal rats froze more than S-controls to the chamber cues, but froze similarly to the tone as compared to the NS-septal rats, thus did not show reduced freezing to the tone in the new chamber. These findings clarify the role of the septum in contextual control of conditioned fear. We are presently investigating the effects of hippocampal lesions on this task as well. Supported by MH46516, MH00956.

442.8

MEDIAL PREFRONTAL CORTEX (mPFC) AND THE EXTINCTION OF FEAR: DIFFERENTIAL EFFECTS OF PRE- OR POST- TRAINING LESIONS M.A. Morgan,* J.E. LeDoux, Dept of Psychology, New York University, NY, NY 10003

In a previous study of fear conditioning, pre-training lesions of ventral mPFC produced resistance to extinction to a CS without affecting acquisition. Stage 1 of the present study looked at the effect of these lesions, now made post-training, on the expression and extinction of the same fear conditioning task. Rats underwent 2 days of tone (CS)-footshock (US) pairings prior to receiving mPFCv (n=16) or sham (n=13) lesions. Two weeks later they underwent extinction training: 2 trials a day of the CS presented alone, until extinction criterion was met. Acquisition and extinction of conditioned fear, as measured by freezing behavior to the CS and to the context, were examined. mPFCv lesioned rats froze significantly less than controls during the test of context acquisition, and showed no other effects. Stage 2 looked at reacquisition and reextinction of the same task. Lesioned rats froze more than controls during reacquisition of the CS and context, and showed resistance to extinction to both. Comparing lesioned rats with their own performance during stage 1, they froze somewhat less to the CS during reacquisition than acquisition, and now showed resistance to extinction, relatively more to the CS than to the context. These results confirm earlier findings that mPFCv is involved in extinction of fear responses, and suggest that an intact mPFCv may be necessary during acquisition in order for normal extinction to occur. mPFCv may be involved in the inhibition of conditioned responding during extinction, perhaps by utilizing appropriate contextual cues. Supported by MH46516, MH00956.

442.9

INACTIVATION OF THE LATERAL AND BASAL AMYGDALA DISRUPTS ACQUISITION AND EXPRESSION OF CONDITIONED FEAR J. Muller, Z. Fridel, and J.E. LeDoux*. Center for Neural Science, New York University, NY, NY 10003.

The lateral and basal nuclei of the amygdala are believed to play an essential role in the acquisition of conditioned fear. However, previous studies involving temporary inactivation of these regions found significant disruption of the expression but not the acquisition of conditioned fear using a contextual conditioning procedure. In the present study we reexamined the effects of inactivation of the lateral and basal amygdala on the conditioning of fear responses to an explicit auditory conditioned stimulus (CS) and to contextual simuli. The $GABA_A$ agonist, muscimol (0.5 μg in 0.5 μl saline), or vehicle was infused in the amygdala through indwelling cannulae about 30 minutes before conditioning and testing. The day after conditioning (pairing of the tone CS with a footshock), rats were exposed to the CS in a novel context. A day later, they were reexposed to the original training apparatus (with no CS presented). On each testing day, conditioned fear was assessed by measuring the percent of time accounted for by freezing behavior during a three minute period. Localization of the cannulae in the lateral and basal amygdala was verified histologically. Rats with muscimol infusions prior to conditioning and vehicle prior to testing exhibited significantly less freezing during exposure to the CS in a novel context and during exposure to the conditioning context than did animals with vehicle infusions before conditioning and testing. Animals given vehicle prior to conditioning and muscimol prior to testing froze less to the CS and context than did controls. Thus, activity in the lateral and basal amygdala appears to play an essential role in both the acquisition and expression of fear conditioning both to the CS and its context. Supported by grants MH 38774, MH 46516, and MH 00956.

442.10

DISRUPTION OF FEAR CONDITIONING TO CONTEXTUAL STIMULI BUT NOT TO A TONE BY LESIONS OF THE ACCESSORY BASAL NUCLEUS OF THE AMYGDALA.

P. Majidishad, D. G. Pelli* , and J. E. LeDoux. Center for Neural Science, New York University, NY, NY 10003.

Lesions of the hippocampus or amygdala interfere with the conditioning fear responses to contextual stimuli, and projections from the hippocampus to the amygdala are believed to be involved in this form of learning. Two major recipients of hippocampal projections are the basal (B) and accessory basal (AB) nuclei. In the present study we examined the effects of electrolytic lesions of these two regions on the conditioning of fear responses to contextual stimuli and to a tone. Twenty days after surgery, rats received two days of tone - shock pairings (2 trials each day). The next day, cumulative time spent freezing to a 60 sec tone presentation (no shock) was measured in a novel test chamber. Contextual conditioning was assessed by measuring the amount of freezing occurring during a 600 sec period in which the rat was in the conditioning chamber (no tones or shocks presented). Lesions were verified histologically. Relative to controls (n=10), lesions of AB (n=6) significantly reduced freezing to the conditioning context but not to the tone. In contrast, neither freezing to the tone nor the context was significantly impaired by lesions of B (n=3). However, the number of animals with acceptable lesions of B was small and the effect approached significance. AB thus seems to be an important link in the pathways involved in contextual conditioning. While B may also contribute, further studies are needed to clarify its role. Supported by grants MH 38774, and MH 00956.

442.11

AMYGDALA LESIONS BLOCK AVERSIONS TO CONTEXTUAL CUES BUT NOT THE ABILITY OF CONTEXTS TO SERVE AS OCCASION SETTERS. D.M. Skinner* and D. van der Kooy. Dept of Anatomy and Cell Biology, University of Toronto, Toronto, Ontario, M5S 1A8.

Occasion setting involves discriminative cues that predict whether or not a target stimulus will be followed by a motivational event (the US). We have previously suggested that most of the discriminative control is not due to simple, Pavlovian associations between the discriminative cue and the US or between the discriminative cue and the target stimulus, but to a more complex, higher-order type of association among all three elements. In a typical conditional discrimination one novel context predicts that access to sacharin will be followed by an injection of LiCl while a second context predicts that saccharin will be followed by a saline injection. In a Pavlovian control procedure one novel context is paired directly with LiCl and the second context is paired with saline injections. Rats trained on the conditional task suppress fluid consumption in the first relative to the second context and also avoid the context associated with LiCl on a place choice test, suggesting that Pavlovian associations and occasion setting are acquired simultaneously. Rats trained on the Pavlovian task also show a large aversion to the context associated with LiCl on a place test but never show fluid suppression in the aversive context, suggesting that the Pavlovian properties of the context are not sufficient to produce occasion setting. In the present study rats with bilateral ibotenic acid lesions of the amygdala were compared to sham controls on a contextual discrimination task. Both groups learned to suppress fluid consumption in the first, relative to the second, context. Sham lesioned rats also showed a large aversion to the first context on a choice test whereas the amygdala lesioned rats did not significantly avoid the context associated with LiCl. Amygdala and sham Pavlovian control groups were given direct pairings of context 1 with LiCl and context 2 with saline. Again, sham lesioned rats showed large aversions to context 1 while amygdala lesioned rats showed no hint of a place aversion. These findings suggest that the Pavlovian aversive properties of the context are not necessary for acquisition of occasion setting and support previous behavioral data suggesting that simple Pavlovian conditioning and occasion setting are fundamentally different.

Supported by MRC Canada

442.12

AMYGDALA AND PERIAQUEDUCTAL GRAY SUBDIVISIONS NECESSARY FOR THE ACQUISITION AND PERFORMANCE OF DISCRETE AND CONTEXTUAL FEAR CONDITIONING.

P.S.F. Bellgowan*, F.J. Helmstetter and D.J. Bailey

Department of Psychology, University of Wisconsin, Milwaukee, WI 53201.

A neural circuit that includes the amygdala and periaqueductal gray (PAG) has been shown to be essential for the expression of Pavolvian fear conditioning to both discrete unimodal and complex contextual cues. However, the role of specific amygdala and PAG subdivisions in these forms of learning is not clearly defined. Independent test for the roles of the lateral (LA) and central (CeA) nucleus of the amygdala, or dorsal (dPAG) and ventral PAG (vPAG) in the acquisition and performance of fear conditioning using contextual and discrete cues were conducted. Rats were prepared with chronic cannula in either the LA, CeA, dPAG, or vPAG and injected with the $GABA_A$ agonist muscimol (MUS; 0.25 μg). Injections were given either before training, before testing conducted 24 and 48 hrs after training, or both in a factorial design. During training subjects received four paired presentations of white noise (WN; 10 sec / 72 dB) with shock (1 mA / 1sec) in a distinctive observation chamber. Testing consisted of measuring fear responses both in the shock-associated training environment and to a single 5 min WN presentation independently during extinction. MUS applied to the LA prior to training reduced freezing to both discrete and contextual cues. Performance to both CS's was blocked by pre-testing MUS injections in the CeA, however, pre-testing injections in the LA only blocked performance to the discrete CS. Performance to the discrete CS was also attenuated by pre-testing MUS in the vPAG but not the dPAG. The present results suggest that differential intra-amygdala neural circuitry is necessary for the acquisition and performance of conditional fear to discrete and contextual cues.

Supported by NIDA grant DA09429

442.13

AMYGDALA KINDLING AND FEAR CONDITIONING

T. J. Hoeppner*, M. C. Smith, L.deToledo-Morrell, T. E. Reimschisel, J..A. Wolter. Departments of Neurological Sciences and Neurosurgery, Rush Medical College, Chicago, IL 60612

Since amygdala kindling produces permanent changes in the excitability of the amygdala with spread of electrical abnormalities to distant neural structures and fear conditioning is blocked by bilateral amygdala lesions, amygdala kindling might interfere with subsequent fear conditioning. Amygdala electrodes were implanted in rats. Half of the rats were kindled, while the other half were handled in the same way, but received no electrical stimulation. All rats received i.p. saline prior to each trial for comparison with the effects of anticonvulsants in other experiments. Kindling proceeded for 15 days or until 3 stage 5 seizures occurred. Two weeks after kindling was completed, rats were given 2 days of sound-footshock fear conditioning followed by 5 days of extinction. Fear conditioning was assessed by the duration of freezing during the 20 second conditioned stimulus (sound).

Kindled seizures started locally in the amygdala and progressively engaged the contralateral amygdala. Kindled rats showed impaired fear conditioning compared to the unkindled controls. Thus unilateral epileptogenic foci may produce behavioral deficits indicative of bilateral dysfunction long after the occurrence of seizures.

(Supported in part by Abbott Laboratories.)

442.14

AMPA RECEPTOR BLOCKADE IN THE BED NUCLEUS OF THE STRIA TERMINALIS (BNST) BUT NOT THE CENTRAL NUCLEUS OF THE AMYGDALA (CNA) DISRUPTS LIGHT-ENHANCED STARTLE: A NOVEL PARADIGM FOR THE ASSESSMENT OF ANXIETY IN RATS.

D. L. Walker* and M. Davis. Dept. Psychiatry, Yale University, New Haven, CT 06508.

The amplitude of the acoustic startle response is reliably increased if elicited in the presence of a cue previously paired with shock (fear-potentiated startle). We recently observed that startle is also increased, without prior conditioning, if testing occurs in a brightly lit vs. darkened environment (light-enhanced startle). For these experiments, startle was assessed for 20 min in the dark (phase I) followed by a second test (phase II) either in the dark or in a well-lit chamber. Although a small increase, from phase I to II, often was observed on dark->dark sessions, a large and significant increase was reliably observed on dark->light sessions. This increase was linearly related to light intensity, did not habituate either within or across sessions, and was blocked by the anxiolytic compound, buspirone. We believe that this phenomenon reflects the anxiogenic properties of bright lights in nocturnal animals.

To evaluate the anatomical substrates of light-enhanced startle, the AMPA antagonist, NBQX, was infused into two structures which project to the startle circuit. Infusions into the BNST disrupted the phase I to phase II increase for light-enhanced startle, but had no effect on fear-potentiated startle. In contrast, infusions into the CNA blocked fear-potentiated startle but had no effect on light-enhanced startle. This double dissociation may reflect complementary roles for the BNST and CNA in unconditioned anxiety vs. conditioned fear. [Supported by MH 47840, MH 00004 and AFOSR F49620-93-1-0293 DEF].

442.15

MEMORY MODULATION AND THE STRIA TERMINALIS.

A.M.Schneider*, E. Thomas, R. Ayyagari, M. Grossman and A. Kakoyannis. Depts. of Psychology, Swarthmore College, Swarthmore, PA 19081 and Bryn Mawr College, Bryn Mawr, PA 19010.

It is generally agreed that there are two systems involved in the formation of recently-acquired memories, an information system, devoted to the content of memory, and a modulatory system, regulating the strength of memory. Several brain areas have been implicated in the modulatory system, the stria terminalis (ST) among the more notable.

Evidence for ST involvement comes from studies that have shown that ST lesions affect retention, but the effect appears to be selective: ST lesions prevent enhancement or impairment of retention produced by drugs, but they do not affect retention produced by the training experience itself. The purpose of the present experiment was to reexamine the effect of ST lesions on retention produced by the training experience.

We gave ST-lesioned rats (male, Long Evans) two types of passive-avoidance training. In one procedure the animals were given a single training trial, and shock was set at 0.8 mA, 2 sec; in the other procedure the animals were given 3 training trials, 1 trial per day, and shock was set at 0.4 mA, 2 sec on each trial.

We found that ST lesions impaired retention produced by the single-trial procedure but had no effect on retention produced by the multitrial procedure, and we attributed the different effects to differences in arousal produced by the two training procedures. In the single-trial procedure when shock is intense, arousal is high, and thus ST lesions impair retention; in the multitrial procedure when shock is weak, arousal is low, and thus ST lesions do not impair retention.

Supported by a grant from the Howard Hughes Medical Institute.

442.16

REGIONAL PATTERNS OF c-*fos* EXPRESSION IN THE AMYGDALA SHIFT BETWEEN THE SEQUENTIAL STAGES OF ACQUIRING AN ODOR DISCRIMINATION.

U.S. Hess*, G. Lynch, and C.M. Gall. Dept. of Psychobiology, Univ. of CA at Irvine, 92717.

Expression of the activity-dependent gene c-*fos* was used to assess relative levels of neuronal activity in the amygdala of rats engaged in odor learning. Five groups of rats, each at a different point of the learning sequence, were used: 1) "Naive" controls were left in their home cage, 2) "Exploration" rats explored the novel training apparatus, 3) "Nose poke" rats performed a familiar nose-poke response for water rewards, 4) "Initial learners" were exposed for the first time to a pair of odors, response to one of which was reinforced with water, and 5) "Overtrained" rats performed the same, but well-learned discrimination. Comparisons (both absolute and internally normalized) of the experimental groups to the naive controls indicated that the basolateral nuclei had elevated levels of c-*fos* mRNA in all stages of the learning paradigm except for overtraining, the medial nucleus was elevated only in the exploration group, and the central nucleus was not significantly different from control in any of the experimental groups. Comparisons *between* the experimental groups produced the following results: **i)** exploration and initial learning had the largest overall effects on c-*fos* mRNA levels, **ii)** the medial nucleus was unusually active during exploration, and **iii)** the basolateral amygdala was disproportionately active during the shift from free to cued responding (i.e., initial learning). Activation of the medial nucleus may be part of a stress response triggered by a novel environment, while the unusual basolateral activation during initial learning could reflect the necessity of assigning positive and negative valences to discriminative cues. Supported by MH10510 and HD24236.

442.17

c-fos EXPRESSION IN ANTERIOR OLFACTORY NUCLEUS AND AMYGDALA ASSOCIATED WITH ODOR-GUIDED FEAR CONDITIONING IN THE RAT.

L.F. Schettino* and T.A. Otto. Dept. of Psychology, Rutgers University, New Brunswick, NJ 08903.

The activity of olfactory pathways and amygdaloid nuclei during an odor-fear conditioning paradigm was traced through immunochemical staining for the proto-oncogene c-fos.

Eight naive male Sprague-Dawley rats weighing 225-249g were used. The animals were handled for 5 minutes on 3 consecutive days. Experimental animals were trained on a fear conditioning paradigm with forward pairings of pyridine odor (Pyr) as the CS and shock as the US. The parameters (ten pairings of Pyr infused into the cage for 20s and shock (0.8mA) overlapping the last 2s. ITI=2 min.) were determined by pilot studies to produce robust "freezing" behavior to subsequent presentations of the odorant alone. Control subjects were not trained. Thirty min after training, all animals were deeply anesthetized by an IP injection of sodium pentobarbital (160 mg/kg) and perfused with PBS/paraformaldehyde 4%.

The brains were sectioned coronally (50μm) and immunostained for c-fos expression. The nuclear staining was quantified by light microscopy counts on a 0.5mm grid at 100X.

The results showed that, compared to controls, trained animals exhibited robust c-fos expression in the anterior olfactory nucleus (AON) and medial nucleus of amygdala ($p<0.05$). These preliminary data are consistent with the notion that the AON and the amygdala may participate in the acquisition or expression of odor-guided conditioned fear.

442.18

STRESS INCREASES PKC TRANSLOCATION IN THE AMYGDALA THROUGH NMDA RECEPTOR ACTIVATION, AND CONTEXTUAL CUES REINTRODUCE TRANSLOCATION.

T. J. Shors*[1], S. Elkabes[2], J. C. Selcher[1], I.B. Black[2]. Dept. Psychol, Prog. Neurosci., Princeton Univ., Princeton, NJ 08544, Dept. Neurosci. Cell Biol., UMDNJ/RWJ Med. Sch., Piscataway, NJ 08854.

The long-term consequences of acute stress on the [3H] phorbol 12,13-dibutyrate (PDBu) binding, an activator of protein kinase C (PKC), were investigated. Exposure to a brief episode of restraint and intermittent tailshock increased [3H] PDBu binding in the amygdala. The increase was persistent, lasting 24 hrs after stressor cessation. The contribution of NMDA receptor activation to the increased PKC translocation was evaluated. Rats injected with an NMDA antagonist prior to the stressor did not exhibit the increase in PKC activity in the amygdala 24 hrs later. Finally, we tested whether exposure to contextual cues associated with the stressor would reintroduce the increase in PKC translocation. Two groups of rats were stressed, one was briefly returned to the stressful context 96 hrs later. A group was exposed only to context and another was naive to stress and context. Stressed rats reexposed to the context 96 hrs later displayed an increase in PKC translocation in the amygdala, thalamus, and area CA1 of the hippocampus. These results suggest that a "psychological" manipulation, namely context reexposure, can induce long-lasting increases in PKC activity. These effects may represent biological indices of stress-related disorders, such as post-traumatic stress disorder (PTSD).

[McDonnel-Pew Foundation, Whitehall Foundation, NSF (IBN-9511027), ONR (N00014-92-J-1897) to TJS].

442.19

LESIONS OF THE NUCLEUS ACCUMBENS CORE, BUT NOT BASOLATERAL AMYGDALA OR SUBICULUM, DISRUPT STIMULUS-REWARD LEARNING IN A NOVEL AUTOSHAPING PROCEDURE. J.A. Parkinson, T.W. Robbins, B.J. Everitt. (SPON: European Neuroscience Association). Dept. of Experimental Psychology, University of Cambridge, UK.

This autoshaping paradigm involves the presentation of two identical visual stimuli on either side of a computer screen, one of which always predicts reward (CS+), the other never (CS-). Although there is no contingent reinforcement, animals show discriminated approach to the CS+ with decreasing latency (see T.J. Bussey Soc. Neurosci. Abstr. 1996). The underlying mechanism for this appears to be one of Pavlovian CS-US association, as animals continue to approach the CS+ even when this action prevents reward (omission schedule).

Animals with selective excitotoxic lesions of the core subregion of the nucleus accumbens were disrupted in this procedure; they took significantly longer to learn the discriminated approach never reaching a level of learning comparable to that of sham-operated controls. The nucleus accumbens receives major projections from cortical and limbic structures such as the basolateral amygdala, medial prefrontal cortex and hippocampal formation which have themselves been implicated in aspects of reward-related learning. We therefore investigated the effects on autoshaping of lesions of the cortical elements of these limbic-ventral striatal systems. However, excitotoxic lesions of the basolateral amygdala, ventral subiculum or dorsal subiculum had no effect on the acquisition of approach behaviour. This is in contrast to the effects of lesions of the anterior cingulate cortex which greatly impaired discriminated approach (see T.J. Bussey Soc. Neurosci. Abstr. 1996).

These results will be discussed in terms of the connectivity of afferents to the nucleus accumbens and also their potential functional significance in terms of cortico-striatal learning systems.

(Supported by Wellcome Trust Programme Grant)

442.20

A ROLE FOR THE ANTERIOR CINGULATE CORTEX OF THE RAT IN STIMULUS-REWARD LEARNING USING A NOVEL AUTOSHAPING PROCEDURE. T.J. Bussey*, B.J. Everitt and T.W. Robbins. Dept. of Experimental Psychology, University of Cambridge, UK.

Recent results from our laboratory suggest that the anterior cingulate cortex may be involved in stimulus-reward learning (Bussey et al., in press). For example, quinolinic acid-induced lesions of this region disrupt the acquisition of a task in which rats were required to learn 8 stimulus-reward associations concurrently (Muir et al., Soc. Neurosci. Abstr. 20:1211, 1994). While suggestive, this effect could have been due to factors other than the stimulus-reward learning requirements of the task, such as the high degree of inter-item interference, the discriminability of stimuli, or the relatively long inter-stimulus intervals in this task. We have therefore developed a method for studying stimulus-reward learning which attempts to eliminate these possibilities. The procedure is carried out in an apparatus which allows for the automated presentation of computer-graphic stimuli (Bussey et al., 1994). White vertical bars are presented on a VI40 sec schedule on either the left or the right of a computer screen. One of these stimuli, (the CS+), is always followed by a sucrose pellet. The other, the CS-, is never followed by reward. As a result of this training, rats come to approach the CS+ much more often than the CS-, and with decreasing latency. The introduction of an omission schedule, under which approaches prevent the delivery of reward, does not disrupt discriminated approach, suggesting that rats' behaviour in this task is governed by knowledge of the stimulus-reward association acquired via Pavlovian conditioning.

This procedure was used to assess stimulus-reward learning in animals with cytotoxic lesions of the anterior cingulate, posterior cingulate and medial frontal cortices. Anterior cingulate-lesioned animals did not demonstrate normal Pavlovian approach, making significantly more approaches to the CS- than sham-lesioned animals. Furthermore, these animals approached the CS+ with significantly longer latencies than did shams. Medial frontal cortex-lesioned animals demonstrated normal task acquisition, but had longer overall approach latencies. Posterior cingulate lesions had no effect on this task. The implications of these results for the neurobiology of motivation and reward will be discussed. (Supported by a Wellcome Trust Programme Grant.)

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS VI

443.1

PERIRHINAL AND POSTRHINAL CONNECTIONS WITH THE RAT ENTORHINAL CORTEX R.D. Burwell*[1] and D.G. Amaral[2]. [1]Center for Behavioral Neuroscience, State University of New York at Stony Brook, Stony Brook, NY 11794-2575, and [2]University of California, Davis, Department of Psychiatry and Center for Neuroscience, Davis, CA 95616.

We have previously demonstrated that entorhinal projections to septal versus temporal levels of the dentate gyrus (DG) of the rat originate from largely non-overlapping bands of the entorhinal cortex (EC). A caudolaterally situated band projects to the septal half of the DG while intermediate and medially situated bands project to the third and fourth quarters, respectively (Dolorfo, C.L. and Amaral, D.G., 1996). Intrinsic entorhinal connections also respect this band-like organization such that the associational projections preferentially terminate within the band of origin (Dolorfo, C.L. and Amaral, D.G., 1996).

In light of this new partitioning of the EC into DG projecting bands, the present study examined the topography of the EC connections with the regions that provide its primary cortical input, the perirhinal (PR) and postrhinal cortices (POR). The study is based on anterograde and retrograde injections placed in the PR, POR, and EC. The PR to EC projections were strongest to the laterally situated (and septally projecting) band; progressively lighter projections terminated in the intermediate and medial bands. The return entorhinal projection to the PR appeared to arise from a somewhat larger portion of the entorhinal cortex. The PR to EC projections are also topographically organized in that rostral PR projects more strongly to rostral EC and caudal PR projects more strongly to caudal EC. The POR to EC projection is similar to the perirhinal projection but appears to more extensively involve the laterally situated band.This pattern of connectivity suggests that the POR may exert a stronger influence than the PR over the septal half of the DG. Funded by Grant NS 16980 to DGA and NS 09247 to RDB.

443.2

HIPPOCAMPAL-DEPENDENT MEMORY CONSOLIDATION: AN EVALUATION OF THREE HYPOTHESES. A. Koerner*, M.J. Thomas, M.P. Weisend, and R.J. Sutherland. Depts. of Psychology and Physiology, University of New Mexico, Albuquerque, NM 87131.

Hippocampal damage is thought to cause temporally-graded retrograde amnesia by interrupting a long-lasting memory consolidation process. We examined 3 factors which have been posited to influence this consolidation process: time, practice, and memory load. Each factor was examined using different training regimens in the hidden platform version of the Morris water task before the hippocampus was damaged. Afterwards, each group of rats was retested in the pool. The influence of time was examined by providing 24 training trials and waiting 10 weeks before surgery. The effect of practice was evaluated by overtraining (240 trials over 10 weeks). The influence of memory load was evaluated by providing training in 10 different rooms. Hippocampal damage was produced with intrahippocampal injections of kainate/colchicine. Hippocampal damage produced equivalent, severe retrograde amnesia for the original pool problem in all training conditions. There is no support for the idea that extensive hippocampal damage produces temporally graded retrograde amnesia. Likewise, anterograde amnesia was also severe and not affected by prelesion experience. This study provides no evidence for a long-lasting, hippocampal-dependent memory consolidation process. [Supported by UNM]

443.3

THE SPECIFICITY AND TEMPORAL CHARACTERISTICS OF RETROGRADE AMNESIA AFTER HIPPOCAMPAL LESIONS. M.P. Weisend*, R.S. Astur, & R.J. Sutherland. Depts. of Psychology and Physiology, University of New Mexico, Albuquerque, NM 87131.

Anterograde and retrograde amnesia are consistent findings after hippocampal lesions. Anterograde amnesia is known to be specific for certain types of memories. It is less clear if retrograde amnesia shares this specificity. Further, the severity of retrograde amnesia is sometimes reported to vary as a function of the interval of time between learning and lesion. The specificity and temporal characteristics of retrograde amnesia were examined in the following experiments. Rats were trained on configural and elemental versions of the Morris water task, Pavlovian fear conditioning, or appetitive operant conditioning. Rats were then returned to their home cages for periods of time that varied between 1 day and 36 weeks. Lesions were produced by intrahippocampal injections of quaint/colchicine. Rats with hippocampal damage showed retrograde amnesia for both configural and elemental versions of all tasks. In contrast, anterograde amnesia was observed for only configural information in these rats. The severity of retrograde amnesia did not vary as a function of time for any version of any task. These results indicate that the hippocampal formation may play a more general role in memory than previously thought. In addition, these results yield no evidence for a hippocampal-dependent memory consolidation process. Supported by UNM's RAC, SRAC, & RPT funds.

443.4

TEMPORALLY GRADED RETROGRADE AMNESIA FOLLOWING SEPARATE AND COMBINED LESIONS OF THE PERIRHINAL CORTEX AND FORNIX IN THE RAT. K.A. Wiig*, L.N. Cooper, and M.F. Bear. Department of Neuroscience, Brown University, Providence, RI, 02912, USA.

The involvement of the perirhinal cortex and fornix in retrograde and anterograde amnesia in the rat was investigated. Male, Sprague-Dawley rats were trained on a series of five visual discrimination problems at distinct time intervals prior to receiving bilateral, electrolytic lesions of the perirhinal cortex, fornix, combined lesions of both these structures, or sham operations. Following recovery from surgery, rats were re-tested on the preoperatively learned discrimination problems, as well as learning a new discrimination and discrimination reversal. Results indicated that all animals with lesions exhibited temporally graded retrograde amnesia, whereby memories acquired in the recent past (1-3 weeks) were impaired, and memories acquired in the remote past (6-8 weeks) were spared. There was no difference in the magnitude of retrograde amnesia between the three lesion groups. Animals in the perirhinal, fornix, and combined lesion groups were able to learn a new discrimination problem at a rate comparable to control rats, however, the animals with lesions were impaired at learning the discrimination reversal. The perirhinal, fornix and combined lesion animals also exhibited a significantly faster forgetting rate over a two week retention interval than control rats. These results suggest that medial temporal structures including the perirhinal cortex and fornix are involved in the consolidation of mnemonic information, and that their involvement in this process occurs over a discrete period of time.

Supported by NIH and the Charles A. Dana Foundation.

443.5

RETROGRADE MEMORY FOR OBJECTS AND PLACES FOLLOWING LESIONS OF THE HIPPOCAMPUS OR PERIRHINAL CORTEX IN RATS. L.H. Francis, M.J. Glenn, and D.G. Mumby*. Department of Psychology, Concordia University, Montreal, Quebec, Canada.

Retrograde and anterograde memory for object information and retrograde memory for place information was assessed in rats with bilateral aspiration lesions of the perirhinal cortex (PRh) or NMDA lesions of the hippocampal formation (HPC; including hippocampus proper, dentate gyrus, and subicular complex). Rats learned three different object discrimination problems at 72, 24, and 1 hr prior to surgery, and a water-maze place navigation task at either 72 or 3 hr prior to surgery. Retention of all problems was assessed postsurgery. Lesioned rats did not differ from controls in performance on 30 postsurgery object discrimination trials, however PRh but not HPC rats made more errors on the first 10 trials than did control rats. PRh but not HPC rats were impaired at acquisition of a new object discrimination. HPC but not PRh rats were impaired on the place navigation task that had been learned prior to surgery. The findings suggest that HPC and PRh belong to dissociable memory systems that deal with information about places and objects, respectively. (Supported by the Natural Sciences and Engineering Research Council of Canada).

443.6

HIPPOCAMPAL DAMAGE DOES NOT IMPAIR OBJECT RECOGNITION IN RATS WITH NO PRESURGERY TRAINING. J. Yates *, L. Schattmann and D.G. Mumby. Dept. Psychology, Concordia Univ., Montreal, QC, Canada H4B 1R6.

Previous lesion experiments indicated that the hippocampus plays a nonessential role in the performance of object-recognition tasks by rats that receive extensive presurgery training. The present experiment examined whether hippocampal functions are critical for normal acquisition and performance of an object-recognition task in rats with no presurgery training. Rats with bilateral hippocampal lesions made either by aspiration or transient cerebral ischemia were trained on the nonrecurring-items delayed nonmatching-to-sample (DNMS) task at a 4-sec retention delay. Neither group performed worse than controls in terms of the number of trials or errors to reach a criterion of 76% over 3 consecutive sessions. Both groups of rats with hippocampal damage continued to perform as accurately as controls as the retention delay was increased to 15, 30, and 60-sec. The only exception to the foregoing observations was a single ischemic rat that failed to perform above chance levels of accuracy throughout 1000 DNMS trials at the 4-sec delay; notably, this was also the only rat that displayed postischemic seizures. The results suggest that the hippocampus plays a nonessential role in the performance of object-recognition tasks by rats. The findings are also consistent with those of previous studies, which suggest that postischemic seizures play a significant role in mediating ischemia-induced object-recognition deficits in rats.

Supported by Fonds pour la Formation de Chercheurs et L'Aide à la Recherche (FCAR).

443.7

MAPPING RECOGNITION MEMORY: PERIRHINAL AND HIPPOCAMPAL NEURONAL EXPRESSION OF C-FOS EVOKED BY NOVEL AND FAMILIAR VISUAL STIMULI. H. Wan[1], X.O. Zhu[1], J.W. Crabtree[1*], J.P. Aggleton[2], B.J. McCabe[3] and M.W. Brown[1]. [1]Anatomy Department, University of Bristol, Bristol BS8 1TD; [2]School of Psychology, UWCC, Cardiff CF1 3YG; Zoology [3]Department, University of Cambridge, Cambridge CB2 3EJ, UK.

The results of recording and ablation experiments in both rats and monkeys indicate that cortical areas near to the rhinal sulcus are important for judgement of the prior occurrence of individual stimuli; such judgement is central to recognition memory. We have determined the relative numbers of neurons activated by novel and familiar visual stimuli in different rat brain regions by immunohistochemically staining for the products (Fos) of the immediate early gene c-fos. Rats viewed two objects simultaneously: a novel object in the monocular field of one eye and a familiar object in the monocular field of the other eye. By this means sets of novel and familiar objects were viewed under the same conditions of alertness and with similar eye movements. The number of Fos stained neurons in area TE of the hemisphere first receiving information concerning familiar stimuli was only 74% ($P < 0.01$) of that in the contralateral hemisphere which had first received information concerning the novel stimuli; it was only 80% in perirhinal cortex ($P < 0.05$). No other sampled cortical areas, including the hippocampus, demonstrated significant differences. Moreover, the number of activated neurons in the hippocampal formation was very low. When rats were given either lorazepam or diazepam to block the acquisition of stimulus familiarity, the novel and repeatedly seen objects each activated similar numbers of neurones in TE and perirhinal cortex (± 4%). Further, preliminary results indicate a threefold increase in the ratio of the number of hippocampal to perirhinal neurons activated by compound stimuli (scenes or groups of items) rather than individual objects. Thus perirhinal cortex is involved in detection of the relative familiarity of individual items while the hippocampus becomes involved for groups of items. *Supported by the MRC & BBSRC.*

443.8

BILATERAL NEUROTOXIC LESION OF THE RHINAL CORTEX IN BABOONS IMPAIRS VISUAL RECOGNITION BUT NOT HABIT MEMORY. X. Blaizot, K. Meguro, C. Le Mestric, A. Rossard, F. Hansen, F. Mézenge, J.C. Baron and C. Chavoix*. INSERM U320, Centre Cyceron, Univ. of Caen, BP 5229, 14074 Caen, France.

Ablation studies in non-human primates have shown that the rhinal cortex (Rh) may play a major part in the visual recognition impairment observed after larger damage to the temporal lobe. In addition, we have shown that neurotoxic Rh lesions induce long-lasting brain glucose hypometabolism in young adult *Papio anubis* baboons (Meguro et al., Soc Neurosci Abstr, 1995, 21:1723) suggesting a crucial role of the affected Rh in both memory deficits and brain hypometabolism in Alzheimer's disease (AD). We report here, from 6 of our previously studied baboons, the effects of entorhinal (ERh) and perirhinal (PRh) neuronal loss induced by stereotaxic ibotenic acid lesions on visual recognition and habit memory; these memories were assessed by automated tasks of visual delayed nonmatching-to-sample (DNMS) with trial-unique drawings (delay and list length conditions) and ten-pair concurrent drawing discrimination, respectively. Pre-operatively, the baboons were trained on the DNMS task and tested on the delay condition with single targets of mixed delays (1 to 60 sec). Following MRI-guided neurotoxin injections into the Rh, at ~D30 and D95, the Rh group (n=3) was unimpaired on the DNMS delay condition but performed significantly worse than the sham-operated baboons (n=3) for the multiple-stimuli memorization (list condition with mixed list lengths up to 15 stimuli). However, at ~D45, no significant difference between groups was observed on the concurrent discrimination task. These results suggest that severe ERh+PRh neuronal loss (confirmed in each baboon) 1) induces similar memory performance dissociation than observed in AD, and 2) affects the storage capacity but not the 60 sec single-item memorization. Relationships between memory impairment and brain hypometabolism are currently being investigated.

443.9

MUSCARINIC RECEPTOR BLOCKADE IN PERIRHINAL CORTEX IMPAIRS VISUAL RECOGNITION MEMORY IN MONKEYS. Y. Tang* and T. G. Aigner. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892

Acetylcholine efflux in the inferior temporal gyrus (IT), perirhinal cortex (PR), and dentate gyrus (DG) of the hippocampus increases quickly in response to visually mediated behaviors (Tang & Aigner, *Soc. Neurosci. Abstr.* **21**:1447, 1995). To clarify further the contributions of acetylcholine in IT, PR, and DG to visual recognition memory, the effects of muscarinic receptor blockade by scopolamine were examined in three rhesus monkeys performing a computer-automated version of delayed nonmatching-to-sample (DNMS) with a list length of 20 trial-unique graphic symbols. Muscarinic receptors were blocked by 3 µl of 1, 5, 10, or 50 mM scopolamine injected unilaterally through a needle at a rate of 0.15 µl/min into the left IT, PR, or DG over a 20-min interval just prior to DNMS. Scopolamine administered into PR impaired significantly the monkeys' performance. The percent correct responses after all but the lowest dose were significantly lower than for saline control (RM ANOVA with Dunn's method, $n=6$, $p<0.05$). The best-effect dose was 10 mM, which reduced correct choices by approximately 10%; the lowest dose (1 mM) caused approximately a 5% reduction in correct choices. Scopolamine unilaterally injected into either IT or DG induced no significant change in performance (RM ANOVA, $n=6$, $p>0.05$). The results suggest that the cholinergic system in PR, but not in IT or DG, is critically involved in visual recognition memory in nonhuman primates, and provide the first direct pharmacological evidence to support the recently proposed role of rhinal cortex in this form of memory. Support: IRP/NIMH NIH.

443.10

FURTHER EVIDENCE OF A DISSOCIATION BETWEEN THE NEURAL SYSTEMS UNDERLYING OBJECT RECOGNITION AND SPATIAL MEMORY IN THE RAT. J.P.Aggleton, E. C. Warburton and R.K. Nakamura*. School of Psychology, University of Wales, Cardiff, U.K. CF1 3YG and NIMH, Rockville, MD20857

In two separate experiments we examined the effects of lesions in i) the fornix and the anterior thalamic nuclei and ii) the perirhinal, postrhinal and TE cortices. Rats were tested on a series of object recognition tests that used spontaneous preference for exploring novelty as a measure of recognition. Following exposure to the sample stimulus and a delay of 15 mins, it was found that radiofrequency lesions of the fornix or cytotoxic lesions of the anterior thalamic nuclei had no effect on the standard task i.e. they showed heightened exploration of the novel object similar to that in a group of control rats. In contrast, rats with extensive cytotoxic lesions of the rhinal cortices failed to discriminate the novel objects and differed significantly from their control animals. These same rhinal rats were, however, able to perform normally on a T-maze alternation task, even when increasing delays were added between choice run and test. In contrast, both the fornix and anterior thalamic lesioned rats were impaired on spatial memory tasks. This double dissocation contradicts the notion that the hippocampus is reliant on inputs from the rhinal cortices for spatial memory, and highlights a marked degree of independence in the functioning of these two regions. (Supported by U.K. MRC and Wellcome Trust)

443.11

PLACE- AND OBJECT-RECOGNITION DEFICITS FOLLOWING LESIONS OF THE HIPPOCAMPUS OR PERIRHINAL CORTEX IN RATS: A DOUBLE DISSOCIATION. M.J. Glenn* and D.G. Mumby, Dept. Psychology, Concordia Univ., Montreal, QC, Canada H4B 1R6

A within-subjects experiment tested the hypothesis that the hippocampus and perirhinal cortex are specialized to deal with information about places and objects, respectively. Rats were trained on a delayed matching-to-place (DMTP) task in a water maze at delays of 4, 60, 300, and 600 seconds, and a trial-unique delayed nonmatching-to-sample (DNMS) task, with objects as stimuli, at delays of 4, 15, 30, 60, and 120 seconds. Following presurgery training, the rats received either bilateral NMDA lesions of the hippocampal formation, bilateral aspiration lesions of perirhinal cortex, or sham surgery. Rats with hippocampal lesions were impaired on the DMTP task at all retention delays, whereas rats with perirhinal cortex lesions performed normally. By contrast, rats with lesions of the perirhinal cortex were impaired on the DNMS task, whereas rats with hippocampal lesions performed normally. This double dissociation suggests that the functions of the hippocampus and perirhinal cortex contribute independently to rats' ability to recognize places and objects, respectively.

This research was supported by a grant from the Natural Sciences and Engineering Research Council.

443.12

EFFECTS OF LATERAL vs. MEDIAL ENTORHINAL CORTEX ASPIRATION ON THE ACQUISITION OF ODOR-PLACE ASSOCIATIONS. T. Otto*, C. Ding, G. Cousens, K. Schiller. Dept. of Psychology, Rutgers University, New Brunswick, NJ, 08903.

We have recently developed a task in which successful performance requires acquisition and retention of associations between specific, spatially neutral odorants and unique spatial locations. Briefly, water deprived rats were placed in a square arena with a combination water- and odor- port in the center and in each of the four corners, and were trained to associate one odorant with one corner and a second odorant with a different corner; visits to the either of the other two corners were never reinforced. Rats were reinforced when they visited the corner that was "paired" with that particular odor.

Errors were divided into two types: "spatial memory errors" consisted in visiting one of the two corners that were never reinforced, and "odor-place association errors" consisted in visiting the incorrect one of the two corners at which reinforcement was available. Both sham-operated rats (n=19) and rats with lesions limited to lateral entorhinal cortex (n=4), but NOT rats with *combined* lesions of lateral and medial entorhinal cortex (n=4), rapidly acquired the spatial components of the task as reflected in the number of spatial memory errors across sessions.

While there was little difference in the performance of sham-operated rats and rats with lesions limited to lateral entorhinal cortex in spatial memory errors, rats with selective lesions of lateral entorhinal cortex were dramatically and persistently impaired in learning the associations between odors and spatial locations. These data suggest that, while lateral entorhinal cortex may not play a necessary role in spatial learning, it is critically involved in the acquisition of the relationships between odors and places, and are consistent with predictions based on both the anatomical projections to this area and their putative mnemonic functions.

Supported by NIH 1R03MH53880-01

443.13

EFFECTS OF RHINAL CORTEX LESIONS ON RETENTION OF PREOPERATIVELY LEARNED OBJECT DISCRIMINATIONS IN RHESUS MONKEYS. J.A. Thornton[1,2]* and E.A. Murray[1]. [1]Lab. of Neuropsychology, NIMH, NIH, Bethesda, MD 20892 USA, and [2]Dept. of Psychology, The George Washington University, Washington, DC 20052 USA.

To test whether the rhinal cortex (i.e., entorhinal cortex and perirhinal cortex) plays a time-limited role in information storage, eight rhesus monkeys were trained to criterion on two sets of 60 object discrimination problems, one set at each of two different time periods (16 weeks and 1 week), prior to bilateral ablation of the rhinal cortex (Group Rh; n=4) or no surgery (Group Con; n=4). Following a two-week rest period, monkeys were assessed for their retention of the object discriminations in two ways: 1) by administering 120 critical trials, one per object discrimination problem; and 2) by measuring the savings exhibited in relearning. Preoperatively, there was no group difference in the rate of acquisition, nor was there a difference in the rate at which the two sets were learned. Postoperatively, monkeys in Group Rh were significantly impaired relative to those in Group Con (68 vs. 91% correct) on the critical trials ($F(1,6) = 25.34$, $p < 0.05$). The ANOVA also yielded a significant main effect of set, with slightly better retention of the discriminations from the more recently learned set ($F(1,6) = 7.41$, $p < 0.05$), but there was no interaction of group and set. In addition, the mean savings for Group Rh was lower than that for Group Con (61 vs. 100%). Finally, monkeys in Group Rh subsequently learned a new set of 10 object discriminations as quickly as monkeys in Group Con, thus ruling out the possibility of a gross impairment in visual perception or discrimination abilities. These findings indicate that the rhinal cortex is critical for the retention of object discrimination problems that were learned up to 16 weeks preoperatively. The results provide no support for the idea that the rhinal cortex plays a time-limited role in information storage. *This work is supported by the NIMH, NIH.*

443.14

EFFECTS OF EXCITOTOXIC HIPPOCAMPAL LESIONS ON LEARNING AND RETENTION OF TOUCHSCREEN VISUOSPATIAL TASKS IN RHESUS MONKEYS. F.Y. Doré*, J.A. Thornton, N.M. White[1] and E.A. Murray, Laboratory of Neuropsychology, NIMH, NIH, Bethesda, MD 20892. [1] Department of Psychology, McGill University, Montréal, Québec, Canada.

Early as well as more recent studies (Angeli, Murray & Mishkin., 1993; Gaffan, 1994; Parkinson, Murray & Mishkin., 1988) in monkeys converge with the research on rodents: spatial learning and memory are impaired by damage to the hippocampal formation or fornix. In order to define further the conditions and the neural basis for this impairment in monkeys, the present study used a new automated allocentric spatial task. On each trial, naive rhesus monkeys were required to search for and to find a specific unmarked location (target square) within stimulus arrays presented on a touchscreen monitor. Each array was composed of 5 different objects, had a unique asymmetrical shape, and a unique location of the target square. Further, each array was presented in various positions and rotations across trials. Measures included: distance from, and number of touches and latency to find, the target square. After learning the task, two groups (n=3), matched for preoperative scores, either received bilateral ibotenate lesions of the hippocampus (fields CA1-CA4 plus dentate gyrus) based on MRI from those animals or were retained as unoperated controls. Hippocampectomized monkeys showed very good retention of the arrays learned preoperatively but were impaired in the first sessions of acquisition of new arrays. They learned additional arrays composed of identical instead of different objects at the same rate as controls. The transient and specific nature of the impairment suggests that the hippocampus plays a more limited role in allocentric visuospatial tasks than in navigational tasks. This work is supported by the McDonnell-Pew Program in Cognitive Neuroscience and the NIMH, NIH.

443.15

LONG-TERM EFFECTS OF NEONATAL HIPPOCAMPAL LESIONS ON SPATIAL RECOGNITION MEMORY IN RHESUS MONKEYS. M.C. Alvarado* J.W. Barnhill, and J. Bachevalier, Dept. of Neurobiology and Anatomy, Univ. Texas Houston Medical School, Houston, TX 77225.

Adult monkeys with early damage to the hippocampal region were unimpaired on visual delayed nonmatching-to-sample (DNMS) using trial-unique objects (Beauregard & Bachevalier, Soc. Neurosci., 23, 1993). However, these same animals were severely impaired on a test of relational memory, the transverse patterning problem (Alvarado et al., Soc. Neurosci., 25, 1995). In the present study, we further tested this group of animals on a location DNMS task. Subjects were four monkeys with neonatal aspiration lesions of the hippocampal formation and subjacent cortex (H), and five age-matched controls (N), 9-11 years old. Animals were trained to a criterion of 90% or 1000 trials. They were then given a performance test of 100 trials at each of four delays (30 s, 60 s, 120 s, and 10 min.). Group N learned the task in an average of 480 trials (one required additional correction training), whereas Group H reached criterion in an average of 835 trials (3 required additional correction training). A comparison of performance across delays for both Groups on object DNMS and location DNMS revealed no Group difference for object DNMS [$F(1,7)=2.24$, n.s.]. By contrast, Group H was severely impaired on location DNMS [$F(1,7)=40.52$, $p< .0001$]. Performance scores of each Group were affected by delay in both tasks, and that effect was greater in the location task. As a function of delay, performance on object DNMS dropped 2% for Group N and 4% for Group H, whereas on location DNMS, it fell 7% and 16%, respectively. These results confirm that early hippocampal formation damage produces a permanent and specific memory loss. They further suggest that the hippocampal formation is not required for visual recognition memory for delays of up to 10 min., but is required for aquisition and retention of relational information, even at short delays.

Supported by NIMH grant MH49728 to J.B.

443.16

EFFECTS OF FORNIX LESIONS ON THE TRANSVERSE PATTERNING TASK. J.L. Muir*, T.J. Bussey and J.P. Aggleton. School of Psychology, University of Wales College of Cardiff, CF1 3YG, Wales, UK.

Configural association theory (Sutherland and Rudy, 1989) is one of the more influential theories of hippocampal functioning, as it attempts to account for a wide range of disparate data. However, subsequent studies have failed to provide unanimous support for this theory. Recently these authors have provided a modification of their original theory, in which the hippocampus is hypothesised to increase the salience of configural representations, relative to elemental ones. One of the predictions of this theory is that disconnection of the hippocampus should impair the acquisition of the transverse patterning task, in which animals learn three "ambiguous cue" discrimination problems of the type A+B-, B+C-, C+A-. Animals must respond directly to the elements A, B and C, thus creating a conflict between associations involving representations of these elements, and configural representations comprised of these elements (Rudy and Sutherland, 1995).

Recently a method has been developed for testing rats using computer-graphic stimuli (Bussey et al., 1994) which provides an ideal opportunity for investigating this suggestion further. In this apparatus, animals must respond by nose-poking a small touch-sensitive response area in the centre of the stimulus. Thus the transverse patterning task, when administered using this method should, according to the analysis of Rudy and Sutherland (1995), be especially sensitive to hippocampal dysfunction. However, pilot studies in our laboratory have shown that animals with complete fornical transection were not only unimpaired on this task, they showed a *facilitation* of acquisition.

In the present study we examine subsequent data from fornix-lesioned animals on the transverse patterning task, with particular attention to the nature of learning by normal animals. We also examine session-by-session data from fornix-lesioned animals in an attempt to shed light on the precise effects of fornix lesions in discrimination learning in general. (Supported by MRC, U.K.)

443.17

THE EFFECTS OF LESIONS TO THE PERIRHINAL AND ENTORHINAL CORTICES OR FORNIX ON THE TRANSITIVE INFERENCE TASK.

Jeff Dusek* & Howard Eichenbaum.
Center for Behavioral Neuroscience, SUNY-Stony Brook, NY 11794.

Bryant & Trabasso (Nature 267:694, 1971) hypothesized that the ability to make a transitive inference (if A > B and B > C, then A > C) is dependent on the ability to recall previously learned premise information. The present experiment examined whether memory impairments consequent to hippocampal system damage would decrease transitivity levels on a nonspatial ordering of olfactory stimuli.

Male Long Evans rats underwent a combined lesion of the perirhinal and entorhinal cortices (PRER), which provide much of the sensory input to the hippocampus, a fornix lesion (FX), which disrupts subcortical connections of the hippocampus, or a sham surgery (SHAM). All rats were trained with four adjacent pairs of odors (A+ vs B-, B+ vs C-, C+ vs D-, D+ vs E-) that formed a sequential hierarchy (A > B > C > D > E). After acquiring all premise problems, rats were subsequently tested with the non-adjacent pair, BD, to assess their capacity for making a transitive inference (B > D). All subjects were able to acquire the four problems; however, only the SHAM subjects were able to develop a flexible representation of the odor series as evidenced by their ability to choose correctly on the pair BD. Conversely, both the FX and PRER subjects were limited to nonrelational strategies that permitted only a repetitive expression of the premise problems. These results extend previous findings linking transitivity to hippocampal function (Bunsey & Eichenbaum, Nature 379:255, 1996) and strongly support the conclusion that the hippocampus subserves declarative memory as characterized by the encoding and flexible expression of relational information. Supported by ONR, NIMH, NIA.

443.18

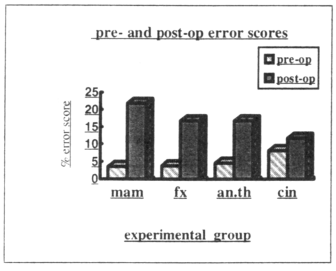
HOMING PIGEON VISUAL RELATIONAL LEARNING IS UNAFFECTED BY HIPPOCAMPAL LESIONS. V. P. Bingman*, C. Baker, L. V. Riters and R. Strasser.
Dept. of Psychology, Bowling Green State Univ., Bowling Green, OH, 43403.

Eichenbaum and his colleagues have proposed that the role of the hippocampal formation and neighboring structures in learning and memory can be best explained by assuming these structures participate in relational learning processes. To test the generality of their hypothesis, homing pigeons were trained on a visual relational learning task modeled on the olfactory task used by Bunsey and Eichenbaum (Behav. Neurosci. 107: 740-747, 1993). Specifically, 8 visual stimuli (A-F, X and Y), varying in color and shape, were presented on a video monitor in an operant chamber. Untreated control, neostriatal lesioned and hippocampal lesioned homing pigeons were trained to recognize and respond to 6 stimulus pairs (drawn from stimuli A-F) with the two stimuli of a pair being presented sequentially (e.g., A-B, B-A, C-D etc., paired associates) and NOT to respond to other stimulus pairs made up from the same stimulus pool (e.g., A-C, A-D etc., mispair sequences) as well as pairs that contained either stimulus X or Y (non-relational sequences). All groups quickly learned not to respond to stimulus pairs that contained X and Y. Across 40 test sessions, pigeons from all groups continued to respond vigorously to paired associates while responses to mispair sequences dropped significantly indicating that the birds had learned the relational nature of the paired associates. No difference was found in the performance of control/neostriatal pigeons compared to birds with hippocampal lesions. Further, a subsequent correlational analysis revealed no relationship between the extent of hippocampal damage among the hippocampal lesioned birds and performance on the task. The data suggest that homing pigeons can rely on a relational strategy to learn to preferentially respond to paired associates and that the hippocampal formation plays no necessary role in this process. This finding contrasts with results from rats and is at least consistent with the conclusion that, for birds, the hippocampal formation is specifically dedicated to spatial cognitive processes. However, the difference between the rat and pigeon results could be a consequence of the different stimuli used (olfactory versus visual) or, alternatively, lesion locus (the rat study used parahippocampal lesions while the pigeon study relied on focal lesions to the hippocampus proper). We gratefully thank NIH for their support.

443.19

THE EFFECT OF ANTERIOR THALAMIC AND CINGULATE CORTEX LESIONS ON 'OBJECT-IN-PLACE' MEMORY IN NON-HUMAN PRIMATES Amanda Parker* & David Gaffan, Dept. of Experimental Psychology, University of Oxford, England, UK.

Macaque monkeys (Macaca mulatta) were trained in an object-in-place memory task, designed to capture the 'whole scene' nature of episodic memory. In the object-in-place memory task the correct, rewarded, response in each scene is to a particular object of a pair, which always occupies a particular position in a unique background which has been generated using randomly chosen colours and shapes. In each session, the monkey learns a new list of these unique scenes. Following stereotaxic lesions of the anterior thalamic nuclei, 3 animals showed a severe impairment in performance. In contrast, following surgical ablation of cingulate cortex a second group of 3 animals were only mildly impaired at the task. These results are compared with those of animals with mamillary body lesions and fornix transection who have been tested using the same behavioral paradigm (Gaffan,J.CogNeurosci 1994, 6, 305; Parker & Gaffan, in press). The results show that cingulate lesions produce a milder amnesia than that caused by anterior thalamic or mamillary body lesions or fornix transection.



This research was funded by the Medical Research Council (UK).

443.20

PERIRHINAL CORTEX ABLATION AND DISCONNECTION OF FRONTAL LOBE FROM TEMPORAL LOBE DO NOT IMPAIR OBJECT-REWARD ASSOCIATION LEARNING ACROSS A DELAY IN MONKEYS. S.A. Gutnikov*, Y.-Y. Ma, M. Buckley & D. Gaffan. Department of Experimental Psychology, Oxford University, England, OX1 3UD, UK.

In monkeys information about the features of visually perceived objects is processed in area TE of the temporal cortex. Area TE projects to the perirhinal cortex (PRC) and, via uncinate fascicle (UF), to the prefrontal cortex (PFC). A widely accepted hypothesis of long-term potentiation (LTP) as a neural mechanism of associative memory requires simultaneous depolarisation of two neurons: the presynaptic neuron which conveys information about the object properties (a neuron in TE) and the postsynaptic neuron which holds information about the reward. Most importantly, in order to form associative LTP, depolarisation of these two neurons must coincide with the temporal gap of no longer than 100 ms. Neurons in TE have been shown to fire during presentation of the object but not after object offset. Thus, in the view of the memory-LTP hypothesis, if object-reward association is formed when the reward is delayed for longer than 100 ms after object offset, the information about the object must be kept somewhere else in order to bridge the delay until the information about the reward is available. In the present study monkeys learned concurrent visual object-reward discriminations with reward delayed for up to 1000 ms after object offset. The rate of learning with 1000 ms delay of reward was no slower than the rate of learning with zero delay. Thus, either the mechanism of associative memory is different from that of associative LTP, or information about objects is kept somewhere outside TE. Ablations of PRC or disconnection of PFC from TE (by transection of UF) did not impair learning, indicating that information about the visually perceived objects is not kept in PRC or PFC across the delay. Supported by UK Medical Research Council.

443.21

HIPPOCAMPAL LESIONS WITHOUT INJURY TO THE RHINAL CORTEX DO NOT IMPAIR AUDITORY RECOGNITION MEMORY IN DOGS

D.M. Kowalska and P. Kuśmierek, (SPON: European Brain and Behaviour Society) Nencki Institute of Experimental Biology, Pasteur St. 3, 02-093 Warsaw, Poland

Three male mongrel dogs were trained to perform a delayed matching-to-sample (DMS) task with auditory trial-unique stimuli with an inter-stimulus delay of 1.5 s. To reach the criterion of 90% correct in 90 consecutive trials, they required an average of 1565 trials (T; range 1300-2075) and 507 errors (E; range 346-718). After a 2 week break, they were retrained to criterion (mean: T=153, E=17). They were then given performance tests with delays 10, 30, 60 and 90 s in blocks of 90 trials (mean for all delays=70% correct, range: 68%-74%). Next, they were retrained on the original 1.5 s delay (mean: T=270, E=40) and then received surgery. The hippocampus was removed bilaterally through a small opening in the parietal cortex (Kowalska & Kosmal, *Acta Neurobiol. Exp.*, 1992, 52:119). The rhinal cortex was not damaged. Postoperatively, the criterion was reattained on the 1.5 s delay in an average of T=227 and E=33. Mean score on the performance tests was 79% correct (range: 73%-83%).

The results indicate that, in dogs, bilateral removal of the hippocampus does not impair performance on an auditory recognition DMS task with trial-unique stimuli.

Supported by Polish KBN Grant No. 6 6330 9203 p/01 and US-Polish Joint Found No. MZ/HHS-93-124.

443.22

HIPPOCAMPAL AND PARIETAL CORTEX PROCESSING OF OBJECT SCENES. W.E. DeCoteau* and R.P. Kesner. Department of Psychology, University of Utah, Salt Lake City, UT 84112.

Using a successive go/no-go paradigm with object scenes, rats were trained on object-based discrimination (OBD), location-based discrimination (LBD), or object/location-based discrimination (O/LBD) tasks. In each of these tasks, rewarded trials consisted of a standard scene of four objects placed over baited food wells. Non-rewarded trials for the OBD consisted of replacing any one of the four objects of the standard scene with a novel object. Negative trials for the LBD consisted of displacing any one of the four objects in the standard scene. Negative trials for the O/LBD consisted of replacing and displacing any one of the four objects in the standard scene. After reaching a criterion of at least a four sec. difference between rewarded and non-rewarded trials, rats were given either hippocampal, parietal cortex or cortical control lesions. None of the lesion groups showed an impairment on the OBD task. Parietal cortex and hippocampal lesions produced impairments on the LBD and O/LBD tasks. On the O/LBD, parietal cortex lesions produced a profound impairment, but the hippocampal lesions resulted in a mild impairment followed by recovery. Based on transfer tests, the recovery appeared to be due to use of an object-oriented strategy. These results indicate that the hippocampus and parietal cortex are important in processing the spatial properties of scenes. Additionally, unlike the parietal cortex, the hippocampus seems unable to flexibly switch from an object/place to an object strategy in solving the O/LBD task.

NSF IBN9511635

444.1

PRACTICE ON AUDITORY TEMPORAL-INTERVAL DISCRIMINATION PRODUCES LEARNING THAT GENERALIZES TO DIFFERENT FREQUENCIES BUT NOT OTHER INTERVALS. M.M Merzenich*, D.V. Buonomano, H.W. Mahncke, B.A. Wright. Keck Center, UCSF, San Francisco, CA 94143-0732

The sensory encoding of the interval, duration, and order of different stimulus features provides vital information to the nervous system. For example, data indicate that temporal processing in the range of tens to hundreds of milliseconds is important to the perception of speech, and that deficits in temporal processing may underlie language impairments. The present study focuses on the influence of practice on temporal processing. The goals of the experiment were to determine: (1) whether practice improved the ability to discriminate between temporal intervals marked by tones of one frequency, and, if so, (2) whether this improvement generalized to a different tonal frequency and/or temporal interval. These issues were examined in eleven human subjects using a two-alternative, forced-choice procedure: two brief tones were separated by a standard temporal interval in one presentation, and by a standard plus a variable interval in the other presentation. Subjects were required to indicate whether the longer interval occurred during the first or second presentation. The duration of the variable interval was adjusted adaptively over 60 trials to determine the discrimination threshold. Each subject was trained on 900 trials per day for ten days using a standard interval of 100 ms presented at 1 kHz. The average discrimination threshold decreased from 20 ms on Day 1 to 11 ms on Day 10. A one-way ANOVA of variance revealed a significant learning curve over the ten days of training ($F_{9,90}$=6.7, p<0.001). Before and after the training period, subjects were tested on a standard interval of 100 ms presented at 4 kHz, and on a standard interval of 200 ms presented at 1 kHz, in addition to the trained condition. Comparison of the pre- and post-test results revealed that temporal-interval discrimination improved significantly in the 100-ms/4-kHz condition, but not in the 200-ms/1-kHz condition. These results indicate that (1) auditory temporal-interval discrimination improves with practice, and that (2) this learning is temporally, but not spectrally, specific. [Supported by McDonnell-Pew and the Dana Foundation.]

444.2

PERCEPTUAL LEARNING OF TEXTURE DISCRIMINATION: SUBJECT AND TASK DEPENDENT VARIABLES. A. Karni*, G. Bertini, P. De Weerd, R. Desimone and L.G. Ungerleider. NIMH, NIH, Bethesda MD 20892.

Several paradigms of perceptual learning suggest that practice can trigger long-term, experience-dependent changes in early processing stages within the adult visual system. In a texture target discrimination task, learning was specific for stimulus parameters such as the target's location and the background elements' orientation as well as the eye receiving training (Karni & Sagi *PNAS* 1991), suggesting mechanisms of figure-ground segmentation in striate cortex as a possible locus of plasticity. To study the responses of V1 neurons in an awake monkey, a modified version of the task was developed (Bertini et al *Soc. Neurosci. Abs.* 1995, 1996). Another version of the paradigm was set up to train human subjects for an imaging study. Here we report our findings using the "monkey" and the "imaging" versions of the task.

The task required the discrimination of the shape of a small parafoveal target consisting of 3 oblique line elements embedded in a background texture made of identical but horizontal line elements. Discrimination performance was measured as a function of the time interval between the briefly presented target stimuli and a patterned mask. Fixation was enforced by a concurrent letter discrimination task at the fovea. The main changes made in the "monkey" task were: no letter discrimination task at fixation, target location was pre-cued, the area within which the targets appeared (spatial uncertainty) was reduced, and the amount of practice per session (for human subjects only) was more than halved. The last two changes were also made in the "imaging" task.

A rhesus monkey and 15 humans (6 of them in the "monkey" task) were tested. In the monkey, as well as in 13 humans, experience-specific learning effects were found. Nine subjects showed clear monocular learning. Of the 6 subjects who showed no monocular learning, 4 showed learning specific for orientation or retinal location but in 2 (both trained on the "monkey" task) learning was not specific to either. In the monkey, learning did not transfer across visual-field quadrants, and was critically dependent on background element orientation. The effects of training, however, transferred between eyes. Our results confirm that perceptual learning is highly specific for the training experience but raise the possibility that task and subject variables are related to different degrees of specificity of training effects. *Supported by NIMH-IRP.*

444.3

PERCEPTUAL LEARNING OF AN IMPLICIT SEQUENCE. J. Salidis, D.B.Willingham*, P.B.Sederberg, and J.A. Hollier. Dept. of Psychology, University of Virginia, Charlottesville, VA 22903.

Motor skills tasks are learned normally by amnesic patients, defining these tasks as implicit rather than explicit tasks. Learning outside the awareness of normal subjects is also considered implicit. Subjects' ability to learn an implicit pattern was tested in two experiments in which they tracked with a joystick a target moving in a one dimensional pattern for the mid portion of each trial; this sequence was embedded between two segments of random movement. Subjects showed greater improvement on the repeating segment than on the random segments. The basis of this learning, perceptual or motor, was evaluated by comparing the performance of a group who initially only watched the target with one who tracked. Subjects who watched the embedded repeating sequence showed more pattern specific knowledge than those who had watched only random movement, but less than those who tracked for the entire experiment. In contrast, a third experiment, in which the pattern was apparent (the target moved within the outline of a square or diamond), did not indicate any watching-facilitated sequence learning.

444.4

VISUAL RECALL FROM LONG-TERM MEMORY INTERFERES WITH PERCEPTION Alumit Ishai* and Dov Sagi, Dept, of Neurobiology, Brain Research, Weizmann Institute of Science, Rehovot 76100, Israel

Visual imagery is the ability to create a perceptual experience in the absence of retinal input. Using a lateral masking detection paradigm of a Gabor target, flanked by two high-contrast Gabor masks placed at different eccentricities, we have shown an imagery-induced facilitation that was subserved by a stimulus-specific short-term memory (STM) (Ishai and Sagi, *Science* 1995, 268: 1772). No interference was seen when the observer imagined Gabor stimuli under STM conditions. To obtain an imagery-induced interference (Perky effect), new experiments were designed, in which the observer had to detect a Gabor target and to imagine lines, using either long-term memory (LTM) or STM. The results showed an orientation-specific interference under LTM conditions. When recall of lines was based on STM, neither interference nor facilitation were seen. These results suggest that imagery-induced interference and facilitation are memory dependent - visual recall from LTM can interfere with perception, while in STM tasks facilitation can be obtained, and support the distinction between low-level and structural representations in visual memory.

444.5

HUMAN WORKING MEMORY IN A RADIAL MAZE PERSISTS OVER A LONG INTERRUPTION: GENERAL MAMMALIAN NEURAL WM MECHANISM? R. B. Glassman*, K. M. Leniek and T. M. Haegerich. Dept. Psychol., Lake Forest College, Lake Forest, IL 60045.

Our studies have shown that spatial working memory (WM) capacity of humans in radial mazes having 13 or 17 arms is the same as that of lab rats; with correction for probable guesses, capacity is within the range of traditional verbal measures of human short-term memory ("the magical number" 7±2 items). We now report that humans doing a radial maze task, like lab rats, sustain memory over surprisingly long interruption-delays. Twenty-one undergraduates were asked to retrieve place markers hidden at the end of each arm of an 8-arm radial maze, 15 m dia., painted on the grass in an area with many nearby visual cues. Subjects were asked to choose arms unsystematically, and to depend on memory to avoid repeating. Most subjects accomplished this task with no repeat-errors even when a delay of 15 minutes, filled with a spatial interfering task, was interposed after the fourth arm-choice (mean=7.67). Somewhat less capacity was observed in testing the same people with an analogous recitation task - to say the letters A through H in random order with no repeats - with an interruption-delay of 30 seconds at the halfway point, occupied by a subtraction task (mean=7.14). The radial maze WM persistence over delay is additional evidence for a common mammalian brain mechanism, perhaps an aspect of cortical structure (also see Soc. Neurosci. Abstr. 1991-1994; Brain Research Bulletin, 1993, 1994). Supported in part by the Lake Forest College Richter Scholar Fund.

444.6

AN IMPLICIT SEQUENCE LEARNING TASK ACTIVATES STRIATUM AS MEASURED BY FUNCTIONAL MRI. P.J. Whalen*, C.R. Savage, T. Curran, A. Kendrick, H.C. Breiter, G. Bush, H.D. Brown, B.R. Rosen & S.L. Rauch. Psychiatric Neuroscience Program & MGH-NMR Center, Harvard Medical School & Massachusetts General Hospital, Charlestown, MA 02129.

The serial reaction time (SRT) paradigm is a well characterized measure of implicit sequence learning. Using a noncontrast functional MRI (fMRI) technique, we sought to replicate PET studies in our lab implicating the striatum as a key structure involved in this task. Subjects were 8 healthy, right-handed, adult males and were instructed to press one of four keys; each key corresponding to one stimulus position of an asterisk appearing at one of four spatial locations at a fixed interstimulus interval. Stimuli were presented during two runs while reaction times (RT) were measured. Each run consisted of three Baseline condition blocks (30 secs) where the order of the stimulus locations was random, alternating with three Implicit Learning (IL) condition blocks (90 secs) where, unbeknownst to the subjects, a twelve item repeating sequence was introduced.

All subjects exhibited a decrease in mean RT between the Baseline and IL conditions ($p < .05$). FMRI data were acquired as 15 contiguous 8 mm thick horizontal slices using a 1.5T MRI instascan device and an asymmetric spin echo T2*-weighted sequence (TR/TE/Flip = 2750/70/90). Structural data were obtained via a T1-weighted echo planar sequence. Foci of significant activation ($p < 10^{-4}$) across all subjects for the motion-corrected, Talairach transformed and concatenated IL vs Baseline comparison were found bilaterally in striatum, in right premotor cortex, and in visual cortex. Inspection of statistical non-parametric maps from individuals revealed that 8/8 subjects demonstrated significant striatal activation. The SRT is shown here to be a reliable fMRI probe of striatal activity and may prove useful for studying neuropsychiatric conditions believed to involve cortico-striatal systems, such as obsessive-compulsive disorder and Tourette syndrome.

Supported by NIMH (MH01215), TSA, Inc. & the David A. Judah Research Fund

444.7

EFFECTS OF FEATURE MANIPULATIONS ON BRAIN POTENTIALS DURING A LEXICAL DECISION TEST. C.A. Joyce*†, K.A. Paller§, H. McIsaac°, & M. Kutas†‡. Cognitive Science† and Neuroscience‡, Univ. of California at San Diego, La Jolla, CA 92093-0515; Psychology, Northwestern Univ.§; Psychology, Univ. of British Columbia, Canada°.

We report on a series of studies in which event-related potentials (ERPs) were recorded while words were presented during lexical decision tests. In the first experiment, the physical presentation was manipulated by presenting some words letter-by-letter and others as whole words at study, but all as whole words at test. Reaction time (RT) showed a repetition priming effect (RT faster for repeated than novel words) as well as a perceptual effect (RT faster for words presented as whole words on both occasions than those presented letter-by-letter at study and whole at test). Recognition scores did not differ for the two study conditions. ERP responses to repeated words were more positive than those to novel words between 300 and 600 msec. There was also a marginally significant perceptually-modulated ERP effect between 400 and 500 msec. In another experiment, font was changed from study to test. RT's again showed repetition priming and perceptual effects with no differences in recognition scores. The ERP repetition effect was also replicated, however the perceptual effect was diminished from that in the first experiment. In order to ascertain whether the diminished perceptual effect was a consequence of the greater perceptual ease of the font compared to form manipulation, a third study utilized a font that was more perceptually challenging. Preliminary RT data revealed a repetition priming effect, but no perceptual effect. Repeated words also elicited more positive potentials between 300 and 400 msec than novel words. Because the ERP repetition priming effect seems relatively unaffected by physical feature changes, it may index declarative memory processes.

Funded by: A grant to M. Kutas from NIMH:#52893 and a fellowship to C.A. Joyce from the McDonnell-Pew Center for Cognitive Neuroscience, San Diego.

444.8

PATTERN PRIMING, INTACT IN ALZHEIMER'S DISEASE, REVEALS PERCEPTUAL NONDECLARATIVE LEARNING THAT IS INSENSITIVE TO ENCODING TIME. B.R. Postle*[1], J.H. Growdon[1,2], & S. Corkin[1]. [1]*Dept. of Brain and Cognitive Sciences and the Clinical Research Center, Mass. Institute of Technology, Cambridge, MA 02139;* [2]*Dept. of Neurology, Mass. General Hospital, Boston, MA 02114.*

This experiment was designed to answer two questions about pattern priming: 1) Does pattern priming represent perceptual, motor, or haptic learning? and 2) Are pattern priming and pattern recognition memory differently sensitive to study encoding time? Subjects (AD = 12, NCS = 14) copied each of six target figures onto dot patterns, and then a) were given the same dot patterns and asked to draw the first figure that came to mind by connecting the dots with straight lines (priming), or b) viewed each of the same six patterns of dots and were asked to indicate which of four possible completions corresponded to the figure that they had just copied (recognition). Confirming previous results (Postle et al. 1995; 1996), the AD and NCS groups achieved comparable priming scores, but AD subjects were significantly impaired on the recognition test. On the priming test, sequence and direction of pen strokes were considerably different between study and test, ruling out motor learning or haptic learning as plausible explanations of the learning in either group, and confirming that pattern priming represents *perceptual* nondeclarative learning. Analysis of mean encoding time for "correct" trials versus "incorrect" trials suggested that pattern priming was not sensitive to differences in encoding time at study (AD mean encoding time for "correct" trials = 9.0 sec, for "incorrect" trials = 11.3 sec; NCS "correct" mean = 5.8 sec, NCS "incorrect" mean = 5.6 sec), but that pattern recognition *was* sensitive to encoding time differences (AD mean encoding time for "correct" trials = 13.8 sec, for "incorrect" trials = 9.4 sec; NCS "correct" mean = 6.0 sec, NCS "incorrect" mean = 5.3 sec). This result suggests that pattern priming, like some forms of verbal nondeclarative perceptual priming, is insensitive to the manipulation of encoding time, while the declarative memory analog of this test is sensitive to this manipulation. *Supported by NIH grant AG06605 and by an NSF Graduate Research Fellowship to BRP.*

444.9

CONCEPTUAL PRIMING WITH BRIEF MULTIPLE PRESENTATION TECHNIQUE: PRESERVATION IN AMNESIA.

M. Beauregard*, H. Chertkow, D. Gold, S. Karema, J. Benhamou, A. Faucher. Lady Davis Institute, Jewish General Hospital; Centre de Recherche du Centre Hospitalier Côte-des-Neiges; St-Anne de Bellevue Veterans Hospital, Montreal, Quebec, Canada.

Considerable controversy surrounds the status of implicit memory processes in patients with amnesia; the original claim that such processes were spared has not been confirmed in recent studies demonstrating decreased conceptual priming for words. Possible contamination of most implicit tasks by impaired explicit strategies, however, may be responsible for such results. We have developed a method of circumventing explicit strategies by using brief duration repeated primes below the awareness threshold of subjects. We have used this approach to evaluate the status of implicit memory in amnesic patients. One group of fifteen amnesic patients with alcoholic Korsakoff's syndrome (KS) and one group of fifteen normal elderly control subjects (NCS) were tested for word priming on a speeded category membership decision task carried out in two blocks. In each block, groups KS and NCS first studied 10 category members, and were then tested on a set of 40 word stimuli. Of these, 10 were the previously studied category members, 10 were new category members (20 "Yes" responses), and 20 were category non-members. List study conditions were manipulated in two experiments. In Experiment 1, subjects were presented in each block with 10 study words. Word presentation consisted of a brief duration (17 ms) pattern masked exposure of the word. Each word was presented 10 times in succession at this exposure duration. No subject or patient could reliably report the words presented. In Experiment 2, subjects of the same two groups were presented in each block with 10 different study words, each shown twice for a one second duration. In both experiments, the average reaction time for "Yes" decisions to the primed words was significantly faster than that to the unprimed words for both KS and NCS groups. Moreover, there was no significant difference in the magnitude of the priming effect between these groups in the two experiments. These findings demonstrate that amnesic patients can exhibit normal levels of conceptual word priming. Furthermore, they are consistent with a previous study from our laboratory showing that priming effects can occur even in the absence of explicit recognition or recall of presented material in normal individuals.

444.10

REPEATED VERB GENERATION IN AMNESIC PATIENTS: NORMAL PRIMING AND VERB SPECIFICITY. C. A. Seger[1]*, L. A. Rabin[1], M. M. Keane[2], M. Zarella[2], & J. D. E. Gabrieli.[1,2] [1]Dept. of Psychology, Stanford University, Stanford, CA 94305 and [2]Memory Disorders Research Center, Boston VA, Boston, MA 02130.

In the verb generation task subjects are presented with nouns and generate for each one an appropriate verb; the nouns can be repeated across blocks, with the result that generation latencies are reduced and different patterns of brain activation are present (Raichle et al., 1994). Research in our laboratory has indicated that reduced generation latency occurs when a verb is repeated, regardless of whether the stimulus noun is repeated or novel. These results suggested that in repeated verb generation verbs are primed as a result of being previously generated. Since many priming tasks are dependent on nondeclarative memory, we decided to test whether verb generation is impaired or preserved in patients with amnesia, who manifest impaired declarative memory but preserved nondeclarative memory. Amnesic patients and matched controls were tested in a verb generation task with 4 repeated blocks of nouns followed by a novel block. The amnesic patients showed the same pattern of performance as controls: they became faster across blocks on repeated nouns and returned to original performance levels when novel nouns were introduced. An examination of patients' responses indicated that the verb generation effect was verb specific: when patients responded to a novel noun with a repeated verb from an earlier block, they showed reduced generation latency equivalent to that found when a repeated verb was generated in response to a repeated noun.

Supported by grants from NIMH and NINDS.

444.11

CONDITIONAL DISCRIMINATION LEARNING IN SCHIZOPHRENIA

K. Dantendorfer*, D. Maierhofer, I. Daum, M. Schugens, P. Anderer, H. V. Semlitsch, H. Katschnig. Dept. of Psychiatry, Univ. of Vienna, Austria 1090.

Recent reports suggest, that memory and learning deficits seem to be more specific to schizophrenia (SZ) than previously accepted. Conditional discrimination learning based on eyelid conditioning has been shown to be selectively sensitive in testing temporal lobe function and has the advantage of making minimal demands on attentional capacities and motivation.

An eyelid conditional discrimination learning task (Daum et al., Behavioral Neuroscience 1991) is used to examine healthy controls and two groups of SZ patients (DSM-III-R paranoid type SZ with predominant positive symptoms and disorganized type with predominant thought disorders). The occurrence of the first conditioned response (FCR) and response frequency to reinforced (CRR) and unreinforced trials (CRU) are quantified.

17 controls showed a FCR after 5 ±2,25 trials as well as a frequency of 32% ±5,5% CRRs and 14% ±3% CRUs. Two paranoid type SZ patients tested up to now, showed high rates of CRRs as well as CRUs (CRR/CRU; 23%/25% and 29%/21%). On the other hand, the two disorganized type patients showed low rates of CRRs and CRUs (CRR/CRU; 2%/4% and 4%/4%). All four patients had the FCR on unreinforced trials and three of them showed delayed FCRs (trials 9, 14, 17) compared to controls.

Our preliminary data suggest, that while paranoid as well as disorganized type schizophrenics show reduced discrimination learning capacity, the two subgroups might be differentiated by conditioned response frequency probably due to differences in temporal lobe functions.

Supported by grant no. 5657 Jubiläumsfonds der Österr. Nationalbank

444.12

EARLY COGNITIVE IMPAIRMENTS IN SCHIZOPHRENIA: A COMPARISON OF DRUG NAIVE AND NEUROLEPTIC TREATED PATIENTS. I. Lussier, E. Stip, M. Babaï, J.B. Debruille and I. Peretz*. Centre de recherche Fernand Seguin, Département de psychiatrie, Université de Montréal, 7331, rue Hochelaga, Montréal, Québec, Canada, H1N 3V2.

In the present study, we examined attention and memory functioning in schizophrenic patients. In order to evaluate the effects of the illness per se, drug naive patients were compared to patients treated with traditional neuroleptics, and to a group of matched normal controls. All subjects groups were submitted to a series of cognitive measures. For memory functioning, verbal (span) and attentional (dual task) working memory components and explicit and implicit recall were measured. For attentionnal functioning, reaction time (alertness), sustained attention (CPT) and selective attention were examined. Results showed that, overall, drug naive patients performed better than patients treated with traditional neuroleptics. Moreover, not all cognitive processes were disturbed in drug free patients: Alertness, working memory, and implicit recall were in the normal range. These observations suggest that there are impairments in information processing early in the disease process but that other factors, such as medication, contribute to the cognitive impairments reported in the schizophrenic population. The effect of illness related factors, such as diagnostic, positive and negative symptoms, and hospitalization will be discussed.

445.1

NEUROPHYSIOLOGICAL MECHANISMS OF CORTEX-SUBCORTEX INTERACTIONS DURING ORGANIZING OF BEHAVIORAL REACTIONS. V.T.Shuvaev,V.I.Shefer* and A.V.Michailov,Lab.of Higher Nervous Activity, Pavlov Institute of Physiology,St.Petersburg,Russia.

A correlational analysis of neuronal activity in cortex and subcortex (neostriatum,VA,VL-thalamus and amygdala) of dogs and cats was carried out. The changes of synchronization level and of the temporal parameters of correlating periods in spike activity of neurons during the formation and realization of different behavioral reactions were investigated. The following scheme of these changes was developed: the maximum of correlation corresponds at first to the interactions neostriatum-amygdala, than to the interaction cortex-amygdala and 100-300 ms later to the interactions cortex-neostriatum (before the formation of a reaction); at the begining of the realization of a reaction this maximum can be observed in the interaction thalamus-cortex and thalamus-neostriatum, but later the simultaneous increase of synchronization cortex-neostriatum and cortex-amygdala becomes more important. New data of the plastic changes of neuronal activity during the formation of the reactions were also obtained. The data suggest that on one hand activity of the neurons is determined by a particular brain structure, which they belong to, but on the other hand, they may form a neuronal networks constituting a kind of net with properties allowing qualitatively a new functions to be implemented.

G.R.F.F.I. 96-07-89112; 96-04-58005

445.2

ENCODING SERIAL ORDER IN A MODEL OF THE PREFRONTAL CORTEX AND BASAL GANGLIA. D.G. Beiser, J.C. Houk*. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

Several lines of evidence suggest that the prefrontal cortex (PFc) and basal ganglia play an important role in the cognitive aspects of serial behavior. We present a modular neural network model of these areas that encodes serial inputs within spatial patterns of PFc activity. The model is based on the topographically-specific circuits linking the PFc with the basal ganglia. Each module traces a pathway from the PFc, through the basal ganglia and thalamus, and back to the PFc. The complete model consists of an array of modules interacting through recurrent corticostriatal projections and collateral inhibition between striatal spiny units. Other computational features include: sharp firing thresholds for effective context detection by spiny units and bi-stable corticothalamic loops for sustaining contexts in working memory.

The model was tested in a simulated version of a delayed-sequencing task. Experimentally, this task begins with the presentation of a sequence of target lights. After a short delay, the monkey must touch the targets in the order by which they were presented. In the simulated paradigm, the untrained model produces different patterns of PFc activation in response to different target sequences -- giving an unambiguous spatially-distributed representation of the sequence. The codes are stable and largely independent of the target presentation rate. In addition, responses from the model's PFc and caudate units, many of which display responses that depend on the serial-order of the target inputs, qualitatively resemble those from the relevant single-unit studies. The model offers several predictions regarding the response characteristics and location of sequential activity in the brain.

Supported by: NIMH Center Grant #P50-MH48185-05.

445.3

MODEL OF CORTICO-HIPPOCAMPAL MEMORY CONSOLIDATION PROCESS BY ACCUMULATION OF CASE MEMORIES AND RE-ORGANIZATION INTO MAPS. T. Omori*, BASE, Tokyo Univ. of Agriculture & Technology / PRESTO,Japan Research and Development Cooperation, Nakamachi, Koganei, Tokyo 184, Japan

A memory formation process has three time constants of short, middle and long. We suppose a hippocampal LTP as the middle term memory that encodes an events by a configural association. We propose a model of memory consolidation process from the hippocampal events to a cortical long term memory in a feature map structure, and discuss about their theory.

The model has two different typed memory systems between an input and an output layers. One is a case memory system that has fast configural association ability. Another is a self organizing feature map(SOFM) with slow learning rate. Given an input, it is memorized in the case memory system, and associated to an output neuron by a teacher signal. When the cases are accumulated, the cases that correspond to an output are back projected to the input layer, and averaged to extract essential features for the output. When, the averaged patterns are given to the SOFM iteratively, multiple feature maps that have same dimensions with the teacher signal are formed. The model can explain the primary brain map formation as a memory re-organization process.

445.4

INFORMATION DYNAMICS IN ASSOCIATIVE MEMORIES WITH SPIKING NEURONS

F.P. Battaglia*[1], and A. Treves[1]. [1]SISSA, Cognitive Neuroscience, via Beirut 2, 34013 Trieste, Italy.

Processing speed is a crucial constraint on the organization of sensory modules. Here, a simulation study is presented of the dynamics of a recurrent network with spiking neurons. The network is composed of excitatory and inhibitory units, with shunting inhibitory synapses. Excitatory-excitatory synapses encode memories in an hebbian fashion, with a boundary forgetting mechanism (Parisi G., 1986, *J. Phys. A* **19** L617). Shunting inhibition has proved to be helpful in stabilizing the dynamics of the network allowing for a larger stability window in parameter space and for more variance in synaptic strengths, resulting in a much larger memory capacity. We show analitically that shunting inhibition, unlike subtractive inhibition, stabilizes network dynamics for memory loading up to the theoretical capacity limit. As a measure of retrieval speed two kinds of information-related quantities are considered: the full information contained in the steady, attractor state reached by the network following the presentation of a partial cue (Treves A., 1990, *Phys. Rev. A* **42** 2418); and the information about which one of the encoded memories has been associated to the cue, which is what can be measured in multicellular recording experiments (see related Abstract by Treves *et al.*). The dependence of the time scales for these quantities on the parameters is studied. It is seen that synaptic conductance time constants have a major effect on retrieval speed (Treves A., 1993, *Network* **4** 259). For realistic parameters values, it is shown that recurrent modules can operate fast enough to contribute to rapid perceptual processes.

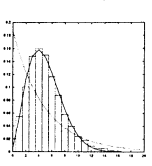
445.5

DO FIRING RATE DISTRIBUTIONS REFLECT ANYTHING BEYOND JUST CHANCE?

S. Panzeri*[1], M. Booth[2], E.A. Wakeman[2], E.T. Rolls[2] and A. Treves[1]

[1]SISSA, Cognitive Neuroscience, via Beirut 2, 34013 Trieste, Italy and [2]Univ. of Oxford, Experimental Psychology, OX1 3UD, UK.

The firing rate responses of a typical cortical pyramidal neuron to large, *ecological*, sets of stimuli usually follow a graded unimodal distribution, with a tail extending to high rates, as seen in many plots of the number of spikes emitted in a fixed time window. The mode of the distribution is close to the spontaneous firing rate (sometimes, and more often the further from sensory inputs, at zero firing) and the tail is close to exponential. A *full* exponential distribution would maximise, under the constraint of fixed average rate, the information transmitted by an artificial unit that used a definite number of spikes as a noiseless code for each stimulus. This has led to the recent suggestion (R.Baddeley) that the exponential tail might be an attempt to optimize information transmission. However most of the information would be transmitted at low rates, where the distribution is often far from exponential and moreover real within-stimulus variability makes the noiseless coding theorem inapplicable. We therefore explore the alternative hypothesis that typical distributions merely reflect a *random* (Gaussian) input activation, taking into account a physiological input-output transform and real noise levels. This leads to better and more economical fits:



Supported by the MRC (UK), INFM and INFN (Italy) and the HCM program of the EC.

445.6

MODELING OF ARTIFICIAL NEURONS WITH COMPLEX DENDRITE STRUCTURES. M. Ichikawa, H. Yamada, T. Iijima and G. Matsumoto*. Molecular and Cellular Neuroscience section, Electrotechnical Lab. Tsukuba, Ibaraki 305 Japan.

Generally, neuron models in artificial neural networks were too simple in structure, however each living neuron forms dendrites of very complex structure. Some physiological results shows that spikes are generated not only at cell body but also in the proximal dendrites. The results suggest that the conventional neuron model used in artificial neuro-network may not mimic living neural network. We propose a novel method of modeling an artificial neuron having multiple dendrites of various properties. Our model consists of four components, each of which will be called basic primitive, "begincell", "dendrite", "link" and "endcell". Any cell should be declared with a nesting of "begincell" and "endcell" primitives, and the other primitives should be inserted between this nesting according to the design of cell. For instance, CA1 pyramidal cells in hippocampus will have at least three "dendrite" primitives, each of which may correspond to the Schaffer collateral, commissural fibers and Perforant path. Each "dendrite" primitive has its own property such as synaptic connectivity, learning rule, scaling factor and so on. Based on the new idea, we recently developed a novel processor. The processor can execute calculations using huge number of synthesized neurons and networks composed of those neurons, effectively. We will present an application for reconstruction of neural activities detected with optical imagings in rat hippocampal slices. *Part of this work was supported by NEDO.*

445.7

DEVELOPMENTAL PLASTICITY FORMS STABLE ATTRACTOR NETWORKS. S. E. Hua*, F. A. Mussa-Ivaldi and J. C. Houk. Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611

Neural network models with purely feedforward connections have been used effectively to characterize sensory systems. On the other hand, networks with feedback connections have additional properties that are potentially more capable of characterizing motor systems and behavioral problems; however, such networks may exhibit chaotic or oscillatory dynamics which are difficult to regulate. Feedback networks which satisfy a symmetry requirement or a detailed balance condition do have stable dynamics, and these "attractor" networks have been used to model associative memory and the generation of motor commands. Experimental findings supporting the existence of attractor dynamics have been found in single unit recordings. However, the acceptance of the attractor architecture as a plausible model of CNS function has been impeded by concerns that symmetry conditions may be too demanding to be biologically realistic.

Here we report that recurrent networks with random initial connections will develop into attractor networks when guided by biologically plausible developmental rules. We assume only a Hebbian learning rule with either weight decay or weight normalization. Hebbian learning with simple weight decay results in symmetric connections. Similarly, Hebbian learning with concurrent pre- and postsynaptic normalization also leads to a symmetric network. Furthermore, Hebbian learning with either pre- or postsynaptic normalization alone leads to a network that satisfies a weaker form of symmetry known as a detailed balance condition. These analytic results hold for both linear and nonlinear networks and are valid for networks with continuous or discrete learning. We conclude that Hebbian learning with various biologically plausible forms of weight normalization leads to the natural development of dynamically stable networks. This work supported by NINDS grant 5-P01-NS17489, NIH/HIMH grant 5-P50-MH48185, and grant ONR N00014-95-1-0571.

445.8

TEMPORAL SYMMETRY OF SPIKE PATTERNS IN ACTIVE MEMORY Yongdi Zhou*, Mark Bodner, Gordon L. Shaw, and Joaquín M. Fuster. Dept. Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024; Dept. Physics and Center for Neurobiology of Learning and Memory, UCI, Irvine, CA 92717.

The importance of symmetry in biological systems is well recognized. The purpose of present research is to determine whether symmetry plays a role in the temporal domain of neuronal spike discharge. A related aim is to find out whether specific patterns in the firing of cortical neurons exhibit symmetries during the short-term retention of sensory information. Using a new method of time-series analysis, single-unit spike trains were scrutinized for specific patterns and symmetries. The spike trains were recorded from parietal cortex of monkeys performing a tactile short-term memory task (delayed matching to sample). The results indicate that specific temporal patterns are present in the spike trains during the memory period of the task. These patterns can be almost 2-sec long and may repeat within a given spike train. That a pattern repeats in the period during which the memorandum must be retained is consistent with the hypothesis that short-term memory networks are maintained active by reverberation of impulses in closed circuits (Zipser et al., 1993). More generally, the patterns encountered are related to one another by symmetry operations that have been predicted by a highly structured model of cortical activity (Shaw et al., 1985).

Supported by NIMH grants.

445.9

COMPUTER MODEL OF INFORMATION TRANSFER FROM THETA TO SHARP WAVE (SPW) STATES DURING MEMORY CONSOLIDATION IN THE HIPPOCAMPUS. Andrea Bibbig*, Thomas Wennekers. University of Ulm, Department of Neural Information Processing, D-89069 Ulm, Germany.

In rats hippocampal EEG activity is clearly related to behavior. Two states can be distinguished: (1) theta-concurrent exploratory behavior characterized by small active cell assemblies in dentate gyrus (DG), CA3 and CA1 firing in response to specific environments ('place neurons'), and (2) immobility, consummatory behavior and slow wave sleep with large arhythmical population bursts (SPW) of almost all cells in CA3 and CA1. G. Buzsáki proposes that memory consolidation involves both stages [1]: During theta synapses between 'place cells' are weakly and transiently potentiated, whereas strong and longer lasting potentiation is achieved during SPW. Our computer model investigates how specific information can be transferred from theta to SPW states and eventually learned in spite the fact that SPW activity is extremely unspecific.

Our network consists of enthorhinal cortex, DG, CA3 and CA1 implemented as associative memories [2] with bursting principal cells and integrate-and-fire interneurons. Learning is implemented as a Hebbian learning rule to mimic LTP-like plasticity: The efficacy of a synapse is strengthened, if (1) the postsynaptic Ca^{2+}-concentration, given as an average (50 ms-range) of the presynaptic spike activity, exceeds a given threshold, *and* the postsynaptic cell activity is larger then a certain value, too. For this postsynaptic cell activity we compared several measures: the local subsynaptic EPSP, the 'global' membrane potential V or spike activity O, or short-time averages of those ($\overline{V}$,$\overline{O}$). Learning is very unspecific in the purely local case, at least some neighbouring synapses should be taken into account. Best results are achieved with averages (20 ms-range) of postsynaptic spike activity. Furthermore we need some mechanism to prevent that former learned assemblies are always stronger than 'new ones'. We choosed a cell facilitation, which raises spike probability after spiking. But cells of 'new assemblies' don't have to fire more frequently during SPW than others but just earlier. We will discuss biological plausibility of all our assumptions.

[1] Buzsáki, Neurosci. 31, 551ff (1989). [2] Bibbig, Wennekers, Proc. 13th Europ. Conf. Cybernetics and Systems Res., 1078ff (1996). Support by DFG.

445.10

SYNCHRONIZED NEURAL ENSEMBLES ACROSS THE ENTORHINAL-HIPPOCAMPAL CORTICES OF THE FREELY-BEHAVING RAT J.J. Chrobak* & G. Buzsáki, CMBN, Rutgers Univ., Newark, NJ 07102

Patterns of activity in neuronal ensembles have computational relevance to the cognitive operations of the mammalian brain. We have described slow synchronizing potentials (theta, sharp wave and dentate spikes), their associated high-frequency oscillations (gamma band [40-100 Hz] and 200 Hz), and the relationship of specific neuronal populations to each pattern in the entorhinal-hippocampal cortices. The present findings describe the coordination of oscillations between distant locations within this circuitry.

Our observations indicate that there is greater synchronization between sites within the dorsal CA1 region than between dorsal and ventral CA1 sites during 200 Hz oscillations. Further, the coherence between entorhinal cortex neurons and coupled CA1 cells may be higher than the coherence between distal CA1 sites. Thus, there can be greater synchronization across structures than within a single lamina of the same structure. Similar findings were observed with regards to synchronization of the entorhinal-hippocampal input pathway (layer II-III to dentate/hilar region) during gamma oscillations. These findings indicate the importance of "physiological connectivity" of neuronal ensembles in the hippocampal formation.

Supported by ADRDA (JJC) and NIH, NSF and HFSP (GB)

445.11

REPEATING SPATIO-TEMPORAL PATTERNS OF NEURONAL ACTIVITY IN THE HIPPOCAMPUS DURING SLEEP. Z. Nádasdy*, A. Bragin and G. Buzsáki, CMBN, Rutgers University, Newark, NJ 07102.

Parallel recordings of unit activity from the CA1 and CA3 areas of the rat hippocampus were analyzed by a "spatial clustering" method during slow wave and REM sleep episodes. The temporal structure of spatially distinct neurons was determined and combined to a "spatio-temporal" spike chart. The time structure of the spike chart was scanned by various sequence searching algorithms. Since the number and complexity of patterns depended on the search parameters, we investigated the parameter space in order to determine an optimal time window. The probability of by chance repetition was determined analytically and by using a simulated (shuffled) database. The patterns multiple spike sequences were (1) temporally coordinated with better than 10 msec precision, (2) stable (3) Markov-chain type and (4) correlated with the sharp wave bursts during slow wave sleep. The functional connectivity of the subnetwork was also determined on the basis of relative position within the spike sequences. The finding revealed that complex but reliably repeating spatio-temporal patterns are present during sharp wave bursts which are determined by the coincident discharge of neurons during preceding REM sleep episodes.

(Supported by NIH, NSF, and HFSP)

445.12

A PLAUSIBLE LEARNING ALGORITHM FOR THE ADJUSTMENT OF THE NOCICEPTIVE WITHDRAWAL REFLEX. C. Fåhraeus*, H. Holmberg and J. Schouenborg. Dept. of Physiology and Neuroscience, Lund University, Sölvegatan 19, S-223 62 Lund, Sweden.

The nociceptive withdrawal reflex is responsible for a complex sensorimotor transformation, adapted to protect the organism from harmful stimuli. In the hindpaw of the rat pups, we have recently shown that alteration of (a) single muscle's movement patterns and (b) cutaneous afferent innervation, both lead to appropriate adjustments of the reflex input-output function. It is therefore plausible that the polysynaptic withdrawal reflex is the result of a tuning algorithm rather than of detailed genetic programming.

Here we present a learning algorithm, founded, trained and tested on biological data, capable of learning sensorimotor transformation fast and accurately. Key features of the model is :

1. Spontaneous activity of premotoneurones in deep dorsal horn.
2. Muscles twitch by motoneurones causing unloading of skin.
3. Afferent cutaneous sensory feedback terminating on interneurones in the superficial dorsal horn.
4. Broadcasting by 1st order interneurones in superficial dorsal horn to premotoneurones in deep dorsal horn.
5. Hebbian correlation of 1st order interneurones in superficial dorsal with spontaneously active premotoneurones.

From model simulations, it can be concluded that one computational layer is sufficient for the highly non-linear (in cartesian coordinates) sensorimotor transformation of the withdrawal reflex. Also, in this model, activity of single muscles is a superior learning base compared to complex muscle activity. Further simulations, implications, and experimental foundation of the model are discussed.

Supported by Swedish MRC proj. 401013, 10596 and by BLANCEFLOR Boncompagni-Ludovisi

445.13

OPTICAL IMAGING OF NEURONAL ACTIVITY WITH VOLTAGE-SENSITIVE DYES IN THE ISOLATED GUINEA PIG BRAIN. Toshio Iijima*, Ichiro Takashima and Marco de Curtis(†) Electrotechnical Laboratory, 1-1-4 Umezono, Tsukuba, Ibaraki 305, Japan and (†) Istituto Neurologico, via Celoria 11, 20133 Milan, Italy.

The neuronal activity of limbic and olfactory cortices was analyzed with high resolution optical imaging (Iijima et al., *Science* 1996, in press) in the isolated guinea pig brain preparation maintained *in vitro* by arterial perfusion (de Curtis et al., *Hippocampus* 1, 341, 1991). The utilization of optical recordings in the isolated brain preparation allows to study without spatial restrictions the distribution and propagation of the activity generated by large assemblies of neurons in an intact brain. Moreover, because of the complete brain surface exposure, optical recordings are facilitated in those structures difficult to reach in *in vivo* conditions, such as the ventral cortical regions. The study focuses on the activity evoked by afferent stimulation in the piriform, entorhinal and perirhinal cortices.

Optical signals recorded after staining the brain with the voltage-sensitive dye RH-795, were compared to the electrophysiological recordings performed simultaneously with extracellular electrodes. Diffuse brain staining was achieved by circulating 1 mg of the dye for 5 minutes at a perfusion rate of 5 ml/min. Optical recordings were performed for several hours after a single exposure to the dye. Electrical stimulation of specific afferents and cortio-cortical associative fibers gave rise to optical signals that propagated along anatomical pathways. Lateral olfactory tract stimulation induced throughout the piriform cortex two peaks of optical signal corresponding to the afferent monosynaptic and the associative disynaptic responses, with a latency of circa 5 and 35 msec, respectively. Dentate gyrus stimulation was followed by the activation of the entorhinal cortex with a latency of about 25 msec, suggesting that a polisynaptic circuit was activated. Further experiments are in progress to define the depth of the optical signal generators.

445.14

INDEXING THE NEOCORTEX THROUGH ASSOCIATIVE LEARNING IN THE HIPPOCAMPUS: A QUALITATIVE APPROACH. J.L. Olds*, J.L. Krichmar and L. Hunter., Krasnow Institute for Advanced Study at George Mason University, Fairfax, VA.

The unique anatomy and physiology of the hippocampus appears appropriate for associating cortical columns that are related by an episode (i.e. a cell assembly). Input to the hippocampal region derives from many areas of the neocortex and a wide range of modalities. We propose a computational model that investigates the hippocampus' ability to index cell assemblies. The dentate granule (DG) cells process the multimodal input from the entorhinal cortex (EC) and create a key for the CA3 pyramidal cells. The CA3 pyramidal cells, which make up an associative memory matrix, learn combinations of cortical column indices through classical conditioning. It is assumed that learning occurs during an exploration period corresponding with the theta rhythm. The output of the CA3 region is a population of activity representing indices to cortical columns in the neocortex. The CA1 region compares input from CA3 with input from EC. If the match is appropriate, the output of CA1 is a set of indices to the neocortex. Otherwise, the model repeats the DG to CA3 to CA1 cycle with additional information from EC. We employ the Qualitative Reasoning Neuron (QRN) methodology to create a highly detailed model of the hippocampal pyramidal and basket cells. AMPA, GABA, NMDA, metabotropic and muscarinic receptors are modeled in detail. The technique is extremely efficient because no quantitative values need to be calculated. However, properties of the hippocampus described above emerge from QRN simulations. The results of the model suggest the role of the hippocampus is to process partial cortical information and return a completed cortical activation.

This research was supported by the Krasnow Institute for Advanced Study.

445.15

CELLULAR EVENTS AND CURRENTS RESPONSIBLE FOR EVOKED NEUROMAGNETIC SIGNALS FROM THE HIPPOCAMPAL SLICE OF THE GUINEA PIG. J. Wu*, and Y. C. Okada, Depts. Neurol. & Physiol., Univ. New Mexico Sch. Med., Albuquerque, NM 87131.

Synchronized population activities in the hippocampal slices produce magnetic fields detectable outside the tissue. The cellular events and currents generating the magnetic evoked field (MEF) were analyzed using longitudinal CA3 slices obtained conventionally from guinea pigs (250-350 g) under barbiturate anesthesia. Synchronized activities were elicited by depolarizing the apical dendrites of the pyramidal cells across the entire slice with an array of bipolar electrodes in the presence of 0.1 mM picrotoxin in the Ringer. The MEF consisted of a spike lasting 5-10 ms followed by a slow wave lasting about 100 ms, both produced by longitudinal currents in the pyramidal cells directed from the apical to the basal side. Field potentials recorded simultaneously with the MEF consisted of a train of population spikes during the MEF spike followed by a slow wave similar in latency to that of the MEF. Increasing $[Ca^{2+}]_o$ from 2.5 mM up to 7 mM increasingly reduced the later part of the slow wave of the extra- and intracellular potentials as well as the MEF, indicating that this part was predominantly due to polysynaptically mediated currents. Adding a non-specific blocker of excitatory amino acid neurotransmitters (kynurenic acid, 1 mM) to the bath eliminated the slow wave and some of the spike train, suggesting that they were generated predominantly by monosynaptically mediated currents. Part of the initial component of the MEF spike and the first 1-3 spikes in the field potential remaining in Ringer with kynurenate appear to have been generated by direct activations of voltage-sensitive conductances by the apical depolarization. The results provide a new insight into the genesis of magnetic signals in a mammalian CNS structure. Supported by NIH grant RO1-NS21149.

445.16

PURINERGIC MODULATION OF CA2 ACTIVITY IN SIGNAL TRANSMISSION BETWEEN CA3 AND CA1 IN THE RAT HIPPOCAMPAL SLICES.

Y. Sekino[1]*, K. Kato[2] and K. Obata[3], [1]PRESTO, JRDC, Dept. of Neurobiol. & Behav., Gunma Univ. Sch. of Med., Maebashi 371, [2]Lab. of Physiol., ERATO, JRDC, Meguro-ku 153, [3]Lab. Neurochem, National Inst. for Physiol. Sci., Okazaki 444, Japan.

In order to further understand signal processing in the hippocampus, we are studying the polysynaptic transmission from mossy fibers to CA1 cells in the rat hippocampal slices with optical recording using a voltage sensitive dye (RH482). We have demonstrated a slow transmission between CA3 and CA1 mediated by CA2 cells in addition to a conventional pathway via Schaffer collateral (Sekino, Y., et al., Soc. Neurosci. Abstr. 1992 and 1995). The mossy-fibers induced optical signals and population spikes (PSs) in CA1 were observed on the slices dissected at an angle of 30-45 degrees to the long axis of hippocampus but rarely in the transversely dissected slices. However, treatment with an antagonist of adenosine A1 receptor, 8-cyclopentyltheophylline (8-CPT; 2 μM), revealed these responses of CA1. They were eliminated by microsurgical removal of the CA2 cell layer or the local application of 0.1 % pentobarbital to the stratum oriens of CA2. These results suggest that CA2 modulates the activity of mossy fiber-CA1 pathway through adenosine A1 receptor. Facilitatory effects of 8-CPT on the strength-response relation of PSs and miniature excitatory postsynaptic currents were confirmed by field potential and patch clamp recording respectively from CA2 cells.

LEARNING AND MEMORY: PHARMACOLOGY VI

446.1

THE ALPHA-2A NORADRENERGIC AGONIST GUANFACINE REVERSES THE WORKING MEMORY DEFICITS INDUCED BY PHARMACOLOGICAL STRESS (FG7142). S.G. Birnbaum* and A.F.T. Arnsten, Interdepart. Neurosci. Prog. and Sect. Neurobiol., Yale Univ. Sch. Med., New Haven, CT 06520

The $\alpha 2_A$ adrenergic agonist, guanfacine, appears to be beneficial in the treatment of prefrontal cortical (PFC) deficits in patients with Attention Deficit Hyperactivity Disorder (ADHD), and has fewer side effects than the $\alpha 2_{A,B,C}$ agonist, clonidine. Interestingly, guanfacine also may relieve ADHD symptoms associated with the stress of child abuse. In animals studies, stress exposure has been shown to impair PFC cognitive function by a hyperdopaminergic mechanism. For example, the pharmacological stressor FG7142 have been shown to increase dopamine (DA) turnover in the PFC and impair delayed alternation performance in the rat (Murphy et al., 1996). Pretreatment with clonidine blocks the deficit induced by FG7142 (see Murphy, Arnsten, Jentsch and Roth, this volume).

In this study, we explored the subtype of noradrenergic $\alpha 2$ receptor effective in reversing the FG7142 induced impairment by comparing clonidine with the more selective $\alpha 2_A$ agonist, guanfacine. Pretreatment with clonidine (0.1 mg/kg) blocked the FG7142 (30 mg/kg) induced impairment in most rats. The rats which were not improved by this dose of clonidine exhibited fairly severe clonidine side-effects (ataxia and sedation). Pretreatment with an equimolar dose of guanfacine (0.11 mg/kg), completely blocked the FG7142-induced impairment without any observable side effects. Neither clonidine nor guanfacine significantly altered performance when given alone. Guanfacine also appears to be equally effective in blocking the stress-induced rise in PFC DA turnover (Morrow, George and Roth, this volume), consistent with an $\alpha 2_A$ mechanism modulating the meso-PFC DA stress response. This mechanism may contribute to guanfacine's beneficial clinical effects, protecting PFC cognitive function from the detrimental effects of stress. This research was supported by PHS grant AG06036.

446.2

ALPHA-1 NORADRENERGIC STIMULATION IMPAIRS, WHILE ALPHA-2 STIMULATION IMPROVES, PREFRONTAL CORTEX SPATIAL WORKING MEMORY FUNCTION. J.C. Steere*, B.-M. Li, J.D. Jentsch, R. Mathews, J.R. Taylor and A.F.T. Arnsten. Shanghai Institute of Physiology, Shanghai, China 200031; and Sect. Neurobiology and Dept. Psychiatry, Yale Medical School, New Haven, CT, USA 06510.

Studies in animals and humans indicate that norepinephrine (NE) improves prefrontal cortex (PFC) cognitive functioning through actions at post-synaptic, alpha-2 adrenergic receptors in the PFC (see Arnsten, Steere and Hunt, Arch Gen Psych 53: 448-455, 1996 for review). For example, systemic administration of the alpha-2 adrenergic agonist, guanfacine, to young or aged monkeys significantly improves delayed response performance, a spatial working memory task dependent on PFC function. However, few studies have examined the effects of increasing stimulation of alpha-1 adrenergic receptors on PFC cognitive abilities. The current study examined the effects of systemic administration of the alpha-1 agonist, cirazoline, on delayed response performance in aged monkeys. Cirazoline is the first alpha-1 agonist to readily penetrate the brain; however, it has additional actions at imidazoline receptors. Results indicate that low doses of cirazoline impaired delayed response performance in a delay-dependent manner. The impairment was blocked by the alpha-1 antagonist, prazosin, consistent with actions at alpha-1 receptors. Additional findings indicate that impairment resulted from actions in the PFC. Infusion of the selective alpha-1 agonist, phenylephrine, into the principal sulcal PFC of a young monkey significantly impaired delayed response performance in a delay-dependent manner. In contrast, intra-PFC infusion of the alpha-2 agonist, guanfacine, improved performance following longer delays. Parallel studies in the rodent with PFC infusions are in progress. These data suggest that NE has opposing actions at post-synaptic alpha-2 vs. alpha-1 receptors in the PFC. This research was supported by PHS grant no. AG06036 to AFTA and NSFC grant no. 39000032 to BML.

446.3

CHANGES IN AMYGDALA NOREPINEPHRINE RELEASE FOLLOWING FOOTSHOCK OR SYSTEMIC ADMINISTRATION OF EPINEPHRINE. C.L. Williams*, P.E. Gold and D. Men. Dept. of Psychology, University of Virginia, Charlottesville, VA 22903.

It is well established that elevations in peripheral hormones in response to emotionally arousing experiences influence memory storage. Although peripheral hormones such as epinephrine (EPI) posses a limited capacity to cross the BBB to produce direct effects on the brain, recent evidence indicates that EPI's effects on memory are mediated in part, through noradrenergic (NE) activation of the amygdala. The present study examined whether experimental manipulations designed to increase EPI concentrations in the periphery, produce corresponding elevations in extracellular levels of NE in the amygdala. A microdialysis probe (2 mm) was inserted into a guide cannula positioned 1 mm above the amygdala in male Sprague Dawley rats 1 hr before testing. Throughout the experiment, aCSF was perfused through the probe at 1 µl/min and dialysate samples were collected every 20 min. After 3 baseline samples were collected, saline was given ip to control for possible injection effects on NE levels. EPI (0.3 mg/kg) was given ip 1 hr after saline and a single 0.8 mA footshock (1 sec) was administered 1 hr and 40 min after the injection of EPI. NE levels were determined using HPLC and EC detection. NE levels after ip injection of saline did not differ from basal levels. EPI produced a significant elevation in extracellular NE that persisted for 40 min after injection. Footshock also produced elevated NE levels although the magnitude and duration of the increase was not as pronounced. These results extend previous findings indicating that peripheral hormones influence memory storage processes via activation of NE in the amygdala. Supported by NIA (AG07648) and NINDS (NS32914).

446.4

DIFFERENTIAL HOUSING HAS SEXUALLY DIMORPHIC EFFECTS ON ODOR PREFERENCES, IMMUNE CELL PHENOTYPES AND WEIGHT LOSS IN CONTROL AND DSP-4 TREATED CD-1 MICE. C. Cornwell*, J. Gavalchin, T. Dewey, H. McFarlane, M.F.E.M. Sarao, C. Coticchia, W. Gerlach, H. McLeod and K. O'Leary. Depts of Psychology and Biology, Syracuse Univ., and Dept. of Medicine, SUNY Health Science Center, Syracuse, NY, 13244.

Norepinephrine (NE) neurotoxins impair behavioral and neurochemical responsiveness by rats and mice to changes in the olfactory and social environments (Cornwell et. al., 1996, Brain Research; Dewey et al., 1995, Soc. Neurosci. Abstr.) The present study evaluated immune status in differentially housed NE depleted CD-1 mice. On postnatal day 61, mice in same-sex living groups of 8 were weighed and assigned to one of three housing conditions: untreated (UNT), laminar flow (LF) or isolated olfactory enclosure (IOE). LF and IOE mice were placed in smaller cages, with a new bedding type, in groups of 4 and injected with 50 µg/g of the NE neurotoxin, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) or water vehicle. Preferences for male vs. female nest odors were tested 3 days later. The next day, the mice were weighed, sacrificed, and hippocampal and splenic tissue was dissected for assay of monamine concentrations and lymphocyte populations respectively. Only IOE males (in both treatment groups) showed significant odor preferences (for female odor). Only LF, DSP-4 and IOE, vehicle and DSP-4 females lost significant weight (5-7%). Immune cell phenotypes were altered in both injection groups as measured in differences of percentages of T-cell and B-cell populations in UNT vs. experimental mice of both sexes. Thus, DSP-4 treatment influenced housing effects on weight loss, but not immune measures. The results also suggest that sex influences the biobehavioral effects of differential housing. *Supported by the Graduate School, Biology and Psychology Depts., Syracuse University.*

446.5

MOTORIC AND ASSOCIATIVE LEARNING EFFECTS OF 5-$HT_{2A/2C}$ ANTAGONISTS IN THE RABBIT. S. Welsh, A.G. Romano* and J.A. Harvey. Div. of Behavioral Neurobiology, Dept. of Pharmacology, The Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19129.

Drugs classified as partial agonists at the 5-HT_2 receptor (e.g., LSD) are known to enhance associative learning. We previously reported that ritanserin, a 5-$HT_{2A/2C}$ antagonist, retarded acquisition of the rabbit's classically conditioned nictitating membrane (NM) response and reduced the amplitude of the unconditioned reflex response. We now report the effects of several other 5-$HT_{2A/2C}$ antagonists on acquisition of NM conditioning and on elicitation of the NM reflex. Ritanserin and MDL-11,939 but not LY-53,857 produced a dose-dependent retardation of acquisition to a tone CS and a reduction in the amplitude of the NM reflex. None of these three drugs had an effect on nonassociative learning. We then examined the effects of these and other drugs on elicitation of the unconditioned NM reflex. The 5-$HT_{2A/2C}$ agonist, LSD and the 5-$HT_{2A/2C}$ antagonists, BOL and LY-53,857 had no effect on the NM reflex. However, four other 5-$HT_{2A/2C}$ antagonists significantly reduced the amplitude of the NM reflex with the magnitude of their effects being mianserin > ritanserin = MDL-11,939 > ketanserin. These data suggest that mianserin, ritanserin, MDL-11,939 and ketanserin may be inverse agonists at the 5-$HT_{2A/2C}$ receptor, while BOL and LY-53,857 are neutral antagonists. These data also demonstrate that activation of the 5-$HT_{2A/2C}$ receptors in the brain are necessary for normal learning and motor performance. Supported by NIMH MERIT award MH16841.

446.6

CHANGES IN CA1 HIPPOCAMPAL CELL FIRING PATTERNS AND THEIR THETA RHYTHM PHASE RELATIONSHIPS FOLLOWING 5-HT3 RECEPTOR ANTAGONISM.

J. Reznic* and U. Staubli, Center for Neural Science, New York University, New York, NY 10003.

5-HT3 receptor antagonism has been shown to facilitate induction of long-term potentiation in CA1 and CA3 hippocampal subfields, to increase the frequency of the hippocampal theta rhythm *in vivo*, and to enhance performance in learning paradigms. Recent work from our lab has demonstrated that the majority of recorded interneurons decrease, and pyramidal cells increase, their mean firing rate following systemic injection of the 5-HT3 receptor antagonist ondansetron. The present study investigated the effects of 5-HT3 blockade on 1) the firing patterns and rhythmicity of 19 CA1 hippocampal cells (14 interneurons and 5 pyramidal cells) as well as 2) changes in their phase relationship with the theta rhythm. For part 1, changes in both the number of single events and in burstiness (number of bursts, burst duration, number of components and inter-component duration per burst) were examined. Autocorrelograms were computed to assess changes in rhythmicity of both cell types. To accomplish part 2, a computer program extracted sequential one second segments of EEG and spike data from five minute recordings and constructed a phase-of-firing histogram to evaluate phase changes between pre- and post-drug administration. Preliminary data suggest that the firing patterns, rhythmicity, and theta-phase relationships of both cell types are modified as a consequence of 5-HT3 receptor blockade; these changes are currently being quantified.

446.7

BLOCKADE OF 5-HT_{1A} RECEPTORS COMPENSATES LOSS OF HIPPOCAMPAL CHOLINERGIC NEUROTRANSMISSION INVOLVED IN WORKING MEMORY OF RATS. M. Ohno* and S. Watanabe. Dept. of Pharmacology, Faculty of Pharmaceutical Sci., Kyushu Univ., Fukuoka 812, Japan.

To clarify roles of hippocampal 5-HT_{1A} receptors in regulating working memory function of rats, the effects of intrahippocampal administration of the agonist and antagonist at this receptor type on working memory performance were examined with a three-panel runway task. The 5-HT_{1A} receptor agonist 8-OH-DPAT, injected bilaterally at 1-10 µg/side into the dorsal hippocampus, dose-dependently increased the number of errors (attempts to pass through two incorrect panels of the three panel-gates at four choice points) in the working memory task, but it had no effect on reference memory errors. NAN-190, a selective 5-HT_{1A} receptor antagonist, did not affect the number of working memory errors, when injected intrahippocampally at 0.32 or 1.0 µg/side. Intrahippocampal administration of the muscarinic receptor antagonist scopolamine at 0.32-3.2 µg/side or the competitive NMDA receptor antagonist CPP at 3.2-32 ng/side caused a dose-dependent increase in the number of working memory errors without affecting reference memory errors. The increase in working memory errors induced by intrahippocampal scopolamine (3.2 µg/side) was reduced by concurrent infusion of 1.0 µg/side NAN-190. In contrast, NAN-190 at 1.0 µg/side did not affect the increse in working memory errors, when infused intrahippocampally together with 32 ng/side CPP. These results indicate that activation of hippocampal 5-HT_{1A} receptors interferes with working memory processing. Also, it is suggested that blockade of hippocampal 5-HT_{1A} receptors can compensate deficiency of septohippocampal cholinergic activity involved in working memory function of rats, but it does not affect impairment of working memory resulting from block of NMDA receptor-mediated neurotransmission.

446.8

INHIBITION OF PROTEIN KINASE A IN THE NUCLEUS ACCUMBENS BLOCKS AMPHETAMINE-PRODUCED CONDITIONED PLACE PREFERENCE IN RATS. R.J. Beninger*, P.L. Nakonechny and M. J. Todd. Depts. Psychology and Psychiatry, Queen's Univ., Kingston, Canada, K7L 3N6.

The nucleus accumbens is an important structure for place conditioning and some data implicate D1-like dopamine receptors, those that activate the cyclic AMP pathway. The present study further evaluated the role of D1-like receptors by inhibiting cyclic AMP dependent protein kinase (PKA) during conditioning. Rats were pre-exposed (15 min/day for 3 days) to an apparatus with two distinct chambers connected by a tunnel. During conditioning (30 min/day for 8 days) one side was paired with intra-accumbens drug injections (0.5 µl) on alternate days and the other side with vehicle injections. Drugs included amphetamine (20 µg/side), amphetamine co-injected with the PKA inhibitor Rp-cAMPS (2,5, 25,0, 250.0 ng/side) or the same doses of the inhibitor alone. Testing sessions were like pre-exposure sessions. Results revealed a significant increase in time spent on the drug-paired side from pre-exposure to test for animals treated with amphetamine or amphetamine plus 2.5 ng/side of Rp-cAMPS; 25.0 ng/side of Rp-cAMPS partially blocked the effect and 250.0 ng/side totally blocked it. Rp-cAMPS alone had no significant effect. Results suggest that place preference conditioning produced by intra-accumbens amphetamine depends on stimulation of D1-like dopamine receptors and activation of cyclic AMP dependent protein kinase. (Funded by N.S.E.R.C.)

446.9

SYSTEMIC BUT NOT INTRA-ACCUMBENS INJECTIONS OF 7-OH-DPAT PRODUCE A PLACE PREFERENCE IN RATS. D. P. Nocent*, P. E. Mallet and R. J. Beninger. Depts. Psychol. and Psychiat., Queen's Univ., Kingston, K7L 3N6, Canada.

Dopamine neurotransmission in the nucleus accumbens has been implicated in place preference conditioning but little is known regarding the function of D3 receptors in the accumbens. The present experiment evaluated place conditioning following intra-accumbens 7-OH-DPAT, a dopamine D3-receptor preferring agonist. The paradigm involved three phases: preconditioning (three 15-min drug-free exposures to an apparatus with two distinctive compartments connected by a tunnel); conditioning (four 30-min pairings of one compartment with drug and four 30-min pairings of the other compartment with saline on alternating days); and test (like preconditioning). During the conditioning phase, rats (N=112) implanted with bilateral chronic indwelling cannulae received intra-accumbens injections (0.5 µl) of amphetamine (10 µg/side) or 7-OH-DPAT (0.1, 1.0, 5.0, 10.0 or 15.0 µg/side); in addition, uncannulated rats (N=12) were treated with 7-OH-DPAT (5.0 mg/kg, s.c.). Intra-accumbens amphetamine and systemic 7-OH-DPAT produced a significant place preference. Intra-accumbens 7-OH-DPAT failed to produce a place preference at 0.1, 1.0, 10.0, and 15.0 µg/side; a significant place aversion was found at 5.0 µg/side. The place preference with systemic 7-OH-DPAT replicates previous findings; results with intra-accumbens 7-OH-DPAT suggest that this structure may not mediate the preference produced by systemic 7-OH-DPAT. (Supported by NSERC).

446.10

SUPRANORMAL STIMULATION OF DOPAMINE D1 RECEPTORS IN THE PREFRONTAL CORTEX IMPAIRS SPATIAL WORKING MEMORY PERFORMANCE IN RATS. J. Zahrt, J.R. Taylor, and A.F.T. Arnsten*. Sect. Neurobiology, Dept. Psychiatry, Yale Med. School, New Haven, CT 06510.

Previous research has shown that increasing endogenous dopamine (DA) turnover in the prefrontal cortex (PFC) by administering the anxiogenic beta carboline FG7142 results in impaired PFC cognitive performance on a spatial working memory task (Murphy et al., 1994; 1996). This impairment correlated with increased DA turnover in the PFC, and was blocked by pretreatment with the D1 receptor antagonist, SCH23390 (ibid). Similarly, low concentrations of D1 receptor antagonists can enhance delayed-related firing in PFC neurons (Williams and Goldman-Rakic, 1995). These results suggest that supranormal D1 receptor stimulation in the PFC can impair working memory performance. The current study tested this hypothesis by infusing the full D1 agonist, SKF81297, into the medial PFC in rats performing the delayed alternation task. In the first experiment, bilateral infusions of SKF81297 (0, 0.01 or 0.1 ug in 0.5 ul) were made through indwelling cannula aimed at the PFC. SKF81297 produced a dose related reduction in working memory performance; only the 0.1 ug dose significantly impaired performance. This dose had no significant effect on response time in the maze, and no effect on behavioral ratings of anxious behaviors, consistent with effects on PFC cognitive function. A second experiment tested for D1 receptor mechanisms underlying the impairment in working memory performance. Rats were pretreated with saline or SCH23390 (0.01-0.03 mg/kg, i.p.) 60 min before receiving PFC infusions of SKF81297 (0.1 ug) or saline into the PFC. As before, 0.1 ug SKF81297 significantly impaired performance in saline pretreated animals. SCH23390 pretreatment, which had no effect on its own, significantly blocked the impairment induced by intra-PFC SKF81297 infusion. These results are consistent with supranormal DA D1 receptor stimulation impairing PFC cognitive function. This research was supported by PHS grant AG06036 to AFTA.

446.11

HIPPOCAMPAL D1 AND D2 SYSTEMS AND WORKING MEMORY FUNCTION. A. Wilkerson and E.D. Levin*, Neurobehavioral Research Laboratory, Psychiatry Department, Duke University Medical Center., Durham, NC 27710 USA.

The hippocampus has long been known to be important for memory function. However, the involvement of hippocampal dopamine (DA) systems has received little attention. Bilateral local infusions of DA D1 and D2 agonists and antagonists were made into the ventral hippocampus of female Sprague-Dawley rats. Then working memory function was assessed in the radial-arm maze. The D1 agonist dihydrexidine (1.1 ug/side) caused a significant ($p<0.05$) improvement in radial-arm maze choice accuracy. Higher doses of 3.3 or 10 ug/side were not effective. The D1 antagonist SCH 23390 was ineffective over a dose range of 0.19-1.67 ug/side. The D2 agonist quinpirole caused a significant ($p<0.05$) dose-related improvement in choice accuracy over a dose range of 1.1-10 ug/side. In a complementary fashion, the D2 antagonist raclopride caused a significant memory deficit at 1.67 ug/side. Lower doses of 0.19 and 0.56 ug/side were ineffective. This study provides clear evidence that hippocampal D2 activity is positively related to working memory performance. Evidence for D1 systems is less compelling. DA systems in the hippocampus may work with the well-characterized hippocampal cholinergic systems in the basis of working memory function. (This research was supported by the National Alliance for Research on Schizophrenia and Depression.)

446.12

DOPAMINE D2, AND NOT D3 OR D4, RECEPTORS ARE RESPONSIBLE FOR THE REINFORCING EFFECT OF BRAIN STIMULATION IN THE RAT. Shinshu Nakajima* Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada.

Self-stimulation of the medial forebrain bundle can be suppressed by injection of either SCH 23390 or raclopride into the nucleus accumbens. The involvement of D1 dopaminergic receptor in the reinforcing effect is evident because SCH 23390 is known to act selectively on D1 receptors. The involvement of D2 receptor is not so clear because raclopride has about equal affinity for D2 and D3 receptors. In the present study, rats were implanted with a bipolar electrode into the medial forebrain bundle, and a cannula into the nucleus accumbens, and trained to press a bar for electrical stimulation. The frequency-response function was plotted 30 min. before and 10 min. after injection of 1 µl of dopamine antagonist solutions or physiological saline through the cannula.

The threshold of reinforcement (the frequency for 50% of maximal pre-injection rate) significantly increased after injection of 10 nmol haloperidol or 10 nmol raclopride, but not after 30 nmol of UH 232 (selective D3 antagonist) or 20 nmol of clozapine (antagonist with high affinity for D4 receptors). Among dopamine receptors in the nucleus accumbens, D2 receptors, along with D1 receptors, seem to be critically involved in producing the reinforcing effect of medial forebrain stimulation, but D3 and D4 receptors are not.

(Supported by NSRC of Canada, OGP0000233.)

446.13

TYPICAL AND ATYPICAL NEUROLEPTICS DIFFERENTIALLY AFFECT PSYCHO-MOTOR SKILLS ACQUISITION IN SCHIZOPHRENIA: FURTHER CONSIDERATION ON THE ROLE OF THE STRIATUM IN PROCEDURAL LEARNING. H. Scherer, M.A. Bédard, E. Stip and H. Cohen *, Lab. de Neurosciences de la Cognition, UQAM, Montreal, Canada.

Thirty-nine schizophrenic patients were divided in 3 groups according to their pharmacological treatment: 1) drug naïve 2) haloperidol 3) clozapine. They were all assessed on psychiatric and extrapyramidal clinical scales (BPRS, PANSS and ESRS). Procedural learning was assessed with the Mirror Drawing task, in which the subject must draw in outline a picture (a star) by looking only to its mirror reflect. Results revealed that drug-naïve patients showed a normal and gradual procedural learning, up until they reached and maintained their optimal level of performance. Patients treated with clozapine showed also a gradual learning as a function of trials, although performances *per se* in the first trials were significantly worse than the two other groups. Finally, patients treated with haloperidol showed large fluctuations in performance between trials that indicates a procedural learning difficulty. Further analysis using ANCOVA indicates that these effects may not be explained by clinical or sociodemographic differences between groups. This suggests that classical and atypical neuroleptics can differentially affect procedural learning in schizophrenia, presumably by a differential dopamine D_2 blocking action of these substances in the striatum.

Suported in part by the "Fonds de la Recherche en Santé du Québec".

447.1

DRUGS OF ABUSE DISRUPT MEMORY MEASURED UNDER A DELAYED MATCHING-TO-SAMPLE PROCEDURE IN SQUIRREL MONKEYS. S.P. Baron, W.D. Wessinger* and G.R. Wenger. Dept. of Pharmacology and Toxicology, Univ. of Arkansas for Medical Sciences, 4301 W. Markham, Little Rock, AR 72205.

The abilities of drugs of abuse from several pharmacological classes to disrupt memory were examined in four adult male squirrel monkeys responding under a delayed matching-to-sample schedule of food presentation. Subjects were required to emit 20 responses on a sample key transilluminated by either a constant white or a flashing blue light. The twentieth response initiated a 3-second delay followed by presentation of two comparison stimuli. If a response was made to the key that matched the sample stimulus (correct match), a single food pellet (97 mg) was delivered. Pentobarbital (0.32 - 10 mg/kg), diazepam (0.1 - 5.6 mg/kg), phencyclidine (0.01 - 0.32 mg/kg), and cocaine (0.1 - 3.2 mg/kg) dose-dependently reduced accuracy of matching performance towards chance levels. Amphetamine (0.01 - 1.0 mg/kg) administration resulted in a small, but statistically significant, reduction in accuracy at a dose of 0.56 mg/kg, while 1.0 mg/kg completely suppressed responding. Signal-detection analyses indicated that decrements in discriminability of stimuli accounted for a greater proportion of error than the did the development of color- or key-response biases. These results may indicate that drugs of abuse share detrimental effects on cognitive processes. This research was supported by NIDA Grant # DA 05815.

447.2

A TWO DAY PROCEDURE TO ASSESS DRUG EFFECTS IN THE SPATIAL WATER MAZE. L. Rajachandran*, A.B. Johnson, M.R. Gates, A.C. Enders, D.L. Gambini, and J.V. Cassella. Neurogen Corporation, Branford, CT 06405.

The Morris water maze has been used extensively as a tool to study spatial learning. In this task, rats must navigate using distal extramaze spatial cues to find a submerged platform in order to escape from water. Typically, multiple training days are employed. We are describing a two day procedure employing either a four or six trial training (acquisition) procedure. The four trial procedure best detects cognitively enhancing properties of drugs while the six trial procedure best detects drugs that produce cognitive deficits. To illustrate these differences male Sprague Dawley rats were pretreated with either physostigmine or scopolamine prior to training in this task. Animals began each training trial from a different quadrant of the tank and were allowed to remain on the platform for ten seconds once they found it. Twenty-four hours later animals were tested for retention on one trial. Latency to locate the platform as well as other measures were taken during acquisition and retention testing. Physostigmine produced significant improvements in retention (MED .003 mg/kg,IV) whereas scopolamine produced deficits in this task (MED .125 mg/kg,IP). These data illustrate the sensitivity of this two day procedure to detect amnestic or memory enhancing effects of drugs. (Funding provided by Neurogen Corporation.)

447.3

SELECTIVE DISRUPTION OF CS AND US INPUTS BY PHARMACOLOGICAL AGENTS: THE BRAINSTEM AS A SITE OF CS-US ASSOCIATIVITY IN TASTE AVERSION LEARNING. M.W. Swank, A.E. Ellis, and W.D. Blaker*. *Furman University, Greenville, SC 29613.* *(Supported by NIH/NICHHD #HD33138-01 to MWS)*

A recent report from this lab describes the disruption of taste aversion learning in mice by 4th ventricular infusions of c-Fos antisense. These data suggest that antisense disrupts associative events by blocking US-mediated events in the nucleus. Here we tested a variety of pharmacological agents and examined their effect on taste aversion learning when infused into the fourth ventricle. The tyrosine kinase inhibitor genistein blocks acquisition of taste aversion learning when administered before the CS saccharin. Genistein had no effect on saccharin intakes during conditioning, suggesting that it does not impair basic sensory processing of taste. The drug had no effect on learning when infused after CS saccharin, suggesting that the effect of genistein is to disrupt intracellular events necessary for association of the CS taste with the antecedent US toxin. Although high doses of genistein can inhibit serine/threonine kinase activity, higher doses were ineffective in blocking taste aversion learning. Furthermore, H-7, a serine/threonine kinase inhibitor which blocks protein kinase C, A and G, was ineffective in blocking learning at any dose tested. Current work is focussed on identifying the signal transduction pathways activated by both the CS and US in the brainstem of the mouse.

447.4

EVALUATION OF EMOTIONAL MEMORY IN THE ELEVATED T-MAZE: IMPORTANCE OF A MULTIPLE-TRIAL TRAINING-TO-CRITERION PROCEDURE. C. A. Conde; V. Costa and C. Tomaz*. Laboratory of Psychobiology and Center for Neuroscience and Behavior, University of São Paulo, Ribeirão Preto, Brazil.

The present study investigate the importance of a multiple-trial, training-to-criterion procedure to provide a more detailed analysis of the mnemonic component in the elevated T maze test. For this purpose, two experiments were undertaken. In the first one, we compared the standard (three-trials) test procedure versus the training to criterion-continuos trial procedure. The second one, the effects of diazepam (i.p. doses of 1, 2 and 4 mg/Kg administered 30 min. before training) was assessed on acquisition and retention of the elevated T maze in the training-to-criterion procedure. The training was conducted using either multiple trials in which the rat was free to shuttle back and forth between open and closed arms until it stayed in the closed arm for 300 seconds or placing the rat 3 times (1 minute intertrial intervals) at the end of the enclosed arm and the time taken to withdraw from this arm was recorded. Following this avoidance training (1 min), the same rat was placed at the distal end of the right open arm, and the time taken to withdraw from this arm was recorded (escape training). To assess memory, inhibitory avoidance and escape were measured again three days later without drug. The results of the first experiment indicate that the criterion-continuos-trial procedure warrant a confinable evaluation of memory, probably because it offer an good level of information acquisition. The second experiment, showed a dose-dependent amnesia in the retention tested 72 hours later on inhibitory avoidance without differences on acquisition performance. The escape latency showed a trend for a decrease in every animal tested 72 h after. These results indicate the importance to use a training-to-criterion procedure when using pre-trial drug treatment to evaluate mnemonic processes in the elevated T-maze test.

447.5

EFFECTS OF SPECIFIC OPIATE RECEPTOR ANTAGONISTS ON THE HABITUATION OF NOVELTY-INDUCED HYPOALGESIA. E. Spreekmeester* and J. Rochford, Douglas Hospital Research Center, Department of Psychiatry, McGill University, Montreal, P.Q., H4H1R3.

Exposure to novel stimuli has been shown to activate endogenous antinociceptive substrates. This novelty-induced hypoalgesia (NIH) habituates with repeated exposure to the stimuli. Previous studies have shown that the habituation of NIH can be attenuated by systemic or intracerebroventricular (ICV) naloxone administration. In order to determine the opiate receptor subtype that is involved in this effect, CTOP, naltrindole and nor-binaltorphimine were used as specific mu, delta and kappa receptor antagonists, respectively. Male Wistar rats were exposed to a hot-plate apparatus which was heated to 48.5°C once a day for 8 days. The latency to lick the hind paw was used as an index of pain sensitivity and a cut-off period of 90s was imposed to avoid tissue damage. CTOP (0.5, 1.0 or 2.0 µg), naltrindole (2.5, 12.5 or 31 µg), nor-binaltorphimine (4, 20 or 50 µg) or vehicle were injected ICV 30 min. prior to each plate exposure. Both 1.0 and 2.0 µg of CTOP prevented the habituation of NIH. Naltrindole and nor-binaltorphimine failed to prevent the habituation of NIH at the doses used. These results suggest that the mu, but not the delta or kappa, receptor is involved in the habituation of NIH. (Funded by the Natural Sciences & Engineering Research Council of Canada)

447.6

NALOXONE HAS OPPOSITE EFFECTS ON PAVLOVIAN CONDITIONED BRADYCARDIA AND AMYGDALA NEURONAL ACTIVITY. L.L. Hernández*, K.L. Watson and S.L. Thompson. Neuroscience Lab., Dorn VA Medical Center, Columbia, SC 29209.

Pretraining, intravenous treatment with the opiate antagonist naloxone-HCl promotes Pavlovian cardiac conditioning in rabbits, delays extinction, and inhibits associated multiple unit neuronal activity in amygdala central nucleus (ACN; Behav. Brain Res., 1990, 41, 71-79). We further investigated naloxone (0.5 mg/kg, i.v.) effects on Pavlovian conditioning and extinction of heart rate and ACN neuronal discrimination, using one 4-s tone (the CS+) to signal a shock US, and another (the CS-) to signal no US. In saline-treated rabbits, increases in CS-evoked neuronal activity, that were larger during the CS+ than the CS-, accompanied development of cardiac discrimination. Naloxone promoted bradycardiac CRs, delayed extinction of cardiac discrimination, and reduced evoked neuronal activity during both CSs, compared to saline. These findings extend our previous conclusions that the increase in CS-evoked ACN neuronal activity early in training normally serves to inhibit conditioned bradycardia, whereas conditioned decreases in ACN neuronal activity disinhibit bradycardiac CRs, and that the circuitry driving these changes is inhibited by endogenous opioids acting afferent to the ACN.

Supported by DVA General Medical Research funds

447.7

MUSCIMOL INACTIVATION OF CEREBELLAR CORTEX DURING ELECTRICAL MICROSTIMULATION CLASSICAL CONDITIONING OF DISCRETE MOTOR MOVEMENTS. A. J. Annala*, R.E. Clark, A.T.M. Moro, P.G. Shinkman, R.A. Swain, and R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

New Zealand White rabbits (*Oryctolagus cuniculus*) acquire, extinguish, and reacquire conditioned responses when exposed to low intensity electrical stimulation (conditioned stimulus, CS) at the base of cerebellar lobule HVI in place of exteroceptive tone conditioned stimulus and high intensity electrical stimulation (unconditioned stimulus, US) at the base of cerebellar lobule HVI (through the same electrodes) in place of exteroceptive air puff unconditioned stimulus. Repeated presentation of low intensity electrical stimulation paired with high intensity electrical stimulation results in robust acquisition of conditioned responses. Repeated presentation of low intensity electrical stimulation in well trained animals results in extinction of conditioned responses. Subsequent repeated presentation of low intensity electrical stimulation paired with high intensity electrical stimulation results in robust reacquisition of conditioned responses. Acquisition is completely prevented by infusion of muscimol into cerebellar lobule HVI during paired training. Extinction is completely prevented by infusion of muscimol into cerebellar lobule HVI during CS alone training. Infusion of muscimol has no significant effect on the amplitude of unconditioned responses. Extensive pre-exposure through repeated presentation of electrical microstimulation where the delivery of CS and US are uncorrelated has no significant effect on subsequent acquisition of conditioned responses. These results strongly support the view that the site of plasticity underlaying acquisition and extinction is highly localized in cerebellar lobule HVI. (Supported by NSF IBN-9215069, ONR N00014-95-1-1152, and NIMH 5-P20-MH-52194 to R.F. Thompson.)

447.8

AUTOPHOSPHORYLATION OF Ca^{2+}/CALMODULIN DEPENDENT PROTEIN KINASE II IS INVOLVED IN FORMATION OF SHORT TERM MEMORY FOLLOWING PASSIVE AVOIDANCE LEARNING W. Q. Zhao* and K. T. Ng Dept. of Psychology, Monash University, Clayton, Victoria 3168, Australia

We have previously reported an elevation of Ca^{2+}calmodulin-stimulated Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) activity in short-term memory (STM) formation induced by a passive avoidance training experience in day-old chicks (Zhao et al., *Neurobiol. Learning and Memory*, **65**: 1996 in print). In the present study, in order to identify training-induced calcium-independent activity of CaMK II, we investigated, using immunobiochemical methods, changes in *in vivo* autophoshorylation of CaMK II associated with memory formation. One day-old chicks were trained on a single trial passive avoidance task. CaMK II were isolated by immunoprecipitation from a specific brain region, the intermediate medial hyperstriatum ventrale (IMHV), dissected from both trained and control animals. Autophoshorylation of CaMK II was examined by anti-phosphoserine and anti-phosphothreoine antibodies in the Western blotting. An increase in autophosphorylation of CaMK II was detected in IMHV tissue from the right hemisphere 10 min following training in comparison with that from the control animal. In the left hemisphere, however, this increased autophosphorylation of CaMK II was not seen unless the animal was subject to the test trial during which a memory retrieval process is theoretically involved. No increase in autophosphorylation of CaMK II was shown in IMHV tissue from chicks subjected to training but did not remember the experience when tested. Furthermore, when KN-62, a CaMK II inhibitor shown to impair STM, was administered into the IMHV immediately after training, the increases in both autophosphorylation and Ca^{2+}/calmodulin-stimulated activity of CaMK II were abolished. These results suggest that changes in autophosphorylation of CaMK II are required for formation of STM, and that these changes may play different roles in the two hemispheres during memory processing.

This research was supported by Monash University.

447.9

ENHANCEMENT OF MEMORY-BASED LEARNING WITH D-AMPHETAMINE TREATMENT IN RAT PUPS WITH HIPPOCAMPAL GRANULE CELL HYPOPLASIA: A POSSIBLE MODEL FOR ADHD. D.A. Highfield*, D. Hu and A. Amsel Univ. of Texas at Austin, Austin, Tx. 78712

Selective exposure to x-irradiation during infancy, from postnatal days (PND) 2-11 in the rat, results in hippocampal granule cell hypoplasia (e.g., Altman, 1986). Preweanling rats with such hippocampal granule-cell agenesis show deficits in patterned single alternation (PSA), a form of memory-based learning (Diaz-Granados et al., 1992), and in the partial-reinforcement extinction effect (PREE), reflecting, particularly, absence of the normal persistence in extinction after partial-reinforcement training (Diaz-Granados et al., 1994). Deficits in attention and persistence, along with increased arousal, have also been discussed in relation to learning deficits observed in children diagnosed with Attention Deficit-Hyperactivity Disorder (ADHD, Amsel, 1990). The present study examined the effects of d-amphetamine, a drug commonly used in the treatment of ADHD, on PSA, after infantile exposure to x-irradiation (PND2-15). Following i.p. injections of 0.3 mg/kg d-amphetamine, x-irradiated preweanling rats (PND 17-18) were trained on PSA. Initial results indicate that d-amphetamine enhances PSA in x-irradiated rats relative to x-irradiated controls. These results, showing alleviation of x-irradiation-related deficits in short-term memory by d-amphetamine injections, along with our earlier results showing deficits after x-irradiation (Diaz-Granados et al., 1992, 1994), encourage the hypothesis that hippocampal granule cell hypoplasia, Altman's model of "minimal brain dysfunction," may be a contributing factor in ADHD, and may provide a good animal model of the disease.

This work supported by NIAAA Grant AA07052 and NIAAA Training Grant AA07471.

447.10

THE IMPROVING EFFECTS OF COCAINE ON MEMORY CONSOLIDATION IN MICE ARE ANTAGONIZED BY THE NON-COMPETITIVE NMDA RECEPTOR ANTAGONIST MK-801. C. Rossi-Arnaud*[1], V. Cestari, C. Castellano. Istituto di Psicobiologia e Psicofarmacologia (C.N.R.) Via Reno 1, 00198 Roma, Italy - [1]Facoltà di Psicologia, Università degli Studi di Roma "La Sapienza", Via dei Marsi 78, 00185 Roma, Italy.

The interaction between cocaine and MK-801 was studied in CD1 mice injected intraperitoneally immediately after training with these drugs and tested in two tasks (one-trial inhibitory avoidance and active avoidance). In the inhibitory avoidance task cocaine (1, 2.5 and 5 mg/kg) dose-dependently improved memory consolidation while MK-801 (0.1, 0.25 and 0.5 mg/kg) impaired it. The administration of a per se ineffective dose of MK-801 (0.1 mg/kg) antagonized the memory improving effects of cocaine (2.5 and 5 mg/kg). In the active avoidance task cocaine improved memory consolidation at 5 mg/kg, while 2.5 and 10 mg/kg were ineffective. The administration of a per se ineffective dose of MK-801 (0.1 mg/kg) also in this case antagonized the facilitating effect of cocaine. These results suggest the existence of an interaction between the excitatory amino acid and catecholaminergic systems in modulating memory consolidation. Since dopaminergic mechanisms are involved in the effects of both MK-801 and cocaine on memory consolidation, a main role of the dopaminergic systems in the effects observed can be envisaged.

447.11

CHANGES IN RESCALING TEMPORAL RESPONDING ON THE TRI-PEAK PROCEDURE AFTER MODULATION OF COCAINE REACTIVITY. M.S. Matell[1]*, W.H. Meck[1], & G.R. King[2]. Depts. of Psychology:Expt & Psychiatry[2], Duke University, Durham, NC 27708.

The "tri-peak" procedure, a modified version of the peak-interval timing procedure, was developed allowing the determination of multiple peaks of temporal responding within a single trial without relying on different signal modalities. Three times of possible reinforcement (10, 30, & 90 s) were mapped to three spatially distinct response levers allowing separate responding for each duration during a single trial. Food reward is given on 50% of the 10- and/or 30-s durations, and 100% of the 90-s durations, thus allowing from one to three food rewards per trial. In order to assess the validity of this procedure and to assess the role of cocaine sensitzation and tolerance in rescaling temporal perception after dopaminergic drug administration, male Sprague Dawley rats (n=15, 12 mo of age) were trained and tested. After reaching criterion levels of peak time responding, 20 mg/kg cocaine HCL was administered for 14 days via either daily subcutaneous injections (.5cc) or continous infusion from an osmotic minipump (5 µL/hr, 100mg/ml)). Previous work has demonstated that these two modes of delivery result in either sensitvity (injection) or tolerance (continuous) to the behavioral activating effects of a challenge injection of cocaine (15 mg/kg) 7 days after withdrawal. Preliminary data indicate that daily cocaine injections cause an immediate horizontal rightward shift in peak times. In contrast, continuous cocaine did not cause any initial changes in peak times. These data can be interpreted in terms of changes in clock speed by examining the pattern of changes in peak time and spread seen during drug administration, withdrawal and challenge phases. (Supported by DA-08899).

447.12

EFFECT OF Δ^9-TETRAHYDROCANNABINOL MICROINJECTION INTO THE RAT BRAIN ON DELAYED MATCHING-TO-SAMPLE PERFORMANCE. A. Miyamoto, T. Yamamoto, S. Watanabe and C. Hara*, Dept. Pharmacol. Fac. Pharmaceut. Sci. Kyushu Univ. Fukuoka 812-82, Japan, *Dept. Pharmacol. Daiichi-pharmaceut. Univ. Fukuoka 815, Japan.

Administration of Δ^9-tetrahydrocannabinol (THC), a major psychoactive constituent in marijuana, has been reported to produce an impairment of short-term memory in human and animals. However, the brain regions through which THC affects short-term memory remain unknown. Therefore, the effects of THC injections directly into the rat brain on the delayed matching-to-sample (DMTS) performance using 3-lever operant apparatus were examined and were compared with those of the muscarinic receptor antagonist scopolamine.

Intraperitoneal administration of THC at 1.8 mg/kg decreased significantly the number of reinforcements in tne test trial but not in the training trial (specific impairment of DMTS performance). THC at 3.2 mg/kg (i.p.) decreased the number of reinforcements both in the test trial and in the training trial (non-specific impairment of DMTS performance). THC at 5.6 µg/side, injected bilaterally into the dorsal hippocampus, produced a significant decrease in the number of reinforcements only in the test trial. In the same way, the specific impairment of DMTS performance was also observed following microinjection of scopolamine at dose of 18 µg/side in the dorsal hippocampus. Scopolamine at 10 µg/side injected into either the medial prefrontal cortex or basolateral amygdala showed the specific impairment of DMTS performance. However, THC at doses up 32 µg/side injected into the medial prefrontal cortex or at doses up 10 µg/side into the basolateral amygdala failed to produce the impariment of DMTS performance. THC at dose of more than 32 µg/side or 10 µg side injected into the medial prefrontal cortex or the basolateral amygdala, respectively, showed the non-specific impairment of DMTS performance.

These findings indicate that THC impairs the DMTS performance, at least in part, via hippocampus.

447.13

INJECTIONS OF Δ^9-THC, BUT NOT ANANDAMIDE, PRODUCE A CONDITIONED PLACE AVERSION IN WISTAR RATS.

P. E. Mallet* and R. J. Beninger. Departments of Psychology and Psychiatry, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

Although exogenous cannabinoid ligands such as Δ^9-tetrahydrocannabinol (THC) have been implicated in reward-related learning, the role of endogenous cannabinoid agonists such as anandamide (arachidonylethanolamide) has never been tested. Thus, the effects of anandamide were tested in a place conditioning task, consisting of three distinct phases: preconditioning (three undrugged 15-min exposures to an apparatus consisting of two visually distinct compartments joined by a tunnel); conditioning (four 30-min pairings of one compartment with drug and four pairings of the other compartment with vehicle); and test (one undrugged 15-min exposure to the same apparatus). Time spent in each compartment was recorded during preconditioning and test; locomotor activity was measured during all experimental phases. Male Wistar rats (N=122) received THC (0.1, 0.5, 1.0, 1.5, 2.0, 4.0 or 8.0 mg/kg) or anandamide (0.5 or 2.0 mg/kg) during the conditioning sessions. The half-life of anandamide was increased by pretreatment with the protease inhibitor phenylmethylsulfonyl fluoride (2.0 mg/kg) during conditioning. A significant place aversion was found at the 1.0 mg/kg dose of THC. No significant place conditioning effect was found with anandamide. Locomotor activity during conditioning was significantly decreased by the 1.0, 1.5, 2.0, 4.0 and 8.0 mg/kg doses of THC and the 0.5 mg/kg dose of anandamide. These preliminary results fail to implicate anandamide in reward-related learning. (supported by NSERC)

447.14

DELTA-9-TETRAHYDROCANNABINOL INFLUENCES SEQUENTIAL MEMORY IN RATS PERFORMING A DELAYED-NONMATCH-TO-SAMPLE TASK. Robert E. Hampson*, Douglas R. Byrd, Joanne, K. Konstantopoulos, Terence Bunn, and Sam A. Deadwyler. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

Previous studies have demonstrated that the cannabinoid, delta-9-THC disrupts processing of sequential memory in rats (Hampson *et al.* JPET, 1989). In rats trained to perform a spatial delayed-match-to-sample (DMS) task, within-trial, delay-dependent memory was also disrupted in the presence of 2.0 mg/kg THC (Heyser *et al.* JPET, 1993). Recently an aspect of sequential memory has been demonstrated in the form of proactive interference in a delayed-nonmatch (DNMS) form of the task (Hampson *et al.* SN Abstr. 1995). The degree of proactive interference was dependent on the length of the delay interval on the preceding DNMS trial. Hippocampal lesions eliminated the delay-dependency of proactive interference.

Administration of THC (1.0-2.0 mg/kg) produced the *same* effect as hippocampal lesions on within-trial DNMS performance *and* on between-trial proactive interference. Performance following vehicle-only injections was not significantly different from control sessions. Behavioral effects of THC were completely blocked by coadministered SR141716A (Sanofi), the cannabinoid receptor antagonist. Results suggest that THC disrupted cognitive processing via cannabinoid receptor-mediated effects on hippocampal cellular activity (Heyser *et al.* 1993), providing further evidence for the role of endogenous cannabinoids in modulating hippocampally-dependent memory processes. *[Supported by NIDA grants DA08549 (REH), DA03502 & DA00114 (SAD)]*

447.15

ENHANCEMENT OF RAT HIPPOCAMPAL ENSEMBLE ACTIVITY BY CX516 PROTECTS AGAINST ERRORS IN SPATIAL DNMS. Sam A. Deadwyler*, Douglas R. Byrd, Joanne K. Konstantopoulos, Gareth J.O. Evans, Gary Rogers+ and Robert E. Hampson. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157. +Cortex Pharmaceuticals, Irvine, CA 92718.

This laboratory has shown that ensembles of hippocampal neurons encode information utilized during the performance of a spatial DNMS task in rats (Deadwyler et al 1996, *J Neurosci*). Different sources of variance extracted from overall ensemble activity included lever position, phase of the task and performance outcome in all animals (n = 7). Recent analyses of DNMS ensemble activity in the same task make it possible to delineate 1) the sequence of events which lead to an error, 2) the cascade of events which follow that error including obligatory miscoding on the next trial, and finally 3) the process by which correct DNMS performance gets reestablished. Such analyses showed that in hippocampal lesioned animals a proactive interference (PI) effect was present on all DNMS trials regardless of length of delay of the prior trial. In normal animals PI only occurred if the delay in the preceding trial was longer than 15 sec and the trial was an error. However if animals were pretreated before the session with the drug CX516 (*aka* BDP12, Cortex Pharmaceuticals), an AMPA channel modulator (Staubli et al 1994, *Proc Natl Acad Sci*), they were completely protected from PI. Ensemble recording verified that CX516 enhanced task-relevant, as well as delay period firing in all hippocampal cells in the ensemble during the DNMS trial. Such enhanced *within trial* ensemble activity may have been the basis for protection in animals normally influenced by PI in this task. *[Supported by NIDA grants DA03502, DA00119 (SAD) and DA08549 (REH)]*

447.16

IS THE CONDITIONED RESPONSE TO A DRUG CONDITIONED STIMULUS ACTUALLY BASED ON STATE DEPENDENT LEARNING?

C. H. Epstein and D. A. Overton.* Depts. of Psychology and Psychiatry, Temple University, Philadelphia, PA 19122.

Prior studies show that if ethanol injection consistently precedes electric shock, then after several pairings rats will become fearful when injected with ethanol, as indicated by suppression of drinking. This is generally viewed as classical conditioning with ethanol the conditioned stimulus (CS), electric shock the unconditioned stimulus (US), and suppression of drinking the conditioned emotional response (CER). Alternately, one could suggest that rats learned a CER to a conditioning box or technician CS, and that the CER was state dependent and hence only subsequently occurred when the animal was drugged.

In the present study, ethanol was injected just after the onset of shock so that the onset of ethanol's drug actions would occur while shock was still ongoing. The goal was to test whether rats would form state dependent memories of shock which would subsequently be retrieved only when the rats were again drugged. In this study, 5 groups were tested. Groups 1 and 2 were conventional forward-conditioning groups in which ethanol was injected 10 or 20 min., respectively, before onset of shock. Group 3 was the experimental group which received ethanol 1 min. after the onset of shock. Group 4, a control group, received saline 1 min. after shock onset. Group 5, another control group, received ethanol 20 min. after shock offset. Shock was a 15 min. series of 0.5-sec pulses of 1 ma RMS scrambled footshock with an average inter-pulse interval of 15 sec. Ethanol 1000 mg/kg was injected ip.

During subsequent test sessions, ethanol produced suppression of drinking in Groups 1 and 2 but no suppression in Groups 3,4,5. Hence the results fail to show the occurrence of a state dependent CER in Group 3 and are congruent with the prevailing classical conditioning model.

447.17

THE EFFECTS OF PENTOBARBITAL, PHENCYCLIDINE AND DIAZEPAM ON TWO DELAYED ALTERNATION PROCEDURES IN PIGEONS A.E. Nordholm*[1], C. Dayer[2] and G. Wenger[2]. [1]Naval Medical Research Institute-Detachment (Toxicology), WPAFB, OH 45433 and [2]University of Arkansas for Medical Sciences, Department of Pharmacology and Toxicology, Little Rock, AR 72205

We have previously reported on the detrimental effects of drugs of abuse in rats responding under a delayed alternation to position procedure. In this abstract we extend those observations to pigeons responding under a delayed alternation to position procedure (spatial discrimination task), and to a separate group of pigeons responding under a delayed alternation to colors procedure (color discrimination task). In the delayed alternation to position task, pigeons pecked (FR-20) on alternating side keys of a standard three-key operant chamber to receive a food presentation. In the delayed alternation to color procedure, pigeons were required to alternate pecks (FR-20) between two of three keys transilluminated with red or green stimulus lights to obtain a food presentation. In both procedures, delay values were randomly selected among 4, 8 and 12 seconds with a mean delay value of 8 sec. Three drugs of abuse, pentobarbital (0.3-13 mg/kg), phencyclidine (0.03-3 mg/kg) and diazepam (0.03-3 mg/kg) were administered (im), and performance (percent correct and response rate) was compared to saline control values. In both procedures, pentobarbital at doses above 5.6 mg/kg produced dose dependent decreases in percent correct, while response rate was decreased at only the highest dose (13 mg/kg). Phencyclidine was without significant effect on percent correct at all doses tested, yet decreased response rate at the 1 and 3 mg/kg dose levels in both groups. Diazepam decreased percent correct at 1 and 3 mg/kg doses, while response rate was unaffected in animals responding under both conditions. These drug effects are consistent with previously published reports of pigeons responding under a titrating matching-to-sample schedule of reinforcement. The data presented here suggests that the effects of these drugs of abuse on performance are not modality specific (spatial or color) nor are they specific to the behavioral assay (delayed alternation procedure versus titrating matching-to-sample); although in other species, the neural circuitry subservient to similar procedures is thought to be different. Funding Sources: NIDA Grant DA 05815

448.1

SUBCELLULAR LOCALIZATION OF FRAGILE X MENTAL RETARDATION PROTEIN IN DENDRITIC SPINES: AN ELECTRON MICROSCOPIC STUDY

Anthony D. Brazelton, Anna Y. Klintsova, Ivan Jeanne Weiler, Thomas A. Comery, Nicholas Hawrylak*, and William T. Greenough. Depts. of Psych. and Cell & Struct. Biol. and Beckman Inst., Univ. of Illinois, Urbana, IL 61801 and Dept. of Phys.Ther., Sch.of Med., W. Va. Univ., Morgantown, WV 26506.

A monoclonal antibody, P1G11, was made against a C-terminal polypeptide designed from the published sequence of Fragile X Mental Retardation Protein, FMRP. This antibody was shown to stain a protein of about 78 kDa on Western blots of rat synaptoneurosomal protein preparations, and to stain brain sections with the same pattern of immunoreactivity as previously resported for a commercially available antibody (Devys et al, 1993).

P1G11 antibody was used to study the subcellular distribution of FMRP in the visual cortex and cerebellar molecular layer of P12-P15 rats. Colloidal gold post-embedding and HRP pre-embedding labelling has revealed immunoreactivity located in spines near postsynaptic densities, in dendrites, and associated with some polyribosomal aggregates in neuronal somata. Immunoreactivity was not observed in axons or glial cells. Often one or more spines in a field of view will exhibit strong labelling, while others exhibit no detectable signal, suggesting the occurrence of selective expression at some synapses. Localization of the protein in spines is compatible with our reports indicating neurotransmitter-induced FMRP translation in synaptoneurosomes and our conclusion that FMRP is translated at synapses under synaptic control. Supported by MH 35321 and the MacArthur Psychopathology Development Network.

448.2

DENDRITIC SPINE MORPHOLOGY IN THE FRAGILE-X KNOCKOUT MOUSE: A MODEL OF THE FRAGILE-X SYNDROME?

T.A. Comery*, J.B. Harris, P.J. Willems, B.A. Oostra, & W.T. Greenough. Neurosc. Prog., Depts. of Psych., Cell & Struct. Bio., & Beckman Inst., Univ. Illinois, Urbana, IL 61801, Depts. of Med. Gen. & Neurochem. & Behav., Univ. of Antwerp, Belgium, & Dept. of Clin. Gen., Erasmus Univ. Rotterdam, Netherlands.

Fragile-X mental retardation syndrome occurs as a result of a decreased expression of the Fragile-X protein (FMRP). An examination of Gogli impregnated brain tissue from human Fragile-X patients reveals a spine morphology characterized by long, thin tortuous spines resembling those observed during development. It is thought that this dysgenesis may account for at least some of the behavioral symptoms of Fragile-X syndrome. Recently, in an attempt to produce an animal model of this sydrome, a transgenic knockout mouse has been produced thatlacks FMRP expression. These knockout mice display no gross brain abnormalities. We have examined the morphology of dendritic spines in the knockout mice utilizing the Golgi-Cox method. Prelimary results suggest that the spines on the apical dendrites of layer V pyramidal cells in occipital cortex of *Fmr-1* knockout mice have spines that are signifcantly longer than those observed in wild-type controls. Spine density along the apical dendrite is also increased in the knockout mice. These results suggest that the *Fmr-1* knockout mouse may be a good model of human Fragile-X syndrome and that FMRP expression may be essential for the production of dendritic spines with a normal, adult-like, morphology. Supported by Kiwanis Nervous System Research Fund and MH35321.

448.3

mGluR STIMULATION MEDIATES PHOSPHORYLATION OF SOME POLYRIBOSOME-BINDING PROTEINS IN RAT BRAIN SYNAPTO-NEUROSOMES.

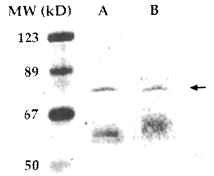
I.J. Weiler*, A.D. Brazelton, W.T. Greenough. Depts. of Psych. & Cell & Struct. Biol. & Beckman Inst., Univ. of IL, Urbana, IL 61801.

Synaptoneurosomes contain postsynaptic polyribosomal aggregates (PRA), in which proteins are translated upon stimulation by agonists for metabotropic glutamate receptors (mGluR). We are studying the PRA-associated proteins by gel analysis of serial salt washes. SDS-PAGE separated 16 prominent bands of approximate molecular weights of 115, 100-105, 95, 80-82, 75-78, 72, 65, 60-62, 55, 50-52, 45, 42, 38, 36, 32 and 28 kDa when proteins were dissociated from PRA in 0.5M K+. After a more stringent wash at 2M K+, additional proteins of MW 88, 73, 57 and 43 were seen in the eluate. Synaptoneurosomes were stimulated briefly by the mGluR-specific agonist 1S,3R-trans-ACPD in the presence of γ-32P-ATP. Stimulation for 1 min. increased the level of phosphorylation of 4 PRA-associated proteins (100-105, 80-82, 75-78 and 60-62 kDa). In the presence of the PKC antagonist calphostin, these 4 phosphorylated bands were much reduced. This suggests that mGluR-mediated phosphorylation of some PRA-associated proteins is dependent on PKC activation. One of these proteins may be the Fragile X Mental Retardation protein (FMRP), since in preliminary experiments, the phosphorylated band at 75-78 kDa was stained by P1G11, a monoclonal antibody against the C-terminal polypeptide of FMRP (see poster by S.A. Irwin et al.). In the presence of calphostin, this band was no longer present in the 0.5 M K+ salt eluate. If these results are confirmed, it would suggest that PKC plays a role in the association between FMRP and PRAs. Supported by FRAXA Research Foundation and MH 35321.

448.4

EVIDENCE FOR TRANSLATION OF THE FRAGILE-X MENTAL RETARDATION PROTEIN IN A SYNAPTONEUROSOME PREPARATION

S.A. Irwin*, I.J. Weiler, B.N. Patel, and W.T. Greenough. Neurosci. and Med. Schol. Progs., Depts. of Psych. and Cell & Struct. Bio., and Beckman Inst., Univ. of Illinois, Urbana, IL 61801.



A. Blot of imppt. protein stained with P1G11. B. Autoradiogram of same blot, showing ^{35}S-met. incorp. into immppt. protein.

The absence of the Fragile-X Mental Retardation Protein (FMRP) in humans leads to fragile-X syndrome, a leading genetic cause of mental retardation. A salient feature of this syndrome is the relative absence of mature cortical dendritic spines in humans and mice. The mRNA for the gene (FMR-1) encoding this protein has been shown to associate with polyribosomal aggregates in response to metabotropic glutamate receptor (mGluR) stimulation of a synaptoneurosome preparation made from P14 rat cerebral cortices. In this study a similar preparation was stimulated with trans-ACPD in the presence of ^{35}S-methionine for 20 minutes, and a single radiolabeled protein (approx. 78 kDa) was immunoprecipitated with a monoclonal antibody (P1G11) to a polypeptide designed from the C-terminal end of both human and mouse FMRP. This finding provides further evidence for the translation of FMRP at synapses in response to mGluR stimulation, and may provide another link between synaptic stimulation and anatomical synaptic change. Supported by MH 35321, MacArthur Psychopath. Dev. Netw., IL State Ment. Ret. Assn., and HD 07333.

448.5

ENRICHED MOTOR LEARNING ENHANCES NEURO-PLASTICITY IN THE YOUNG LURCHER MOUSE.

K.C. Goss[1], L. Coudsi[2], R. Dvorak[2], and R.F. Mervis[2]*. [1]Univ. Tennessee Med Ctr, Knoxville, TN 37920, [2]NeuroMetrix Research, Inc. Columbus, OH 43212.

The Lurcher mutant mouse is characterized by apoptosis of Purkinje cells in the postnatal cerebellum, their dropout being essentially complete by 60 days-old (P60). This study was designed to assess the effects of an enriched motor environment from P10-P40 (i.e., "trained") on motor learning and on cerebellar and cortical histology in Lurcher and normal mice. "Non-trained" Lurcher and normal controls were reared in standard housing conditions for the same 30 day period. "Trained" Lurchers and control mice showed several changes from the "non-trained" mice. Behavioral tests showed that motor enrichment resulted in enhanced performance in both controls and Lurchers. Golgi-impregnation studies of Purkinje cells showed that motor learning resulted in a significantly enlarged dendritic arbor in the normal mice. And, in the somatosensory cortex, motor learning resulted in a more extensive dendritic arbor in the basilar tree of layer V pyramids. Cortical pyramidal cells of non-trained Lurchers also showed a neuroplastic enhancement of their dendritic arbor suggesting involvement of cortical circuits to compensate for loss of cerebellar neurons. Evidence of plasticity in response to motor learning was also reflected by an increase in the depth of the cerebellar molecular layer in both trained normal and Lurcher mice. These results, in combination with the behavioral data, provide evidence that enforced motor learning can reorganize cortical circuits in the Lurcher mutant when demanded by the genetically programmed death of Purkinje cells. (Supported in part by the Physicians Medical Education Foundation of the University of Tennessee)

448.6

SPATIAL LEARNING AND HYPPOCAMPAL SYNAPTIC PLASTICITY IN ADULT RATS WITH INTRAVENTRICULAR IMPLANT OF HYBRIDOMA CELLS SECRETING NEUTRALIZING ANTIBODIES TO NGF

F. Ruberti, RM Costagliola, MC Cenni, E. Cherubini, L. Maffei, A. Cattaneo and N. Berardi. Ist. di Neurofisiologia CNR,I-56127 Pisa, Scuola Normale Superiore, Pisa, SISSA, Trieste (Italy)

Recent results indicate that neurotrophins, such as NGF and BDNF, play a role in neural plasticity not only during development but also in adults. We have assessed spatial learning performance and hyppocampal synaptic plasticity in adult rats after a treatment aimed at blocking endogenous NGF activity. 9 adult Long Evans rats were intraventricularly implanted with αD11 hybridoma cells secreting antibodies preventing NGF binding to its receptors. As a control, 9 littermates were implanted with parental P3U myeloma cells. Antibody titre was determined in samples of CSF with enzyme-immunoassay (ELISA). Performance of αD11 rats was significantly impaired with respect to controls, both in terms of acquisition rate and of final level of accuracy. Impairment in performance correlated with αD11 titre in CSF. In hyppocampal slices prepared from behaviourally characterized P3U and αD11 rats we have then examined long term synaptic plasticity (LTP). Field excitatory postsynaptic potentials (fEPSP) were recorded in stratum radiatum of CA1 area after stimulation of Schaffer collaterals. LTP was elicited by 2 trains of tetani (100 Hz, 1 sec) with an interval of 10 sec. We found that in the majority of slices from αD11 animals LTP was inducible and its early phase (up to 1.5 hours after tetanus) was essentially the same as that found in slices prepared from P3U rats.

Supported by HFSP grant rg 93/93

448.7

Experience related molecular and structural plasticity of glial cells in the chicken brain.

A. Zimmermann* and E. Besemfelder. Zoologisches Institut der THD, Schnittspahnstr. 3, 64287 Darmstadt, FRG.

While work on neuronal plasticity in the context of learning has covered much ground, little is known about glial cell responses. With an interest in gliabiology within the general framework of potential experience related changes, we have used GFAP and E1 antibodies to monitor aspects of glial cell plasticity. E1 is a cell surface glycoepitope defined by E1 monoclonal antibody. It is abundantly present on many GFAP negative glial cells as well as on certain neurons throughout the chicken brain.

Western blot analyses showed that the levels of E1 expression in the tectum dropped precipitously after hatching. Posthatching day 1 (P1) levels were several fold lower than on the last day in ovo (E20). P4 socially-raised chickens (s-chicken) had higher tectal and forebrain E1 levels than same age chickens reared in isolation (i-chicken). Western blots of tectum proteins of i-chickens which had been subjected to an auditorily provoked learning (imprinting) experience in a Y shaped arena with few visual cues often showed significantly higher levels of E1 one hour following two 5-10 min sessions in the arena than their unexperienced counterparts. This detectability of changes in E1 expression following confrontation with an early learning (imprinting) task in Western blots of tissue homogenates suggests that alterations in E1 expression occur in a general or large scale fashion. Thus, although the E1 epitope is not exclusively glia cell associated, it appears necessary to invoke changes in glial E1 expression.

Experience related structural plasticity of astrocytes was apparent in GFAP stainings. Astrocytic processes of extensive length were present in the brains of i- but not s-chickens 4 weeks after hatching, particularly in tectum and hippocampus.

Supported by DFG Zi 455/2-5.

448.8

Effects of protein synthesis inhibition on long-term cholinergic enhancement in somatosensory cortex of anaesthetized rats. D. Verdier, M. Maalouf, N. Tremblay* and R.W. Dykes. Département de physiologie, Faculté de Médecine, Université de Montréal, Montréal, Québec.

Previous work by Verdier et al. (1995) demonstrated that pairing electrical hindpaw stimulation with basal forebrain (BF) stimulation induces a long term increase in the size of the evoked potential in rat somatosensory cortex. This long-term cholinergic enhancement (LTCE) was shown to require NMDA receptor activation and nitrous oxide synthesis. Because LTCE could possibly result from an activation of nociceptive fibers by the electrical stimulus, we developed a analogous preparation where the hindpaw was stimulated using a light mechanical stimulus. In addition, we tested the effects of the protein synthesis inhibitor Anisomycin.

Our results indicate that when a 10 ms cutaneous stimulus is presented 30 ms after a 1 ms BF stimulation during 10 trials, the evoked potential in the somatosensory cortex was significantly enhanced by up to 40% in 7 out of 33 experiments. This enhancement lasted over 90 min and was observed immediately after pairing in all but one animal where the increase in the size of the evoked potential was progressive. Three rats were treated with Anisomycin (60 mg/kg, systemic) 90 min prior to the pairing. In two cases, the evoked potential was not enhanced. In the third case, an enhancement was seen immediately after pairing and lasted until the end of the recording (90 min after paring). We conclude that a light mechanical stimulus is effective in inducing LTCE when it is paired with BF stimulation. However, the effects of Anisomycin remain unclear. Additional experiments will be performed to determine if 1)Anisomycin blocks LTCE completely, 2) does not affect LTCE or 3) inhibits only the long-term component as has been traditionally observed in hippocampal long-term potentiation. (Supported by the Medical Research Council of Canada and Fonds de la Recherche en Santé du Québec).

448.9

SINGLE-CELL CORRELATES OF REPRESENTATIONAL BOUNDARIES IN RODENT SI CORTEX. P.W. Hickmott* & M.M. Merzenich. Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143.

Many regions of adult neocortex are capable of considerable reorganization as a result of changes in the activity patterns of their inputs. The cellular and circuit properties that underlie this plasticity are incompletely understood, but it has been hypothesized that strengthening and/or weakening of already existing intracortical excitatory and/or inhibitory synapses may be one mechanism underlying these cortical reorganizations.

To examine this hypothesis we mapped a ~1-2 mm length of the boundary between the forepaw and lower jaw representation in rat SI cortex using standard extracellular recording with penetrations at ~50 micron intervals. After mapping, the boundary was marked by iontophoresis of pontamine sky blue (2%, in .5 M NaAc), and 400 micron thick coronal slices were taken from the marked region. Current-clamp recordings are made from single layer 2/3 neurons within 300 microns of the mark using blind whole-cell recording. Electrical stimuli are applied at the same cortical depth either across the boundary (CB) or at the same horizontal distance on the other side of the cell (NCB). Stimulus intensities for both sites ranged from the minimum necessary to evoke a PSP to that causing a maximal PSP. In 9/20 cells, PSPs resulting from NCB stimulation had peak and average amplitudes ~20% greater than PSPs from CB stimulation, and also had a >20% more rapid fall time from peak to 1/2 peak amplitude. In an attempt to develop a preparation that shows more robust boundary effects, we have recently begun using a slice in which the supragranular layers are isolated by cutting in the slice at a depth of 500-700 microns, and have preliminary data that a higher percentage of neurons from these slices have greater PSP amplitudes for NCB stimulation.

These experiments indicate that it is possible to detect cellular correlates of representational boundaries in sensory cortex. Using this preparation we are investigating specific differences in EPSPs and IPSPs across these boundaries and hope to use it to investigate plasticity of cortical representations.

[Supported by NIH Grants NSO-7067, NS-09859 and NS-10414.]

448.10

EXPERIENCE-DEPENDENT CHANGES IN CORTICAL CELL MORPHOLOGY ARE AGE-DEPENDENT. B. Kolb*, R. Gibb, G. Gorny, and A. Oullette. Univ. of Lethbridge, Lethbridge, AB, Canada, T1K 3M4.

The morphology of cortical pyramidal cells was examined in rats given 1 of 3 different experiences (tactile stroking, 'enriched' housing, or specific forelimb training). Tactile stroking was given 3X daily for 15 min, beginning in the first few days of life and continuing until postnatal day 21 (PN21). Environmental housing was manipulated by placing rats in large enclosures with novel objects (enrichment) beginning on PN21, PN200, or PN800 and continuing for 90 days. Forelimb training involved either reaching through bars for food or pulling up strings to obtain food, and began on PN120 and continued for 30 days. Morphology of cortical neurons was examined in adulthood.

Both enrichment and stroking during development led to increased dendritic length and decreased spine density in pyramidal cells. In contrast, both enrichment and forelimb training in adulthood increased dendritic length but had no effect upon spine density.

These results suggest that environmental experiences during development effect cortical morphology differently than similar experiences in adulthood. This age-related experience-dependent difference in cortical neuron morphology may account for differences in the effects of earlier vs later experience on behaviour. The failure to observe increased spine density with experience contrasts with the increases in both dendritic length and spine density in response to trophic factors, gonadal hormones or early lesions.

Supported by an NSERC operating grant to BK.

448.11

A HIPPOCAMPUS FOR ALL SEASONS: PHOTOPERIODIC MODULATION OF HIPPOCAMPAL VOLUME IN MEADOW VOLES.

L.F. Jacobs*[1], K.M. Grady[2], K. Olstott[1], F. Marouf[1], K. Allen[1] and T.M. Lee[2]. [1]Dept. Psych., Univ. California Berkeley, CA 94720-1650; [2]Dept. of Psych., Univ. Michigan, Ann Arbor, MI 48104-1687.

Hippocampal size shows seasonal patterns of sexual dimorphism in wild rodents: in spring, but not winter , the hippocampus is significantly larger in males. Other structures, such as cerebellum, show no seasonal cnange in size. The present study replicated this result in laboratory voles, using photoperiod as the seasonal cue. Males and females were raised in either long (LD) (14:10) or short (SD) (10:14) photoperiods. Voles were autopsied at 9.5 weeks of age and brains were postfixed in 10% buffered formalin. Brains were embedded in gelatin-albumin, frozen sections were cut in the horizontal plane at 40 µm intervals and every fourth section was mounted and stained with cresyl violet. Volume of whole brain, CB and HP were determined by stereological assessment of area in mounted sections, using a 45X projected image of the section; all slides were coded. The accuracy of this method was confirmed using a computer-based image measuring workstation. Residual values from a line of best fit between whole brain and substructure (HP, CB) were used to measure the relative size of substructures. Analysis of these residuals revealed, as predicted, a significant photoperiod-by-sex interaction in HP size, but not in brain size or in CB size. In the LD group, hippocampal size was larger in males; in SD, larger in females; CB size did not differ between groups. Thus, the same pattern seen in wild voles was confirmed in laboratory voles. Given the widespread distrbution of this effect in wild rodents, is likely that domestic laboratory rodent species, although normally kept under constant photoperiod, would also show dramatic hippocampal plasticity in response to photoperiod. Supported by the Univ. Calif. Hellman Fund and NIH (RO3 MH53060) to L.F.J. and (NICHD) HD 24575 to T.M.L.

448.12

ENRICHMENT DURING DEVELOPMENT INDUCES ENDURING INCREASES IN RESTING CEREBRAL GLUCOSE METABOLISM IN MONKEYS. M. J. Raleigh*, W. P. Melega, S-C. Huang, S. R. Cherry, M. T. McGuire, and M. Blurton-Jones, M. E. Phelps. Depts. of Psychiatry and Molecular and Medical Pharmacology, UCLA, Los Angeles, CA 90024.

This positron emission tomography (PET) study used 2-deoxy-2[^{18}] fluoro-D-deoxyglucose (FDG) to examine the enduring effects of enrichment on local cerebral metabolic rates for glucose (lCMRglc) in developing vervet monkeys. PET scans were performed while the monkeys were in a conscious, resting state and we determined lCMRglc in nine regions of interest (ROI). The 8 subjects ranged in age from 5 to 7 months, the period of maximal metabolic neuroplasticity in this species. The 4 enriched and the 4 age- and sex- matched controls lived in complex, species typical social groups. During the 45 day enrichment regime, enriched subjects had access to a joy-stick controlled computer and learned to solve delayed response and visual tracking problems. Control subjects could see but not manipulate the computer. Three quantitative FDG PET scans were obtained per subject: prior to enrichment, at the conclusion of the enrichment regime, and eight months later.

Before enrichment, control and enriched subjects did not differ in lCMRglc in any ROI. Immediately after enrichment, relative to control subjects, experimental subjects exhibited significant bilateral increases in lCMRglc in the dorsolateral frontal, orbitofrontal, temporal, and parietal cortices by 34%, 28%, 32%, and 19% respectively ($p < .01$). Eight months later lCMRglc remained elevated in dorsolateral frontal, orbitofrontal, and temporal cortices by 24%, 18%, and 21%. There were no differences in lCMRglc in the visual cortex, somatosensory cortex, brain stem, cerebellum, or cortical white matter. Thus early enrichment induces substantial, enduring, regionally-specific increases in lCMRglc .

Supported by the Dana Foundation, the Veterans Administration, the Department of Energy, and the Mead Foundation.

448.13

ESTRADIOL INCREASES SENSITIVITY OF CA1 PYRAMIDAL CELLS TO NMDA RECEPTOR-MEDIATED SYNAPTIC INPUT. C.S. Woolley* and P.A. Schwartzkroin. Neurosurgery Dept., Univ. of Washington, Seattle, WA 98195

Previous studies have shown that, in the adult female rat, the density of dendritic spines and synapses on hippocampal CA1 pyramidal cells is sensitive to the ovarian steroid, estradiol, and fluctuates naturally as estradiol levels rise and fall during the estrous cycle. Currently, the consequences of hormone-induced changes in spine/synapse density for synaptic transmission are not known. To address this issue, we have compared synaptic responsiveness of CA1 pyramidal cells to the density of dendritic spines on each cell. Hippocampal slices were prepared from adult female rats that were ovariectomized and treated with either estradiol or oil vehicle. EPSPs evoked by Schaffer collateral stimulation were recorded from neurons within the CA1 pyramidal cell layer. Following recording, each cell was filled with biocytin to visualize dendritic spines. The slope of an input/output relationship (EPSP slope vs. stimulus intensity) was compared to spine density for each cell. Analysis of the dendrites of biocytin-filled CA1 pyramidal cells demonstrated that the density of dendritic spines is increased in ovariectomized animals treated with estradiol compared to that in animals receiving vehicle alone. In initial experiments, no correlation between I/O slope and spine density was observed when composite EPSPs were elicited in cells at resting membrane potential. However, when the NMDA receptor mediated component of the EPSP was isolated pharmacologically, a significant correlation between I/O slope and spine density was observed ($R=0.65$, $p<0.01$); the mean I/O slope was significantly increased in cells from estradiol-treated animals compared to vehicle-treated controls ($p<0.01$). These results demonstrate that estradiol increases CA1 pyramidal cell sensitivity to NMDA receptor-mediated synaptic input.

Supported by NS 18895 (PAS) and NS 09787 (CSW)

448.14

CONTEXTUAL FEAR CONDITIONING ACROSS THE RAT ESTROUS CYCLE. Etan J. Markus*, Maja Zecevic, & Michael L. Anderson. Behavioral Neuroscience Div., Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269

The natural 4-5 day female rat estrous cycle has been related to changes in hippocampal structure (CA1 synaptic density - Woolley et. al., 1990; 1992) and physiology (LTP - Humphreys et. al., 1994; Korol et. al., 1994). This is especially apparent during the 24 hour period between the peak of estradiol (proestrus) and the low estradiol part of the cycle (estrus). This increase in synapses and plasticity during proestrus is accompanied by a decrease in the water maze ability of the rats (Frye, 1994; Warren et al. 1995). To examine whether this proestrus-related decline is limited only to the water maze, rats were tested on different paradigm also shown to be sensitive to hippocampal function, contextual fear conditioning.

24 female and 12 male, 4-5 months old Fisher 344 rats were examined for contextual and cued fear conditioning. The rats experienced two tone-foot-shock pairings in a distinctive chamber (3 minute baseline, followed by 30 sec tone, followed by a 2 sec 0.4 mA current, twice). Retention was tested 2-3 weeks later, first the animals were placed in the original conditioning chamber, with no tone or shock administered (3 min., context retention), followed by placement in a novel chamber for 3 min. after which the conditioning tone was presented for 3 min. (cued tone retention). Half the females were conditioned and tested for retention during proestrus and the other half during the estrus part of the cycle. The males were yoked to the female rats conditioning and retention testing schedules.

It was found that proestus rats show less contextual fear conditioning than male or estrus female rats. There was however no difference among the three groups in their conditioning to the tone stimulus. These results suggest that the changes found during the proestrus part of the cycle are related to spatial-contextual information processing and not to a general change in learning ability.

448.15

ROLE OF ESTROGEN IN MODYFING SYNAPTIC FUNCTION

Heather N. Fugger*[1] and Thomas C. Foster.[1,2] [1]Neuroscience Graduate Program and [2]Department of Psychology, University of Virginia. Charlottesville, VA 22903

Anatomical studies demonstrate that dendritic spine number in region CA1 of the hippocampus varies with the level of ovarian hormones. To determine if the increase in synapse number following estrogen treatment represents a population of functional synapses, female (4 mo) Sprague-Dawley rats were ovariectomized, and three days later rats were injected with 100 µg estradiol benzoate in 1 ml sesame oil or 1 ml oil vehicle. A second injection was given 24 hours later. 48 hrs following the second injection CA1 physiology was examined in an *in vitro* hippocampal slice preparation. Paired-pulse stimulation (50ms ISI) was delivered in order to measure fiber potential (fp) amplitude, EPSP slope, and paired-pulse ratio. To examine changes in magnitude and susceptibility to induction of hippocampal synaptic plasticity, long-term depression (LTD) and long-term potentiation (LTP) inducing stimulation were given. The results indicate that fp amplitude and EPSP slope were not different for the OVX (n=11, n=slices) and OVX+E (n=12) groups suggesting no difference in synaptic strength. A small but reliable LTD could be induced, however. LTP was not different between groups (OVX+E (n=5), 1.34±0.08 mean±SEM. OVX (n=5), 1.17±0.07, $p \leq 0.1$). These data suggest that estrogen may alter the potential for LTP.

Supported by NS31830 to TCF.

448.16

VARIATIONS IN LEVELS OF CIRCULATING OVARIAN HORMONES INFLUENCE ASTROCYTES IN THE CEREBRAL CORTEX OF THE ADULT FEMALE RAT. M.L. Forgie*, P.M. Burke and B. Kolb. Dept. of Psychology, The Univ. of Lethbridge, Lethbridge, AB, Canada, T1K 3M4.

Previously we reported that ovariectomy (OVX) of adult female rats increased the surface density (Sv) of glial fibrillary acidic protein-immunoreactive material (GFAP-IR) in certain cortical areas (Forgie et al., Soc. Neurosci. Abst., 1995,74.1). Our results for intact females were inconsistent, however. One explanation is that variations in Sv across the estrous cycle may be as great as those that occur in response to OVX, and rats were sacrificed without regard to the stage of the cycle. In the present study, adult females were either OVX'ed or sham-operated and vaginal lavages were taken for 4 weeks following surgery. Intact rats were sacrificed on the afternoon of proestrus, estrus, or diestrus, and all brains were processed for GFAP immunohistochemistry. Using a stereology system, the Sv of GFAP-IR was quantified in five different cortical areas. Variations in Sv of GFAP-IR according to stage of the estrous cycle, and in response to OVX, were observed in many, but not all, of the cortical areas examined. In some regions, OVX increased Sv of GFAP-IR, although no significant variations in Sv occurred over the cycle. Conversely, in other regions, the changes across the estrous cycle (e.g., diestrus vs. proestrus) were as large as those produced by OVX. These data confirm and extend our previous finding that fluctuations in levels of circulating ovarian hormones can produce alterations to astrocytes in the cerebral cortex of the adult female rat.

This research was supported by an AHFMR Fellowship to MLF and an NSERC operating grant to BK.

448.17

AN INNOCUOUS BIAS IN WHISKER USE IN ADULT RATS ALTERS RELATIVE NMDA AND AMPA RECEPTOR SENSORY TRANSMISSION IN BARREL FIELD CORTEX. [1]M. Armstrong-James*, [1]Jane H. Houchen, [2]Ford F. Ebner. Department of Physiology, QMW, London University, U.K. E1 4NS[1] and I.D.N., Kennedy Center, Vanderbilt University, Nashville, Tn-37203[2].

Cortical plasticity can be induced in adult rat cortex by cutting all except two whiskers, D2 and D3, for 3-30 days ("whisker-pairing"; WP). WP causes Hebbian potentiation of single unit responses in barrel D2 to the center (D2) and "paired" surround (D3) whiskers. Responses to disused (e.g. cut D1) whiskers decrease. We used antagonists to NMDAR (AP5) and AMPAR (CNQX) to demonstrate changes in NMDA/AMPA receptor mediated responses after 30 days of WP for cells in the D2 barrel. (1) For response to the D2 principal whisker, potentiation was restricted to the earliest (<10 ms latency), thalamo-cortical (T-C) discharges, and this was attributable solely to potentiation of AMPA-dependent activity ($p<.001$, Mann-Whitney U test). Later response components (discharge latencies 10-100 ms) exhibited decreased NMDA-dependence. (2) In controls AMPA/NMDA receptor profiles were highly NMDA receptor-dependent and indistinguishable for D1 and D3 surround responses. For WP animals, bias in response to the paired D3 surround whisker was attributable to enhancement of both AMPA- and NMDA-dependent components of late discharges relative to cut D1 whisker responses ($p<.001$ and .01 respectively). Hence, AMPAR transmission is potentiated and NMDAR components are decreased in the direct T-C relay, while both AMPA and NMDA receptor transmission are potentiated between columns for paired surround inputs. These results show that a novel sensory activation pattern can initiate different changes in glutamate receptors of at least two different types; thalamocortical synapses readjust receptor efficacy differently from corticocortical synapses between two columns receiving increased activity. (Supported by theWellcome Trust AJ, JH and NIH-NS-25907 AJ, FE.)

448.18

CHRONIC ORAL ANIRACETAM DOES NOT MODIFY BEHAVICURAL OR CORTICAL PLASTICITY ASSOCIATED WITH "ENRICHED" HOUSING. M. Osborne, J. Chadbourn, M. Campbell, D. Travis and M. Saari*. Neuroscience Research Unit, Nipissing University, 100 College Dr., North Bay, Ontario, Canada, P1B 8L7.

Forty male rats were randomly allocated to a 2 X 2 factorial design. The 41 day old rats were housed for six days in either "enriched" or "isolated" environments. Half of the rats in each housing condition were administered oral aniracetam (50mg/kg) or vehicle (45g cyclodextrin/100ml H_2O). This yielded 4 groups with 10 subjects per cell. "Enrichment" consisted of social housing with daily exposure to an open field scattered with various objects. The "isolated" rats were housed individually in hanging cages (21 X 21 X 28cm) with routine care. Humidity and temperature were controlled and the light cycle reversed. As expected, "enrichment" influenced adaptation to a novel open field and place navigation whereas an unexpected increase in activity was found with the aniracetam treatment. Thickness of the occipital cortex was increased by "enrichment" but not aniracetam. Aniracetam did not influence brain morphology except for the pyramidal cell layer of the hippocampus.

448.19

HANDEDNESS AND NEUROPSYCHOLOGICAL PERFORMANCE IN PATIENTS WITH LEFT TEMPORAL-LOBE EPILEPSY OR LEFT HEMISPHERE ARTERIOVENOUS MALFORMATIONS. R. Roth, Z. Caramanos, G. Leonard* and R. Leblanc. Montreal Neurological Institute, McGill University, Montreal, Canada, H3A 2B4.

It is known that left temporal-lobe epilepsy (LTLE) is associated with a shift in handedness (Leonard et al, 1991), and it has been suggested that left hemisphere arteriovenous malformations (LAVM) can have a similar effect.

In order to examine this directly we compared the handedness of groups of patients with either LTLE (n=34) or LAVM (n=29), as well as their performance on an extensive battery of neuropsychological tests. LTLE patients had significantly lower mean IQ ratings and mean verbal and visuospatial performance than the LAVM subjects. No differences were found on tests of cognitive flexibility, language ability, or sensorimotor function. Six of the LTLE cases, but only two LAVM cases were left-handed. Given the small sample sizes, this distribution did not differ from that expected by chance. Nevertheless, when we compared the LAVM group to a larger group of LTLE patients (in whom 40 of 145 cases were left handed), there were significantly fewer left-handed LAVM cases than expected (Fisher Exact Test, $p = 0.017$).

Thus, our findings suggest that LAVM may be less disruptive, both in terms of cognitive function and cerebral organization, than LTLE.

Funded by the National Science and Engineering Research Council of Canada.

MOTIVATION AND EMOTION: DRUGS

449.1

DOPAMINE (DA) AND ACETYLCHOLINE (ACh) RELEASE IN THE RAT LATERAL HYPOTHALAMUS (LH): OPPOSITE CHANGES IN RELATION WITH DRINKING. M. Puig de Parada*, G. Molina de Chacón, X. Páez, M.A. Parada, and L. Hernández. Department of Physiology, Universidad de los Andes, Mérida 5101-A, Venezuela.

Drinking behavior after local administration of dopaminergic and cholinergic drugs suggests that DA in the LH plays an inhibitory, and ACh a facilitatory role in water intake. A described interaction within the LH is the dopaminergic inhibition of cholinergic elements. Those facts suggest that the basal release of both neurotransmitters should display opposite changes in relation with deprivation-induced drinking. This study explored that possibility. Male Wistar rats were trained for 2 weeks to drink just during one hr daily. The test day animals had access to water while undergoing microdialysis in the LH and usually drank during the first 40 min. DA turnover (n=6) or ACh release (n=5) were monitored. DA increased above 3.9 ± 0.8 pg/20µl in the four 20-min samples taken after water was available, peaking (5.6 ± 1.4 pg) in the 2nd sample (155 ± 47%; F:23.5; p<0.0001). DOPAC (basal: 42 ± 5.4 pg/20µl) increased gradually after water was presented and peaked (55 ± 5.8 pg) 80 min later (132 ± 9.7%; F:25.2; p<0.0001). HVA did not change. ACh release decreased from 2.5 ± 0.7 pmol/20µl to 1.36 ± 0.7 pmol in the 2nd sample post-water presentation, which represented a decrease to 40 ± 18% of the pre-water mean basal level (F:4.9; p<0.05). These modifications confirm the prediction that oriented the present study, but the temporal resolution does not allow a valid interpretation in regard with its physiological significance. We propose that they could represent factors involved in the development of water satiety.

Supported by CDCHT-ULA grant N° M-498-95-03-A.

449.2

THE INFLUENCE OF SOCIAL ISOLATION ON SENSITIVITY TO THE REINFORCING EFFECTS OF D-AMPHETAMINE AND COCAINE. J.K.Smith* J.C.Neill & B.Costall Postgraduate Studies in Pharmacology, University of Bradford, West Yorkshire, BD7 1DP, U.K.

Previous research has demonstrated that rearing rats in social isolation can lead to hyperactivity, enhanced responding for reward related stimuli, and increased sensitivity to psychomotor stimulant drugs (Robbins T.W. 1996; J. Psychopharmacology 10(1):39-47). The aim of the present study was to assess the influence of long term post-weaning social deprivation on the reinforcing effects of both *d*-amphetamine and cocaine.

Female Hooded Lister rats (n=24) were housed individually or in groups of 6 from weaning (21 days) for a period of 12 weeks. The socially reared rats were environmentally enriched with various toys, and handled daily. Rats were trained to respond either for a previously food paired conditioned stimulus (CS) or on a progressive ratio (PR) schedule of food reinforcement in one of four identical operant chambers. Responding was then assessed 30min after administration of amphetamine (0.5mg/kg i.p.), cocaine (5mg/kg i.p.) or saline vehicle (i.p).

Isolated rats responded at a significantly higher level for presentation of the CS ($p<0.01$), and this difference was further enhanced following administration of both *d*-amphetamine (0.5mg/kg; $P<0.05$) and cocaine (5mg/kg; $P<0.01$). In addition isolated rats responded at a higher level ($p<0.05$), and achieved a higher break point under a PR schedule of reinforcement, which was similarly enhanced following treatment with both *d*-amphetamine (0.5mg/kg; $P<0.05$) and cocaine (5mg/kg; $P<0.05$).

Results of the present study, in agreement with previous studies (Jones et al Psychopharmacology 102:364-372; 1990) demonstrate that isolated rats exhibit enhanced responding for rewarding stimuli. In addition results show that isolation rearing leads to an enhanced sensitivity to the reward enhancing properties of both cocaine and *d*-amphetamine.

(Supported by a Bradford University studentship)

449.3

STUDY OF DEFENSIVE REACTIONS INDUCED BY MICROINJECTION OF EXCITATORY AMINO IN THE INFERIOR OF RATS S.H. Cardoso*, N.C. Coimbra, V. Sardaryanst and M.L. Brandão Laboratório de Psicobiologia FFCL-RP/USP Ribeirão Preto-SP Brazil

The inferior colliculus (IC) is primarily involved in conveying auditory information to higher cortical structures. Recently we have shown that this structure may also be part of a brain system commanding defensive behavior. Here we present evidence for the involvement of excitatory amino acids in the expression of these defensive reactions. Microinjections of increasing doses (5, 10, 20 and 40 nmol) of NMDA - an excitatory amino acid - into the central nucleus of the IC of rats placed inside a circular arena induced gradual aversive reactions characterized by runnigs, rearings, and jumps. These reactions were inhibited by the NMDA specific antagonist AP7 previously microinjected within the IC. It is suggested that NMDA receptors mediated mechanisms are called into play during the expression of the defense reaction.

Supported by Fapesp

449.4

SEX STEROID EFFECTS ON SOCIAL BEHAVIOR IN CHICKS. L. Normansell*, M. Holmes, A. Kennedy, & S. Eastek, Program in Neuroscience, Muskingum College, New Concord, OH 43762.

Following chronic testosterone (T) administration, young chicks crow regularly and become very aggressive towards conspecifics. Since T follows two metabolic pathways, aromatization to estradiol (EB) and reduction to dihydrotestosterone (DHT), we investigated the effects of administration of these hormones on social behavior in young domestic chicks. Chicks were obtained at one day post hatch, housed in like-treated flocks, and administered either 2.5mg T, 2.5mg EB, 2.5mg DHT, 2.5mg of both DHT & EB, or oil vehicle control. Animals in the various experimental conditions did not differ from each other in either body weight or testes weight. When isolated, DHT-treated chicks emitted slightly fewer distress calls than the other chicks, but significantly more calls of other types. When animals were observed in their home cages, T-treated animals were significantly more aggressive than the other groups. While they displayed no aggression towards their flockmates, the EB-treated chicks directed more aggressive acts towards the experimenters' hands when they were systematically placed within the cages. Both the T and EB/DHT animals emitted crows, while neither the EB, DHT, or Veh chicks ever did. When tested on a social approach task, where a separated animal had to move to rejoin the flock, there were no differences between the groups. In summary, while testosterone treatment induces many adult-like patterns of behavior in young chicks, which of those are modulated by each of the separate metabolic pathways will require additional testing.

449.5

DIFFERENTIAL EFFECTS OF INTRA-SEPTAL R(+)8-OH-DPAT IN TWO ANIMAL MODELS OF ANXIETY. J. Menard* and D. Treit. University of Alberta, Edmonton, Alberta, CANADA T6G 2E9

Previous studies (Pesold & Treit, 1992; 1994) have shown that septal lesions or intra-septal infusions of the $GABA_A$-benzodiazepine agonist midazolam produced anxiolytic effects in two animal models of anxiety (i.e., decreased open-arm avoidance in the elevated plus-maze test and decreased burying in the shock-probe burying test). Because similar anxiolytic effects are seen after PCPA-induced depletion of serotonin (5-HT), and lesions of the dorsal raphe, which innervates the septum (Treit et al., 1993), it seems possible that 5-HT participates in the septal regulation of these fear behaviors. The present study was designed to examine the effects of intra-septal infusions of the selective and highly potent 5-HT_{1A} receptor agonist R(+)-8-OH-DPAT in these tests. Intra-septal infusions of R(+)-8-OH-DPAT (5 or 10 μg) produced a selective decrease in rats' burying behavior in the shock-probe burying test, but did not impair rats' open-arm avoidance in the plus-maze test. Combined with our earlier results, these results suggest that distinct receptor sub-types within the septum mediate certain fear responses (burying behavior), but not others (open-arm avoidance). [*Funded by NSERC Canada*]

449.6

THE ANXIOLYTIC EFFECTS OF R(+)-8-OH-DPAT DIFFER IN THE SEPTUM AND IN THE AMYGDALA. D. Treit* and J. Menard. University of Alberta, Edmonton, Alberta, T6G 2E9.

Previous studies (Pesold & Treit, 1994) showed that intra-septal infusions of a $GABA_A$-benzodiazepine type anxiolytic (midazolam) decreased shock-probe burying but not shock-probe avoidance, whereas intra-amygdaloid infusions of midazolam decreased shock-probe avoidance but not shock-probe burying. These site-specific effects suggest that the amygdala and the septum exert differential control over different fear reactions. It is not known, however, whether these structures also mediate the effects of non-benzodiazepine anxiolytics, such as 5-HT_{1A} agonists (e.g., 8-OH-DPAT). In the present experiment, anesthetized rats were chronically implanted with guide cannulae in either the septum or the amygdala. Intra-septal infusions of the selective and highly potent **[R]** enantiomer of 8-OH-DPAT (5 or 10 μg) produced a selective decrease in rats' shock-probe burying without affecting the number shock-probe contacts. Conversely, intra-amygdaloid infusions of R(+)-8-OH-DPAT (5 μg/side) increased the number of shock-probe contacts without decreasing shock-probe burying. These results extend the generality of our previous findings to 5-HT_{1A} type compounds, and further reinforce the contention that the septum and amygdala exert parallel but distinct control over different fear reactions. [*Funded by NSERC Canada*]

449.7

CHRONIC CHANGES IN SOCIAL BEHAVIORS OF THE RAT FOLLOWING SHORT-TERM TREATMENT WITH A BRIDGED ORGANOPHOSPHATE. M. Y. V. Bekkedal*, J. Rossi III, and J. Panksepp. Dept. of Psychology, Bowling Green State Univesity, Bowling Green, OH 43403.

Previous research reports long-term behavioral changes resulting from short-term treatment with inverse agonists of the benzodiazepine receptor. In this research, a putative inverse agonist, trimethylolpropane phosphate (TMPP) was evaluated for its short- and long-term effects on social behaviors in laboratory rats.

Long Evans rats received 3-4 daily treatments of TMPP (0.1 mg/kg i.p.), and the following behavioral measures were investigated several days post-treatment: rough-and-tumble play, gregariousness, open field exploration,-and elevated plus-maze investigation. No group differences were found in measures from the plus-maze. In the open field there was a main effect for elevated activity in the TMPP group. Measures of social behavior showed group differences such that play, as measured with pins and dorsal contacts, and gregariousness, as measured with an automatic social investigation appartus, increased in animals previously treated with TMPP. Most of these chronic changes in social behaviors were observed in female rats only, and were demonstrated to interact with the environment in which TMPP exposure occurred (ie: positive vs. negative). The effects of TMPP were most evident if the drug had been experienced in a negative environment which was chilled (55° F), brightly lit (550 lux), and a loud sound was presented for 30 sec at 15 min intervals for 2 hrs.

These results indicate that short-term exposure to the compound TMPP can lead to long lasting changes in social behavior, and possibly altered neurochemistries or brain structures. Preliminary results show that some chronic effects are blocked by the co-administration of TMPP and the benzodiazepine, chlordiazepoxide, suggesting antagonist or inverse agonist actions of TMPP at the benzodiazepine receptor.
Supported by Naval Medical Research and Development Command

449.8

BEHAVIORAL EFFECTS OF ANABOLIC ANDROGENIC STEROIDS IN PREPUBERTAL RATS
Augustus R. Lumia, Marilyn Y. McGinnis*, Jenna Calabrese and Kirsten Legg
Biopsychology Program, Skidmore College, Saratoga Springs, NY 12866, Department of Cell Biology and Anatomy, Mt. Sinai School of Medicine, CUNY, New York, NY, 10029

Anabolic androgenic steroid (AAS) use has increased among adolescents and is associated with increased aggression and violence. This study assessed the effects of chronic AAS exposure on male sexual behavior, sexual preference and aggression in prepubertal male rats. Males were assigned to one of three groups: Group 1 received three weekly injections of 1mg testosterone propionate (TP); for eleven weeks, Group 2 received TP for five weeks and was then given propylene glycol (PG) for six weeks (withdrawal condition: TPw), Group 3 received PG for eleven weeks. Beginning after the eleventh week of injections, weekly behavioral tests were conducted. Males received 30 min tests for sexual behavior, ten min tests for sexual preference and a ten min test for aggression. Mounts, intromissions and ejaculations were recorded for male sexual behavior tests. For sexual preference, males were placed in a three compartment apparatus and the time spent in the presence of either a sexually receptive or non receptive female was recorded. For aggresssion tests, males were placed with either an intact or castrated opponent, under three environmental conditions: home cage, opponents cage or neutral cage. The frequency of attacks/fights initiated and submission were recorded. Prepubertal AAS did not effect either sexual behavior or sexual preference. TPw males fought significantly more with intact opponents. TPw males also showed the least amount of aggression against castrated opponents. PG treated males were the least aggressive. Aggression was the highest in the home cage against intact opponents for TP males. The results suggest that the potentiation of aggression by AAS is modulated by both the environmental context and the stimulus qualities of the opponent.
Supported by a grant from the Harry Frank Guggenheim Foundation

449.9

EFFECTS OF 5-HT_2/5-HT_{1C} RECEPTORS BLOCKADE ON FEAR-INDUCED ANALGESIA ELICITED BY ELECTRICAL STIMULATION OF THE DORSAL PERIAQUEDUCTAL GRAY. M.L. Brandão* and N.C. Coimbra. Lab. of Psychobiology, FFCLRP-USP, Brasil, 14049-901.

The dorsal periaqueductal gray matter (DPAG) has been implicated in the control of defensive behaviors. Electrical and chemical stimulation of this structure elicits fear and escape behaviour, expressed by immobility (freezing) and wild running and jumps. There is evidence that the neural substrates responsible for defensive behaviour in this level of the midbrain tectum may also be responsible for fear-induced analgesia. This study was aimed to examine the characteristics of the analgesia that follows the defense reactions induced by electrical midbrain tectum stimulation at freezing and escape thresholds. The animals were submitted to the tail-flick test, following the induction of the defensive behavioral responses. The obtained results show that the antinociception that follows the freezing and escape behaviors were not antagonized by central microinjections of the opioid antagonist naltrexone. On the other hand, the fear-induced analgesia was inhibited by microinjections of the serotonergic blockers, methysergide and ketanserin in the DPAG. Since methysergide is a non-specific antagonist of 5-HT receptors and ketanserin acts with high degree of specificity at 5-HT_2/5-HT_{1C} receptors the present results suggest that activation of 5-HT_2/5-HT_{1C} receptors may be implicated in the antinociception induced by DPAG stimulation. Immunohistochemical evidence is also presented for the occurrence of 5-HT-positive perikarya and nerve terminals in the DPAG.

FAPESP

449.10

REVERSIBLE INACTIVATION OF PREFRONTAL CORTEX DECREASES SPONTANEOUS AND AMPHETAMINE-INDUCED LOCOMOTION IN RATS. M. F. Dent* and D. B. Neill. Department of Psychology, Emory University, Atlanta, GA 30322.

Ablation or dopaminergic denervation of prefrontal cortex (PFC; Cg1 and Cg3) in rats generally yields an increase in locomotion with systemic amphetamine. However, such lesions require a recovery period during which denervation supersensitivity or other reorganizations could develop, thereby enhancing the effects of the amphetamine. Temporary inactivation of PFC with lidocaine has been shown to decrease extracellular DA levels in nucleus accumbens and to suppress burst firing of VTA cells; these effects were the opposite of those obtained from the infusion of glutamate into the PFC. This study examined the effect of reversible inactivation of the PFC on the locomotor response to amphetamine, thought to be mediated by the mesocorticolimbic DA system. Lidocaine (20 μg in 1 μl) infusion into PFC significantly decreased locomotion in a photocell box and attenuated the effect of amphetamine (1 mg/kg IP) in increasing locomotion. These results are consistent with the neurochemical and electrophysiological results and different from the behavioral effects of irreversible ablation.

Supported by grant IBN-9412703 from the National Science Foundation.

449.11

VENTRAL HIPPOCAMPAL INFUSION OF 2% LIDOCAINE ENHANCES AUDITORY STARTLE. J.H. Evans*, C.W. Harley and G. Snedden. Psychology Dept., Memorial Univ., St. John's, NF A1B 3X9 Canada.

In earlier work we observed that large aspiration lesions of hippocampus increased auditory startle. In contrast, ibotenate lesions which affected only the dorsal hippocampus failed to elevate the startle response. Since the large aspiration lesions included much of the ventral hippocampus and since this area is preferentially connected to amygdalar structures we hypothesized that ventral not dorsal hippocampus might modulate auditory startle.
Following a 5 minute adaptation to the test chamber, 11 male rats were exposed to 15 8kHz tones of 115dB at 30 s intertrial intervals against a 70dB background noise. The subjects were then implanted with bilateral guide cannulae directed at ventral CA1. Following recovery from surgery they were tested under the same conditions; once following a 1μL injection of 2% Lidocaine hydrochloride (Astra) and once without an injection. Exposure to lidocaine and no lidocaine conditions was counterbalanced. On completion of startle testing subjects were anaesthetized and india ink injected to localize cannulae . One subject was discarded based on lateral placement.
The results show that ventral hippocampal lidocaine injections significantly increased auditory startle for the first half of the testing, the probable duration of lidocaine effectiveness. We interpret this finding in terms of a possible inhibitory modulation of amygdala by ventral hippocampus and the known role for amygdala in fear enhanced startle.

Funding source: NSERC grant 9791(CWH) & Memorial University.

449.12

NALOXONE REVERSES DISINHIBITORY/AGGRESSIVE BEHAVIOR AND SYNAPTIC HYPOACTIVITY IN 5-HT DEFICIENT RATS. B. Söderpalm*, A.I. Svensson, G. Andersson, H. Billig and J.A. Engel. Inst. of Physiol. and Pharmacol, Göteborg Univ., Medicinaregatan 7, S-413 90 Göteborg, Sweden.

Disinhibited, impulsive behavior of e.g. violent offenders, suicidal patients and patients suffering from antisocial personality or substance abuse disorders may involve deficient brain serotonin (5-HT) systems. Brain 5-HT depletion produces aggression/-disinhibition also in experimental animals, as exemplified by the 5-HT depletion induced anticonflict effect in conflict models, an effect that may reflect anxiolysis and/or poor impulse control (Soubrié, 1986). Pharmacological manipulations reversing the 5-HT depletion induced anticonflict effect, e.g. negative modulators of $GABA_A$/benzodiazepine receptors (*cf.* Söderpalm & Engel, Life Sci 49:139-153, 1991), also decrease aggressive and alcohol self-administrating behaviors in rats. Naloxone reduces ethanol self-administration and using the stable analogue naltrexone this effect has been confirmed in humans. These findings and indications of $GABA_A$ antagonistic actions of naloxone prompted us to investigate the effects of naloxone on 5-HT depletion induced disinhibitory behaviors, and on the expression of brain *c-fos* after conflict exposure.

1) The anticonflict effect observed after selective 5-HT depletion (5,7-DHT, i.c.v.) in a modified Vogel's conflict model was counteracted by naloxone (0.5-5.0 mg/kg, s.c.). This effect was in turn reversed by a low dose of amobarbital (2.0 mg/kg, i.p.). 2) The number of attacks made by 5,7-DHT-lesioned residents, and the total time spent fighting, was reduced after pretreatment with naloxone (5.0 mg/kg) or the partial inverse benzodiazepine receptor agonist Ro 15-4513 (2.0 mg/kg, p.o.). 3) Exposure to conflict induced less *c-fos*, measured with Northern blot, in the amygdala and frontal cortex in 5,7-DHT-lesioned animals than in controls. This effect was reversed by naloxone.

The present results suggest that naloxone, its stable analogue naltrexone, or other weak negative modulators of $GABA_A$/benzodiazepine receptors may alone or in combination with indirect or direct 5-HT receptor agonists represent a new treatment principle for disorders chracterized by poor impulse control and/or low 5-HT function.

Supported by the Swedish MRC (grants no 11583 and 4247).

449.13

ABECARNIL ANTAGONIZES THE ENHANCED ACETYLCHOLINE RELEASE IN RAT BRAIN DURING THE ANTICIPATION BUT NOT CONSUMPTION OF FOOD. C.A.Ghiani*, L. Dazzi, E. Maciocco, G. Maira, G. Flore and G. Biggio, Dept. of Experimental Biology, Chair of Pharmacology, University of Cagliari, 09123 Cagliari, Italy.

The effect of food deprivation, food intake and satiety on acetylcholine (ACh) release from prefrontal cortex and hippocampus was investigated in freely moving rats trained to eat their daily food in 2 h (10-12 a.m./light phase). ACh release increased in cortex (+42%) and hippocampus (+55%) 40-60 min before food presentation. Food intake further increased ACh release 40-80 min from food presentation in hippocampus (+75%) and cortex (+91%). The increase in ACh output from cortex and hippocampus persisted during the two hours of food consumption returning to basal values at different time in cortex and hippocampus after food removal. The increase in ACh output measured before food presentation was prevented by the anxiolytic drug abecarnil (0.05 - 0.1 mg/kg i.p.). In contrast, this drug, also at a dose able to reduce (-50%) markedly ACh release in both brain areas, failed to antagonize the increase of ACh output elicited by food consumption. ^{35}S-TBPS binding to the $GABA_A$ receptors was higher in the brain of fasted rats when compared to fed animals. Our results suggest that while activation of $GABA_A$ receptors by abecarnil is crucial to antagonize the increase of ACh release during anticipation of food it failed to prevent the enhancement of ACh output elicited by food consumption suggesting that different mechanisms (GABAergic and nonGABAergic) are involved in the regulation of cortical and hippocampal cholinergic function before and during feeding behaviour.

449.14

VENTRAL TEGMENTAL AREA NON-DOPAMINE NEURONAL ACTIVITY AND RESPONSIVENESS TO ANESTHESIA S.C. Steffensen, S.A. Raymond*, R.-S. Lee, and S.J. Henriksen. Scripps Research Institute, La Jolla, CA 92037 and Anesthesia Research Labs, Harvard Medical School, Boston, MA 02115

The ventral tegmental area (VTA) is an important component of the mesocorticolimbic system and has been implicated in reward, locomotor activity and cognition. Anesthesia alters the firing of VTA dopamine (DA) neurons as well as their responsiveness to dopaminergics and to drugs of abuse. Inhibition of VTA DA neurons by GABAergic (putative non-DA) neurons may be particularly sensitive to specific anesthetics which may contribute to the difficulty in studying the physiology and interdependence of both VTA DA and non-DA neurons in isolation. Therefore, we characterized VTA non-DA neurons in vivo and observed their activity during freely-behaving and anesthetized conditions. The following criteria were invoked to distinquish VTA DA and VTA non-DA neurons. VTA DA neurons were driven antidromically from the nucleus accumbens (NAcc) with conduction velocities ranging from 0.6-0.8 m/s and spike durations > 500 µs. VTA non-DA neurons were characterized by the following criteria: 1) Exhibited non-bursting spontaneous activity characterized by negative-going action potentials with spike duration < 500 µs; 2) Inhibited by stimulation of the NAcc; 3) Driven antidromically by stimulation of the thalamus, but not NAcc; and 4) Not inhibited by in situ DA. In freely-behaving rats, VTA non-DA neurons were characterized by high firing rates (72 ± 5 Hz) that increased 85 ± 6 % before and during the onset of movement. Administration of halothane (1%) while recording from VTA non-DA neurons decreased VTA non-DA firing rate by 71 % and changed the firing pattern into an intermittent oscillation consisting of alternating 0.5-2s on and 0.5-2.0 s off periods (mean=0.43 ± 0.07 Hz), identical to the pattern we had observed for these units in the anesthetized preparation. Chloral hydrate (60 mg/kg) suppressed VTA non-DA activity. These results demonstrate that VTA non-DA neurons are strongly affected by anesthesia and are differentially affected by two anesthetic agents that each produce adequate systemic anesthesia. This work was supported by PHS AA10075 to SCS and DA08301 to SJH.

449.15

EFFECTS OF ESTROGEN AND PROGESTERONE ON DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS OF THE FEMALE RAT IN RESPONSE TO SEXUALLY RELEVANT ODORS AND PALATABLE FOOD. E.P. Bless* and J.B. Mitchell. Department of Psychology, Boston College, Chestnut Hill, MA 02167.

Existing evidence indicates that activity in the mesolimbic dopamine (DA) system (MLDA) is associated with reward and motivation. One method of recording activity within this system is by measuring DA release in a terminal region, the nucleus accumbens (NACC). Previously, our laboratory has found that DA release in the NACC of the female rat in response to odors from a sexually active male rat is greatest when the animal is in estrus. Conversely, DA release in response to a palatable food (Froot Loops) is greatest during diestrus. The present experiment examined the involvement of the specific ovarian hormones in this effect. DA release in the NACC was measured using electrochemistry in ovariectomized female rats injected with one of the following (each injection was 20 hours apart): estrogen and oil (E), estrogen and progesterone (E+P), oil and progesterone (P) or oil and oil (Oil). Electrochemical recordings were performed while subjects were presented with either male odors or with a palatable food. Subjects were tested 4, 24, 48 and 72 hours after the first hormone injection with each stimulus. Preliminary data indicate that the greatest DA release in response to bedding occurred 24 hours after E+P as compared to any other time point and hormone treatment. E alone and P alone at the same time point increased release over Oil but not to the same extent as E+P. On the contrary, DA release in response to palatable food was lowest 24 hours after E+P compared with any other time after E+P injection. The largest DA release in response to palatable food occurred 72 hours after E alone. Therefore, it does appear that estrogen and progesterone or either hormone alone alters MLDA activity. Interestingly, the modulation is not to produce a general increase or decrease, but rather is dependent on the stimulus with which the animal is presented.

Supported by intramural funds

449.16

EFFECTS OF NON-SELECTIVE AND BZ-1 (ω1) SELECTIVE, BENZODIAZEPINE RECEPTOR LIGANDS IN THE MOUSE DEFENSE TEST BATTERY. G. Griebel*, D.J. Sanger and G. Perrault. CNS Research Department, Synthélabo Recherche, 31, avenue Paul-Vaillant Couturier, 92220 Bagneux, France.

The behavioral effects of several BZ (ω) receptor ligands were compared using the Mouse Defense Test Battery (MDTB) which assesses defensive reactions of Swiss mice confronted with a natural threat (a rat) and behavior in situations associated with this threat. Primary measures taken before, during and after rat confrontation were escape attempts, flight, risk assessment and defensive threat and attack. The drugs used included non-selective BZ (ω) full (clonazepam, clorazepate, chlordiazepoxide and diazepam) and partial (bretazenil and imidazenil) agonists, and BZ-1 (ω1) selective (abecarnil, CL 218,872 and zolpidem) receptor ligands. The non-selective BZ (ω) receptor compounds only partially reduced some flight measures in response to the approaching rat, except clonazepam which attenuated all flight reactions. In contrast to their mild and inconsistent actions on flight, the non-selective BZ (ω) receptor agonists displayed clear effects on risk assessment when subjects were chased by the rat, but did not affect risk assessment responses when mice were trapped in a straight alley. When contact was forced between the subject and the rat, the agonists reduced defensive threat and attack reactions, while the partial agonists imidazenil and bretazenil only weakly attenuated defensive attack. Similarly, after the rat had been removed from the test area, the non-selective full agonists displayed greater efficacy than the partial agonists in reducing escape attempts. The selective BZ-1 (ω1) receptor ligands produced no specific effects on defensive behavior. Taken together, these data demonstrate that (1) non-selective BZ (ω) agonists displaying high intrinsic activity affect a wider range of defensive behaviors than non-selective BZ (ω) receptor partial agonists; (2) the defense system does not involve primarily BZ (ω) receptors containing the α1 subunit.

450.1

EFFECTS OF ACUTE AND CHRONIC SOCIAL ISOLATION IN FISCHER-344 AND SPRAGUE-DAWLEY RATS. S.M. Siviy*, B.J. Brubaker, J.H. Birkholz, M.J Pineault & T.S. Gilbert, Department of Psychology, Gettysburg College, Gettysburg, PA 17325.

Fischer-344 (F344) rats exhibit a number of neurobehavioral characteristics which make them especially interesting for studying the neural basis of behavior in juvenile rats. In particular, we have found that juvenile F344 rats engage in significantly less play behavior than either Buffalo or Sprague-Dawley rats. Since these differences are exaggerated following longer periods of social isolation, we assessed the extent to which acute and chronic social isolation affected behavior in both F344 and Sprague-Dawley (SD) rats. Rats arrived on postnatal day 21 (P21) and were immediately housed either individually (isolates) or in groups of four (socials). After 7 days, rats were placed in an open field for 60 minutes and activity was automatically monitored. While SD isolates were significantly more active than SD socials, there were no differences in activity between F344 isolates and F344 socials. Housing conditions were maintained and the rats were tested again as adults (P80-P85). At this later time, the isolates of both strains were found to be slightly less active and reared less than the socials. These data show that while these two strains differ in their acute response to isolation-housing, they respond similarly to more prolonged isolation. The extent to which strain differences after acute isolation may reflect age-specific alterations in dopaminergic functioning is currently being pursued and will also be presented. (Supported by a Gettysburg College Professional Development and Research Grant to S.M. Siviy)

450.2

CORRELATIONAL ANALYSIS OF ANIMAL MODELS OF ANXIETY, FEAR, AND DISAPPOINTMENT. Charles F. Flaherty*, Anna Greenwood, and Cynthia Coppotelli. Rutgers University, New Brunswick, NJ

Sixty rats were tested in each of four procedures: Elevated plus maze; emergence from home cage to novel open field; conditioned fear to a context (shock US); and successive negative contrast (shift from 32% to 4% sucrose). Correlational analysis revealed only a few interrelationships. For example, rats which tended to emerge sooner into the open field and rats which tended to spend more time in the open arms of the plus maze also tended to show larger decrements in licking following reward reduction -- all of which suggested a bias toward exploratory behavior. Factor analysis (oblique rotation) indicated four factors: Elevated plus maze Trial 1 (Generalized anxiety?); Elevated Plus Maze Trial 2 (Phobia?); Conditioned fear and open field emergence (Fear/Timidity?); and negative contrast -- degree of behavioral decrement following reward reduction (Disappointment?). The independence of negative contrast and fear was further illustrated by the failure of electrolytic lesions of the central nucleus of the amygdala to affect negative contrast, although fear conditioning, in the same animals, was largely eliminated by this lesion. The data suggest that different emotional states are generated by these tests and that they can be isolated neurobiologically as well as behaviorally.

Funded by NIMH grant to C. F. Flaherty.

450.3

EFFECTS OF FOOD RESTRICTION ON MESOACCUMEBENS DOPAMINE RESPONSE TO STRESS

Stefano Puglisi-Allegra*, Rossella Ventura and Simona Cabib, Dip. Psicologia, Universita' di Roma "La Sapienza" and Istituto di Psicobiologia e Psicofarmacologia (CNR), Roma, Italy

Exposure to stressors produces a time-dependent biphasic alteration of dopamine (DA) release in the nucleus accumbens septi (NAS): an initial increase of DA release is followed by a decrease below control levels.This response has been reported in two species, rat and mouse, under different stressful conditions, and using different technical approaches (intracerebral microdialysis and ex vivo tissue levels of 3-methoxytyramine (3-MT)). In mice, 10 but not 3 min of restraint stress induce a significant increase of 3-MT levels in the NAS. A return to basal levels is observed within 20 min of stress exposure and a significant decrease of 3-MT levels below basal vaues is evidend following 30 min of restraint. Mice food restricted for 13 days and then free fed for the following 48 hrs showed a significant increase of 3-MT levels in response to 3 min of retraint. A return to basal levels was observed within 10 min of stress exposure, whilst 30 min of restraint were unable to significantly reduce 3-MT levels below basal values. These results suggest that food restriction is a chronic stressful condition that renders animals more susceptible to stress-induced activation and less susceptible to stress-induced inhibition of mesoaccumbens DA release.

This research was founded by Italian CNR and MURST

450.4

DIFFERENT MEASURES OF EMOTIONALITY IN CXB MICE.

D. F. Peeler*, Department of Neurosurgery, Univ. of Mississippi Medical Center, Jackson, MS 39216.

Various measures have been used as indices of emotionality in mice. Different behavioral measures may give different, sometimes conflicting, indications. The contributions of contextual and genetic factors to measures of emotionality were assessed by observing adult male mice (n=51) of the CXB set (2 progenitor, 7 RI strains) in an open-field. Behaviors observed included locomotor activity, grooming, rearing, leaning (rearing against sides), defecation, illumination preference, contact with objects in the field and responses to contextual change. Mice were observed over 3 sessions; 1/3 of the field area was in deep shadow. Objects in the field were relocated between sessions 2 and 3.

There were complex interactions involving genetic and contextual variables. Strain was a pervasively significant factor which interacted with amount of locomotor activity, sector in which locomotion occurred (field center or periphery), illumination level, leaning, defecation, contact with objects and response to object relocation. Behaviors often interpreted as indicative of emotionality in rodents (e.g., defecation) were in some strains contradicted by other supposed measures of emotionality (e.g., exploration). ,The results indicate that different strains may react differently within a given context and/or that different strains may exhibit different manifestations of emotionality. The interpretation of any behavioral measure must accommodate the potential influences and interactions of both contextual and genetic variables.

(Support: Neurosurgery Dept., UMMC.)

450.5

EFFECTS OF SATIATION ON MONKEY SEPTAL NEURAL ACTIVITY

T. Ono*[1], H. Nishijo[1], T. Kita[2], K. Terasawa[2], R. Tamura[1]. Depts. [1]Physiol. and [2]Japn. Orient. Med., Fac. Med., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, Japan.

Single neuron activity in the septal nuclei (SP) was recorded from water- and food-deprived monkeys during discrimination of objects associated with and without juice. Motivational state of the monkeys was assessed by a clinical test based on behavioral responses to the object associated with juice and raisin. Of 349 neurons recorded from two monkeys, 67 responded in the ingestion phase. Of these 67 neurons, 31 were further tested with the clinical-liquid (juice or water) test in which liquid was provided until the monkey became satiated. These 31 SP neurons were classified into 2 groups: type-I neurons (n=10) responded to juice ingestion with inhibition, and type-II neurons (n=21) with excitation. Spontaneous activity of the type-I, but not type-II, neurons was higher in deprived condition, and decreased as the monkey became satiated by intake of liquid. When the monkey was further tested with juice after satiation with raisin, both spontaneous neural activity and motivational state resumed. Nine type-II neurons responded to the sight of the object associated with juice as well as ingestion of juice in the operant discrimination task. The responses of these type II neurons to both the sight of the object associated with juice and ingestion of juice also decreased by satiation. Both the neural and behavioral responses resumed when the monkey was further tested with raisin. These types-I and II neurons were located mainly in the anterior part of the SP. The results suggest that the activity of type-I neuron might reflect thirst or hunger drive levels, and that responses of type-II neurons might be related to reward perception.

450.6

DEFENSIVE QUIESCENCE TO SOCIAL STRESS: RELATIONSHIP TO OTHER "IMMOBILE" DEFENSES R.J. Blanchard*, and D.C. Blanchard. University of Hawaii, Honolulu, HI 96822.

The mammalian defense repertory encompasses a range of responses, some of them long-lasting. In addition to active, oriented behaviors (e.g. flight and defensive threat and attack) and immobility with orientation and enhanced responsivity to startle stimuli (freezing), there are response patterns involving reduced activity or performance without an orientation component. These have often been labelled in accord with their presumed functions (e.g. submission, as an attack-limiting signal) or psychological processes (e.g. helplessness) which they are presumed to parallel. Relatively little attention has been given to the topography of the responses involved, or to their potential interrelationships.

An emerging body of research on the immediate and long-term consequences of subordination and social stress in rats, mice, and insectivores suggests that subordinate or defeated males may show patterns of reduced activity characterized by hyporesponsivity to a variety of external stimuli, including lowered movement celerity; lack of orientation to danger sources; and an increased duration of immobility in to neck pinch. These may involve specific (midbrain?) neuroanatomic mechanisms with reciprocal inhibitory relationships to those involved in active or oriented defensive responses. We describe these behaviors and suggest the inclusive term of "defensive quiescence" to cover the variety of related behaviors previously described by similar and dissimilar labels. Supported by NSF IBN95-11349 and NIH RR08125

450.7

INDIVIDUAL DIFFERENCES IN THE DEVELOPMENT OF DEFENSIVE BEHAVIORS IN RHESUS MONKEYS: IMPLICATIONS FOR PSYCHOPATHOLOGY. S. Shelton*, N. Kalin . Dept. of Psychiatry, Univ. of Wisconsin Sch. of Med., Madison, WI 53719

Twenty-six rhesus monkeys were tested repeatedly at 4, 8, and 12 months to characterize the expression and development of their defensive responses induced by maternal separation and exposure to a potential threat. By 4 months of age, monkeys showed adult-like adaptive context dependent responses. When separated and alone, infants emitted frequent coo vocalizations; when exposed to the facial profile of a human intruder, infants engaged in freezing behavior. By 8 months of age, separation-induced coos were dramatically reduced, whereas the duration of threat-induced freezing remained unchanged. At 12 months, a further decrease in cooing occurred without a reduction in freezing. Individual differences in responding to the different contexts occurred and remained stable. However, no relation was found between the amount of cooing emitted during separation and the duration of threat-induced freezing. Over the entire period of testing, separation-induced coos demonstrated a logarithmic decrease in all but 3 of the animals tested. These 3 animals may provide a model to study factors associated with a vulnerabillity to develop psychopathology induced by loss. Greater individual variability in the patterns of change in freezing behavior occurred over the period of testing; 17 animals were unchanged, 6 animals decreased, and 3 increased their amount of freezing from 4 to 12 months. Taken together, these results demonstrate stable individual differences in separation-induced cooing and threat-induced freezing and lend support to data suggesting that defensive responses elicited by attachment bond disruption are regulated differently than those elicited by exposure to threatening situations. In addition, this developmental approach provides a strategy to identify, early in life, individuals at risk to develop stress-related psychopathology.

BIOLOGICAL RHYTHMS AND SLEEP: CIRCADIAN RHYTHMS II

451.1

ARE THERE MORPHOLOGICALLY AND ELECTROPHYSIOLOGICALLY DISTINCT SUBPOPULATIONS OF CHICKEN PINEAL CELLS? Theresa D'Souza* & Stuart E. Dryer Prog. Neurosci., Florida State University, Tallahassee, Fl 32306.

Chicken pineal cells express an intrinsic circadian oscillator that persists in dissociated cell culture. In previous studies we noted that acutely-isolated chick pineal cells exhibit marked variability in the nature and amplitude of voltage-evoked K^+ currents (1). Here we have examined the electrophysiological properties of chicken pineal cells maintained in dissociated cell culture for five days under 12:12 light-dark cycles. Whole-cell recordings were made under normal illumination 4-6 hr after light-on or under infrared illumination 4-6 hr after lights-off. In addition to fibroblasts, these cultures contained two types of cells that could be distinguished on the basis of size and shape. The first type had a small cell body (7-9 μm in diameter) with a single rod-shaped process extending a few microns from the cell body. The second type was spherical and larger (10-14 μm diameter), and did not elaborate any processes. These cells also had a larger nucleus and a more granular appearance under modulation contrast optics. Currents were evoked by a series of depolarizing voltage-steps from a holding potential of -60 mV. Peak outward currents measured at +20 mV in the small cells as a group were 318 ± 26 pA (mean ± s.e.m.). Peak currents during the day were 340 ± 35 pA (n. s.). Peak outward currents in the larger cells as a group were 84 ± 10 pA ($P < 0.001$ compared to small cells). Peak currents during the day were 92 ± 18 pA, whereas currents during the daytime were 80 ± 12 (n.s.). These results suggest that voltage-activated K^+ currents of chick pineal cells are not under circadian control and they raise the possibility that the pineal gland contains morphologically and functionally distinct cell types. (1) *Brain Res.* 572: 182-189 (1992). SUPPORTED BY AFOSR F-49620.

451.2

ULTRASTRUCTURAL DISTRIBUTION OF POLYSIALYLATED NEURAL CELL ADHESION MOLECULE (PSA-NCAM) IN THE SUPRACHIASMATIC NUCLEI OF THE ADULT MOUSE. H. Shen[1], M. Watanabe[2] & J. D. Glass[1]*. [1]Dept. of Biological Sciences, Kent State Univ., Kent, Ohio 44242; [2] Division of Pediatric Cardiology, Dept of Pediatrics, Rainbow Babies & Children Hospital, Case Western Reserve Univ., Cleveland, Ohio 44106.

In a previous study we demonstrated robust expression of polysialylated neural cell adhesion molecule (PSA-NCAM) in the hamster suprachiasmatic nuclei (SCN). In the present study, this characterization was extended to the ultrastructural level using immunoelectron microscopy. Neuronal and glial elements both expressed immunoreactive PSA-NCAM on the cell membrane. In neurons, reaction product was unevenly distributed on the plasmalemma of somata, dendrites and axons. The closely packed unmyelinated axon fascicules were particularly immunoreactive. For glia, immunostaining was sparse on the somata and main shafts of astrocytes. However, fine astrocytic processes were frequently immunopositive in regions where they were interposed between adjacent neuronal somata. Labeling of glial processes was also associated with the neuropil and their investment of outer capillary walls. Consistent with an anti-adhesion property of PSA-NCAM, direct neuronal and/or glial cell surface contacts were existent in membrane regions not expressing PSA-NCAM. In view of the hypothesized role of PSA-NCAM in modulating contact-dependent cell-cell interactions and facilitating neuroplasticity in the adult brain, these findings are evidence for a role of this molecule in regulating cellular interactions in the mammalian biological clock (AFOSR F49620-93-0086 JDG).

451.3

ZINC SENSITIVITY OF $GABA_A$-MEDIATED SYNAPTIC CURRENTS IN THE RAT SUPRACHIASMATIC NUCLEUS.
G.J. Strecker* and F.E. Dudek. Dept. Anatomy and Neurobiology, Colorado State Univ., Fort Collins, CO 80523.

GABA is the main neurotransmitter in the suprachiasmatic nucleus (SCN). Little is known, however, about which form of GABA receptor exists in this region. The SCN appears to contain significant levels of Zn^{2+}, which could modulate GABAergic function. Zn^{2+} is known to block some $GABA_A$-receptor channels, but not others. We have used whole-cell voltage-clamp recordings from acute hypothalamic slices containing the SCN to determine the Zn^{2+} sensitivity of SCN neurons from rats aged 22-28 days.

Miniature $GABA_A$-receptor-mediated IPSCs (mIPSCs) were recorded in a mixture of TTX (1 μM) and glutamate receptor channel antagonists AP5 (15 μM) and DNQX (30 μM). The addition of $ZnSO_4$ (200 μM) significantly reduced the amplitude of mIPSCs in 7 of 8 cells (Kolmogorov-Smirnov test, 2-sample) to 57% ± 4% (S.E.M.) of control mean amplitude. Zn^{2+}-induced amplitude reductions were significantly reversed after Zn^{2+} removal (n = 3), but only partially.

Zn^{2+} also reduced the apparent frequency of mIPSCs in 7 of 8 cells (χ^2 test, 2-sample) to 57 ± 9% of control. All PSCs remaining in the presence of Zn^{2+} were completely and reversibly blocked with the $GABA_A$-receptor antagonist bicuculline methiodide (30 μM, n = 3). Zn^{2+}-induced frequency reductions were significantly reversed after Zn^{2+} removal (n = 3). These results suggest that $GABA_A$-mediated synaptic transmission in the SCN can be attenuated by Zn^{2+}, and raise the possibility that Zn^{2+} is a modulatory element in the function of the circadian timing system
Supported by a grant from the AFOSR.

451.4

AFFERENT CONNECTIONS OF THE INTERGENICULATE LEAFLET OF THE GOLDEN HAMSTER: A COMBINED RETROGRADE AND ANTEROGRADE TRACING STUDY. N. Vrang*[1] and J. D. Mikkelsen[2]. Inst. of Med. Anat., Univ. of Copenhagen, Denmark and [2] Dept. Neurobiol., Res. and Dev., H. Lundbeck A/S, Copenhagen, Denmark.

Recently, it has been proposed that the NPY-containing geniculohypothalamic tract plays a central role in mediating non-photic phase-shifting. However the central areas conveying non-photic information to the IGL are unknown. To investigate the afferent connections of the IGL in the golden hamster the retrograde tracer Cholera toxin, subunit B were injected into the IGL. Subsequently, the anatomical organization of fibers projecting to the area of the IGL were studied in a series of anterograde tracing experiments using *Phaseolus vulgaris*-leucoagglutinin. The tracings revealed projections to the IGL from relatively few but widespread areas in the fore- and mid-brain. *Limbic system*: A projection to the IGL arising from cells in layer 5 and 6 of the ipsi-lateral prefrontal cortex (cingulate areas CG1 and CG2) was identified. A dense ipsilateral projection to the IGL was found from the rostral part of the zona incerta. *Hypothalamus*: Neurons with a bilateral distribution in the dorsal cap of the SCN and in the retrochiasmatic area project to the SCN. Additionally the IGL is bilaterally innervated from the ventromedial hypothalamic nucleus. *Visual system*: Besides a dense innervation from the contralateral IGL, the IGL receives a predominantly ipsilateral projection from the olivary, posterior and medial pretectal nuclei. *Brainstem*: Ipsilateral projections from the cuneiform nucleus and the dorsal raphe nucleus were identified. The present data demonstrates, that in addition to its direct retinal input, the IGL receives inputs from other visual areas as well as regions belonging to the limbic system, the latter possibly involved in non-photic (arousing) mechanisms. Located upstream and reciprocally connected to the clock the IGL is therefore in a position to integrate/modulate photic and non-photic information before it is relayed to the clock itself.
This study was supported by a grant from the Danish Medical Research Council.

451.5

DIURNAL OSCILLATION OF FOS-LIKE IMMUNOREACTIVITY IN AFFERENT CELL POPULATIONS OF THE RAT SUPRACHIASMATIC NUCLEUS. M. M. Moga.* Dept. Anatomy, Indiana Univ. School of Medicine, Terre Haute, IN 47809

In the rat brain, the proto-oncogene c-fos shows a spontaneous oscillation in its basal expression corresponding to the sleep-wake cycle. Increased levels of Fos protein and c-fos mRNA are observed in specific cell populations during periods of wakefulness (e.g., subjective night in the rat). In the present study, we examine whether any of these state-dependent Fos-like immunoreactive (Fos-lir) cell populations project to the suprachiasmatic nucleus (SCN), the mammalian circadian pacemaker. Cholera toxin subunit B (CTB) injections were placed into the SCN of albino rats. Five days later, animals were sacrificed at one of two timepoints, ZT 3/4 or ZT 15/16 (12:12 L:D cycle; lights on at ZT 0). At ZT 3/4, a few double-labeled cells (i.e., cells containing both Fos-lir and CTB) were found in the anterior part of the paraventricular thalamic nucleus. At ZT 15/16, a small number of double-labeled cells were observed in several different SCN afferent cell populations, including the paraventricular thalamic nucleus, the posterior hypothalamic area and the medial pretectal area. These findings show that the SCN receives input from cell groups which are metabolically active at specific circadian timepoints, and suggest that the SCN receives state-dependent feedback from a variety of neural sources. Supported by Indiana Univ. School of Medicine.

451.6

EXPRESSION OF FOS-LIKE IMMUNOREACTIVITY IN SYRIAN HAMSTER HYPOTHALAMUS IN RELATION TO BEHAVIORAL STATE. C. Henschel and F.C. Davis*, Dept. of Biology, Northeastern Univ., Boston, MA 02115.

The expression of Fos-like immunoreactivity has been shown to be highly correlated with sleep and sleep duration prior to sacrifice in the ventrolateral preoptic area of the rat hypothalamus (Sherin et al.,1995). Using immunohistochemistry we examined the expression of Fos-IR in the brains of adult Syrian hamsters (*Mesocricetus auratus*) that were observed to be in different behavioral states in the hour preceeding sacrifice. Hamsters were individually housed in wheel-running cages maintained in constant dim light (~1 lx) and there wheel-running activity rhythms were measured for several weeks prior to observation. On the day of sacrifice, 7 hamsters were observed every 5 minutes for at least an hour. Three hamster were awake (by behavioral criteria) for the preceeding hour, one was asleep for at least the last 35 minutes before sacrifice, and three were asleep for at least 45 minutes. Hamsters were deeply anesthetized with sodium pentobarbitol before being intracardially perfused with phosphate buffered saline followed by 4% paraformaldehyde. Brains were postfixed overnight, then cryoprotected with 20% sucrose in PBS. 60µm coronal sections were cut and put through an IHS procedure. Preliminary results indicate that in sleeping hamsters, cell nuclei showing Fos-IR were most common in an area just lateral to the paraventricular nucleus of the hypothalamus with a few also observed in the dorsal suprachiasmatic nucleus (SCN). In awake hamsters, Fos expression was common throughout much of the hypothalamus with particularly high density of stained nuclei in the ventral SCN. These results suggest that Fos expression in the hypothalamus, particularly the SCN, is correlated with behavioral state. The observed differences in Fos expression are not likely be determined by the time in the animals circadian cycle since both sleeping and awake hamsters were sacrificed during their subjective nights. Differences in SCN Fos expression reported in the literature may have been influenced by the animals' behavioral states which might not have been noted in those studies. Supported by NIH grants HD18686 to FCD.

451.7

ELECTROPHYSIOLOGICAL EVIDENCE FOR THE ROLE OF SUBSTANCE P IN RETINOHYPOTHALAMIC TRANSMISSION IN RATS. Y.I.Kim*, H.C.Shin[1], J.-M.Chung[2] and S.K.Hong. Dept. Physiol., Korea Univ. Coll. Med., Dept. Physiol., [1]Hallym Univ. Sch. Med. and [2]Dept. Biol., Ewha Womans Univ., Seoul, Korea.

In mammals, the retinohypothalamic transmission is a key step for entrainment of circadian rhythms to the environmental light-dark cycle. Although excitatory amino acids are thought to be the neurotransmitters utilized in this transmission, other neuroactive substances such as substance P (SP) have also been suggested. SP-like immunoreactivity has been reported to be present in the retinal ganglion cell fibers projecting to the hypothalamic suprachiasmatic nucleus (SCN). Furthermore, the phase-shifting effects of exogenously applied SP on the circadian rhythms of SCN neuronal activity have been demonstrated employing rat hypothalamic slice preparations. In the present study, we sought to provide physiological evidence for the role of SP by examining the effect of a SP antagonist on the phase delay of the circadian unit activity rhythm induced by optic nerve stimulation. Male S-D rats (80-150g), kept in 12-h L/12-h D environment (light started at ZT0) for ≥2 wk, were used in this study. Under Nembutal anesthesia (100 mg/kg, ip.; at ZT11-12), the brain was removed and parasagittal brain slices (550 µm) containing the SCN and the optic nerve were cut. These slices were transferred to a modified Haas-type gas interface recording chamber perfused with HEPES-buffered artificial cerebrospinal fluid (pH 7.4, 34-36°C, bubbled with 100% O_2). At ZT15, the optic nerve was stimulated (50V, 0.5 ms, 2 Hz, 10 min) with or without bath application (~15 min) of the SP antagonist L-703,606 (10 µM, NK1 type; RBI, USA). For control slices, sham stimulation was performed. On the next day single-unit recordings were obtained from the SCN. In control slices, the peak of the unit activity rhythm was detected at ZT5.67±0.67 (SEM, n=3). In slices stimulated in the presence and absence of the antagonist, the peaks were at ZT7.17±0.79 (n=6) and 9.0±0.71(n=5), respectively. These data demonstrate partial blockade of the optic nerve stimulation-induced phase delay by the SP antagonist, and therefore, support the hypothesis that SP plays a role in retinohypothalamic transmission in the rat. (This research was supported by The Hallym Academy of Sciences, Hallym University.)

451.8

THE DIURNAL RHYTHM OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) GENE EXPRESSION IN THE SUPRACHIASMATIC NUCLEI (SCN) IS DIFFERENT IN MALE AND FEMALE RATS. K. Krajnak*, K.L. Rosewell and P.M. Wise. Dept. of Physiology, Univ. of Kentucky, Lexington, KY 40536.

In female rats, the timing of the surge of luteinizing hormone (LH) is synchronized to the light/dark (LD) cycle. Studies suggest that VIP neurons in the SCN influence both the timing and amplitude of the LH surge by regulating gonadotropin releasing hormone (GnRH) neurons. If true, we would speculate that VIP gene expression changes during the morning, just prior to the LH surge to signal a change in GnRH release. Such a pattern of expression would be dramatically different than that reported in males, in which VIP mRNA levels are suppressed during the light period. To test this, we examined VIP gene expression in the SCN of female rats that were housed in a 14:10 LD cycle (lights on 0400h), ovariectomized for one week and treated with estradiol to induce a daily LH surge. VIP mRNA was measured at various points over the LD cycle, using *in situ* hybridization. A riboprobe was transcribed using a 1kb fragment of the VIP cDNA that encodes for exon 6 and VIP mRNA was assessed in individual cells. VIP gene expression was low at 0300h peaked at 1200h prior to the afternoon surge of LH and then decreased by 2300h. These results indicate that the diurnal rhythm of VIP gene expression is different in male and female rats, and suggest that changes in VIP mRNA within the SCN of females are correlated with the activation of GnRH neurons. (Supported by NIH AG02224 to PMW).

451.9

RETINAL SYNAPSES ON CALBINDIN-IR CELLS OF THE HAMSTER SUPRACHIASMATIC NUCLEUS: A DOUBLE LABEL IMMUNO-ELECTRON MICROSCOPY STUDY. D.N. Bryant*[1], J. LeSauter[2], R. Silver[2] and M-T. Romero[1]. [1]Depart. of Psychol., SUNY at Binghamton, Binghamton, NY 13902; [2]Barnard Col. Columbia Univ. NYC, NY 10027.

It is well established that pacemaker cells of the suprachiasmatic nucleus (SCN), a heterogeneous hypothalamic region, regulate circadian rhythmicity, and that light acts as an entraining signal. In the search for the identity of the pacemaker cells within the SCN, one method involves following the pathway of photic input from the eye to specific cells of the SCN. We recently described a subregion within the hamster SCN which expresses calbinindin-D_{28K} (CaBP) (Silver et al., Neuroreport, 1996). Several lines of evidence suggest that these cells may be pacemaker neurons: First: approximately 75% of the CaBP-ir cells co-express fos in hamsters exposed to light at CT 18. Second, destruction of the CaBP-ir subnucleus renders animals arrythmic in their locomotor activity, even when other parts of the SCN are spared (LeSauter et al., Soc. Neurosci. Abstr.,1995, 21: 178).

Light microscopic evidence indicates that retinal inputs visualized following bilateral intraocular choleratoxin B subunit (CT) injections terminate in the CaBP rich area of the SCN. In order to assess whether the CaBP-ir cells receive direct retinal input, we used double label immunoelectron microscopy to identify retinal terminals on CaBP neurons or dendrites. A random sampling at a single level of CaBP-ir neurons and dendrites shows that labeled CT retinal terminals appose both CaBP perikarya and dendrites. CT labeled terminals also contact other, nonidentified neurons and dendrites. CaBP labeled terminals are found in close apposition with other CaBP-ir perikarya and dendrites. The results indicate that CaBP-ir cells of the core region of the SCN may be important in the entrainment effects of light in the hamster. Furthermore, there is reciprocal innervation among CaBP elements, consistent with the integration if activity among pacemakers necessary for controlling circadian rhythms.
Supported by the Research Foundation of SUNY (MTR)

451.10

IMMUNOCYTOCHEMICAL ANALYSIS OF THE SUPRACHIASMATIC NUCLEUS OF COMMON MARMOSET *Callithrix jacchus* . J. Lu*[1], L. F. S. Moreira[1,2], V. Alones[1], M. S. O. Costa[2], A. A. L. Menezes[2], M. Menaker[1]. Dept. of Biology[1], Univ. Virginia, VA 22903, USA ; Depto.de Fisiologia[2], Univ. Fed. Rio Grande do Norte, RN, Brazil.

The common marmoset, *Callithrix jacchus*, a new world diurnal primate, shows a free-running locomotor circadian rhythm in constant dim light. The animals also display a phase response pattern similar to other mammals. In this study, we tried to identify the suprachiasmatic nucleus (SCN), the biological clock using immunohistochemical techniques. Perfused marmoset brains were shipped from Natal, RN (Brazil) to UVa where histology was performed. 30 µm frozen transverse sections were collected in a series of six and stained for vasopressin (VP), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), glial fibrillary acidic protein (GFAP), mammalian GnRH and GABA, and visualized with standard ICC techniques. The SCN is located above the optic-chiasm, lateral to the third ventricle in the anterior hypothalamus, which is consistent with a previous tract-tracing study done in our laboratory. A few small VP immunostained cells were found in the SCN. In contrast, numerous large VP-IR cells were found in the supraoptic nucleus (SON). GFAP, the astrocyte marker, heavily labeled the SCN. NPY-IR fibers were found in the SCN, but no NPY-IR neurons were found in the presumed intergeniculate leaflet (IGL) which has been shown to project the NPY to the SCN in many species in mammals including primates. Intraventricular injections of colchicine may be necessary to visualize NPY cells in the IGL. Supported by NIH T32-HD07328 (JL), CNPq(LFSM) and AFOSR F49620-1-0356 (MM and VA)

451.11

NEUROTROPHIN AND NEUROTROPHIN RECEPTOR EXPRESSION IN THE SUPRACHIASMATIC NUCLEUS (SCN) OF RATS AND HAMSTERS. M.N. Lehman*, H.T. Jansen, M. Wortman, P. Stevens[2], C. Kim, R.B. Norgren[1] and P. Zeitler[2]. Dept. Cell Biol., Neurobiol. & Anat. and Neurosci. Program, Univ. Cincinnati, Cincinnati, OH 45267; [1]Dept. Cell Biol., U. Nebraska Med. Ctr., NE 68198; [2]Div. Endocrinol., Children's Hosp. Res. Foundation, Cincinnati, OH 45267

In addition to their trophic actions, neurotrophins (NT) may act as neuromodulators in the adult brain (Kang and Schuman, Science, 267:1658). One example may be in the SCN circadian pacemaker, since NGF injections can phase shift circadian activity rhythms in the hamster (Golombek et al., Physiologist, 38:A22). Neurons in the rat SCN express BDNF (Liang et al., Neurosci. Abstr., 21:453) and p75 (Sofroniew et al., Brain Res., 476:358), but other NTs and NT receptors have not been examined. We used reverse transcription-polymerase chain reaction (RT-PCR) to screen the rat and hamster SCN for mRNAs encoding NGF, BDNF, and NT-3, and the receptors p75, TrkA, and TrkC. Total RNA (2μg) was reverse transcribed from microdissected SCN, forebrain and hippocampus of juvenile male rats (n=3) and hamsters (n=5). Primers specific for each NT and NT receptor were used to identify corresponding cDNAs. A constitutive ribosomal mRNA species (S12) served as an internal control. Forebrain and hippocampal extracts of both rat and hamster contained PCR products of appropriate size. The rat SCN region contained NGF, BDNF, NT-3, and TrkC, but not TrkA mRNAs. By contrast, the hamster SCN region contained NT-3, TrkA, and TrkC mRNAs; NGF, BDNF and p75 mRNAs were not detected. Cloning and sequencing of cDNAs for hamster NGF, NT-3 and TrkC demonstrated strong homology to NT sequences in other species. Immunocytochemistry confirmed the species differences in TrkA and p75 in the SCN: the hamster SCN contained TrkA-positive cells but not p75, while the converse was true for the rat SCN. Despite these differences, it appears that both rat and hamster SCN regions contain TrkC and NT-3 mRNAs. The precise localization of these NTs and receptors, and their role in circadian pacemaker function, are under investigation. Supported by Univ. Cincinnati.

451.12

BRAIN-DERIVED NEUROTROPHIC FACTOR AND TrkB RECEPTORS IN THE RAT SCN: DISTRIBUTION AND FUNCTIONAL IMPLICATIONS. F.-Q. Liang*, R. Miranda, F. Sohrabji, C. Brooks and D. Earnest. Dept. of Human Anatomy, Texas A&M Univ., College of Medicine, College Station, TX 77843.

Since light regulates expression of the mRNAs for brain-derived neurotrophic factor (BDNF) and its cognate receptor, TrkB, in other retinorecipient targets and TrkB tyrosine kinase receptors have been localized in retinal ganglion cell axons within the optic chiasm, the present study examined anatomical and functional associations between this neurotrophin and its receptor in the rat suprachiasmatic nucleus (SCN).

Cytoplasmic expression of hybridization signal for BDNF mRNA and BDNF-immunoreactivity was separately observed within a number of cells in the rat SCN. Cells expressing BDNF mRNA and BDNF-immunoreactive profiles were distributed in similar patterns throughout the entire SCN. Expression of BDNF mRNA and protein was also evident within some cells in the optic chiasm. Immunostaining for the TrkB receptor, which preferentially binds BDNF, was mainly observed in terminals and small fibers within the ventrolateral SCN and within the optic chiasm. Dual application of *in situ* hybridization and immunocytochemical techniques revealed that cells expressing BDNF mRNA in the ventrolateral SCN were often closely apposed to TrkB-positive fibers emanating from the optic chiasm. Some cells within the dorsomedial SCN were uniquely characterized by the coexpression of both TrkB-immunoreactivity and BDNF mRNA.

Since interactions between BDNF and the TrkB receptor have been implicated in the short-term regulation of synaptic transmission within the CNS, continuing research will examine the possibility that similar mechanisms may play a role in the circadian and/or photoentrainment functions of the SCN.

451.13

PEPTIDERGIC INNERVATION OF GONADOTROPIN RELEASING HORMONE (GnRH) NEURONS IN FEMALE SYRIAN HAMSTERS. Kim L. Huhman* & Eline M. van der Beek. Dept. Psych., Georgia State Univ., Atlanta, GA 30303; Dept. Human & Animal Physiol., Agricultural Univ. Wageningen, Haarweg 10, Wageningen 6709 PJ, Netherlands.

The GnRH cells that control the preovulatory surge of luteinizing hormone (LH) are known to receive timing information from the circadian clock in the suprachiasmatic nucleus (SCN). In rats, SCN projections to GnRH neurons contain vasoactive intestinal peptide (VIP). Other SCN efferents contain vasopressin (AVP) and perhaps peptide histidine isoleucine (PHI). The purpose of the present experiment was to examine the peptidergic innervation of GnRH neurons that are activated during the LH surge (as evidenced by colocalization of Fos-ir) in female hamsters. Groups of 3 hamsters were killed at 4 time points on the afternoon of proestrus (1100, 1300, 1500, 1700 hr with lights off at 1800 hr) and triple-stained immunohistochemically for GnRH, Fos and either VIP, PHI or AVP. PHI fibers were apposed to 12-28% of GnRH neurons including those activated during the LH surge, and the distribution of these fibers appeared to be very similar to that seen in rats. In contrast to rats, only a small percentage of the GnRH neurons were found in association with VIP-ir fibers. Finally, GnRH neurons in the caudal preoptic area were consistently innervated by AVP fibers. These data suggest that PHI, and to a lesser extent VIP and AVP, might signal the LH surge via SCN efferents in hamsters.

(Supported by NIH NS34896)

451.14

CHARACTERIZATION OF RECEPTORS MEDIATING EFFECTS OF SEROTONIN (5-HT) ON PHOTIC RESPONSES OF HAMSTER SUPRACHIASMATIC NUCLEUS (SCN) CELLS. S.-W. Ying and B. Rusak* Dept. of Psychology, Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4J1.

5-HT has been shown to phase-shift rodent circadian rhythms, but the receptors mediating these effects remain uncertain. We demonstrated previously that 5-HT agonists active at the 5-HT_{1A} receptor suppress photic responses of hamster SCN cells, but these drugs also have high affinity for the recently cloned 5-HT_7 receptor. We therefore assessed drugs with differential affinities for 5-HT_7 and 5-HT_{1A} receptors for their effects on photic responses of hamster SCN cells. Hamsters were anesthetized with urethane and responses of cells to retinal illumination, and their sensitivity to drugs were studied using multibarrel micropipettes to record single-unit activity and to apply drugs iontophoretically.

The 5-HT agonists 5-HT, 8-OH-DPAT and 5-carboxamidotryptamine all depressed spontaneous activity and photic responses of SCN cells. Drugs with high affinities for the 5-HT_7 receptor, but not the 5-HT_{1A} receptor, clozapine and ritanserin, were able to antagonize these effects of 5-HT agonists, while cyanopindolol was ineffective. The putative 5-HT_{1A} antagonists WAY100635 and *p*-MPPI did not antagonize the effects of 8-OH-DPAT on SCN cells, but did antagonize those on hippocampal cells. In the cerebellar cortex, where there are few 5-HT_7 receptors, ritanserin was not an effective antagonist for the effects of 8-OH-DPAT. These results support the hypothesis that the influence of 5-HT on SCN cells is mediated by a receptor that is pharmacologically most similar to the 5-HT_7 receptor.

Supported by grants from the U.S. AFOSR and NSERC of Canada.

451.15

SEROTONIN (5-HT) RECEPTOR BINDING AND mRNA EXPRESSION IN THE RAT SUPRACHIASMATIC NUCLEUS (SCN). P.A. Scott*, M. Cagle, & M.A. Rea. Krug Life Sci. and BRAIN Res. Inst., Armstrong Lab., Brooks AFB, TX 78235, and Dept. of Pharm., UTHSCSA, San Antonio, TX, 78284.

Circadian rhythms are entrained to the environmental light-dark cycle as a consequence of daily light-induced adjustments in the phase of the circadian clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus. Previous work has shown that serotonin (5-HT) serves to modulate the response of the circadian clock to light through a mechanism involving activation of a 5-HT_{1A}-like receptor (Rea et al, 1994). To further elucidate the nature of the 5-HT receptor(s) responsible for the regulation of the photic response of the SCN, we have characterized the pattern of expression of 5-HT receptors in the SCN using *in situ* hybridization and quantitative receptor autoradiography.

In situ hybridization using an antisense cRNA probe for the rat 5-ht_7 receptor revealed the presence of a dense cluster of 5-ht_7-positive cells localized to the anterior SCN. At more caudal levels, the major band of 5-ht_7-expressing cells extended from the dorsal lateral border of the SCN into the subparaventricular zone. Only a few scattered 5-ht_7-positive cells were observed in the ventral aspect of the SCN throughout its rostro-caudal extent.

Quantitative receptor autoradiography was performed on SCN sections prepared at circadian time 19 using [^{3}H]-8-OH-DPAT (0.6-0.8 nM) in the presence of either pindolol (1 μM) or ritanserin (0.5 μM). Nonspecific binding was defined using 10 μM 5-HT. Results showed that approximately 80% of [^{3}H]-8-OH-DPAT binding was pindolol-sensitive, while approximately 20% was ritanserin-sensitive. This suggests that the majority of [^{3}H]-8-OH-DPAT binding in the SCN is to the 5-HT_{1A} receptor subtype.

Research supported by AFOSR #2312CA (MAR).

451.16

LOCAL SEROTONIN AGONISTS DOSE-DEPENDENTLY ATTENUATE LIGHT-INDUCED PHASE ADVANCES OF CIRCADIAN ACTIVITY RHYTHMS IN HAMSTERS. E.Todd Weber*, Anna.M. Michel, Robert L. Gannon and Michael A. Rea. Biological Rhythms and Integrative Neuroscience Research Institute, Armstrong Laboratory (CFTO), Brooks AFB, TX.

Light-induced phase shifts of the suprachiasmatic nuclei (SCN) are modulated by serotonin (5-HT). In order to elucidate the site of action of 5-HT agonists on light-induced phase advances and the receptor subtype(s) responsible for serotonergic modulation of phase advances, 8-OH-DPAT and 5-carboxyamidotryptamine (5-CT), compounds with high affinities for 5-HT_{1A} and 5-HT_7 receptors, were injected into the region of the SCN of Syrian hamsters prior to light pulses sufficient to induce phase advances of the circadian clock. Animals received 0.3 μl of either vehicle, 5-CT or 8-OH-DPAT, 10 minutes prior to 10 minutes of 20 lux white light at circadian time (CT) 19 and returned to DD for 10-14 days. Pretreatment with either 5-CT (1 μM= 72 ± 15, 10 μM= 40 ± 8, 100 μM= 28 ± 7 mins, mean ± SEM) or 8-OH-DPAT (1 μM= 47 ± 14, 10 μM= 33 ± 6, 100 μM= 23 ± 11 mins) dose-dependently attenuated light-induced phase advances (vehicle controls= 73 ± 9 minutes). Furthermore, systemic 8-OH-DPAT was equally effective at inhibiting light-induced phase shifts in 5,7-dihydroxytryptamine-lesioned hamsters (21 ± 7 mins versus control, 87 ± 8 mins). Systemic 8-OH-DPAT (0.5 mg/kg i.p.) attenuation of light-induced phase advances (53± 12 mins vs. control 79 ± 13 mins) was blocked by pretreatment with (+)WAY-100,135 (5 mg/kg; 73 ± 11 mins), clozapine (2.5 mg/kg; 76 ± 10 mins) or ritanserin (5 mg/kg; 80 ± 13 mins). In a separate experiment, 8-OH-DPAT (1 mg/kg i.p.) attenuation of light induced phase advances (63 ± 17 mins vs. control 127 ± 29 mins) was blocked by WAY 100,635 (5 mg/kg, 98 ± 16 mins), but not by pMPPF (5 mg/kg, 65 ± 16 mins), both reportedly 5-HT_{1A}-specific antagonists. Together, these results strongly support serotonergic modulation of photic input at the level of the SCN. Furthermore, the serotonin receptor(s) that mediate the inhibitory effect of 8-OH-DPAT in the hamster has pharmacological properties of both 5-HT_{1A} and 5-HT_7 receptors.

This work is supported by AFOSR 2312CA(MAR). ETW is a NRC Research Associate.

451.17

ACTIVITY OF 8-OH-DPAT ENANTIOMERS AT THE 5HT7 RECEPTOR IN SCN AND TRANSFECTED CHO CELLS. J. D. Miller*, Z. W. Liu, and T. Tenner. Deptartment of Pharmacology, Texas Tech University School of Medicine, Lubbock, TX 79430.

A new member of the 5HT receptor family, 5HT7, has recently been cloned and characterized. This receptor is positively coupled to adenylate cyclase. It has been suggested that this receptor is responsible for 5HT-induced phase advances in the suprachiasmatic nucleus (SCN). A complicating factor is the presence of 5HT1A receptors in the SCN, which may also be occupied by the most selective known ligand for 5HT7, 8-OH-DPAT. To further investigate the pharmacology of the 5HT7 receptor, we have examined the activity of the enantiomers of 8-OH-DPAT in 5HT7-transfected CHO cells. We have found that (+) 8-OH-DPAT (EC50=75 nM) is about 25 times more potent at the 5HT7 receptor than (-) 8-OH-DPAT (EC50=1.9 μM) in elevating the activity of the cyclase. In contrast, the published (Cornfield et al., 1991) potency ratio (for inhibition of hippocampal adenylate cyclase) of the 8-OH-DPAT enantiomers at 5HT1A is about 2.4 (EC50(-) / EC50(+) =135 nM / 57.4 nM), suggesting that the (+) isomer is roughly equipotent at the two receptors and that the (-) isomer is much more potent at 5HT1A. The 5HT7/5HT2 antagonist, ritanserin (10 μM), suppresses cyclase activity in the transfected CHO cells (57% reduction in basal cyclase activity). In cultured SCN cells (21 days postnatal) preliminary results indicate that (+) 8-OH-DPAT (10 μM) elevates an outward, voltage-dependent, TEA-sensitive current that appears to be I_K. (-) 8-OH-DPAT (10 μM) and the 5HT1A antagonist, pindolol, have no effect on this current, but it is potently suppressed by ritanserin (10 μM). These results support the hypothesis that the 5HT7 receptor is the functionally important 5HT receptor for 5HT-induced phase advances in the SCN and that the phase shift may involve the opening of a potassium channel. Supported by NIA AG11084-03.

451.18

$5HT_{1B}$ PRESYNAPTIC RECEPTORS ON RETINAL AXONS MEDIATE 5HT INHIBITION OF PHOTIC RESPONSES IN THE SCN. G.E. Pickard*, P.A. Scott, A.F. Riberdy, E.T. Weber and M.A. Rea. BRAIN Research Institute, Armstrong Lab. (CFTO), Brooks AFB, TX 78235 and *Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

5HT fibers arising from the median raphe nucleus innervate the suprachiasmatic nucleus (SCN). To test the hypothesis that 5HT modulates SCN retinal input via $5HT_{1B}$ presynaptic receptors on RHT axons, we examined the effects of $5HT_{1B}$ agonists on light-induced behavioral phase shifts and Fos expression in hamster and mouse SCN. The effect of enucleation on SCN $5HT_{1B}$ receptors was determined by quantitative autoradiography using [^{125}I]-ICYP to label $5HT_{1B}$ binding sites.

Injection of the $5HT_{1B}$ agonists TFMPP and CGS 12066A, 30 min prior to light (10 min at 20 lux) at CT 14 and CT 19 (hamster) or CT 16 (mouse) inhibited light-induced phase shifts of wheel-running activity in a dose-dependent manner. Injection of (+)WAY 100135, a selective $5HT_{1A}$ antagonist, or mesulergine, a $5HT_{2A/2C}$ antagonist, 30 min prior to TFMPP injection had no effect on TFMPP inhibition of light-induced phase shifts. Methiothepin, a non-selective $5HT_1$ antagonist, attenuated the TFMPP effect. TFMPP (1mM) infused into the SCN 10 min prior to light stimulation blocked phase shifts. Systemic injection of TFMPP dose-dependently inhibited SCN Fos expression although some cells in the dorsolateral caudal SCN were TFMPP insensitive. Enucleation reduced [^{125}I]-ICYP binding sites in the ventral SCN by 35%. The results are consistent with the interpretation that $5HT_{1B}$ presynaptic receptors on RHT axon terminals inhibit retinal input to the SCN. Thus, 5HT tone in the SCN may play an important role in the regulation of circadian phase by serving to modulate the response of the circadian oscillator to photic stimuli. Supported by AFOSR University Resident Research Program (GEP), an NRC post-doctoral fellowship (ETW) and AFOSR 2312CA (MAR).

451.19

LABEL AND ELECTRICAL COUPLING OF NEURONS IN RAT SUPRACHIASMATIC NUCLEUS (SCN). Z. G. Jiang*, Y. Q. Yang and C.N. Allen Center for Research on Occupational and Environmental Toxicology and Department of Physiology and Pharmacology, Oregon Health Sciences University, Portland, OR 97201.

Whole-cell recording from single neurons of the suprachiasmatic nucleus in horizontal brain slices with an electrode containing the label neurobiotin resulted in staining of single and multiple neurons in 55% and 30% cases, respectively (n=71). Typically, the recorded neuron soma was darkly stained with the HRP-DAB reaction method and its dendritic processes and an axon were clearly visible. In cases of multiple cell staining, additional adjacent 1-5 neurons were lightly stained and termed as the coupled neurons. The resting membrane potential, input membrane conductance, membrane capacitance, the decay time constant and the maximum H-current amplitude of the recorded neurons having coupled cells were not significantly different from those of neurons not showing label coupling. Stimulation of the preoptic area activated an antidromic action potential or an all-or-none small slow inward current in some neurons when the synaptic transmission was blocked by a calcium-free/Mn^{2+} solution. The small slow inward current did not "collide" with an orthodromically activated action spike suggesting that the current represents the signal from an electrotonically coupled neuron. In addition, the frequency of biphasic field currents from a neighboring cell firing were increased by depolarization and decreased by hyperpolarization of the recorded cell in half the cases. These data demonstrate a chemical and electrical low-resistance coupling between the SCN neurons, which could be important in synthesizing a circadian rhythm in the principal pacemaker of the biological clock in mammalians. (Supported by grant AG10794)

451.20

METABOTROPIC GLUTAMATE RECEPTOR mRNA, INCLUDING MGLUR6 AND NEW SPLICE VARIANTS OF MGLUR7, ARE EXPRESSED AND DEVELOPMENTALLY REGULATED IN SCN. A.N.van den Pol*, P.K.Ghosh, N.Baskaran. Sect.Neurosurgery, Yale Med. Sch., New Haven, CT. 06520.

Using RT-PCR, we studied the expression of metabotropic glutamate receptors (mGluR) in the rat suprachiasmatic (SCN) and arcuate (ARC) nuclei. Glutamate is the primary excitatory transmitter innervating both the SCN and ARC. Metabotropic glutamate receptors have not received much attention in the hypothalamus. Some reports indicate greater physiological responses to metabotropic agonists in the developing brain than in the adult. Unique non-overlapping primers were chosen from the C-terminal regions of mGluRs1-8. mRNA was isolated from punches of SCN, ARC, and whole brain, converted to cDNA by reverse transcription, and amplified by PCR. In contrast to previous reports that mGluR6 was only expressed in retina, we found it also expressed in the adult SCN and ARC with RT-PCR, but not with conventional Northern blots. We found two new mRNA splice variants of mGluR7, mGluR7B, and mGluR7C. Sequencing the mGluR7B cDNA revealed a unique 92 bp insert. Within the mGluR7B C-terminal insert is a stop codon (UAG) that would generate a C-terminal amino acid sequence different than mGluR7. All splice variants of mGluR7 were strongly expressed in adults, but only weakly in neonates. Two of the mGluRs (4 and 5) were expressed more strongly in the P10 neonatal SCN and ARC than in adults. Others (mGluR2,3,6) were expressed more strongly in adults. These data indicate that all cloned mGluRs are expressed in the hypothalamus. Each is regulated independently on the basis of hypothalamic region and developmental age.

NEUROETHOLOGY: INVERTEBRATES

452.1

RESPIRATORY PUMPING IN *APLYSIA FASCIATA* HAS A VENTILLATORY FUNCTION IN NATURAL AND ARTIFICIAL TIDE POOLS. M. Levy & A.J. Susswein*. Dept. of Life Sciences, Bar Ilan University, Ramat Gan, 52 900 Israel.

Respiratory pumping in *Aplysia* is a behavior whose neural circuitry has been explored, but whose natural functions are incompletely understood. Although the form of the movement suggests that it has a ventillatory function, previous studies trying to establish such a function have been inconclusive. Respiratory pumping has been shown to function in a defensive context, to act as an adjunct to osmoregulation, and also to have a sexual function, perhaps in dispersal of pheromones. We have now examined whether respiratory pumping might have a ventillatory function in natural and artificial tide pools.

The rate of respiratory pumping was found to be increased when *Aplysia fasciata* are trapped in natural tide pools, as well as in artificial tide pools in which the oxygen concentration is systematically decreased, and temperature, salinity and CO_2 concentration are systematically increased. Artificial tide pools also lead to an increase in the time spent immobile, and decreases in the time spent feeding and mating. The increase in respiratory pumping contributes to the ability of *Aplysia* to persevere in artificial tide pools, since ablation of the osphradium and cutting the pleural-abdominal connectives, procedures leading to a decrease in the respiratory pump rate, also cause an increase in the number of animal observed to collapse (characterized by the animal's losing contact with the substrate and lying on its side with no sign of movement). Respiratory pumping in artificial tide pools is triggered by a combination of all four stimuli that are changed, but the effects of change in the oxygen concentration and temperature are the largest. Changes in other behaviors were also observed in response to all of the stimuli that are present artificial tide pools.

Supported by Israel Science Foundation Grant No. 561/93

452.2

HYPOXIA INDUCED RESPIRATORY PATTERNED ACTIVITY IN *LYMNAEA* ORIGINATES AT THE PERIPHERY: ROLE OF THE DOPAMINE CELL. T. Inoue*, Z. Haque, M. Takasaki, K. Lukowiak and N. Syed. Neuroscience and Respiratory Research Groups. University of Calgary, Calgary, Alberta, T2N 4N1 and Arabian Gulf University/CMMS, Manama, Bahrain.

A central pattern generator (CPG), appears sufficient for respiratory rhythmogenesis in both isolated and semi-intact preparations of the fresh water snail *Lymnaea stagnalis*. Identified respiratory CPG neurons, VD4 and IP3I, control inspiration and expiration respectively, whereas RPeD1 initiates the respiratory cycle in these cells. The source of excitatory input that drives RPeD1 is, however, unknown. In this study, we demonstrate that the hypoxia drive underlying respiratory rhythmogenesis in *Lymnaea* originates at the periphery. Specifically, patterned respiratory activity was monitored electrophysiologically in both semi-intact (CNS-peripheral organs attached) and isolated brain preparations that were maintained either under normoxic (21% O_2), hypoxic (10% O_2) or anoxic (100% N_2) saline conditions. Superfusion of semi-intact preparations with hypoxic saline, either initiated the respiratory activity in a previously quiescent preparation, or significantly increased its frequency in spontaneously active preparations. Anoxic conditions, on the other hand, suppressed respiratory discharge in the semi-intact preparations. The spontaneously occurring respiratory activity in isolated brain preparations, however, remained unchanged under hypoxic conditions, suggesting that the hypoxia induced respiratory activity in *Lymnaea* has peripheral origin. Utilizing semi-intact and intact animal preparations, we present evidence to support the hypothesis that synaptic interactions between the peripheral chemoreceptors and RPeD1 are necessary for respiratory rhythmogenesis in *Lymnaea*. Together, our data suggest that although central CPG neurons appear sufficient for the basic respiratory rhythm in *Lymnaea*, the peripheral chemoreceptor are essential for the initiation of the respiratory activity in this animal. Supported by MRC (Canada).

452.3

THE ROLE OF SENSORY FEEDBACK IN CRAWLING BEHAVIOR IN NORMAL AND SURGICALLY MANIPULATED MEDICINAL LEECHES. T.W. Cacciatore,* R. Rozenshteyn, J. Gharakhani, W.B. Kristan Jr. Department of Neuroscience, UCSD, La Jolla, CA 92093.

The medicinal leech crawls to locomote across a solid substrate. A crawling step consists of an elongation wave followed by a contraction wave which pass from the anterior to posterior of the animal. The front and rear suckers attach and detach from the substrate in coordination with the segmental movements to propel the animal forward. The crawling behavior is variable: steps vary in length, coordination between elongation and contraction, and may include searching movements in the anterior during elongation. However, stereotyped movement patterns underlie all crawling steps. We performed a detailed analysis of stereotyped crawling movements in normal and surgically manipulated animals to help gain insight into the nature of the underlying neuronal circuitry. Animals with markers sewn between most midbody segments were filmed crawling to determine the length of segments throughout a step. Each segmental length profile was then parameterized by a sum of 2 sigmoidal functions. The amplitude and timing of these movements, along with sucker movements, completely characterize the behavior and were used to quantitate the coordination between segments. Leeches with surgical manipulations were also analyzed to test the role of sensory feedback in coordinating the motor pattern. Leeches denervated in midbody segments 7-12 crawled normally suggesting that sensory feedback is not required for producing the stereotyped crawling pattern. However, animals which were stretched during a crawling step showed abnormal coordination, including prolonged step duration and back-to-front elongation and contraction waves. The role of the head and tail brains was examined by analyzing animals which had the connectives to each brain cut. Supported by NIH Training Grant #GM08107 and Research Grant #MH43396.

452.4

CHANGES IN SEROTONIN ARE CORRELATED WITH PARASITE-INDUCED ALTERATIONS IN HOST BEHAVIOR. B.J. Maynard, L.J. DeMartini, and W.G. Wright*. Dept. of Biology, Colorado State University, Fort Collins, CO 80523.

The acanthocephalan parasite *Polymorphus paradoxus* appears to manipulate the behavior of its crustacean intermediate host, the amphipod *Gammarus lacustris*. Amphipods infected with *P. paradoxus* show an abnormal preference for brightly lit environments and respond to disturbance by clinging to floating objects. These altered behaviors bring the amphipod to the water surface, thus increasing transmission of the parasite to its surface-feeding definitive host, mallard ducks. A related species of parasite, *P. marilis*, also uses *G. lacustris* as its intermediate host, but induces a much milder behavioral change, i.e. a preference for only slightly brighter environments, and no change in response to disturbance. Once again, these altered behaviors likely increase transmission, since *P. marilis* uses a diving duck, the lesser scaup, as its definitive host, rather than surface-feeding mallards. The question of how these two parasites induce behavioral changes in their amphipod hosts remains unanswered. Both parasites exist as free-floating cysts in the hemocoels of their hosts, causing no mechanical damage. It is known that injections of serotonin (5HT) into the hemocoel of non-parasitized amphipods are able to mimic the behavioral effects of *P. paradoxus*, suggesting the possibility of neurochemical manipulation by the parasite. We compared the pattern of 5-HT immunoreactivity in whole mounts of the ventral nerve cords of uninfected amphipods with those from amphipods infected with *P. paradoxus* or *P. marilis*. All nerve cords showed one bilateral pair of 5HT immunoreactive cell bodies in the third thoracic ganglion, but the fine structure varied dramatically, depending on the infection state of the host. Nerve cords from amphipods harboring *P. paradoxus* showed a 2.8 fold increase in the number of visible 5HT storage sites in comparison with non-infected preparations processed in the same dish; no such increase was observed with the *P. marilis*-infected preparations. These results further implicate 5HT in the dramatic alterations of host behavior induced by *P.paradoxus*. Support provided by the Whitehall Foundation.

452.5

ALTERED EXCITABILITY OF CRAYFISH ESCAPE REFLEX DURING AGONISTIC ENCOUNTERS A. Shamsian, R. Kulkarni, & F. Krasne*. Dept. Psychology & Brain Res. Inst., UCLA, LA, CA, 90095.

In crayfish 5-HT, which is suspected of being released during agonistic encounters, *increases* lateral giant (LG) escape reflex excitability in social dominants and *reduces* it in subordinates (Yeh et al, *Science*, 1996, 271, 366). Hoping to gain insight into the functional significance of this counter-intuitive finding we studied the effect of agonistic encounters on excitability of the LG reflex in freely behaving crayfish with chronic electrodes. We found that during encounters escape is inhibited substantially in subordinates but only slightly and in some cases not at all in dominants, a finding rather analogous to the 5HT result. Escape in crayfish can be generated both by the reflex circuitry studied here, which produces highly stereotyped and prompt responses, and by a "voluntary" system that produces less stereotyped, visually guided tail-flip swimming but at longer latency. We suggest that subordinates, under constant threat of attack, continuously engage voluntary decision circuitry and inhibit reflex circuitry whereas dominants go about their business, relying on reflex escape to protect them from surprise attack. Consistent with this view, escape responses of subordinates, which are common during fights, are not mediated by reflex circuitry. We believe that both GABA and 5-HT contribute to the modulation of LG reflex excitability during social interactions.

Supported by NIH Grant NS8108.

452.6

BEHAVIORAL SEQUENCING DURING ADAPTATION TO CURRENT AND SURGE IN THE AMERICAN LOBSTER. L. Schlichting and J. Ayers*. Marine Science Center, Northeastern University. East Point. Nahant, MA 01908

We are developing a detailed object-based computational model for the control of omnidirectional ambulation based on the lobster walking system. We use reverse kinematic and electromyographic analysis to segment rheotaxic behavior into stereotypic units to establish the sequences utilized to adapt to translational flow and surge. Walking movements in flow and surge involve yaw corrections as well as rotations. When walking into flow specimens compensate by lowering the body and increasing the period of stepping. In addition to leg mediated yaw compensation, lobsters use their claws, abdomen and swimmerets as hydrodynamic control surfaces. As flow increases, specimens lower the chelipeds and elevate the abdomen and telson to generate a force vector to the substrate.

These findings are integrated into our ambulation controller and extend the library of object classes to include sensors, command interactions, behavioral hierarchies and sequences. The goal of these studies is a robotic control architecture capable of operation in the turbulent littoral environment. Supported by ONR.

452.7

INFLUENCE OF HIGHER CENTERS ON THORACIC CIRCUITS OF THE ESCAPE SYSTEM IN COCKROACH. P.L. Schaefer and R.E. Ritzmann*. Department of Biology, Case Western Reserve University, Cleveland, OH 44106-7080.

The cockroach escape system has been studied at the level of behavioral observations as well as individual cell circuitry. Most studies on cell circuitry have focused upon connections to giant interneurons in the terminal ganglion and there output to thoracic interneurons and ultimately to leg motor neurons within thoracic ganglia. These studies have produced a fairly complete description of neural circuitry from sensory inputs to motor outputs. Other observations by C.M. Comer et al. have documented other forms of phasic sensory input that include tactile stimulation of antennae. However, little emphasis has been placed upon tonic influences from higher centers which may modulate sensory inputs from other regions, even thought several pieces of evidence suggest that descending tonic activity may play an important role in the escape system.

These observations prompted us to compare the leg movements associated with the escape response in decapitated animals to those movements seen in intact individuals. Observations were made both on free ranging animals and on tethered animals walking on a lightly oiled plate. The movements of critical joints in the metathoracic legs of decapitated animals were normal. In the mesothoracic legs the direction of movement of leg joints was normal, but the magnitude of movement was significantly lower. However, prothoracic legs were dramatically affected by decapitation. Subjects made little or no movement of prothoracic legs.

These data suggest that prothoracic circuitry is influenced to a greater extent than circuitry in other thoracic ganglia. Ongoing experiments will attempt to localize the origin of this tonic effect within higher centers.

Supported by Whitehall Foundation Grant #J95-02 to RER.

452.8

LOCALIZATION OF VIBRATIONAL SOURCES BY THE FIDDLER CRAB *Uca Pugilator*. T. Haresign*, J. LaPolla, and B. Vemulapalli. Biology Program, Richard Stockton College, Jimmy Leeds Road, Pomona, NJ 08240

Fiddler crabs (genus *Uca*) have long been thought to be sensitive to substrate-borne vibrations. The ability to localize vibrational signals would provide an adaptive mechanism that would be useful to the crab in terms of predator avoidance and possibly also in localization of conspecifics. To test localization ability crabs were placed in the center of a circular behavior arena. These crabs all had their vision occluded by prior application of an opaque paint over the eyes. A vibrational source located at a specific position in the arena was activated and the crab's direction of movement relative to the source was quantified. Results in U. pugilator indicate that there is a significant movement away from the vibrational source, suggesting that the crabs are able to localize the source. Furthermore, our results suggest that the localization task is easier when the long axis of the body is aligned perpendicular to the oncoming vibrational waves. This may indicate that the crab is comparing arrival times of the wavefronts between the legs on either side of the body. Comparison of arrival times would entail time measurements of less than 1 millisecond. Further experiments are being conducted to further explore the computational basis for localization in these crabs.

452.9

JUVENILE HORMONE REDUCES AUDITORY SENSITIVITY IN THE PRAYING MANTIS, *TAUMANTIS EHRMANNII*. A.L.Harron and D.D.Yager*, Dept of Psychology, Univ. of MD, College Park, MD 20742.

Juvenile hormone (JH) is present in insects throughout their life. Within each instar, or developmental stage, there is variation in the amount present. In all nymphal instars except the last, a high level is present at the time of ecdysis, or molting. In the final instar, the level is low at ecdysis. Thus, high JH levels present at ecdysis result in maintenance of juvenile characteristics into the next instar. When administered during the final instar, hydroprene (a synthetic JH) results in adult insects that retain some juvenile characteristics.

Taumantis ehrmannii adults demonstrate sexual dimorphism for wing length and hearing, such that long wings are associated with low auditory thresholds. This raises the question of whether JH, which can produce shortened wings, can also raise auditory thresholds. Males, which have long wings, have a single ear on the ventral metathorax midline with physiological best-frequency (70 kHz) threshold of 70 dB SPL and demonstrate an in-flight escape response at this frequency. Females, which have short wings, are functionally deaf, as they have a physiological best-frequency (30kHz) threshold of 92 dB SPL and demonstrate no behavioral response to sound. Hydroprene-treated adult males have a best-frequency (70 kHz) threshold of 90 dB SPL, display abnormal ear anatomy, and have shortened, deformed wings that prevent flight. Life span, feeding behavior, and general activity level of these treated males are not affected. The auditory sensitivity reduction indicates that the final instar is crucial for normal auditory sensitivity and leads to the question of what differences occur in auditory development of deaf versus hearing mantises.

Supported by research grant #5-R29-DC01382-04 from the National Institute on Deafness and other Communication Disorders, National Institutes of Health to DDY.

452.10

EFFECTS OF UNNATURAL CALLING SONGS ON PHONOTAXIS AND AN AUDITORY INTERNEURON IN THE CRICKET. G. Atkins*, H. Bingo[1], D. Kim, N. Carlson and J. Stout Dept. of Biology, Andrews University, Berrien Springs, MI 49104.

Phonotaxis of young female *Acheta domesticus* is selective for the syllable period of the males conspecific calling song, with syllable periods of 50-70 ms being most attractive. This selectivity decreases with age (Walikonis et al. 1991).

L3 is an ascending, prothoracic, auditory interneuron that responds in a band-selective manner that matches the selectivity of phonotaxis. In young females, L3 shows a strong decrementing response to calling songs (the first syllable of the call produces twice as many spikes by L3 than the third syllable) that have syllable periods of 50-70 ms. Shorter and longer syllable periods do not induce a strong decrement. L3s in older females do not show this decrement at any syllable period tested and thus are unselective (Henley et al. 1992).

We have formed a working model of the circuit by which L3 exhibits a band-selective response and how this might change with age. The model proposes excitation and delayed inhibition on the side ipsilateral to L3s axon and contralateral inhibition of both ipsilateral inputs (Atkins et al. 1995).

Our current experiments center around phonotaxis and the responses of L3s during stimulation with unnatural calling song models. The paradigm consists of songs in which parameters of the syllables can be changed within a given chirp. For example, what happens phonotactically in old females, which are unselective for syllable period, when a loud syllable is follow by 2 less intense ones in a given chirp. This should produce a "decrementing-like" response in L3 and thus, does it cause changes in selectivity in old females? With these and similar types of experiments, it is our intent to learn more about the function of L3 in selectivity of females and to elucidate further how the L3s response is shaped.

Funded by: NSF - IBN 92-22127 and Andrews University faculty grants.

452.11

HORMONAL AND EXTERNAL EFFECTS ON PHONOTACTIC AND NEURONAL THRESHOLDS OF FEMALE CRICKETS. J. Stout*, M. Bronsert, J. Hao, D. Mbungu, T. Standish and G. Atkins. Biology Dept., Andrews Univ. Berrien Springs, MI 49104.

Juvenile hormone III (JHIII), applied to the abdomen of 1-day-old female *Acheta domesticus* caused a significant decrease in phonotactic thresholds. Removal of the corpora allata from 5-day-old females with low phonotactic thresholds caused increased phonotactic thresholds 2 to 5 days later. After a temporary increase of about 25 dB, the phonotactic thresholds drop to 10 dB above preallatectomy levels. Application of JHIII to allatectomized females, with a mean increase in thresholds of 20 dB, results in decreased thresholds (mean of about 15 dB) over the next 12 hours. Also, a variety of external factors influence phonotactic thresholds of females (e.g. exposure to males, time of day, previous phonotactic testing, Stout et al. 1996).

In 1-day-old females with high phonotactic thresholds, the threshold of the L1 auditory interneuron can be 30 or more dB lower than their phonotactic threshold. In females with phonotactic thresholds of 70 dB or lower, the L1 threshold is within 10 dB of their phonotactic threshold. Thus factors other than the threshold of the L1 auditory neuron influence phonotactic thresholds.

The JHIII levels that influence phonotactic thresholds also influence the thresholds of the L1 auditory interneuron and the expression of a gene in the L1 neuron like the alpha subunit of nicotinic receptors in locusts. The causal relationships between expression of this gene in the L1 (or other) auditory interneuron and JHIII levels will be evaluated as a means for translating external influences into changes in phonotactic thresholds.

Funded by: NSF - IBN 92-22127 and Andrews University faculty grants.

452.12

FORAGING BEHAVIOR AND MUSHROOM BODIES IN ALLATECTOMIZED HONEY BEES. Joseph P. Sullivan, Gene E. Robinson, and Susan E. Fahrbach* Dept. of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL 61801 U.S.A.

Foraging behavior in honey bees is associated with high juvenile hormone (JH) titers and reorganization of the mushroom bodies, the region of the insect brain that mediates learning. To test the hypothesis that juvenile hormone influences the development of both foraging behavior and mushroom body structure, the corpora allata, the glands that produce juvenile hormone (JH), were surgically removed from day-old worker bees. Allatectomized (CA^-), sham, and control bees were individually marked and introduced into a colony within a glass-walled hive. Radioimmunoassay was used to verify that CA^- bees had undetectably low JH titers. Regular behavioral censuses showed CA^- bees performed the full repertoire of tasks inside the hive to support colony growth and maintenance. Observations at the hive entrance revealed flight activity between CA^- and sham bees was not significantly different. The CA^- bees demonstrated flight capability but foraged significantly less than shams. Brains from 1 day old, foraging shams and controls, and non-foraging CA^- bees were dissected and sectioned to investigate whether the absence of JH during adult life affects the reorganization of the mushroom bodies. Measurements of the volume occupied by the neuropil and intrinsic cells of the mushroom bodies (Kenyon cells) using the Cavalieri method are in progress. (Supported by NSF IBN 94-23164 to S.E.F. and G.E.R.)

452.13

FASCICLIN II AND DROSOPHILA MYOCYTE ENHANCER BINDING FACTOR 2 IN THE MUSHROOM BODIES OF THE HONEY BEE. S.M. Farris[1], S.E. Fahrbach[1], G.E. Robinson*[1] and R.L. Davis[2]. [1]Dept. of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, and [2]Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

The mushroom bodies are centers of learning and memory in the insect brain. The Kenyon cells are intrinsic neurons of the mushroom bodies, receiving olfactory and visual input in the calyces and relaying information to other parts of the protocerebrum via the alpha and beta lobes. Antibodies directed against two proteins expressed in the mushroom bodies of the fly *Drosophila melanogaster* show robust staining patterns in the brain of the honey bee, *Apis mellifera*. Drosophila myocyte enhancer binding factor 2 (Dmef2) is a transcription factor first recognized for its role in myogenesis. In the mushroom bodies, nuclei of the non-compact Kenyon cells stain intensely for Dmef2, while those of the compact Kenyon cells do not. A subset of neuronal nuclei in the antennal lobes and subesophageal ganglion also show intense Dmef2 immunoreactivity. Fasciclin II (fasII), an insect cell adhesion molecule homologous to vertebrate NCAM, is detected as a discreet band of staining in the calycal, alpha, and beta lobe neuropils of the mushroom bodies. Neurons in the central bodies also show fasII immunoreactivity, as do several large fiber tracts in the deuterocerebrum. Dmef2 and fasII will be used as markers to study neuronal plasticity in the mushroom bodies that is associated with behavioral development in the adult honey bee. *Supported by an NIH Cell and Molecular Biology Training Grant and NSF Award IBN 94-23164.*

452.14

SEX PHEROMONE RESPONSE SPECIFICITY IN TWO RACES AND HYBRIDS OF THE EUROPEAN CORN BORER MOTH. C. E. Linn Jr.*[1], M. S. Young[2], T. J. Glover[2], and W. L. Roelofs[1]. [1]Dept Entomology, NY State Agric. Exp. Station, Cornell University, Geneva, NY [2] Dept. Biology, Hobart and William Smith Colleges, Geneva, NY.

Two races of the European corn borer moth, *Ostrinia nubilalis*, use opposite ratios of Z- and E11-tetradecenyl acetate in their sex pheromones. Behavior/genetic studies demonstrated that male antennal receptor responses are controlled by an autosomal gene, but that blend recognition and upwind flight are controlled by a sex-linked factor. As part of a program to understand how these genetic factors influence neurophysiological processes and behavior, response specificity of males in the two races and their hybrids to a large array of ratios/concentrations was studied in the sustained-flight tunnel. Males of each race completed flights to the source to a wide range of concentrations (0.3 to 3000 µg on rubber septa), but maximal response was exhibited to a narrow concentration range of the natural ratios of isomers (3:97 and 99:1 E:Z). The races differed significantly in their sensitivity to increasing proportions of the minor isomer, with higher concentrations of off-ratios eliciting source contact in the race using 99:1 E:Z with the complete set of ratios tested. Finally, F_1 hybrids exhibited no specificity, with high levels of response occurring to a wide range of ratios/concentrations. In agreement with previous studies, response specificity was the result of a lower concentration/lower ratio threshold on orientation to the odor plume, and a higher concentration/higher ratio threshold on arrestment of upwind flight. The results also agree with the current integrated model explaining the orientation mechanisms and neurophysiological processing involved in perception of odor quality and response to pheromone, suggesting a link between the sex-linked factor and antennal lobe processing. Supported by Howard Hughes Medical Foundation summer intern grant to Hobart and William Smith Colleges.

452.15

NOVEL FEATURES OF EXTRINSIC MUSHROOM BODY NEURONS IN COCKROACH BRAIN Y.-S. Li* and N.J. Strausfeld. ARLDN, Univ. Arizona. Tucson, AZ 85721

The paired mushroom body (MB) neuropils of insects are believed to underlie olfactory associative learning and memory (Menzel, R. [1993] Associative learning in honey bees. *Apidologie*, 24 (3) 157-168). However, although the MBs receive a substantial afferent supply from olfactory interneurons, in many insects the main receptive regions of the MBs (the calyces) are also invaded by visual and mechanosensory relays, suggesting that MBs are multimodal integrators. So far, previous models of MBs suggest that computations are performed by networks of parallel intrinsic neurons, the axons of which are transected by, and presynaptic to efferent (output) neurons which then lead to premotor neuropils or other brain regions. New intracellular recordings and dye fills from efferent neurons in the cockroach *Periplaneta americana* have revealed novel features of the MB's internal organization and relationships with other brain areas. Efferent neurons arising in either of the MB's two lobes respond to a variety of sensory stimuli (odor, light, sound and touch) and many efferents have complex morphologies consisting of multiple dendritic trees both within and outside the MBs. Furthermore, afferent neurons have been identified that supply the MB lobes rather than the calyx. Comparisons with anosmic taxa, whose MBs lack calyx and pedunculus, show that their lobes exclusively receive the MB's afferent sensory supply. These features, and ongoing intracellular recordings, suggest that the MBs are not relatively isolated neuropils, as previously supposed, but are highly integrated with other brain areas in modulating the activity of premotor pathways supplying locomotion circuitry in the thoracic ganglia.

Supported by grant IBN 9316729 from the National Science Foundation and an NIH training grant, NS07309.

452.16

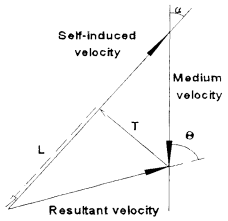
NEURAL ARRANGEMENTS ARE EVOLUTIONARILY CONSERVED IN OLFACTORY AND SENSORY ASSOCIATION CENTERS OF SEGMENTED INVERTEBRATES. Nicholas J. Strausfeld*. Robert S. Gomez, and Lars Hansen. ARLDN. Univ. Arizona. Tucson, AZ 85721

Comparative studies of arthropods and annelids reveal unexpected levels of conservation of brain structures. Based on the presence or absence of specific neural characters amongst 30 taxa, phylogenetic analysis using parsimony has suggested evolutionary relationships amongst invertebrates that closely match trees obtained by others from both classical morphological observations as well as comparisons of conserved DNA. Two specific neural architectures occur as leitmotivs throughout most of the arthropods, annelids, Onychophora and Platyhelminthes, but not in mollusks. In insects, these architectures are known as the antennal lobes and mushroom bodies: the former being glomerular olfactory neuropils supplying the latter --- paired lobed neuropils comprising thousands of intrinsic neurons derived from extremely small and tightly backed basophilic perikarya ("globuli cells"). The possibility that mushroom bodies might have arisen only once in evolution is supported by their intrinsic architecture which, whether in an annelid, insect, or chelicerate, follows the same organizational rules with respect to detailed arrangements of processes, afferents, and outputs. Even in many crustaceans, where large populations of globuli cells supply folded, rather than lobate, centers just proximal to the optic lobes, these regions (called hemi-ellipsoid bodies) nevertheless have architectures that conform to the general organizational principles of mushroom bodies in other arthropods. The results suggest a high degree of conservation of neural arrangements and hence operational principles in olfactory neuropils and the neuropils to which their interneurons project. *Supported by a John D. and Catherine T. MacArthur Foundation Fellowship to NJS.*

452.17

A multimodal control system for navigation in a moving fluid medium: Visual flow fields are not enough. Jim H Belanger.* ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

An organism attempting to navigate while suspended in a moving fluid medium faces a task of considerable complexity. Its motion through the environment is the vector sum of its self-induced motion, and that of the medium (see figure). Because it is suspended in the medium, the organism is unable to detect the velocity of the medium using mechanosensory cues, but instead must rely on visual input to deduce this. (Visual inputs are shown in the figure as T, for transverse visual flow, and L, for longitudinal visual flow). However, consideration of this problem reveals that, in the absence of independent knowledge of the velocity of either the organism or the medium, visual cues alone are insufficient to steer a desired course (θ) with respect to the flow. Based on behavioral and neurophysiological data, largely derived from studies of olfactory-guided locomotion in insects, I have developed a model which is capable of solving this problem. The essential addition to the control system is information on the animal's self-induced speed. In flying insects, this is the animal's air speed, which is available via mechanosensory receptors. The values of air speed, T, and L are sufficient to define all sides and angles of the 'triangle of velocities', and thus can form the basis of a control system. Such a controller has been tested using simulations of olfactory-guided locomotion in moths, and can successfully steer any desired course across a range of wind velocities. [Supported by NSF Grant IBN-9216532]



'Triangle of velocities' for an organism in a moving fluid medium.

452.18

LOOMING SENSITIVE INTERNEURONS IN THE OPTIC LOBES OF THE HAWK MOTH *MANDUCA SEXTA* (SPHINGIDAE, LEPIDOPTERA). Martina Wicklein* and Nicholas J. Strausfeld, ARLDN, Univ. Arizona. Tucson, AZ 85721

Hummingbird hawkmoths are rapid and highly maneuverable diurnal insects that use their large compound eyes to maintain exact position in front of a food source (flowers) even if this moves and irrespective of the direction of movement. Similarly, the tobacco hornworm moth *M. sexta* (a crepuscular American sphingid) performs precise hovering behavior. Electrophysiological investigations show that the optic lobes of *M. sexta* possess several types of neurons that respond to different looming stimuli. These include expanding objects in front of a contrasting or textured background, turning spirals and moving gratings. Each of these simulations makes it possible to test different properties of the looming stimulus (moving contrast edges, figure-ground stimuli, motion parallax) and thereby reveal which component of the looming stimulus is relevant for an identifiable neuron. Two types of responses can be observed when recording from the lobula plate of *M. sexta*. Type 1 neurons (looming edge detectors) react to expanding discs in front of a background and to moving spirals. Moving edges are the relevant stimuli for these neurons: Both the turning spiral and the expanding disk provide the cell with edges that move from the periphery to the center or vice versa. Type 2 neurons (looming object detectors) respond only to expanding discs and show no reaction when stimulated with a turning spiral. For Type 2 neurons, components of the looming stimulus other than moving edges must also be relevant. The responses of Type 2 neurons suggest that figure-ground stimuli and/or motion parallax are the crucial components of the looming stimulus for this type of neuron. *Supported by a Feodor Lynen Fellowship for MW and a grant from the National Center of Research Resources (NCRR), NIH RR08688 to NJS.*

452.19

VISUAL MOTION PROCESSING IN THE LOBULA PLATE OF BLOWFLIES: DIRECTION- AND NON-DIRECTION SENSITIVE OUTPUTS TO THE LOBULA AND CENTRAL BRAIN. John K. Douglass* and Nicholas J. Strausfeld. ARLDN, Univ. Arizona. Tucson AZ 85721, USA.

An important feature of insect as well as primate visual systems is the functional and anatomical segregation of parallel retinotopic pathways for processing distinct features of the visual world. In the fly brain, there is a major separation of achromatic, motion sensitive pathways to the lobula plate from wavelength-sensitive pathways to the lobula. Anatomical and physiological observations suggest a further subdivision of retinotopic, motion-sensitive pathways to the lobula plate into directionally-selective (DS) and non-DS channels (Douglass and Strausfeld [1996] J. Neurosci., in press). Within the lobula plate, there is evidence for continued segregation and integration of motion information. In addition to the well-known DS giant tangential neurons at this level, there exist at least 15 morphologically distinct types of small-field retinotopic neurons which relay information among the lobula plate, lobula and lateral deutocerebrum. Consistent with earlier recordings from this diverse group (Gilbert and Strausfeld [1992] J. Comp. Neurol. 316:72-86) we find that some small-field lobula plate efferent neurons having outputs to the lateral deutocerebrum are DS, while others, though non-DS, nevertheless exhibit distinct responses to motion and flicker. In contrast with these, limited data from medullary inputs to the lobula so far provide no evidence for DS activity within this neuropil other than by large male specific neurons (Gilbert and Strausfeld [1991] J. Comp. Physiol. 169:395-411). *Supported by a grant from the National Center for Research Resources (RR08688).*

452.20

ACTION SPECTRA OF THE BIOLUMINESCENT RESPONSE IN FIREFLY *Photinus pyralis* FEMALES (COLEOPTERA: LAMPRIDAE). K. M. Worthy and A. B. Lall,* Department of Biology, Howard University. Washington, D.C.

The action spectra S(λ) of the female-response to the photic stimulation of a simulated bioluminescent (BL) optical signal of the species male were determined in *P. pyralis*. In nature, *P. pyralis* males emit a 500 ms J-flash every 4 to 4.5s. A xenon arc in conjunction with a monochromator and a electromagnetic shutter provided the simulated male-flash whose color varied from 400 to 640nm at 20 nm intervals, intensity controlled by neutral density filters, flash-duration varied from 500ms and delivered every 4s. The intensity of the simulated flash was varied to determine the threshold of the female-response at thirteen stimulus wavelengths. The firefly females respond only to green-yellow stimuli >500 nm and not to stimuli in the near-UV and blue (380-480nm). S(λ) functions which had a narrow peak in the yellow-green (λ_{max} 565nm) region. The evidence that the a narrow yellow receptor in the female mediates the detection of BL of the conspecific male is based upon the presence of a correspondence in shape and in λ_{max} between species BL emission spectrum (λ_{max}=564nm), the spectral sensitivity of a narrow yellow receptor by electroretinographic S(λ) data (λ_{max}=565nm) and the S(λ) function of an intracellular response from individual retinular cells (λ_{max}=565nm), *in situ* absorption spectrum of *P. pyralis* P545 visual pigment overlaid by species specific screening pigment (λ_{max} 517nm) and the shape and the λ_{max} of S(λ) functions of the female-response. Hence the information in the species BL optical signal is processed exclusively by the green/yellow visual receptor in *P. pyralis*, and should be taken as evidence for an achromatic detection of the bioluminescent optical signal. Support: NSF grant # BNS 90-13076 & NIGMS grant # SO-66-MO-8016

452.21

NORTHERN BUTTERFLIES ARE THE BRIGHTEST: THE UV-SENSITIVITY AS A FACTOR FOR VISUAL ECOLOGY.

M. Järvilehto* and V.B. Meyer-Rochow, Univ. of Oulu, Dept. of Biol., Unit for Animal Physiol., P.O. Box 333, 90571 Oulu, Finland.

Many butterflies make use of UV-absorbing or -reflecting patches on their wings in courtship, mate recognition, and mate selection (Obara, Y. & Hidaka, T. (1968) Proc. Jap. Acad. 44, 829-832; Rutowski, R. (1981) Z. Tierpsychol. 55, 325-334; Meyer-Rochow, V.B. (1991) J. Roy. Soc. N.Z. 21, 169-177). We examined 28 female butterflies *Pieris napi*, common in Finland, under UV-light of 366 nm wavelength and found that the pattern in UV-reflectivity on the wings of females show variation, which is correlated with geographic latitude. Photographic cross-correlations show that in ca. 75% of all female specimens from the northern latitudes 66.5° in Finland (near the polar circle) had wing patterns that were more strongly reflecting than those of individuals caught ca. 600 km further south (61°). Seasonal influences were ruled out when the phenomenon was demonstrated in early as well as late summer generations. The sunlight's UV-component at higher latitudes decreases due to the lower angle of the sun and if the differences of UV-patterns in intra- or interspecific communications have a significance for the butterfly, it will either have to increase the sensitivity of UV-receptors or has to generate more powerful UV-signals. It seems that *P. napi* has more selectional pressure on the second evolutional solution.

STRESS II

453.1

STRESS INDUCIBLE 70kDa HEAT SHOCK PROTEIN (HSP72) EXPRESSION IN ADRENAL GLANDS OF FAWN-HOODED RATS WITH CONGENITAL SEROTONIN ABNORMALITIES. S. Mulugeta, B. Tedeschi, C-T Hsu, S.R. Shenoy, T. Coon, J. McCormick, M-T Maa, G.E. Goode* & P.E. Aravich. Eastern Virginia Med. School, Norfolk, VA 23501; VAMC, Hampton, VA 23667; Governor's School for Sci. & Tech., Hampton, VA 23666; Christopher Newport Univ., Newport News, VA 23606.

Various stressors activate the hypothalamo-pituitary-adrenal system (HPA) and an intracellular stress-response system related to the 70kDa heat shock protein family. The HSP70 system is thought to play a role in cellular maintenance by "chaperoning" proteins. Several studies demonstrate that acute HPA activation induces stress-inducible HSP72 expression in the adrenal cortex. The purpose of this investigation was to determine if Fawn-Hooded (FH) rats, which have chronic HPA activation, have adrenal stress-inducible HSP72. FH rats have congenital brain and platelet serotonin abnormalities and are an animal model of depression and alcoholism. They have a variety of other behavioral abnormalities (see M-T Maa et al., this meeting) and eventually develop hypertension and glomerulosclerosis. The subjects were female FH rats (n=6, 5-mo. old) and weight-matched Wistar strain controls (n=5). Adrenal glands were homogenized in denaturation buffer and proteins separated by SDS-PAGE. HSP72 expression was assessed by SDS-PAGE immunoblots probed with specific monoclonal antibody (Stressgen). It was found that stress-inducible HSP72 occurred in the adrenal glands of all six FH rats and in none of the five Wistar controls. These data suggest that stress-inducible HSP72 is constitutively expressed in the adrenal glands of Fawn-Hooded rats. Further studies are needed to determine if this is due to increased synthesis or decreased turnover.

This research supported by EVMS institutional funds.

453.2

REGULATION OF t-[^{35}S]TBPS BINDING IN INESCAPABLY STRESSED RATS WITH LEARNED HELPLESSNESS BEHAVIOR.

J. H. Wu, G. L. Kramer, I. L. Crawford* and F. Petty. VAMC and Depts. of Psychiatry and Neurology, Univ. of Texas Southwestern Med. Sch., Dallas, TX 75216.

$GABA_A$ receptor complex is postulated to mediate the neurochemical responses in stress and in human depression. t-[^{35}S]-butylbicyclophosphorothionate (TBPS) binding is a sensitive marker to changes in the function of the $GABA_A$ receptor complex. We investigated the alteration of [^{35}S]TBPS binding in several mood responsive areas of rat brain by quantitative receptor autoradiography. We found that rats with learned helplessness behavior (LH) after inescapable stress, [^{35}S]TBPS binding was significantly decreased in dorsal hippocampus, hypothalamus and amygdala (P=0.031, P=0.001 and P=0.025, respectively) compared to tested controls or to naive controls. There were no significant differences among experiment groups and control groups in either prefrontal cortex or septal area. Also, no significant differences were found in rats without LH after inescapable stress compared to control groups in any of the investigated brain areas. It is concluded that the function of $GABA_A$ receptor complex is regulated differently in various brain areas of learned helpless rats. The decrease of [^{35}S]TBPS binding, concomitant with the decreased release of GABA in learned helpless animals, is therefore related to the learned helplessness behavior.

Dept. of Veterans Affairs

453.3

SEROTONERGIC NEUROTRANSMISSION IN THE PREFRONTAL CORTEX OF ROMAN HIGH-AVOIDANCE (RHA) AND LOW-AVOIDANCE (RLA) RATS: A BRAIN MICRODIALYSIS STUDY.

M.G. Corda *, O. Giorgi, D. Lecca, G. Piras, P. Driscoll [1] and G. Di Chiara. Department of Toxicology, University of Cagliari, *ITALY* and [1] ETH Zentrum, Zurich, Switzerland.

RHA and RLA rats are selected and bred for rapid versus poor acquisition of two-way avoidance behavior in a shuttle box. RHA and RLA rats show multiple behavioral differences related to emotional factors, RLA rats being emotionally more reactive. In addition, a number of differences in dopaminergic, GABAergic and serotonergic (5HTergic) function in the CNS have been reported in these two lines. Given the role of serotonin (5HT) in the regulation of the reactivity to stressors and the above mentioned line-related differences in emotivity, the present study was undertaken to characterize 5HTergic neurotransmission in the prefrontal cortex (PFCX) of RHA and RLA rats using brain microdialysis. The basal release of 5HT (fmol/20 μl) was significantly lower in the PFCX of RHA rats (5.37 ± 0.65) than in RLA rats (7.73 ± 0.60, n = 12 rats per group, $p < 0.05$). Consistent with recent data indicating that the binding density of [^{3}H]paroxetine is larger in the PFCX of RHA rats than in their RLA counterparts, the increase in 5HT output produced by the systemic administration of the 5HT uptake inhibitor chlorimipramine (10 mg/kg, IP) was more pronounced in RHA rats than in RLA rats. Thus, the peak effect of chlorimipramine 40 min after treatment was + 269 % and + 184 % in RHA and RLA rats, respectively. In addition, a 2-way ANOVA revealed a significant difference across rat line ($F_{1,124} = 46,8$; $p < 0.001$) and across time after treatment ($F_{12,113} = 4,0$; $p < 0.001$). Taken together, the above results may provide functional significance to the line-related differences in biochemical markers of serotonergic neurotransmission observed under *in vitro* conditions. Studies aimed at comparing the effects of environmental stressors and anxiogenic drugs on 5HTergic neurotransmission in these two lines are underway in our laboratory.

453.4

EFFECTS OF INESCAPABLE AND ESCAPABLE STRESS ON THE RELEASE OF SEROTONIN IN THE VENTRAL HIPPOCAMPUS OF THE RAT.

J. Amat, S. Maswood, P. Matus-Amat, J. Barter, L. R. Watkins and S. F. Maier*. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Organisms exposed to inescapable stress show a constellation of behavioral and physiological alterations that do not occur if the stressor is escapable. Both pharmacological and lesion experiments suggest that these effects may occur in part because inescapable stressors produce a more intense activation of serotonergic neurons within the dorsal raphe nucleus (DRN) than do escapable stressors. Here we report experiments using in vivo microdialysis to directly measure serotonin (5-HT) efflux in the ventral hippocampus, a projection region of the DRN. Pairs of male Sprague Dawley rats were exposed to escapable 1.0 mA tailshock (ES) or exactly equal yoked inescapable tailshock (IS). Samples of extracellular fluid were collected at 15 min intervals before, during, and after the treatment. Levels of 5-HT and 5-HIAA were measured by HPLC. Both IS and ES produced increases in 5-HT, but the increase was much larger in IS subjects. Extracellular 5-HIAA decreased in association with the 5-HT increases, and increased after 5-HT returned to near normal levels. Thus stressor controllability does appear to modulate DRN 5-HT activity and 5-HIAA extracellular levels do not reflect 5-HT release, as has been previously reported.

Supported by MH 00314 and Universidad Central de Venezuela.

453.5

EFFECTS OF CONTROLLABILITY OF STRESS ON HIPPOCAMPAL PHARMACOLOGY. C.L. Wellman*[1], M.J. Cullen[2], and M.A. Pelleymounter[2]. [1]Dept. Psychology, Indiana University, Bloomington, IN 47405 and [2]Dept. Neurobiol., Amgen, Thousand Oaks, CA 91320.

Previous studies have shown that exposure to uncontrollable shock produces deficits in behaviors mediated by the hippocampus. To begin to correlate these behavioral effects with pharmacological changes in hippocampus, we examined effects of controllable and uncontrollable shock on hippocampal pharmacology. To assess the role of mu opioid and cholinergic receptors in mediating effects of uncontrollable stress, we trained rats in a controllability paradigm and assessed pharmacological changes in hippocampus. Rats who could terminate shock (N=7) were paired with rats who could not terminate equivalent shock (N=7); a group of control rats did not receive shock (N=7). When rats who could terminate shock responded at an 80% rate, all brains were removed and sectioned (16 μm), incubated with tritiated ligands and processed with autoradiographic procedures appropriate for visualizing muscarinic, $GABA_A$, and mu opioid receptor binding, as well as serotonergic uptake and noradrenergic reuptake sites. Density of label in hippocampal CA1 and CA3 fields and dentate gyrus was assessed using an image analysis system (MCID). Quantitative densitometry revealed a 14-22% decrease in muscarinic and mu opioid receptor binding in CA3 and dentate gyrus in rats receiving uncontrollable shock relative to rats receiving no shock; no significant changes were observed in rats that could terminate shock. Thus, ability to control shock prevents stress-induced changes in the opiate and cholinergic systems in hippocampus. Supported by ADAMHA grant to MAP.

453.6

AMYGDALAR SEROTONIN MEDIATES LONG-TERM SOCIAL ROLES FOLLOWING AGGRESSIVE INTERACTION Tangi R. Summers*, Earl T. Larson, Amy L. Hunter, Kenneth J. Renner, Neil Greenberg, and Cliff H. Summers. Dept. of Biology, University of South Dakota, Vermillion, SD 57069, Dept. of Zoology, University of Tennessee, Knoxville, TN 37996.

Stressful aggressive interaction stimulates, and social roles are distinguishable by, central serotonergic activation in telencephalon as well as brainstem. Pairs of male lizards, *Anolis carolinensis*, were allowed to fight and form dominant/subordinate relationships. In brainstem, subordinate males have reduced 5-HT, but increased 5-HTP, 5-HIAA and 5-HIAA/5-HT at 1 hour following the fight, gradually decreasing over a week of cohabitation. In micropunched regions of telencephalon, the greatest serotonergic changes also occur in subordinate males. The paleostriatum (medial amygdala), shown to play a role in regulation of aggression, develops serotonergic activity more slowly; greatest following a week of interaction. Turnover, 5-HT and 5-HIAA levels escalate over the first week of interaction in subordinate males, and return to baseline by 1 month. In dominant males, the pattern is accelerated, most extensive 5-HT system activity at 1 h, decreasing over a month. These data are consistent with rapid neuroendocrine stress modulation in dominant individuals, and delayed serotonergic activity in subordinate males. Supported by NIH grant NICHD-1-T32-HDO 7303-01A1, NSF grant OSR-9108773 and HHMI 7195-539501.

453.7

CIRCLING BEHAVIOR IN EARLY CALLOSOTOMIZED MICE. S.L. Schmidt, Filgueiras, C.C., Krahe, T.E., E.M. Caparelli-Dáquer*, Manhães, A.C. Dep. Fisiologia, Univ. Estado Rio de Janeiro, Brazil, RJ 20551-030.

Adult mice with neonatal section of the corpus callosum or sham lesions were examined for turning preferences in swimming rotation. On the first postnatal day, pups were taken from their mothers, anaesthetized by hypothermia and subjected to a longitudinal transection of the corpus callosum. After recovery from surgery, the animals were returned to their mothers and left undisturbed for 21 days. Sham operated animals were subjected to the same procedures except the callosal section. The normal group consisted of animals that were not handled from birth to the 21 postnatal day. At adulthood, the animals were tested in three different days on a free-swim apparatus which consisted of a plastic container of 21 cm diameter, partially filled with water. The animal's free swim rotations were continuously recorded for 5 minutes with a video camera. All clockwise and counterclockwise full rotations (360°) were counted from the video images of the tests. We also measured 30° turns. After behavioral testings all operated animals and rondom samples of normal and sham operated animals were intracardially perfused with saline followed by a 4% formaldehyde solution. The brains were coronally sectioned at 40μm, and stained with cresyl-violet. In all animals, the behavioral analysis showed that there was a decline in activity with repeated testing. The highest activity always occurred during the first minute of the first test. For each test there was an overall decline in activity from the first to the second minute. In sham-operated animals, there was a significant effect of sex: handled females had a tendency to be more lateralized and did not show a decline for the preferred side of rotation with repeated testing. In mice with callosal defects, there was a significant interaction between sex and consistency of laterality: acallosal females that were consistent left-rotators did not decline their activity to the preferred side with repeated testing. It is conceivable that testosterone and callosal development may suppress cortical enlargement in the left hemisphere. Thus, this study provides support for the idea that a left greater than right cortical asymmetry at the population level of female mice might be responsible for a side-preference in circling behavior.

453.8

HIPPOCAMPAL/SUBICULAR REGULATION OF ANTIDIURETIC HORMONE FOLLOWING PSYCHOLOGICAL STRESS. K.W. Nettles, D.J. Luchins* and M.B. Goldman. Psychiatric Institute, University of Illinois in Affiliation with the University of Chicago, Chicago, IL, 60612.

The hippocampus/subiculum has been hypothesized to restrain hypothalamically-mediated neuroendocrine responses to psychological stress. To assess if this structure similarly influences antidiuretic hormone (AVP) secretion, we subjected lesioned rats to a standard swim stress (which does not increase plasma AVP in intact animals). Following anesthesia with pentobarbital, 60 d male Long-Evans rats received bilateral aspiration of the hippocampus (H) or just the overlying parietal cortex and corpus callosum (C). Aspiration was performed under direct visualization using a hypodermic needle connected to a suction pump. The lesion transects the subiculum and fimbria but leaves the ventral hippocampal tip intact. After being individually housed for 3 wk, rats underwent a 45s forced swim (H_2O = 20°C) between 0900 and 1000 h, and were then sacrificed by decapitation at 30 min intervals for 90 min (n = 5 @ ea. time pt).

Immediately following the stressor, AVP levels did not differ (H = 2.8 ± 1.8 pg/ml; C = 1.7 ± 0.6), but then rose linearly in H (+ 90 min = 4.7 ± 2.4) to a greater extent than in C (+ 90 min = 2.4 ± 0.9) (MANOVA group X time F (3,32)= 2.63 p =.06, linear contrast p <.02). Plasma osmolality (H = 305.9 ± 4.1 mosm/kg; C = 306.7 ± 3.4), sodium, glucose, creatinine and urea, and hematocrit were similar across groups, suggesting recognized AVP stimuli could not account for the finding. ACTH and corticosterone responses did not differ across groups.

While further testing is needed using more discreet lesions, assessing other stressors, and confirming the integrity of recognized regulatory pathways, these preliminary results indicate that hippocampal aspiration is associated with an unexplained increase in plasma AVP following psychological stress. **Sponsored by The Brain Research Foundation of the University of Chicago.**

453.9

ONTOGENY OF BEHAVIORAL AND AUTONOMIC DEFENSIVE RESPONSES FOLLOWING INFUSION OF KAINATE INTO THE DORSAL PERIAQUEDUCTAL GRAY. G.A. Goodwin* and G. A. Barr .Columbia University and NY State Psychiatric Institute 722 W. 168th St. New York, NY 10032.

Adult-typical defensive behaviors emerge independently during the first month of postnatal life in the rat. For example, freezing is seen by about postnatal day 14 (P14), whereas flight in response to a sudden noise does not emerge until around P20. The autonomic control of heart rate is also changing during this period maintained tonically by sympathetic activity during the first postnatal week, with phasic parasympathetic activation occurring in response to stressor exposure. The central systems modulating defensive responses must also undergo dramatic changes during this time. One structure important in the response to stress in the adult is the midbrain periaqueductal gray (PAG). The present study was designed to examine the role of the PAG in the control of defensive responses during early ontogeny. Kainic acid was infused (0.0, 0.03, 0.10, 0.3 nmol) into the dorsal PAG of rats at P7, 14, and 21. Escape like behaviors (running, jumping) were evoked by infusion of the two highest doses at P14 and 21. P7 pups exhibited only a brief (30 sec) increase in activity immediately following drug infusion. A significant increase in heart rate was not observed until P21; respiration rates increased following infusion of kainate at P7 and P14 but not P21. The results suggest that the PAG organizes respiratory, behavioral, and cardiovascular components of defense independently during the first three postnatal weeks in the rat. Supported by grants MH18264, DA05712, and DA07646.

453.10

LONG-TERM SENSITIZATION OF ESCAPE BEHAVIOUR AFTER REPETITIVE ELECTRICAL STIMULATION OF THE RAT SUPERIOR COLLICULUS Sheila M King* Dept Exp Psychology, University of Oxford, UK

Life-threatening events generally result in a set of behaviours which include risk assessment, freezing and escape, as well as the concomitant autonomic and endocrine changes which collectively form the 'fight-or-flight' response. However, when the initial stressor is of sufficient duration, intensity or frequency, the defence system may undergo a long-term increase in sensitivity similar to kindling. Consequently, the person may become hypervigilant, and suffer from symptoms of chronic psychophysiological arousal ie be in a persistent 'fight-or-flight' mode.

It is known that repeated stimulation of structures in the rodent midbrain defence system ie the superior colliculus [SC] and the cuneiform area, increases the duration and intensity of the defensive responses obtained. So far, however, it has not been established whether sensitization of this system results in a long-term alteration of the animal's responses to subsequent stressful events. The purpose of this study was, therefore, to study the behaviour of animals in a threatening environment after direct activation of the midbrain defence circuitry by repetitive electrical stimulation of the SC in both anaesthetized and unanaesthetized animals. 14 days after stimulation, animals were placed on an unstable, elevated and exposed plus maze for 5 mins and their behaviour recorded. Testing was carried out regularly over a 3 month period.

Throughout the testing period, stimulated animals reliably exhibited a range of behaviours designed to escape the aversive environment. These included visual scanning, preparing to jump, and jumping off the apparatus. Unstimulated control animals and animals which had received footpinch whilst anaesthetized did not in general exhibit these behaviours. In contrast, the experimental animals' performance on standard behavioural tests eg the stable elevated plus maze and hole board, did not differ from the controls. These results demonstrate that kindling of the midbrain defence circuitry can produce a long-term change in an animal's reaction to threat, and may lead to the development of a functional model of extreme anxiety states.

Supported by the Robert & Joan Case Royal Society Fellowship.

453.11

CORTICOSTERONE LEVELS IN RAT BRAIN MICRODIALYSATES FOLLOWING EXPOSURE TO NOISE STRESS OR TO LIPOPOLYSACCHARIDE (LPS). R.W. Bonsall*, M.S. Emery and J.M. Weiss. Department of Psychiatry and Behavioral Sciences, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Microdialysis is widely used to measure the local release of monoamines in specific regions of the brain, but it can also be used to measure hormones that are produced peripherally and cross the blood-brain barrier. Using conventional 4-mm probes constructed with regenerated cellulose membranes (Spectra/Por MWCO 13,000, 200-μm i.d.) or 3-channel probes of similar dimensions in which a preservative solution was added to the outflow just distal to the membrane, tissue levels of corticosterone were sampled in hippocampus and prefrontal cortex and then measured in 2.5 μl aliquots of perfusate using a micro-adaptation of a commercially-available radioimmunoassay. Sensitivity was 0.2 pg per tube, with a coefficient of variation of about 5%. At a flow rate of 0.5 μl/min, *in vitro* recovery rates for corticosterone were 13-14%. Perfusate concentrations of corticosterone showed a circadian variation in the range of 0 to 100 pg/30-min sample with peak levels at the beginning of the dark (active) phase and a nadir 3-4 h after the start of the light phase: this implied tissue levels of 0 to 44 ng/ml. Exposure to a noise stress for 30 min produced elevated perfusate concentrations of corticosterone that lasted for 90 min and peaked at 200 pg/30-min in the sample after the noise stress. To assess the adrenal response to immunostimulation in the absence of other stressors such as handling and injections, LPS (10 μg/kg) was infused from a remote location via an indwelling i.p. cannula. This produced elevated perfusate concentrations of corticosterone starting 1 h after the infusion, peaking at about 60 pg/sample at 2-3 h and lasting for 3.5 h. Monoamines and monoamine metabolites were measured by HPLC-EC simultaneously with the steroid. The technique is sufficiently sensitive to permit serial corticosterone sampling rates of up to twelve per hour which could be maintained for prolonged periods. Supported by USPHS Grant MH 50420.

453.12

IN VIVO DIALYSIS REVEALS RAPID GLUCOCORTICOID STIMULATION OF SEROTONIN FROM HIPPOCAMPAL CORTEX
Cliff H. Summers *, Tangi R. Summers, John M. Matter, Jen McKay, Patrick J. Ronan, Earl T. Larson and Kenneth J. Renner.
Dept. of Biology, University of South Dakota, Vermillion, SD 57069.

During stressful situations, central serotonin (5-HT) release coincides with glucocorticoid feedback. To test neuromodulatory effects of stress hormones, 5-HT overflow was measured from the medial (hippocampal) cortex of *Anolis carolinensis*. A concentric dialysis probe (o.d. 250 μm, 1.0 mm length) was inserted a depth of 1.0 mm, 0.6 mm behind the center of the parietal eye. Perfusates were collected at 20 min intervals and analyzed for 5-HT. Probes had 5-HT precalibration recoveries of about 15%. Concentrations of 5-HT stabilized to basal levels after approximately 3.5 h. Tetrodotoxin (1μM) inhibition of approximately 60% of 5-HT overflow suggested that most of the 5-HT signal was neuronally derived. The effect of corticosterone (B) on 5-HT overflow appears to be dose and time dependent: Intracortical infusion of 2 ng/ml evoked transient increases in 5-HT release of approximately 50%, 20 ng/ml B 200%, and 200 ng/ml B 400%. Duration of corticosterone infusion correlated positively with 5-HT response. Short-lived glucocorticoid stimulation of 5-HT release suggests a possible mechanism for endocrine mediation of continuously changing and transient social behavioral events. Supported by NSF grants IBN-9596009 and OSR-9108773.

453.13

EFFECTS OF NMDA AMYGDALOID LESIONS ON GASTRIC EROSION FORMATION DURING EXPOSURE TO ACTIVITY-STRESS. N.S. Morrow,[1,4] C.V. Grijalva,[2] J. Landeira-Fernandez,[2] P.J. Geiselman,[3] T. Garrick.[1,4] CURE: Digestive Diseases Research Center, Dept. of Veteran Affairs Medical Center,[1] Los Angeles CA 90073, Depts. of Psychology[2] and Psychiatry,[4] UCLA, Los Angeles, CA 90024, Dept. of Psychology and Pennington Biomedical Research Center,[3] LSU, Baton Rouge, LA, 70808.

The effects of chemical lesions (N-methyl-D-aspartate [NMDA]) of the central (CE) or basolateral amygdaloid (BL) nuclei on gastric erosion formation, wheel running and survival were examined in male rats exposed to activity-stress. As compared to control rats, gastric erosion formation was attenuated in rats with bilateral NMDA lesions in the BL and exacerbated in rats with lesions in the CE, p's < .05. There were no group differences in wheel running, food intake, body weight or length of survival. These results indicate that chemical lesions of CE or BL alter stress-induced gastric erosion formation in a manner opposite to that observed after electrolytic lesions, suggesting that fibers of passage through the amygdaloid nuclei mediate the effects on the viscera during exposure to experimental stressors. **Supported by a Pilot and Feasibility Award from NIH Center Grant DK 41301 (Animal Core), by VA Merit Review funds and by NSF grants BNS 87-09982 and BNS 9196142.

453.14

HIPPOCAMPAL CHOLINERGIC BLOCKADE INCREASES ANXIETY IN THE BLACK-WHITE BOX AND SOCIAL INTERACTION TESTS.
J.W. Smythe*[1], D. Murphy[1], S.Bhatnagar[2], C. Timothy[1], and B. Costall[1].
[1]Dept. of Pharmacology, Univ. of Bradford, Bradford, U.K., BD7 1DP and
[2]Dept. of Physiology, UCSF, San Francisco, CA 94143-0444, U.S.A.

Hippocampal cholinergic projections mediate attention to arousing stimuli as demonstrated by behavioral, electrophysiological and endocrine studies. We recently reported that peripheral injections of the cholinergic antagonist scopolamine (SCOP) increased anxiety-like behaviour (ALB) in rats and we sought to investigate if this response might be hippocampally-mediated. Adult male, Lister hooded rats were implanted bilaterally with hippocampal cannulae 3 weeks prior to testing. On the test day, rats were injected with vehicle (VEH; 0.9% saline), 15 or 30 μg SCOP, 20 min prior to being placed into the white chamber of the Black-White box (n=7/group). Rats were scored for latencies to exit and re-enter the white chamber. A separate group of rats was injected with VEH (n=12) or 10 μg SCOP (n=18) and tested 20 min later in a social interaction paradigm. Pairs of rats, both injected with VEH or SCOP, were placed into an open field and scored for time spent engaged in interactive behaviours (sniffing, following, physical contact). Data were assessed by ANOVA and Bonferroni corrected t-tests. ANOVA on the black-white box data revealed significant group effects for both times to exit $F(2,17)=3.60$, $P<.05$, and re-enter the white chamber $F(2,17)=4.73$, $P<.03$. SCOP at 30 μg significantly reduced time to exit the white arena ($P<.05$), while the 15 μg dose elevated latencies to re-enter the white chamber ($P<.01$). SCOP also reduced the duration and amount of social behavior compared to VEH rats ($P<.02$). Loss of hippocampal cholinergic function impairs processing of threatening stimuli that manifests itself as increased ALB and reduced social interaction behavior. Paradoxically, in the absence of cholinergic function, animals exhibit heightened sensitivity to the environment. *(Supported by Medical Research Council of Canada and U. of Bradford)*

453.15

MICROINJECTION OF MUSCIMOL INTO THE DORSOMEDIAL HYPOTHALAMIC NUCLEUS ATTENUATES FOS EXPRESSION IN THE PARAVENTRICULAR NUCLEUS FOLLOWING AIR STRESS IN RATS.
S.M. Morin*, E.H. Stotz-Potter and J.A. DiMicco. Dept. Pharmacol. & Toxicol. and Prog. in Med. Neurobio., Indiana Univ. Sch. of Med., Indpls, IN 46202

In mammals, acute stress elicits increases in heart rate (HR) and blood pressure (BP) and stimulates the release of adrenocorticotrophic hormone (ACTH). We have shown that microinjection of the $GABA_A$ receptor agonist muscimol (MUS) into the dorsomedial hypothalamus (DMH) prior to air stress reduces the cardiovascular changes while similar treatment in the paraventricular nucleus (PVN) has no effect. We have also shown that stress-induced increases in plasma ACTH are attenuated by microinjection of MUS into either the DMH or the PVN. These data suggest a primary role for the DMH in the integration of a multifaceted stress response, including mobilization of the hypothalamic-pituitary-adrenal axis. In this study, we investigated the effect of microinjection of MUS into the DMH on air stress-induced expression of Fos, an immediate-early gene product widely used as a marker of neuronal activation, in other hypothalamic regions. Rats implanted with guide cannulae in the DMH and arterial lines for monitoring HR and BP received 80 pmol/100nL MUS or 100 nL saline prior to being subjected to air stress for 20 minutes. One hour following the beginning of the stress the animals were sacrificed and the brains processed for Fos immunohistochemistry. In saline-treated rats, high levels of Fos expression were evident throughout the hypothalamus, especially in the DMH, PVN, ventromedial nucleus (VMH) and the bed nucleus of the stria terminalis. However, in MUS-treated rats Fos expression was virtually abolished in the PVN and was attenuated to lesser degrees in other hypothalamic areas. These results suggest that activation of neurons in the DMH may be necessary for excitation of neurons in the PVN during stress. (Supported by USPHS Grant NS 19883 and American Heart Ass'n., Indiana Affiliate)

453.16

LESIONS OF THE AUDITORY THALAMUS SPECIFICALLY BLOCK CORTICOSTERONE RELEASE AND INDUCTION OF c-*fos* mRNA IN THE FOREBRAIN ASSOCIATED WITH LOUD NOISE STRESS IN RATS.
S. Campeau*, H. Akil, and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Loud noise reliably elicits the release of corticosterone and the induction of the immediate early gene c-*fos* in many rat forebrain areas. The paraventricular nucleus of the hypothalamus is thought to be the final relay essential for stress-induced corticosterone release. To begin to determine the putative pathway through which loud noise activates the paraventricular nucleus, the auditory thalamus was chosen for study because of its obligatory role in relaying auditory information to the forebrain.

Male Sprague-Dawley rats (n = 48) received either neurotoxic (ibotenic acid) lesions aimed at the auditory thalamus (n = 18), sham lesions (n = 15) or were unoperated (n = 11). Four rats served as naive controls. All rats except the naives were handled for 2 min and placed in the experimental cages for 10 min on each of 14 consecutive days following surgery. On Day 15, the rats were placed in the cages for 60 min, and a 105 dBA noise (20 - 20000 Hz) was turned on for the last 30 min. The rats were decapitated immediately upon removal from the cages, their trunk blood collected and processed for corticosterone radioimmunoassay. The brains were rapidly removed, frozen, and processed for c-*fos in situ* hybridization.

Loud noise produced a reliable increase in plasma corticosterone in the unoperated (21.4 ± 2.9 μg/dl) and sham-operated rats (17.2 ± 3.4 μg/dl), but not in the rats sustaining relatively complete auditory thalamic lesions (n = 9; 2.8 ± 1.1 μg/dl). Loud noise induced c-*fos* mRNA in many forebrain areas of the unoperated and sham-operated rats, but not in the auditory thalamic lesioned rats. The auditory nuclei below the level of the auditory thalamus displayed reliable c-*fos* induction in all groups. Restraint and ether stress elicited similar corticosterone release in all groups. These results indicate that the auditory thalamus is an obligatory relay in the pathway involved in the release of corticosterone by loud noise and suggest the mediation of noise stress by forebrain structures. Supported by MRC of Canada and MH42251.

453.17

CRF ALTERS CORTICOSTERONE EFFECTS ON MEDULLARY SENSORIMOTOR INTEGRATION AND NEURAL EXCITABILITY. J. D. Rose[1]*, Glenn Marrs[1] and F. L. Moore[2]. [1]Dept. of Psychology. Univ. Wyoming, Laramie, WY 82071 and [2]Dept. of Zoology, Oregon State Univ., Corvallis, OR 97331.

Previously, we have shown that corticosterone (CORT) rapidly depresses sensorimotor processing and excitability in medullary reticulospinal and non-reticulospinal neurons in the roughskin newt (*Taricha granulosa*), apparently by an action at a CORT neuronal membrane receptor. In contrast, corticotropin-releasing factor (CRF) increases the activity of medullary neurons as it stimulates locomotion in newts. The present study examined effects of sequential medullary CRF application and medullary or systemic CORT administration in immobilized newts, to simulate the sequential release of these hormones in a natural stress response. CRF caused an immediate increase in firing rate and somatic sensory responsiveness of non-reticulospinal neurons and concurrently enhanced several parameters of excitability of reticulospinal neurons. Subsequent CORT administration, within 5-10 min of CRF, was followed by continued increases in excitability of these same neuronal populations. Thus, the neural action of CORT is modulated by immediately preceding actions of CRF, indicating that the temporal pattern of action of these stress hormones is a critical factor in determining their neuronal effects. Supported by NSF grants IBN-9410284 (J.R.) and IBN-9421175 (F.M.).

453.18

REGULATION OF CORTICOTROPIN-RELEASING FACTOR (CRF) ACTIONS ON LOCUS COERULEUS (LC) NEURONS: EFFECTS OF ADRENALECTOMY. L.A. Pavcovich and R.J. Valentino*. Dept. of Psychiatry, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA, 19102.

CRF has been hypothesized to act as both a neurohormone and brain neurotransmitter in the coordination of the stress response. The neurohormone actions of CRF are regulated by corticosteroids, as indicated by the effects of adrenalectomy, which include increased CRF synthesis and release (Dallman et al., 1992; Plotsky and Sawchenko, 1987). The present study tested the hypothesis that adrenalectomy also regulates a putative neurotransmitter effect of CRF, ie., its activation of LC neurons. LC spontaneous discharge rates were significantly higher in adrenalectomized (ADX) vs. sham-operated (SHAM) rats. Intracoerulear microinfusion of a CRF antagonist decreased LC discharge rates of ADX rats to rates comparable to those observed in SHAM rats, but had no effect in SHAM rats. The CRF dose-response curve in ADX rats was shifted in a complex manner, suggestive of additivity or that a proportion of CRH receptors were occupied prior to CRF administration. Hypotensive challenge (thought to activate the LC via CRF release) increased LC discharge rate in ADX rats by a magnitude greater than that which would be predicted on the basis of additivity. In contrast, the magnitude of LC activation by carbachol (i.c.v.) and sciatic nerve stimulation (mediated by an excitatory amino acid input) were similar in both groups. These results suggest that adrenalectomy enhances tonic and stress-induced CRF release within the LC. Thus, the actions of CRF on the pituitary (a neurohormone effect) and on the LC (a neurotransmitter effect) may be regulated in a parallel manner. Such parallel regulation may underly the coexistence of neuroendrocrine and noradrenergic dysfunctions in stress-related psychiatric disorders.Supported by USPHS grants MH42796 and MH00840.

453.19

MIRRORS REVERSE DISTRESS VOCALIZATIONS BUT NOT STRESS-INDUCED ANALGESIA IN THE CHICK SOCIAL-SEPARATION PROCEDURE. G. S. Watson[1]* & K. J. Sufka[1,2]. Departments of Psychology[1] & Pharmacology[2], The University of Mississippi, Oxford, MS 38677.

Chicks separated from social companions displayed an increase in distress vocalizations and a decrease in formalin-induced (0.05 ml of 0.10% formalin injected into the plantar surface of the right foot) nociceptive behaviors (i.e., stress-induced analgesia) relative to chicks tested with two social companions during 3 minute test period. Chlordiazepoxide (3.0-10.0 mg/kg/ml, IM 30 min before tests) dose-dependently reversed both distress vocalization and stress-induced analgesia effects. When mirrors were substituted for social companions, stress effects on distress vocalizations but not stress-induced analgesia were reversed. Physical contact with social companions may be necessary for the prevention of stress-induced analgesia whereas a purely visual stimulus (chick's image in a mirror) is sufficient to reverse distress vocalizations. In subsequent mirror studies exploring the effects of chlordiazepoxide on distress vocalizations only, chlordiazepoxide reversed distress vocalizations when injected IM, but not when injected IP.

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR IV

454.1

AMYGDALA BUT NOT HIPPOCAMPAL LESIONS DISRUPT OLFACTORY MEMORY INVOLVED IN PREGNANCY BLOCK IN FEMALE PRAIRIE VOLES. G.E. Demas*, J.M. Williams and R.J. Nelson. Department of Psychology, Behavioral Neuroendocrinology Group, The Johns Hopkins University, Baltimore, MD 21218.

Mammalian reproduction is influenced by chemosensory information in many rodent species. The presence of an unfamiliar male results in spontaneous abortion by a pregnant female and a return to an estrous state within 48 h (i.e., the Bruce effect). Disruption of pregnancy does not occur in the presence of a familiar male. Unlike most laboratory species, female prairie voles do not exhibit regular estrous cycles; rather, female prairie voles are induced into estrus by chemosensory stimuli contained in the urine of male conspecifics. Females come into contact with these pheromones while grooming the ano-genital (A-G) region of unfamiliar males. After initial contact with a male, female prairie voles maintain a brief 8-10 day "memory" for their previous mate. Subsequent exposure to the same male within this 8-10 day window does not elicit ano-genital investigation by the female and pregnancy block does not result. The present study is an attempt to elucidate which neuroanatomical area(s) are involved in olfactory memory. Female prairie voles received bilateral electrolytic lesions of either the amygdala or hippocampus; females were subsequently exposed to males for 48 h, separated for 3 days, and then reintroduced to their original mate for 24 h. A greater proportion of females receiving amygdala lesions displayed pregnancy block when re-exposed to their previous mates compared to voles receiving hippocampal or sham lesions. Amygdala-lesioned voles also displayed a greater number of A-G investigations compared to the other groups. Olfactory tests suggest that these results were not due to a basic disruption of the main olfactory or accessory olfactory systems. Taken together, these results suggest that the amygdala plays an important role in olfactory memory or reproductive motivation in prairie voles. Supported by USPHS grant HD22201.

454.2

BRAIN MECHANISMS INVOLVED IN POSTPARTUM ESTRUS IN PRAIRIE VOLES. L.F. Katz, G.F. Ball, and R.J. Nelson*Department of Psychology, Behavioral Neuroendocrinology Group, The Johns Hopkins University, Baltimore, MD 21218.

Female prairie voles (*Microtus ochrogaster*) do not exhibit spontaneous estrous cycles; rather, estrus is induced by direct contact with chemosensory stimuli from male conspecifics. Female prairie voles also display spontaneous postpartum estrus. Mating probably occurs most commonly during postpartum estrus in nature, yet little is known about its neuroendocrine or neuroanatomical bases. The goal of this study was to determine the neuroanatomical bases of postpartum estrus, and to compare these pathways to those previously found to be involved in male-induced estrus in this species.

In the present study, perfused brains brains of three experimental treatment groups (0-12 hours, 12-24 hours, and 24-48 hours postpartum) and two control groups (late pregnancy and paired nonpregnant females) were processed using immunocytochemistry for the *fos* protein. Digitized images of brain slices (40μm) were projected from a bright field microscope at 10x and analyzed using a computerized image analysis system (Image 1.4 Mac). A significant increase in the number of *fos*-ir cells was observed in the accessory olfactory bulb (AOB), medial preoptic area, thalamic paraventricular nucleus, primary somatosensory area, and the bed nucleus of the stria terminalis from brains obtained 0-12 hours postpartum. The neural activation seen in the AOB in the 0-12 hour postpartum group resembles the *fos* expression observed in the AOB in female prairie voles immediately following exposure to male urine. The origin of the exogenous cue triggering this activity in the AOB may be from contact with a non-volatile stimulus from either the male or the newborn pups. Supported by NIH grants HD22201and MH50388.

454.3

NEUROPLASTICITY OF THE MATERNAL BRAIN: CHANGES IN SIGNAL TRANSDUCTION AND GLIAL PROTEINS ASSOCIATED WITH POSTPARTUM EXPERIENCE IN RATS. A.S. Fleming*, R. Featherstone, J. Watchus, R. Smyth, M. Lydan, D. O'Day and G. Ivy. Depts. Psychology and Biology, Erindale Coll., Univ. of Toronto, Mississauga, Ont., CANADA L5L 1C6.

When female rats give birth, they adequately care for their offspring with no prior maternal experience. Once females have interacted with young, their maternal responsiveness is sustained past the period of hormonal priming. The long-term retention of responsiveness is mediated by prior experience and learning processes.

A number of neural sites show elevated Fos-like immunoreactivity (Fos-lir) with experience and in response to pups or pup-associated cues, including the medial preoptic area (MPOA), the basolateral amygdala (BLA) and the parietal cortex (PC). These studies explore the upstream signalling elements associated with experience-based Fos expression, including protein-kinase C (PKC) and calmodulin-binding proteins (CaMBPs), as well as other proteins associated with neuroplasticity (e.g. GFAP in Astrocytes).

In different studies, postpartum females were provided with a brief interactive experience with pups or control experiences. Within each experience condition, animals were either sacrificed immediately (exposure phase) or after an interval (retention), and brains were assessed immunocytochemically for Fos-lir, PKC or GFAP or using gel electrophoresis, for CaMBPs. Brain sites were quantified using a light microscope with 10 x 15 magnification.

The MPOA and PC showed higher densities of cells showing Fos-lir and PKC and lower concentrations of CaMBPs following the exposure phase and pup stimulation in postpartum animals. The MPOA and PC also showed elevated experience-based PKC and GFAP at retention, in the absence of additional pup-stimulation. No experience effects were seen in BLA or control sites (e.g. VMH, HIPP). These studies show neuroplasticity within the maternal circuit. Supported by the Medical Research Council of Canada awarded to A.S. Fleming.

454.4

EFFECTS OF KINDLING-LIKE STIMULATION OF THE MEDIAL PREOPTIC AREA AND THE MEDIAL AMYGDALA ON MATERNAL MOTIVATION IN VIRGIN FEMALE RATS. H.D. Morgan*, J.A. Watchus and A.S. Fleming. Department of Psychology, University of Toronto, Mississauga, Ontario, Canada L5L 1C6.

The effects of kindling-like electrical stimulation of the medial preoptic area (MPOA) or the medial amygdala (AME) on maternal and other associated (motivational) behaviors and its interaction with pup exposure were investigated. Previous experiments have shown that kindling the AME and MPOA in pup experienced females enhanced their functional effects. The present study investigated the effects of kindling on the same functional endpoints in virgin animals sustaining a variety of pup experiences. Four conditions of MPOA and AME kindled virgins were used: (1) kindled [K] (and not-kindled [NK] control group) without pup exposure, (2) K (and NK) during pup exposure, (3) K (and NK) 1 week before pup-exposure, and (4) K (and NK) both during pup exposure and 1 week before pup-exposure (16 groups total). Stimulation was applied daily for 2 weeks using 2 second trains of biphasic square wave pulses at 1 ms duration and 400μA. Testing proceeded 1 week after kindling. For groups that had exposure to pups (conditions 2,3 and 4) MPOA K animals showed shorter latencies to become maternal than MPOA NK animals and than AME K and NK animals. For measures of maternal 'motivation', in pup-exposed conditions 2 and 4 AME kindled animals showed a decreased attraction to pup odors in a modified hole board test compared to AME NK animals and MPOA NK animals. AME K animals in pup-exposed conditions 3 and 4 showed also showed a decreased attraction to pup odors in the modified hole board when compared to non-pup-exposed AME NK animals. There was no difference between any groups for the hole board test within the non-pup-exposed condition. The above results confirm that the MPOA and the AME are involved in facilitating and attenuating maternal responsiveness and related (precursor?) behaviors, respectively. However, this effect was produced only in interaction with pup exposure. Kindling possibly enhances neural changes during functional reorganization of neural substrates.
Supported by NSERC of Canada awarded to A.S. Fleming.

454.5

MATERNAL MOTIVATION: THE EFFECT OF MEDIAL PREOPTIC LESIONS ON OPERANT BEHAVIOR IN FEMALE RATS. A.W. Lee, A.S. Fleming, S. Clancy and M.L. Smith*. Dept. of Psychology, Erindale Coll., Univ. of Toronto, Mississauga, Ont., CANADA L5L 1C6.

The present study consists of four studies, in which the dissociation of maternal motivation and maternal consummatory behaviors in female rats was investigated.

The first study determined the effect of reproductive state on both maternal behavior and instrumental bar press response for pup reinforcement. Groups consisted of maternally experienced impregnated or cycling females. Impregnated females showed a significant increase in both bar press response and the expression of maternal behaviors from pregnancy to Day 1 postpartum. Cycling females showed no changes over a comparable time period. Study 2 compared two groups of cycling maternally experienced females. Half were re-induced to become maternal through continuous pup exposure and half were not induced. Maternal females exhibited a significantly higher pup-reinforced lever pressing rate than did the non-maternal females. Study 3 investigated effects of MPOA lesions on operant behavior. Two groups of cycling maternally-experienced females were induced to become maternal. Groups received bilateral electrolytic medial preoptic area (MPOA) or sham lesions. Pre-surgery, both groups showed a high operant rate for pups and all components on maternal behaviors. After surgery, the bilateral lesioned group showed a decrease in bar press response and did not engage in pup-directed maternal behaviors. Sham subjects did not change their maternal or bar press responses for pups and remained maternal throughout the study. Study 4 investigated the effects of MPOA, nucleus accumbens, and lateral amygdala lesions on postpartum rats. After surgery, the MPOA group showed a decrease in bar press response and did not engage in pup-directed maternal behaviors. No differences were found between sham, nucleus accumbens and lateral amygdala lesioned animals. Supported by the Natural Sciences and Engineering Research Council of Canada awarded to A.S. Fleming.

454.6

COMPARISONS OF ARGININE VASOPRESSIN STAINING IN TWO SPECIES OF PEROMYSCUS WITH DIFFERENT LEVELS OF PARENTAL CARE. J.K. Bester and C.A. Marler*. Dept. of Psychology, University of Wisconsin-Madison, Madison, WI 53706

Previous studies have suggested that arginine vasopressin (AVP), a neuropeptide hormone, is involved in parental and aggressive behavior. In this study, we compared the locations and intensity of AVP immunoreactive staining in the brains of two closely related species, *Peromyscus californicus*, a monogamous species with high levels of parental care, and *Peromyscus leucopus*, a promiscuous species with low levels of parental care. We confirmed behavioral differences in parental care between the two species under similar laboratory conditions. Because of these differences, we compared the degree of AVP immunoreactive (AVP-ir) staining using image analysis in brain areas associated with sexual, aggressive, and parental behavior, including the bed nucleus of the stria terminalis, the lateral septum, the amygdala, and the preoptic area. The degree of AVP-ir staining was also compared in brain areas that traditionally have been associated with water balance and non-reproductive functions. These areas include the paraventricular nucleus, the supraoptic nucleus, and the suprachiasmatic nucleus. AVP-ir staining was significantly greater in the medial amygdala of *P. californicus* and no difference was found in the paraventricular nucleus. Ongoing analyses are being performed to compare other brain areas in the two species. Results of these analyses will be compared to other species comparisons of AVP-ir staining.

454.7

INCREASES IN NADPH-DIAPHORASE STAINING IN THE SUPRAOPTIC AND PARAVENTRICULAR NUCLEI DURING LATE PREGNANCY AND EARLY LACTATION IN RATS. N. Popeski*, S. Amir & B. Woodside, Center for Studies in Behavioral Neurobiology , Department of Psychology, Concordia University, Montréal, Canada.

Among numerous putative functions the gaseous transmitter molecule Nitric Oxide (NO) has been implicated in the modulation of a variety of neuroendocrine events. Recently an increase in staining for NADPH-d, a histochemical marker for Nitric Oxide Synthase has been reported in the paraventricular (PVN) and supraoptic (SON) nuclei of late pregnant rats. In this study, the time course of these changes was examined more closely by comparing NADPH-d staining in the PVN and SON of rats at different stages of pregnancy and lactation. Thus, female rats either unmated or on Days 4, 12, 16 or 22 of pregnancy or Days 4, 12 or 20 of lactation were anaesthetized and perfused with ice-cold saline and 4% paraformaldehyde. Brains were removed and processed for NADPH-d histochemistry. Females in early pregnancy showed staining similar to that of virgin animals. Consistent with our earlier study NADPH-d staining was increased in late pregnancy and in some stages of lactation. These data suggest that NO may be involved in the events surrounding parturition and lactation. The role of the hormonal status of the female in producing these effects is presently under investigation. (Supported by Grant # 7938 from NSERC to B.W.)

454.8

DIFFERENTIAL FOS EXPRESSION IN THE BRAINS OF MATERNAL AND NON-MATERNAL VIRGIN FEMALE RATS EXPOSED TO VARIOUS STIMULI. T.P. Sheehan*, E.C. Stack, M.J. Numan, and M. Numan. Psychology Dept., Boston College, Chestnut Hill, MA 02167

Virgin female rats are not spontaneously maternal, but can be sensitized to show maternal behavior after daily exposure to pups. Differences in these behavioral states may be reflected in the activity of different brain areas. To investigate this, we exposed both sensitized and naive (non-sensitized) virgin female rats to pups for two hours. Control groups were exposed to marbles as a non-pup stimulus. After exposure, animals were sacrificed and their brains were immunohistochemically processed for detection of the Fos protein, which is used as a marker for neuronal activation. Our initial results indicate that the levels of Fos in the ventral portion of the lateral septum (LSv) and the central nucleus of the amygdala (CNA) are highest in naive virgins exposed to pups when compared to all other groups. All subjects exposed to pups showed higher levels of Fos in the medial preoptic area (MPOA), with sensitized virgins showing a greater Fos response relative to naive virgins. In naive virgins, Fos expression in the LSv and CNA may represent the novelty of pup odors and/or an aversive response to such odors. Enhanced Fos in the MPOA may be a result of both pup exposure and the expression of maternal behavior. Results will also be presented on Fos expression in other brain areas such as the bed nucleus of the stria terminalis and the periaqueductal gray.

This research was supported by NSF Grant IBN 9319315.

454.9

SATIETY OF PUPS REGULATES DECLINE OF NURSING DURATION WITH TIME POSTPARTUM IN LACTATING LONG-EVANS RATS. S.E. Keer* and J.M. Stern, Department of Psychology, Rutgers University, New Brunswick, NJ 08855.

Rooting and suckling of rat pups is essential for initiating and maintaining their dams' nursing behavior. During the 2nd week postpartum (PP), there is a normal decline in the duration of time spent nursing, attributed previously to maternal hyperthermia rather than milk transfer. In contrast, we hypothesized that the development of satiety reponsiveness in the 2nd postnatal week contributes to the decline in nursing time. In the 1st study, pups received 0, 4, or 8% of their body weight in half-and-half creamer by stomach gavage on days 7, 12, and 15 PP. Dams nursing 4%- or 8%-gavaged pups showed a decreased number of milk ejections (ME); litter weight gain was also decreased. On days 7 and 12 (4-h dam-litter separation and subsequent observation until 30 min after 1st ME) the 1st ME occurred considerably later. On day 15 (undisturbed 24-h video recording) crouch latency was 10x longer and crouch duration 50% shorter than in dams gavaged with nothing. The 2nd study investigated whether inhibition of milk release would increase nursing duration. Dams underwent mammary duct ligations (MDX) or a control procedure (MDC) on day 6-7 followed by 12-h undisturbed video recordings with foster pups on days 7-8 and 13-14. Weight losses were seen in both day 7 (-7.0 g) and 14 (-4.6 g) litters nursed by MDX dams. On days 7-8, hovering over (without actual nursing) was 40% higher in MDX dams, while passsive (supine) crouching was 35% higher in MDC dams. On days 13-14, kyphotic (upright) crouching lasted 7x longer in MDX than in MDC dams. These experiments suggest that the decline nursing duration in rats is regulated by pup satiety since sating pups decreases while preventing milk transfer to pups increases nursing duration on days 13-14 PP.

Supported by MH40459 (JMS)

454.10

PERIAQUEDUCTAL GRAY LESIONS, PRE- OR POSTPARTUM, SPECIFICALLY IMPAIR KYPHOTIC NURSING BEHAVIOR BUT FACILITATE MATERNAL AGGRESSION IN LONG-EVANS RATS. J.S. Lonstein*, D.A. Simmons, and J.M. Stern, Department of Psychology, Rutgers University, New Brunswick, NJ 08903.

Electrolytic lesions performed on day 7 postpartum (PP) encompassing the lateral and ventrolateral regions of the caudal periaqueductal gray (PAG) at the level of the trochlear nucleus (PAG-X) almost eliminate upright crouched nursing (kyphosis) compared to controls (PAG-C), assessed by continuous 60-min observations after a 3-h mother-litter separation on days 8-13 PP. All dams spent similar amounts of time with their litters, but time spent in other nursing postures was increased in lesioned rats. No deficits were seen in other behaviors including retrieval, nesting time, and pup licking. Though milk ejections and litter weight gains were similar for lesion and control groups during the daily observation periods, 24-h litter weight gains were reduced by ~15% after lesioning. Maternal aggression toward an unfamiliar male intruder was tested presurgically on day 5, and on postlesion days 8 and 10 PP. Attack latency was reduced, and attack number and duration were increased, in PAG-X vs. PAG-C dams. When lesions were performed prepartum on gestation days 15-17, and subjects observed for 60 min after a 3-h dam-litter separation on days 1-6 PP, a similar deficit in upright crouched nursing and facilitation of maternal aggression was seen. Deficits in 24-h litter weight gains were larger (~25-30%) after lesioning prepartum. Upright crouched nursing is therefore necessary for normal litter growth, especially during early lactation when pups are small. The present results, along with previous Fos findings (SN '95) and the known medullary, trigeminal, and spinal connections of the PAG, suggest that the PAG may be a component of the pathway by which suckling elicits the postural alterations seen during kyphotic nursing and facilitates maternal aggression. [Supported by MH40459 (JMS)]

454.11

CHANGES IN CENTRAL NEUROTENSIN IMMUNOREACTIVITY ASSOCIATED WITH LACTATION AND MATERNAL BEHAVIOR IN RATS. M. Numan*, S.A. Aviles, M.D. Williams, E.C. Stack, T.P. Sheehan, and M.J. Numan, Dept. Psychology, Boston College, Chestnut Hill, MA 02167.

Neurotensin is a neuropeptide that is found in high concentrations within the basal forebrain. Since the distribution of Fos-expressing neurons within the basal forebrain of maternal rats approximates the distribution of neurotensin-containing neurons, the present study investigated whether changes in neurotensin systems are associated with maternal behavior and lactation. Virgin female and maternal lactating rats were sacrificed and their brains were processed with standard immunocytochemical procedures for the detection of neurotensin-immunoreactive (NTir) cell bodies and fibers. None of the females was treated with colchicine. An image analysis system was used to measure the density of NTir fibers. Preliminary findings indicate that lactating females have significantly more neuronal cell bodies that contain NTir within the central nucleus of the amygdala (CN) and the oval nucleus of the bed nucleus of the stria terminalis (BST). Lactating females also have a higher density of NTir fibers within the retrorubral field (RRF) of the midbrain. These changes may be due to increased synthesis and/or decreased release of neurotensin in postpartum females. Neurotensin within the CN and BST may be involved in regulating either suckling-induced activation of the adrenocortical system or the adrenocortical stress response. Neurotensin fibers within the RRF may be influencing aspects of maternal behavior.

Supported by NSF Grant IBN-9319315.

454.12

THE ROLE OF LATERAL HABENULA NEURONS IN THE NON-HORMONALLY MEDIATED DISPLAY OF MATERNAL BEHAVIOR IN SENSITIZED VIRGIN FEMALE RATS. T. Matthews-Felton*[1], J. S. Rosenblatt,[2], J.I. Morrell[1]. C.M.B.N.[1] and I.A.B.[2], Rutgers University, Newark, N.J. 07102

Our previous studies have shown that the Lhb is necessary for the hormonal onset of maternal behavior (Corodimas et al., 1993; Matthews-Felton et al., 1995), but not for the postpartum maintenance of already established maternal behavior in the rat (Corodimas et al., 1992). Although hormones initiate maternal behavior in the naturally parturient female, it is maintained by pup stimulation and is largely non-hormonal. In contrast, the sensitization model of maternal behavior, can occur by direct pup stimulation with no hormonal interaction at all. Sensitization consists of continuously exposing females to pups and results in maternal behavior in 5-7 days even in ovariectomized or hypophysectomized females. Sensitization was used in the present study to examine the effects of Lhb lesions on non-hormonal, pup-mediated maternal behavior.

Virgin female rats received bilateral kainic acid-induced lesions of the Lhb, or as a control, the dorsal hippocampus. A third group served as unlesioned controls. All animals were tested for pup retrieval, nest building, and crouching behavior for 15 days. General activity and carrying ability were also tested during this period. A fluorescent retrograde tracer (Fluoro-Gold), along with cresyl violet stained sections were used for histological analysis of the lesions.

Preliminary results indicate that lesions produced in the Lhb do not disrupt the initiation of maternal behavior produced by sensitization. There were no significant differences in pup retrieval, nest building, and crouching behavior between animals with Lhb lesions and either the hippocampal or unlesioned control groups. Supported by MBRSG 5 SO6 GM 208223-09 to T.M-F. & J.S.R. and HD 22983 to J.I.M.

454.13

DEFICIENCY IN MOUSE OXYTOCIN PREVENTS LACTATION, BUT NOT FERTILITY OR PARTURITION. W. S. Young, III*, E. Shepard, J. Amico¶, M. E. LaMarca†, C. McKinney†, E. I. Ginns†. Lab. Cell Biology and †Clinical Neuroscience Branch, NIMH, NIH, Bethesda, MD 20892; ¶Div Endocrinol Metab, Univ Pittsburgh, School of Medicine and VA Medical Center, Pittsburgh, PA 15261

To investigate whether oxytocin (OT) is essential for parturition, lactation, various reproductive behaviors, and memory, we eliminated, by homologous recombination, most of the first intron and the last two exons of the mouse OT gene. No gross anatomical or behavioral differences were noted between wild-type (WT), heterozygous (HE) or homozygous (HO) mutant pups. Weights of HO mice were not significantly different from WT mice through at least two months (males: 23.1±1.2g (±sdev) and 20.9±0.7; females: 19.3±0.2 and 17.7±0.6). OT has natriuretic and antidiuretic properties and its receptor is present in the rat kidney, so we measured two parameters that could reflect kidney dysfunction. No differences were noted among males in plasma osmolalities (WT: 297±2.4mOSM (±sem), HO: 297±2.1) or abilities to concentrate urines after 24 hours of water deprivation (WT: 2945±151mOsm (±sem), HO: 3045±360). OT transcripts are seen in the PVN and SON of the WT and HE mice using an exon A probe, with greatly reduced levels in the HO PVN. The mRNA levels for the HE and HO PVNs are 53% and 1% of the WT level. Homozygous mutant mice have no detectable neurophysin or processed oxytocin in the paraventricular nucleus, supraoptic nucleus or posterior pituitary. OT RIA of pituitary extracts was 220 ± 20 (mean ± SEM) ng/pituitary in WT mice and <1 ng/pituitary (800 ± 50 pg/pituitary) in HO mice. Plasma OT levels in the HO mice were undetectable (WT was 15-20pg/ml). VP transcripts are reduced in both nuclei by 30% ($p<0.01$ in the SON). Suprachiasmatic VP expression was unaltered in HO mice arguing against a nonspecific reduction due to disruption of the OT gene which is closely linked to the VP gene. Expression of dynorphin, which is co-expressed with VP, is elevated by 37% in the HO PVN neurons compared to the WT ($p<0.01$). PVN expression of corticotropin-releasing factor and enkephalin is unaltered. Interestingly, HO males and females are fertile and the HO females are able to deliver their litters. However, they do not lactate and their pups die within 24 hours.

454.14

COMPARISONS OF THE BEHAVIORAL EFFECTS OF OXYTOCIN AND VASOPRESSIN IN MONOGAMOUS PRAIRIE VOLES, M. M. Cho, A. Dharmadhikari, J. R. Williams, & C. S. Carter*. Department of Zoology, University of Maryland, College, Park, MD 20742

Oxytocin (OT) and arginine vasopressin (AVP) have been implicated in pair bonding in prairie voles *(Microtus ochrogaster)*. Earlier studies suggested that males respond most readily AVP, which tends to increase the preference for a familiar partner and aggression to strangers; while females are sensitive to OT, which facilitates partner preferences. In other systems there is evidence that OT and AVP may occupy and affect each other's receptors. In the present study, we have compared the behavioral effects of centrally-administered (icv) OT and AVP on behaviors associated with pair bonding and exploration. At comparatively high doses (100 ng) both OT and AVP can stimulate the rapid development of partner preference in both sexes. Lower doses were less effective. In males, exploratory behavior in an elevated plus maze also tended to increase following both OT and AVP, but the effects of AVP were longer lasting (measured for 3 hours or more) than those of OT (lasting approximately 1 hour). These results suggest that high doses of OT and AVP can have apparently similar behavioral effects in both sexes, although the temporal profiles of the two peptides may not be identical. Sponsored by NIH and NIMH.

455.1

ANXIOLYTIC ACTION OF SUBSTANCE P ADMINISTERED SYSTEMICALLY OR INTO THE BASAL FOREBRAIN. R.U. Hasenöhrl[1]*, O. Jentjens[1], M.A. De Souza Silva[1], C. Tomaz[2] and J.P. Huston[1]. [1]Inst. Physiol. Psychology, Univ. Düsseldorf, D-40225 Düsseldorf, Germany; [2]Dept. Psychobiol., Univ. Sao Paulo, Ribeirao Preto, 14049 Brazil.

Reinforcing and memory-promoting effects of substance P (SP) were found upon injection into several parts of the brain and intraperitoneally. Given the close link between fear/anxiety and memory processes for negative reinforcement learning, the aim of the present study was to gauge the effect of SP in the rat elevated plus-maze (EPM) and social interaction test of anxiety. When given peripherally (IP), SP had a biphasic dose-response effect on the behavior in the EPM with an anxiolytic-like action at 50 μg/kg, and an anxiogenic-like one at 500 μg/kg. After microinjection into the region of the nucleus basalis magnocellularis (NBM), SP (1 ng) was found to exert anxiolytic effects, since SP-treated rats spent more time on the open arms of the EPM and showed an increase in time spent in social interaction. These results show that SP can have anxiolytic-like properties in addition to its known memory-promoting and reinforcing effects, supporting the hypothesis of a close relationship between anxiety, memory and reinforcement processes. *Supported by DFG.*

455.2

THE ROLE OF SUBSTANCE P IN EMETIC AND ANTIEMETIC EFFECTS OF RESINIFERATOXIN ON *SUNCUS MURINUS*. M. Toyoda*, S. Kakimoto, H. Saito, N. Matsuki, A. J. Woods[a] and P. L. R. Andrews[a]. Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, JAPAN and [a]Department of Physiology, St George's Hospital Medical School, London, U.K.

To elucidate the role of Substance P (SP) in the emetic response, we examined the effect of resiniferatoxin (RTX) on *Suncus murinus*. RTX, an ultrapotent analog of capsaicin, is considered to release neuropeptides such as SP mainly from primary afferent C-fibers and then deplete them. Both systemic (1-100 μg/kg, s.c.) and central administration of RTX induced emesis dose-dependently. The Emetic effect of 100 μg/kg (s.c.) RTX was not significantly affected by abdominal vagotomy but was completely blocked by a selective NK_1-receptor antagonist, CP-99,994 (30 mg/kg, s.c.). On the other hand, RTX also had antiemetic effect. Pretreatment (- 60 min) with 100 μg/kg RTX reversibly blocked emesis induced by cisplatin, $CuSO_4$, nicotine or motion. Animals that had treated with capsaicin (100 mg/kg, s.c.), a presumptive depleter of SP, 1-2 days after the birth did not respond to RTX tested when they became 3 months old. However, they responded to cisplatin, $CuSO_4$, nicotine and motion. These results suggest that RTX causes emesis probably by releasing SP in the brainstem (eg. NTS) and that the depletion of SP and/or the desensitization of SP receptors are likely to cause subsequent antiemetic effects. The results support an important role for SP in emesis. (Supported partly by Grant-in-Aid 07557311 from the Ministry of Education, Culture and Science of Japan. SB Pharmaceuticals. MSD. Phizer INC)

455.3

ENDOGENOUS NITRIC OXIDE MODULATES SUBSTANCE P-INDUCED ITCH-RELATED BEHAVIOR. T. Andoh, T. Nagasawa, K. Hayashi, Y. Kuraishi*. Dept. of Applied Pharmacol., Res. Inst. for Wakan-yaku, Toyama Med. & Pharm. Univ., Toyama 930-01, Japan.

Substance P (SP) causes an itch sensation in human subjects when applied to the skin. Recently we have found that an intradermal injection of SP elicits itch-related behavior, that is, scratching of the injected site by the hind paws and that such SP action is inhibited by NK_1 antagonist. As stimulation of NK_1 receptors activates nitric oxide (NO) synthase in the endothelial cells and macrophages, the present experiments were conducted to determine whether the NO-cyclic GMP pathway was involved in SP-induced scratching. Male ICR mice (5-6 weeks old) were used and SP was intradermally injected into the rostral back in a volume of 50 μ L. Pretreatment with the NO synthesis inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 0.1-10 mg/kg, i.v. 5 min before), but not its enantiomer D-NAME (0.1-10 mg/kg), significantly suppressed SP (100 nmol)-induced scratching in a dose-dependent manner. Another NO synthesis inhibitor 1-ethyl-2-thiourea (0.1-10 mg/kg, i.v. 5 min before) showed similar inhibitory effects. When injected simultaneously with SP (100 nmol), the NO scavenger hemoglobin (0.01-10 nmol) or the guanylate cyclase inhibitor methylene blue (0.001-0.1 nmol) significantly suppressed SP-induced scratching. Although either L-arginine (300 nmol) or SP (30 nmol) was almost without effects, a concomitant intradermal injection of these agents induced apparent scratching. These results suggest that the NO-cyclic GMP pathway is involved in the SP-induced scratching, probably an itch sensation.

455.4

CORTISTATIN MODULATES CORTICAL AND HIPPOCAMPAL ELECTROPHYSIOLOGICAL ACTIVITY AND SLEEP. O. Prospéro-García*[1], J. R. Criado[1], L. de Lecea[2], S. C. Casalman[1], J. G. Sutcliffe[2], and S. J. Henriksen[1]. [1]Dept. Neuropharmacology and [2]Molecular Biology, The Scripps Res. Inst. La Jolla, CA.

Cortistatin is a novel peptide with structural similarity with somatostatin but is the product of a different gene. Cortistatin can be found in the brain of rats but not in organs such as adrenal glands, liver, spleen, thymus, ovary, testis or anterior pituitary. The regional distribution of cortistatin in brain suggests that the highest levels are found in the cerebral cortex and the hippocampus. The examination of the cellular distribution of this peptide showed that is synthesized in GABA-ergic interneurons. We have tested in halothane-anesthetized rats, the ability of cortistatin to modify both local electroencephalographic (EEG) activity when it is iontophoretically administered into the cerebral cortex; and evoked population spikes and paired-pulse inhibition-potentiation processes in the hippocampus when it is iontophoretically administered into the hippocampus. In addition, cortistatin was intraventricularly infused into the lateral ventricle of freely moving rats in order to observe changes in the spontaneous behavior, EEG and in the sleep patterns up to four hours following administration. Results demonstrated that locally applied cortistatin does not modify the EEG slow wave activity of anesthetized rats; however, it prevents EEG activation (fast activity) induced by the iontophoretic administration of acetylcholine (ACh). In the hippocampus, cortistatin prevents the ACh-induced paired-pulse disinhibition. In freely-moving rats, cortistatin induces behavioral quiescence and slow wave sleep during normal as well as in reverse light-dark cycle. At the higher dose used (10 μg), cortistatin reduced REM sleep in normal cycle, but 500 ng increased REM sleep in reverse cycle. These facts support cortistatin as a new peptide with anti-ACh antagonist activity and potential sleep modulating properties.
This work was supported by grants from GM32355, MH47680and DA08301 to S. J. H. and J. G. S.

455.5

EFFECTS OF THE $5HT_3$ ANTAGONIST, ONDANSETRON, ON PENTAGASTRIN-INDUCED ANXIETY IN PATIENTS WITH ANXIETY DISORDERS. U. McCann*, M. Geraci, C. Morgan, S. Slate, R. Post. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892-1272.

Cholecystokinin (CCK) agonists are anxiogenic in animals and humans, and patients with anxiety disorders are more susceptible to the anxiogenic effects of CCK agonists than healthy controls. Preclinical evidence suggests that $5HT_3$ receptors may play a role in CCK-induced anxiety. In particular, in rats, $5\text{-}HT_3$ antagonists such as ondansetron have been found to attenuate the anxiogenic effects of CCK agonists. Interestingly, ondansetron has also been reported to be effective in the treatment of panic disorder and social phobia. In an effort to determine the role of $5\text{-}HT_3$ receptors in pentagastrin-induced anxiety in humans, patients with panic disorder and social phobia underwent three separate pharmacological challenges in random order: 1) Saline pretreatment followed by pentagastrin infusion (0.6 ug/kg over 1 minute); 2) Ondansetron pretreatment (0.15 mg/kg over 15 minutes, followed by pentagastrin (0.6 ug/kg over 1 minute); 3) Saline pretreatment followed by saline infusion. Subjects received pentagastrin (or placebo) while participating in a structured social interaction task, as previously described, and completed repeated measures of anxiety. As previously, intravenous pentagastrin lead to increased anxiety and panic in patients with anxiety disorders. Pretreatment with ondansetron, at a dose that is clinically effective for antiemesis, did not attenuate the anxiogenic effects of pentagastrin. These studies suggest that the anxiogenic effects of pentagastrin are not mediated via $5HT_3$ receptors.

455.6

EFFECTS OF ONDANSETRON ON PENTAGASTRIN-INDUCED ANXIETY IN PANIC DISORDER PATIENTS.
A.W.Goddard*, S.W.Woods, G.M.Banks, G.R.Heninger, D.S.Charney. Dept. of Psychiatry, Yale U. Sch. of Med., New Haven, CT 06519.

There is preclinical evidence that the $5\text{-}HT_3$ blocker, ondansetron (OND), attenuates fear responses provoked by a mixed cholecystokinin (CCK) receptor agonist. Clinical data implicate dysfunctional CCK neurotransmission in the pathophysiology of human panic disorder (PD). This study examined the effects of acute administration of OND on anxiety induced by the mixed CCK agonist, pentagastrin, in unmedicated PD patients. METHODS: Four PD patients (2 males, 2 females; mean age±SD=33±7 yrs) consented to participate in 2 clinical tests. Test were administered in a randomized, double-blind, cross-over manner and were separated by approximately 1 week. On one test day patients received an infusion of active OND 2 mg IV, given as an infusion over 20 minutes, followed by a bolus of pentagastrin 0.6 ug/kg IV. On a second test day patients were given placebo OND (PLAC) IV followed by pentagastrin 0.6 ug/kg IV. Within test measures included visual analog scale (VAS) anxiety, DSM-IV somatic panic symptoms, blood pressure, heart rate, and plasma cortisol, ACTH, prolactin, and growth hormone levels. RESULTS: Four of the 4 patients reported panic attacks during the OND test, while 3/4 had full panics during the PLAC test. The mean peak change from baseline anxiety levels on the OND test = 59±35 mm, and for the PLAC test = 63±25 mm (paired t-test t=0.3, df=3, p=0.8). Physiological and neuroendocrine data will be presented. DISCUSSION: The data from this protocol at present indicate that there is no attenuation of pentagastrin-induced anxiety with the 2 mg IV OND dose. Further testing at higher OND doses is indicated. If also negative this would suggest that there are not clinically significant 5-HT/CCK interactions in PD occurring via $5\text{-}HT_3$ receptors.
Supported in part by NIMH grant MH-45966 to Dr. Woods.

455.7

BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF PERIPHERALLY ADMINISTERED CHOLECYSTOKININ$_4$. A-K. Gilbert*, I. Jerabek, M. Bolongo, J.P. Boulenger, A. Cadieux and F.B. Jolicoeur. Depts. of Psychiatry and Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Qc, Canada, J1H 5N4.

Peripheral administration of cholecystokinin$_4$ (CCK$_4$) induces panic-like symptoms in humans. To better understand this phenomenon, we have examined the behavioral and neurochemical effects of peripherally administered CCK$_4$ in animals. After an intraperitoneal injection of CCK$_4$ (5µg), male hooded rats (230-300g) were placed in a standard plus maze and their behaviors, in terms of number of entries and time spent in closed and open sections of the maze, periods of motor immobility, and general motor activity were measured for a period of 15 min. Immediately after, dissection of the following regions was performed: nucleus accumbens, striatum, septum, hypothalamus, hippocampus, amygdala, frontal cortex and prefrontal cortex. In each region, concentrations of dopamine (DA), serotonin (5-HT) and of the metabolites HVA, DOPAC and 5-HIAA, were measured by HPLC-ECD. In the same regions, concentrations of neuropeptide Y-Like immunoreactivity (NPY-Li) were determined by radioimmunoassay. Results showed that peripheral administration of CCK$_4$ produces behavioral effects associated with anxiety in animals: decreases in general motor activity, increases in periods of motor immobility and reductions in number of entries and time spent in sections of the maze. Analysis of the biochemical data revealed prominent decreases in utilization of both DA and 5-HT in the prefrontal cortex, as estimated by metabolite-transmitter ratios, as well as a marked decrease in NPY-Li concentrations in the nucleus accumbens The results of the present study clearly demonstrate that peripheral administration CCK$_4$ has important central actions as evidenced by the observed anxiety-like behaviors in animals and by selective and region specific neurochemical changes.(Supported by the Reseau en Santé Mentale du Fonds de la Recherche en Santé du Québec).

455.8

EFFECTS OF INTRAPERITONEAL ADMINISTRATION OF CCK AGONIST AND ANTAGONIST ON ACOUSTICAL STARTLE RESPONSE, AN "ANXIETY" MODEL, IN RATS.

Y. Zhou[1], A. I. Arifuzzaman[2], S. A. Josselyn[2], P. W. Frankland[2], J. Bradwejn*[1] and F. J. Vaccarino[1+2] Clarke Institute of Psychiatry[1], Toronto Ont. M5T 1R8 and Department of Psychology[2], University of Toronto, Toronto, Ont. Canada. M5S 3G3,

It is known that systemic administration of CCK or CCK agonists induce panic attacks in humans and that CCK-B antagonists can inhibit this CCK-induced anxiety. Acoustic startle response has been used as an "anxiety" model both in human and in animals. Few studies have been reported on the effects of systemic injection of CCK with this model.

In this study, rats were administered Pentagastrin, a CCK-B agonist, intraperitoneally followed by trials of acoustic startle at 4 different intensities of acoustic stimuli (80, 90, 100, 110 dB). Startle amplitude was measured and analyzed. Pentagastrin (0.1 mg/kg), resulted in a significant increase of startle response at higher intensities of acoustic stimuli (100 dB, 110 dB); pretreatment of L-365,260 (0.1 mg/kg, i.p.), a CCK-B antagonist, significantly blocked Pentagastrin induced startle response increases.

These results provide more evidence that CCK is involved in the process of anxiety and demonstrate the usefulness of the startle model as a tool for anxiety research.

(This research is sponsored by The Medical Research Council of Canada, *Anxiety Group Grant* to J. B. & F. J. V..)

455.9

INTRA-ARCUATE MORPHINE MICROINJECTIONS INDUCE GRF-DEPENDENT AND PROTEIN SELECTIVE FEEDING.

M. R.Taube and F. J. Vaccarino*. Dept. of Psychology, University of Toronto, Toronto, Canada, M5S 1A1.

Growth-hormone releasing factor (GRF)-induced feeding behavior and GRF-induced growth hormone release (GH) are dependent on opiate actions (Dickson & Vaccarino, 1994; Wehrenberg et al., 1985). Opiates stimulate arcuate nucleus GRF neurons involved in GH release and were hypothesized to activate arcuate GRF neurons mediating appetitive behavior. Further, GRF injections into the suprachiasmatic nucleus/medial preoptic area (SCN/MPOA) during the light period increase protein intake, while carbohydrate and fat intake are unaffected (Dickson & Vaccarino, 1994). The present experiments further investigated the effects of morphine and GRF antibodies on macronutrient food selection, with specific emphasis on the role of the arcuate nucleus.

In both experiments, non-deprived male rats were given a three-choice macronutrient diet (protein, carbohydrate and fat). Experiment 1 was designed to explore the feeding behavior in a macronutrient paradigm resulting from intra-arcuate morphine injections (0, 1, 10, and 20 µg/0.5 µl). Eleven Wistar male rats received all doses and were subsequently tested for overall food intake, water intake, locomotion and feeding activity in four hour testing sessions. Following all three intra-arcuate morphine doses, protein intake was selectively increased. In Experiment 2, 16 Wistar male rats received intra-SCN/MPOA pretreatments (normal rabbit serum or GRF antibodies) followed by intra-arcuate morphine injections (1 µg/ 0.5µl) or saline injections. Results illustrated GRF antibody blockade attenuated morphine-induced protein selective feeding effect. Together, both studies suggest that an endogenous opioid trigger stimulates complimentary central-peripheral actions of GRF via the arcuate nucleus.

(Research sponsored by NSERC grant to F. J.V.).

455.10

MICROINJECTIONS OF NEUROTENSIN-(8-13) IN THE MEDIAL PREFRONTAL CORTEX STIMULATE MIDBRAIN DOPAMINE CELL FIRING. M.D. Fatigati, R.M. Anderson and P.-P. Rompré* Département de Psychiatrie, Université de Montréal, Montréal(Québec), Canada.

In a previous study, we showed that microinjections of neurotensin-(1-13), but not neurotensin-(1-8), into the medial prefrontal cortex (mPFC) stimulate midbrain dopamine (DA) cell firing. These findings suggest that the release of endogenous neurotensin (NT) in the mPFC activates an excitatory input to midbrain DA cells and that this effect is mediated by the high-affinity NT receptor. In this experiment, we further characterized the role of the physiologically-relevant NT receptor by studying the effectiveness of neurotensin-(8-13), the shortest active fragment. Experiments were performed on male rats anesthetized with urethane (1.2 g/kg). Dopamine cells were recorded with a single micropipette (2M NaCl, 0.1% Chicago Blue) and identified according to the previously described classical criteria. Rate of firing was recorded at least 5 min before, and 20 min after, the injection of 0.3 or 3 nmol/0.5 µl of NT-(8-13) or its vehicle; in some cells the sensitivity to the D2-like agonist, quinpirole (100 µg/kg, sc) was tested. A total of 26 cells were recorded, eight before/after vehicle microinjection and 18 before/after NT-(8-13) microinjection (0.3 nmol, n=9; 3 nmol, n=9). Eleven out of 18 cells were stimulated by NT-(8-13), and the increase in firing rate observed after 20 min ranged from 16 to 742% and from 43 to 380% with 0.3 and 3 nmol respectively. At each dose, the percentage increase in firing was inversely related to the basal firing rate. These findings further support a role for the high-affinity NT receptor in the activation of an excitatory input from the mPFC to midbrain dopamine cells.

Supported by a grant from MRC(Canada).

455.11

TYR-PRO-LYS-GLY-NH$_2$ (TYR-K-MIF-1) AFFECTS DRINKING BEHAVIOR IN RATS RELATIVE TO A SOLUTION'S PALATABILITY IN A TWO-BOTTLE CHOICE PARADIGM. G. A. Olson*, R. L. Bell, W. L. Nores, A. L. Vaccarino, A. J. Kastin, & R. D. Olson. Department of Psychology, University of New Orleans, New Orleans, LA 70148 & VA Medical Center, New Orleans, LA 70146.

We studied the effects of Tyr-K-MIF-1 on drinking behavior in male Sprague-Dawley rats presented with a two-bottle choice paradigm. In both experiments, tap water was available 12 hours per day, and food was available *ad libitum* throughout the experiment. Intake was measured in four 30-minute test periods during a two-hour test session after 12 hours of water deprivation. Both experiments had one between-subjects factor (doses) and three within-subjects factors (solutions, days, and test periods). In Experiment 1, thirty rats (n = 5) were injected IP for seven days with one of six doses of Tyr-K-MIF-1 (0.00, .001, 0.01, 0.10, 1.00, and 2.50 mg/kg), and twenty minutes after the injections the rats had access to both a 20% sucrose solution and tap water. Although the dose "effects" were not statistically significant, the analysis revealed that the simple effects of dose for the water data approached significance, $p = .099$, and the simple effects of dose for the sucrose data were clearly not significant, $p = .990$. In Experiment 2, twenty-five (n = 5) rats were injected IP for four days with one of five doses of Tyr-K-MIF-1 (0.00, .001, 0.01, 0.10, and 1.00 mg/kg). Twenty minutes after the injections, the rats had access to both a 9% ethanol solution (a 9% ethanol solution is aversive to naive animals) and tap water. There was a significant 4-way interaction (Dose x Solution x Day x Test Period): $F(36, 162) = 1.74$, $p = .011$, with an effect size of partial $\eta^2 = .279$ and a power of .996. Analyses of the simple effects revealed significant simple effects of dose for the ethanol data: $F(4,18) = 2.92$, $p = .050$, with an effect size of partial $\eta^2 = .394$ and a power of .666, but simple effects of dose for the tap water data were not significant ($p = .590$). Duncan's post hoc analyses revealed that the middle dose groups drank significantly more than the control and the higher dose groups. Tyr-K-MIF-1 was recently isolated from the human brain, and the stimulation of the consumption of less palatable solutions is the first behavioral effect found for this peptide.

455.12

ROLE OF BRAIN AMINES IN THE BEHAVIORAL RESPONSES ELICITED BY THYROTROPIN-RELEASING HORMONE IN RATS. D. Funk*, F.S. Hall, R.M. Post and A. Pert. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

The systemic or intrathecal administration of thyrotropin-releasing hormone (TRH) in rats elicits behaviors including wet dog shakes, jaw movements, tail rattle and locomotion. The neural substrates underlying these effects are not known. Since amine-containing pathways in the CNS, such as the dopamine (DA) and 5-hydroxytryptamine (5-HT) systems mediate the activational effects of many drugs, it was hypothesized that the behaviors elicited by TRH may also result from effects on brain amine function. To address this issue, experiments were carried out assessing the effects of lesions of the DAergic or 5-HTergic systems made via the microinjection of 6-hydroxydopamine or 5,7-dihydroxytryptamine, respectively, on the behavioral responses elicited by TRH. Lesions of the DAergic innervation of the NAC did not affect the behavioral responses to TRH (10 and 20 mg/kg i.p.), a finding in agreement with our observation in an *in vivo* microdialysis study that TRH (20 mg/kg i.p.) did not alter the release of DA in the NAC. Neither did lesions of the 5-HTergic projections ascending from the dorsal and median raphe nuclei affect the behavioral responses to TRH. In contrast, lesions of 5-HTergic neurons in the raphe magnus that project to the spinal cord reduced the numbers of wet dog shakes and jaw movements elicited by TRH. Taken together, these results suggest that changes in the activity of the 5-HTergic projections to the spinal cord, but not to the forebrain mediate some of the behavioral responses elicited by the systemic administration of TRH. They further suggest that changes in the release of DA in at least the NAC do not underlie the behavioral effects of TRH. Funded by NIMH.

455.13

BEHAVIORAL EFFECTS PRODUCED BY INJECTING A NEUROKININ-1 AGONIST INTO THE PERIAQUEDUCTAL GRAY OF THE RAT.

R. Mongeau* and C.A. Marsden. Dept. of Physiology and Pharmacology, Queen's Medical Centre, Univ. of Nottingham, U.K., NG7 2UH.

Substance P, a neurokinin-1 agonist abundant in the periaqueductal gray area (PAG), is a potent analgesic when infused into this region, while the excitatory D,L homocysteic acid (DLH) injected into the ventral and the dorsal PAG produces freezing and flight behaviors, respectively. A recent study indicated that infusion of the neurokinin-1 agonist GR73632 into the dorsal raphe (DR), a serotonergic nucleus located in the ventral PAG, produces behavioral freezing. It was thus of interest to investigate the behavioral effect of injecting GR73632 into subregions of the PAG. Rats were implanted with a guide cannula placed above the PAG. One week after, behaviors were monitored with a video camera and subsequently analyzed by computer. Behavioral freezing was produced when DLH (5 nmol) was injected into the ventral PAG situated rostrally to the DR, but infusion of GR73632 (10 pmol) into this region did not change the behavioral activity. Furthermore, the flight response elicited by stimulating the dorsal PAG with DLH (5 nmol) was not changed by this prior drug administration. In contrast, injection of GR73632 (10 pmol) into the dorsal PAG decreased the amplitude of the DLH-induced flight response (maximum speed: saline=0.80 $\pm$ 0.14 m/s; GR=0.42 $\pm$ 0.14 m/s; N=6), though this change was not found significant with this number of animals. Although the behavioral freezing that usually follow the DLH-induced flight response appeared prolonged, GR73632 injected into the dorsal PAG did not produce by itself behavioral freezing. Taken together these data suggest that neurokinin-1 receptors located outside the DR subdivision of the ventral PAG do not mediate by themselves behavioral freezing. The possibility that neurokinin-1 receptors in the dorsal PAG modulate the DLH-induced defense responses will be further assessed.

455.14

CENTRAL EFFECTS OF TACHYKININ NK RECEPTOR AGONISTS AND ANTAGONISTS ON THE PLUS-MAZE BEHAVIOR IN RATS.

T. C. de Lima* and S. J. Ribeiro. Department of Pharmacology, CCB, Universidade Federal de Santa Catarina, Florianópolis, SC, 88040-900, Brazil.

Previous results (Teixeira et al., 1996, Eur. J. Pharmacol., *in press*) had shown that the i.c.v. injection of agonists for NK_1 neurokinin receptors substance P (SP) and SP methyl ester (SPME) promoted anxiogenic effects in mice observed on a plus-maze. On the other hand, the central administration of the specific NK_1 receptor antagonist, FK 888, produced an anxiolitic profile of action. In the present study we investigated the influence of these treatments on rat behavior in the plus-maze, trying to determine the central site(s) involved in these effects. Cannulas were stereotaxically implanted in the lateral ventricles (AP= - 0.8 mm, ML= $\pm$ 1.5 mm, DV= - 3.5 mm) of adult Wistar rats under Equitesin® anaesthesia. After a recovery period the animals were injected i.c.v. with SP (10 pmol), SPME (10pmol), FK 888 (100 pmol) or the control solution (PBS).. Five min later they were tested on an elevated plus-maze for 5 min. SP caused an significant reduction of frequency and time spent on open arms ($P<0.05$), whereas SPME only reduced the time spent on these arms, when compared to the PBS values. FK 888 treatment did not modify frequency of entries or time spent on open arms. All the other parameters on the plus-maze test were not changed by the different treatments. Other animals were implanted with cannulas directed to the bed nucleus of the *stria terminalis* (BNST; AP= - 0.8 mm, ML= $\pm$ 1.4 mm, DV= - 7.8 mm) or basolateral nucleus of the amygdala (BLA; AP= - 2.0 mm, ML= $\pm$ 3.8 mm, DV= - 7.6 mm) and both received 10 pmol of SP i.c.v. SP decreased the number of entries on open arms when injected into the BNST and both the entries and time spent on open arms when administered into the amygdala, showing that the NK_1 receptor activation promotes an anxiogenic effect in the rat which seems to involve both nuclei.

Supported by Brazilian Goverment (CNPq and CAPES).

455.15

NEUROKININ NK-1 AND NK-3 RECEPTOR AGONISTS IN THE VENTRAL TEGMENTAL AREA (VTA) AND NUCLEUS ACCUMBENS (NAS) INDUCE ANALGESIA IN THE FORMALIN TEST.

N. Altier* and J. Stewart. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Canada.

We have shown previously that intra-VTA infusions of the SP analogue, DiMe-C7, cause analgesia in the formalin test for tonic pain. Here we report on the role of NK-1 and NK-3 receptor agonists injected into either the VTA or NAS on analgesia using this test. Rats were tested in the formalin test following an injection of 0.05 ml of 2.5 % formalin into one hind paw immediately after bilateral intra-VTA infusions of either the NK-1 agonist, GR-73632 (0.01, 0.1, or 1.0 nmol/rat), the NK-3 agonist, senktide (0.01, 1.0, or 3.0 nmol/rat) or saline. Two weeks later, the same rats were assessed in the tail-flick test for phasic pain. Tail-flick latencies were recorded following immersion of the tail in 55 C hot water at 10 min intervals for one hour immediately after intra-VTA infusions of either GR-73632 (1.0 nmol/rat), senktide (3.0 nmol/rat), or saline. In a second group of rats the effects of these compounds were test in the NAS. In both the VTA and NAS, the NK-1 and the NK-3 agonists caused dose-dependent analgesia in the formalin test, although the NK-1 agonist was more effective. Neither compound was effective in the tail-flick test. These findings support our earlier ideas that activation of SP during stress and pain may play a role in pain suppression. Furthermore, they suggest that SP systems in the forebrain, and particularly those that interact with the mesolimbic DA system, play an important role in the inhibition of tonic pain.

Supported by Medical Research Council, Canada

455.16

A COMPARISON OF THE DISTRIBUTION OF μ- OPIOID AND NMDA-R1 RECEPTORS IN THE PERIAQUEDUCTAL GRAY OF THE CAT.

K. Schubert-Reilly*, M.B. Shaikh, E. Faber, T.A. Teran and A. Siegel. Lab. of Limbic Syst. & Behav., Depts. of Neurosciences & Psychiatry, NJ Medical School, UMDNJ, Newark, NJ 07103 and Dept. of Biology, Kean College, Union, NJ 07983.

The midbrain periaqueductal gray (PAG) constitutes the lowest region of the neuraxis of the brain for the integration of defensive rage behavior (DR) in the cat. The PAG receives inputs from the medial hypothalamus that facilitate DR and from the central nucleus of amygdala that suppress DR. The functions of these pathways are mediated by μ-enkephalin and NMDA receptors, respectively. This study sought to identify and characterize the distributions of these receptors within the PAG. Immunohistochemical techniques were used to identify μ-enkephalin and NMDA-R1 receptors in the PAG of the cat. The results indicate that both types of receptors are located throughout the dorsal PAG--the critical region for DR. The distribution of these receptors display significant overlap within the rostral half of the dorsal PAG. However, μ-enkephalin receptors are much more prevalent within the ventral aspect of the rostral half of the PAG as well as in the caudal PAG. These results provide further support for the role of these receptor subtypes in regulating DR.

[Supported by NIH Grant NS 07941-26].

455.17

MUSCIMOL IN THE VENTRAL PALLIDUM DOES NOT BLOCK OPIOID-INDUCED LOCOMOTION IN THE DOPAMINE-DEPLETED NUCLEUS ACCUMBENS .

L. Churchill* and P. W. Kalivas. Dept. of Veterinary & Comparative Anatomy, Pharmacology & Physiology, Washington State University, Pullman, WA 99164-6520.

The output of the nucleus accumbens to the ventral pallidum is critical in mediating locomotion induced by opioids or dopamine in the nucleus accumbens, since locomotion is blocked by stimulating $GABA_A$ receptors in the ventral pallidum (Austin and Kalivas, Japan J Pharmacol **50**:487). To evaluate the role of this pathway in opioid-induced locomotion, male rats were dopamine lesioned bilaterally with 6-hydroxydopamine (4 μgm/3 μl/side over 12 min) in the nucleus accumbens 10 days prior to the start of the behavioral testing. The $GABA_A$ agonist, muscimol (0.02 nmol/side) was microinjected in the ventral pallidum 5 min prior to the μ-opioid agonist, Tyr-D-Ala-Gly-NmePhe-Gly-OH (DAMGO) (0.33 nmol/side) into the nucleus accumbens. Locomotion was measured as horizontal photocell counts for a maximum of 6 trials with a minimum intertrial interval of 3 days. Muscimol did not block DAMGO-induced augmentation of horizontal photocell activity in the dopamine-lesioned rats. In contrast, the motor stimulation produced by dopamine (53 nmol/side) microinjections into the nucleus accumbens of dopamine-depleted rats was significantly blocked by muscimol into the ventral pallidum. These results suggest that dopamine depletion in the nucleus accumbens changes the circuitry for DAMGO-induced motor activity by removing involvement of the GABAergic projection from the nucleus accumbens to the ventral pallidum. This work was supported in part by USPHS grants #DA06612 (LC) and #MH40817, #DA03906, #DA00158 (PWK).

456.1

CONTENT OF β-ENDORPHIN, ENKEPHALIN AND DYNORPHIN AND THEIR SPECIFIC mRNAs IN DISTINCT BRAIN REGIONS OF THE ALCOHOL-PREFERRING C57BL/6 AND ALCOHOL-AVOIDING DBA/2 MICE. N.T. Jamensky and C. Gianoulakis. Douglas Hospital Research Centre, McGill University , Verdun, PQ, Canada H4H 1R3.

The endogenous opioid system, which consists of neuropeptides and receptors, is believed to be implicated in the reinforcing effects of alcohol consumption. Genetically determined differences in this system have been observed between animals who have a preference or aversion to ethanol solutions. The objective of the present studies was to compare the content of endorphins, enkephalins and dynorphins in distinct brain regions using specific radioimmunoassays as well as the content of their specific mRNAs (proopiomelanocortin, proenkephalin, and prodynorphin, respectively) using *in situ* hybridization techniques in animals demonstrating a preference (C57BL/6) or aversion (DBA/2) towards ethanol consumption. Results indicated some differences in the brain β-endorphin and enkephalin systems between the C57BL/6 and DBA/2 mice. The most significant differences were observed in the dynorphin system where significantly higher levels of both peptide and mRNA were observed in the DBA/2 mice. Notably, the DBA/2 mice had higher levels of both dynorphin immunoreactivity and prodynorphin mRNA content in the nucleus accumbens. Interestingly, the release of dynorphin peptides in the nucleus accumbens has been shown to decrease dopamine release, and therefore may be acting as a negative reinforcer. It is possible then that the higher dynorphin and prodynorphin content observed in the DBA/2 mice compared to the C57BL/6 mice may be important in determining their low alcohol consumption. Supported by a grant from NSERC of Canada.

456.2

SEX DIFFERENCES IN THE PTZ-LIKE STIMULUS PRODUCED BY ETHANOL WITHDRAWAL AND NICOTINE. M. E. Jung, H. Lal and C. J. Wallis*, Department of Pharmacology and Substance Abuse Institute of North Texas, University of North Texas Health Science Center, Fort Worth, TX 76107

An animal model of anxiety [pentylenetetrazol (PTZ) discrimination] was used to compare the anxiogenic stimuli induced by ethanol withdrawal (EW) and nicotine (NIC) in male and female rats. Rats were trained to discriminate a neutral stimulus (saline) from the PTZ stimulus (16 mg/kg, IP) under a FR 10 schedule of food reinforcement. Rats were tested for their dose response to PTZ and NIC prior to treatment with a nutritionally balanced liquid diet (4.5% ethanol) for 10 days. Presence of an endogenous PTZ-like cue was tested 12-24 hr after EW (saline, IP). Challenge with nicotine at 36h was used to demonstrate protracted EW (PEW). At 12-24h EW more male rats (82%) responded on the PTZ lever than female rats (65%) after saline injection and the stimulus persisted longer in males than females. NIC (0.16 -2.5 mg/kg, IP) partially substituted for the PTZ cue in males (70%) and females (40%) and this effect was potentiated by PEW (males, 100% and females, 50%). These data suggest that female rats are less sensitive to the anxiogenic stimuli induced by EW and NIC in the PTZ paradigm Supported by NIAAA # AA09567 and #AA10545.

456.3

INDIVIDUAL DIFFERENCES IN LIMITED ACCESS, ORAL SELF-ADMINISTRATION OF ETOH BY RANDOMLY BRED RATS, M. J. Cichelli* and M. J. Lewis, Neurobehavior Lab, Temple University, Dept. of Psychology, Philadelphia., PA 19122

Consumption patterns in randomly bred rats trained to orally self-administer EtOH exhibit markedly bimodal distributions. Ethanol-naive, male Sprague-Dawley rats (with ad lib access to food and water) were presented for 30 minutes each weekday with two drinking tubes, one containing tap water and the other containing water mixed with ethanol. The initial ethanol concentration was 2%; this was gradually increased in 2% increments as each animal reached EtOH preference and consumption criteria. The rate of progression to higher ethanol concentrations, the highest concentration consumed and the amount of ethanol consumed appeared to be neither normally nor randomly distributed. By the end of 30 sessions, two quite distinct subpopulations were identified - one of animals which quickly acquired the ethanol drinking response and drank relatively large amounts of a 10% ethanol solution and another of animals which drank only small amounts of lower concentration ethanol solutions. After 60 exposure slowly acquired ethanol drinking behavior and were beginning to consume larger amounts. These results should be considered in future research using oral self-administration models in randomly bred rats.(Supported in part by Temple University Graduate Research Fellowship and Department of Psychology)

456.4

PERIODIC NALTREXONE AND INTAKE OF ALCOHOLIC BEVERAGE L. D. Reid*, L. R. Gardell and C.L. Hubbell, Lab. for Psychopharm., Rensselaer, Troy, NY 12180.

For over 2 months, 45 male Sprague-Dawley rats were on a daily regimen of presentation of alcoholic beverage designed to achieve moderately high levels of intake of ethanol. That regimen involved presentation for 2 hr a day of a palatable 12% ethanol-solution and water. After 3 weeks, rats took, on average, over 2.0 g/kg of ethanol during 2 hr a day. Then, the daily regimen was suspended and the rats had no opportunity to take ethanol for 30 days (abstinence). After abstinence and for an additional 45 days, the regimen was re-instated. Considering the rats' intakes before abstinence, three groups of rats were selected and one of three dosing regimens instituted. For one group, placebos were given daily for the first 35 days of reinstatement. For one group, naltrexone (NTX), 10 mg/kg, was given for the same 35 days. For the other group, NTX was given on Days 1-5, 11-15, 21-25 and 31-35 and placebos on the other days. NTX, as expected, reduced rats' intakes of alcohol when given daily without apparent decrement in its effectiveness. The effects of abstinence also reduced rats' intakes of alcohol and interacted favorably with NTX's effects to reduce markedly alcohol-intake. The group that received NTX periodically behaved as the group getting placebos when they received placebos and as the group getting NTX when they received NTX. These results have implications for the use of NTX as a pharmacotherapy for alcoholism. One implication, for example, is that NTX may not have effects that endure beyond NTX's administration and, to achieve enduring effects, abstinence and NTX-therapy should be combined. Supported by DA08937, NIDA.

456.5

DIFFERENTIAL EFFECTS OF INTRACEREBRAL μ- AND δ-OPIOID RECEPTOR ANTAGONISTS ON ALCOHOL SELF-ADMINISTRATION IN RATS. P. Hyytiä* and K. Kiianmaa. Department of Alcohol Research, National Public Health Institute, Helsinki, Finland.

Systemically administered μ- and δ-opioid receptor antagonists have previously been shown to suppress alcohol drinking in rats. However, limited information is available on the specific neuroanatomical locations of μ- and δ-opioid receptors relevant for alcohol reinforcement. The purpose of the present experiments was to assess the contribution of μ- and δ-opioid receptors in the nucleus accumbens (NAC), the basolateral amygdaloid nucleus (BLA), and the ventral tegmental area (VTA) to the regulation of alcohol self-administration. Wistar rats were trained to respond operantly for oral alcohol (10% w/v) during daily 30-min sessions using a saccharin fading procedure. Following the establishment of reliable baseline alcohol responding, the rats were implanted stereotaxically with bilateral guide cannulae either above the NAC, the BLA, or the VTA. After postoperative recovery, the rats were given intracerebral microinjections of a μ-opioid receptor antagonist CTOP (0-0.5 μg) or a δ-opioid receptor antagonist naltrindole (0-0.5 μg) and then tested for alcohol responding. Injections of naltrindole into both the NAC and the BLA significantly decreased alcohol-reinforced responding, whereas CTOP suppressed alcohol responding only when injected into the BLA. When injected into the VTA, neither CTOP nor naltrindole had significant effects on alcohol responding at the particular doses used. These findings suggest that opioid receptors at least in the NAC and the BLA are involved in the regulation of alcohol self-administration and that the contribution of μ- and δ-opioid receptors may differ from one brain area to another.

456.6

OPIOID ANTAGONISTS SUPPRESS THE ETHANOL-INDUCED INCREASE IN EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS OF AA RATS. K. Kiianmaa* and P. Hyytiä. Department of Alcohol Research, National Public Health Institute, Helsinki, Finland.

Our previous studies have shown that opioid antagonists administered icv suppress ethanol intake by alcohol-preferring AA (Alko Alcohol) rats. The purpose of the present study was to examine whether this could be related to the effect of opioid antagonists on ethanol-stimulated dopamine release in the nucleus accumbens. Ethanol naive AA rats were injected icv with CTOP (1 μg), a μ-receptor antagonist; naltrindole (1 μg), a δ-receptor antagonist; or naloxone (1 μg), a mixed opiate receptor antagonist, followed by an ip injection of ethanol (1 g/kg) 20 min later. The extracellular levels of monoamines in the nucleus accumbens were examined with *in vivo* microdialysis. Samples were collected from freely moving animals every 10 minutes, and the concentrations of the monoamines and their metabolites were determined in the dialysate with small pore HPLC. Ethanol significantly increased the extracellular levels of dopamine and DOPAC. This effect of ethanol was attenuated by the particular dose of CTOP and naltrindole examined but not naloxone. These results may have implications concerning whether the suppressive effect of opioid antagonists on ethanol intake may be mediated via the blockade of the ethanol-induced increase in dopamine release in the nucleus accumbens.

456.7

THE EFFECTS OF NALTREXONE ON A MULTIPLE SCHEDULE OF ETHANOL AND SACCHARIN SELF-ADMINISTRATION. K.L. Shelton* P.M. Beardsley and R.L. Balster Dept. of Pharmacology/Toxicology, Medical College of Virginia, Richmond, VA 23298-0310

Because naltrexone has show efficacy for the treatment of alcoholism, its effects in animal models of alcohol self-administration are of interest to determine the predictive validity of these procedures. We tested naltrexone using a multiple schedule of ethanol and saccharin self-administration in which selective drug effects on ethanol-reinforced responding could be assessed. Adult male Long-Evans rats were trained to lever press during daily 60-min sessions for 0.05 ml deliveries of 10% ethanol (w/v) and 0.1% saccharin solutions under an alternating fixed-ratio 4 multiple schedule. Doses of 0.3, 1.0 and 3.0 mg/kg naltrexone were then administered, i.p. 30 minutes prior to the experimental session for six consecutive days. Six-day blocks of pre-session saline injections intervened between each naltrexone dose. All three doses of naltrexone significantly ($p < 0.05$) suppressed ethanol self-administration in a dose-related manner. The 3.0 mg/kg dose of naltrexone also significantly suppressed saccharin self-administration. These results indicate that systemic administration of naltrexone can selectively suppress ethanol self-administration at doses and time points that do not decrease saccharin self-administration. We are currently testing morphine in the same multiple schedule model to determine if opposite effects are produced by an opiate agonist. (Research supported by NIAAA grants AA-08473, AA-05357 and NIDA grant DA-01442)

456.8

EFFECT OF A SINGLE DOSE OF DMI ON VOLUNTARY ETHANOL DRINKING AND LATERALIZED MONOAMINE LEVELS IN RATS. W. J. Shoemaker*, A. W. Deckel and D. M. Hebert. Alcohol Research Center, Dept. of Psychiatry, Univ. of Connecticut Health Center, Farmington, CT 06030.

Prior studies indicate that a single large dose of Desmethylimipramine (DMI; 25 mg/kg, i.p.) produces large increases in voluntary ethanol ingestion in Wistar rats. Using a sucrose-fading paradigm, the increased drinking was seen at all levels of ethanol and sucrose solutions. Furthermore, following the 8 weeks of drink testing, DMI-treated rats demonstrated decreases in serotonin and 5-HIAA in the raphe nuclei. The current study confirms a 15% increase in voluntary ethanol intake after 10 mg/kg DMI. In addition, a group of rats were given 25 mg/kg DMI for the first time following completion of the sucrose-fading regimen and stabilization on the 5% sucrose:10% ethanol solution. Again, DMI produced a 17% increase in ethanol intake over 7 days of testing. Brain analysis using HPLC and electrochemical detection revealed that DMI produced large decreases in the serotonin metabolite 5-HIAA in the raphe region. In addition, DMI produced increases in dopamine and DOPAC in the *right* , but not the left, nucleus accumbens. (This work supported by NIAAA grant #P50-AA3510)

456.9

ACUTE NALTREXONE TREATMENT REDUCES ALCOHOL CONSUMPTION AND PALATABILITY IN RATS. K.G. Hill* and S.W. Kiefer. Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506-5302.

Naltrexone, an opiate antagonist, has been shown to reduce alcohol consumption in rats. The presumed mechanism of this effect is that alcohol intake, which would normally produce opiate activity, is blocked and thus no longer reinforcing. In the present experiment, it was hypothesized that naltrexone treatment may also alter the palatability of alcohol. To test this idea, rats were treated with naltrexone (groups were given either 0, 0.5, 1.0, or 3.0 mg/kg, ip) and then tested 30 min and 60 min later for their taste reactivity responses to 10% alcohol. After four, daily reactivity tests, the rats were then tested for 10% alcohol consumption during restricted, 60 min, fluid access periods over four consecutive days. Rats received the same naltrexone dose as previously. Finally, four days of alcohol tests were then done without drug treatment. The results indicated that the 1.0 and 3.0 mg dose clearly decreased ingestive responding and increased aversive responding to 10% alcohol, thus supporting the hypothesis that naltrexone treatment alters the palatability of alcohol by making it more aversive. Naltrexone treatment, regardless of dose, also reduced alcohol consumption across the four test days although alcohol intake immediately returned to control levels once the drug treatments ceased. The present data are consistent with previous findings that naltrexone treatment can reduce alcohol intake in rats and that a contributing factor in this decrease may be reflected by the reduced palatability of the alcohol solutions. Research supported by the Department of Psychology, Kansas State University.

456.10

ETHANOL SELF-ADMINISTRATION IN CONSTANT DARKNESS ALTERS AMPLITUDE AND PHASE OF CIRCADIAN AND ULTRADIAN RHYTHMS. D.G. Harper, W. Tornatzky, and K. A. Miczek, Dept. of Psychology, Tufts University, Medford, MA 02155.

Chronic ethanol self-administration disturbs homeostatic rhythms which are relevant to the drug's physiological and behavioral effects. The purpose of the present experiment was to investigate further the emergence of ultradian rhythms during chronic ethanol self-administration in the absence of a light zeitgeber. We also compare these rhythmic changes to our previous findings (NS 485.6, 1995) in animals under a light-dark cycle. Subjects were 8, adult male Long-Evans rats implanted with a telemetry sender (Data Sciences) which monitored temperature, heart rate and activity. Subjects were studied under 4 basic conditions; 1) light schedule - free feeding, 2) constant darkness - free feeding, 3) constant darkness - restricted feeding and 4) constant darkness - restricted feeding - EtOH drinking. Animals were studied for 18-21 days during each treatment condition. Animals self-administered 10% ethanol at 10:30 for 30 min/day for 3 weeks and then were presented with ethanol at differing concentrations (5%, 10% and 20%) for 5 days at each concentration. Animals were then returned to 10% concentration before withdrawal. During freerunning conditions circadian acrophase of temperature and heart rate advanced. Ethanol self-administration was characterized by significant delays in circadian acrophase of temperature and heart rate followed by significant increases in the hemicircadian (2 cycle/day) amplitude of temperature and heart rate that were not seen during constant darkness and food restriction. These changes were complete at the end of three weeks of ethanol self-administration, and are similar to changes observed under a light schedule. Additionally, the phase times of 2,3,4 and 5 cycle/day rhythms changed to coincide with the time of ethanol availability. Supported by USPHS R37AA05122 and R01DA02632.

456.11

I.V. ETHANOL SELF-ADMINISTRATION IN β–ENDORPHIN KNOCK-OUT MICE. N.J. Grahame*, C.C. Fjeld, M.J. Low, and C.L. Cunningham. Dept. of Behavioral Neuroscience and VIABR, Oregon Health Sciences University, Portland, OR 97201.

The endogenous opioid peptide, β–endorphin, has been implicated in the reinforcing effects of ethanol (e.g., Gianoulakis, 1993). In order to study the importance of this peptide in ethanol reinforcement, while ruling out effects arising from differences in ethanol taste receptivity, we assessed i.v. ethanol self-administration in β–endorphin knock-out and wild-type mice. β–endorphin knock-outs were developed by introducing a premature translational stop codon into the proopiomelancortin (POMC) gene through targeted mutagenesis. The mutant allele was then backcrossed for 7 generations onto a C57BL/6J background. Homozygous mice expressing the mutated POMC allele have normal expression of ACTH and melanocyte-stimulating hormones, but completely lack β–endorphin. Male mice were allowed to nosepoke for 75 mg/kg ethanol in 5*u*l jugular vein infusions during 2-h daily free-operant sessions. Two nosepoke holes were present, only one of which was active. Animals were reinforced on an FR-3 schedule with a 2-s time-out period after each infusion. During 9 sessions, knock-out animals acquired ethanol-reinforced responding, preferentially nosepoking in the active hole, and taking an average of 1.4 g/kg of ethanol per session by Day 9. Wild-type mice, however, did not preferentially respond on the ethanol-reinforced side, and took only 0.6 g/kg ethanol by Day 9, indicating that i.v. ethanol served as a reinforcer only for knock-out mice. Thus, in the absence of taste factors, β–endorphin is not necessary for ethanol reward. AA10760; HD 30236.

456.12

ALPHA-MELANOCYTE STIMULATING HORMONE (α-MSH) AFFECTS ETHANOL INTAKE IN A TWO-BOTTLE CHOICE PARADIGM. R. L. Bell*, W. L. Nores, R. D. Olson, G. A. Olson, & A. J. Kastin. Department of Psychology, University of New Orleans, New Orleans, LA 70148 & V.A. Medical Center, New Orleans, LA 70146.

We previously showed that α-MSH reduced ethanol intake dose-dependently in rats (Nores et al., 1995). Further, Krishnan et al. (1991) showed that an α-MSH fragment ($ACTH_{4-10}$) reduced ethanol intake in rats. α-MSH has been shown to affect attention and learning (Sandman et al., 1980). Consequently, we investigated the role of aversive learning in α-MSH's effects on ethanol intake. Eighteen male Sprague-Dawley rats had access to both tap water and an ethanol solution, in a limited access (1 hour/ day) two-bottle choice paradigm, for nine days. The ethanol solution was 5% (v/v) for the first five days, and 10% (v/v) for the next four days. In naive rats, 5% ethanol is subthreshold aversive and 10% ethanol is aversive. Water and food were available *ad libitum* throughout the experiment. Measures of food intake, 23 hour water intake, and body weight were taken before the treatments. There were two treatment groups (n = 9). Treatment involved an IP injection of either 1.00 mg/kg α-MSH or an equivolume injection of diluent. Injections were made twenty minutes before the one-hour test period (9:20 a.m. to 10:20 a.m.). A 2 x 9 (Treatment x Days) mixed analysis of variance was performed, with treatment as the between-subjects factor and days as the within-subjects factor. Statistical analyses revealed a significant Treatment x Days interaction: $F(8, 128) = 2.11$, $p = .039$, with an effect size of partial $\eta^2 = .117$ and a power of .827. The simple effects of treatment within each day were analyzed, with only that for day nine being significant: $F(1, 16) = 5.27$, $p = .036$, with an effect size of partial $\eta^2 = .248$ and a power of .576. No other significant results were obtained. Thus, while there were no significant differences in group intakes for the first eight days, α-MSH decreased intake of the aversive 10% ethanol solution after repeated administration.

456.13

AMPEROZIDE, A 5-HT-2A RECEPTOR ANTAGONIST, IS MORE EFFECTIVE IN REDUCING ALCOHOL INTAKE THAN FLUOXETINE OR DULOXETINE. Amir H. Rezvani*, D.H. Overstreet, and R.A. McArthur[1], Skipper Bowles Center for Alcohol Studies, Univ. North Carolina, Chapel Hill, NC 27599-7178 and [1]CNS Research, Pharmacia & Upjohn, Nerviano (MI), Italy.

Voluntary consumption of large amounts of alcohol has been demonstrated in several strains of rats, including Fawn-Hooded (FH) rats and the selectively bred alcohol-preferring (P), high alcohol drinking (HAD) and alcohol-accepting (AA) rats. Previously, we showed that these strains exhibit differential responses to a number of serotonergic drugs, including the releaser fenfluramine. The present communication compares the 5-HT-2A receptor antagonist, amperozide (1, 2.5, 5, 10 mg/kg), with the 5-HT reuptake inhibitor, fluoxetine (5, 10, 15 mg/kg) and the 5-HT and NE reuptake inhibitor, duloxetine (5, 10, 15 mg/kg). After establishment of stable baselines, rats were injected subcutaneously with amperozide or intraperitoneally with fluoxetine or duloxetine. The 24-hr intakes of food, water and alcohol were recorded. All drugs dose-dependently reduced alcohol intake, but only amperozide was selective, inhibiting alcohol intake at doses which did not affect food or water intake. Unlike findings with other agents, the AA rats were relatively more sensitive to the effects of amperozide, while the HAD rats tended to be more resistant to the effects of all three agents. These findings indicate that although alcohol-preferring rat strains are differentially sensitive to several serotonergic drugs which suppress alcohol intake, amperozide selectively suppresses alcohol intake in a dose-dependent manner in all strains tested. Therefore, it is a more effective suppressor of alcohol intake than is fluoxetine or duloxetine.

Supported by Pharmacia & Upjohn.

456.14

ETHANOL SELF-ADMINISTRATION BY MALE AND FEMALE C57BL/6 MICE: A COMPARISON OF LIMITED ACCESS AND OPERANT PROCEDURES. B.M. Kelley, A.L. Bandy, W.Q. Boggan* and L.D. Middaugh. Dept. of Psychiatry, CDAP, Med. Univ. of S.C., Charleston, S.C. 29425.

The present study compared ethanol intake in a limited access paradigm with its intake and reinforcing effect in an operant self-administration paradigm. Aside from the operant component the methodologies were identical for the two experiments. Amounts of ethanol consumed (0, 3, 6, 9, 12, and 15%) by mice maintained at reduced body weight during 30 min tests were determined after and before daily feeding as well as when water was present or absent during limited access testing. In both experiments mice consumed large amounts of ethanol (9-13 g/kg, operant; 6-11 g/kg, limited access) and consumed more when fed before rather than after feeding. During limited access testing they consumed more ethanol when water was present than when absent. Under most conditions females consumed more ethanol than males in the limited access experiment, but less than males in the operant experiment. Both male and female mice lever-pressed for ethanol at all concentrations and increased lever-responses as demands increased on FR and PR schedules of reinforcement. The results indicate that ethanol is a positive reinforcer for both male and female C57 mice, that its reinforcing effects are not simply related to caloric value, and that gender differences in ethanol consumption are influenced by the associated response requirements. (DA-08034 & AA-10761.)

456.15

QUANTITATIVE EEG PREDICTS RELAPSE IN PATIENTS WITH CHRONIC ALCOHOLISM G. Winterer, B. Klöppel, A. Heinz, M. Ziller, L.G. Schmidt, W.M. Herrmann and H. Rommelspacher* Department of Psychiatry, Free University of Berlin (Germany)

Objectives: In clarifying the potential use of Quantitative Electroencephalography (QEEG) for the prediction of relapse, we investigated 29 abstainers and 49 relapsers. Methods: Electrode positions according to the 10/20 system. Time constant 0.3 s, 70 Hz low-pass filter, 167 Hz sampling rate. One representative 30 s artefact-free EEG-segment was analysed of each patient. For quantitative analysis (pre-processing) we chose spectral as well as Hjorth's parameters and the correlation dimension of Grassberger-Proccacia. On the basis of these parameters, we first created a discriminant function (approximated F-test for Wilk's lambda) with random and stepwise introduction of variables, first comparing 24 relapsers and 14 abstainers. Afterwards, the best fitting discriminant function was applied to the remaining 25 relapsers and 15 abstainers. For comparison, we analysed the 2x2 groups with an artificial neural network approach (feed-forward multilayer perceptron): Variable input vector, 1 hidden layer (2-20 neurons), two output units. Various input selection methods. Results: Independent testing of the discriminant function (5 variable model), gained from the first groups of relapsers and abstainers, resulted in an overall correct classification of 75% (sensitivity: 88%, specifity 54%) and especially showed a frontally pronounced EEG-desynchronization with decreased absolute frontocentral alpha-activity, relative left frontal theta-activity and an increased left frontal correlation dimension in relapsers. The neural network approach (9 variable model) increased the overall correct classification rate up to 85% for the independent test-set (sensitivity 100%, specifity 60%). Conclusions: QEEG seems to be highly sensitive in the detection of relapsers. Neural network approaches may enhance the discrimination power, when compared with linear statistics. Compared with the abstainers, relapsers obviously show a frontally pronounced functional disturbance.

Sponsored by the DFG (Deutsche Forschungsgemeinschaft) (He916/7-1) and by the European Commission (BMH1-CT-94-1129)

456.16

EFFECTS OF CHRONIC ETHANOL INGESTION ON MID-LATENCY AUDITORY EVOKED POTENTIALS DEPEND ON LENGTH OF EXPOSURE. E.A. Floyd*, J.D. Reasor, E.L. Moore, and H.K. Rucker. Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157 and the Dept. of Physiology, Meharry Medical College, Nashville,TN 37208.

We hypothesized that chronic ethanol ingestion is associated with modifications in components of mid-latency auditory evoked potentials (MAEPs). To test this, male Long-Evans rats were administered 10% ethanol in drinking water as the sole fluid source for three, six, or nine months. MAEPs were obtained and compared to age-matched control groups. MAEPs were obtained from additional rats after four weeks of abstinence. Three months of ethanol exposure was associated with increased latencies and amplitudes of Na and Pa. MAEP components recovered and returned to control values after four weeks abstinence following three months of EtOH exposure. Few significant differences were observed in the ethanol-treated or abstinent group after six months exposure. However, nine months of ethanol exposure revealed a significant increase in latencies and decrease in amplitudes of both Na and Pa components. After 4 weeks abstinence, the Na and Pa component peak latencies appeared earlier than controls. Na and Pa peak amplitudes were slightly greater than the ethanol-treated group, however, they did not recover to control values. These findings suggest that chronic ethanol consumption may produce time-dependent structural and/or neurochemical alterations in substrates for cortical information processing which may be irreversible. In the present paradigm, this irreversibility may occur after 6 or more months of ethanol intake, and may be detected with the use of MAEPs.

Supported by GAANN Award #P200A40516 to EAF, NIH T32MH19843-02, and ONR N00014-91-J-1372

456.17

DOES MENSTRUAL PHASE OR PREMENSTRUAL SYMPTOMS AFFECT ALCOHOL CRAVING OR RELAPSE IN WOMEN SEEKING TREATMENT FOR ALCOHOL DEPENDENCE? L. Williams-Hemby*, A.R. Childress, L. Spaulding, J. Volpicelli and C.P. O'Brien Treatment Research Center, University of Pennsylvania, Philadelphia, PA 19104

Numerous studies suggest that women increase their alcohol consumption at particular phases of the menstrual cycle. Specifically, several reports indicate that women with premenstrual symptoms increase their alcohol consumption during the late luteal phase of the cycle when premenstrual symptoms are most severe. Additional studies suggest that women with moderate to severe premenstrual symptoms are at greater risk for developing alcohol dependence. Taken together, these findings suggest that the late luteal phase of the menstrual cycle may be a vulnerable time for relapse in women seeking treatment for their alcohol abuse, particularly in those women who have moderate to severe premenstrual symptoms. The goal of the present study is to investigate the effect of menstrual cycle phase and symptom severity on the course of treatment in women enrolled in a double-blind, placebo-controlled naltrexone clinical trial for alcohol dependence. Women will be asked to complete a daily symptom rating which will track cycle phase, emotional and physical symptom severity, intensity of alcohol craving and the amount of alcohol use. This instrument will allow us to determine if alcohol craving or relapse occur more frequently during a particular menstrual phase, as well as to determine whether particular menstrual symptoms might be a risk factor leading to excess alcohol craving and/or relapse. Supported by T32 DA 07241 and AA07517

456.18

COGNITIVE DYSFUNCTION AND PLASMA BRANCHED-CHAIN AMINO ACID/AROMATIC AMINO ACID MOLAR RATIO IN ALCOHOLICS. T. Saito*, K. Kobatake, T. Shirasaka, K. Yanbe and H. Ozawa. Sapporo Medical University, Sapporo 060 Japan.

The plasma branched-chain amino acid (BCAA)/aromatic amino acid (AAA) molar ratio was examined in relation to cognitive dysfunction and alcohol withdrawal symptoms in 40 alcoholics. Plasma amino acids were examined with a programmed Hitachi 835-50 Amino Acid Analyzer. Cognitive functions were examined using the number connection test and performance tests (digit symbol test, block design test) of the Wechsler adult intelligence scale (WAIS). Plasma amino acids and cognitive function test were performed after alcohol withdrawal state. The BCAA/AAA molar ratio in alcoholics was significantly lower than that in controls (normal volunteers). The BCAA/AAA molar ratio in patients with transient hallucinations or delirium tremens was significantly lower than that in patients without these symptoms. The BCAA/AAA molar ratio had correlations with the values of liver function tests. The BCAA/AAA molar ratio had positive correlations with the scores of the performance tests of WAIS in patients with delirium tremens. These results indicate that abnormalities of amino acid metabolism caused by liver damage may have an important role in the cognitive dysfunction of alcohol dependence.

456.19

EXPRESSION OF $GABA_A$ RECEPTOR ISOFORMS IN ALCOHOLISM. J. M. Lewohl*, D. J. Crane and P. R. Dodd, *CRC Laboratory, Royal Brisbane Hospital Foundation, Q 4029, and Faculty of Science and Technology, Griffith University, Q 4111, Australia.*

Chronic and excessive alcohol abuse leads to a selective loss of neurones in the superior frontal cortex and is accompanied by alterations in $GABA_A$ receptors. These receptors are pentameric protein complexes composed of a number of isoforms designated α_{1-6}, β_{1-4}, γ_{1-3}, δ and ρ_{1-2}. Combinations of subunits form receptors which differ in their pharmacological properties and regional distribution. Thus, variations in subunit expression may account for the differences in GABA neurotransmission characteristic of long-term alcoholism. The expression of the α isoforms of the $GABA_A$ receptor was analysed by competitive RT/PCR using primers designed from the conserved regions of α_{1-3}. Total RNA was extracted from the superior frontal and motor cortices of 6 control (<20g ethanol/day), 6 alcoholic and 4 alcoholic-cirrhotic cases (>80g ethanol/day) matched for age and post-mortem delay. No significant differences were found in the expression of α1, α2 or α3 between case groups or between the frontal and motor cortices within each case group. However, the profile of α expression was shown to be significantly different between the case groups ($F_{8,52}$=3.37, p=0.0035) with no difference between frontal and motor cortices. The difference in α expression may reflect an alteration in the subpopulation of $GABA_A$ receptors in the brains of alcoholics irrespective of cirrhosis. The subunit composition and functional characteristics of $GABA_A$ receptors in alcoholic and alcoholic-cirrhotic cases is currently being elucidated.

Supported by NHMRC and Griffith University

456.20

DEPRESSION, ALCOHOL USE, AND NEUROCHEMICAL LEVELS IN A SAMPLE OF COLLEGE UNDERGRADUATES. J. Walter, M. D. Anderson, V. Chandhoke, & J. M. Flinn*. Department of Psychology & The Shared Research Instrumentation Facility, George Mason University, Fairfax, Virginia 22030.

This research seeks to examine the relationship between serotonin and dopamine pathways and depression and alcohol use, in a sample of undergraduate male college students from two universities. Subjects were divided into minimal (n=15) and heavy (n=17) drinking groups, based on current alcohol use as evaluated using the Substance Abuse Subtle Screening Inventory (SASSI), Alcohol Use Disorders Identification Test (AUDIT), and self-report of drinking patterns. Some students were drawn from an alcohol education program, others volunteered to meet a research requirement. Of the volunteer group, over 30% were determined to be at high risk for alcohol abuse. Depression was assessed using Beck's Depression Inventory (BDI). Tryptophan and tyrosine pathways were evaluated using HPLC measures of urine.

Significant correlations were observed between alcohol consumption and depression as measured by AUDIT and BDI respectively ($r = .377, p < .025$). Analyses of neurochemical levels to date show that depression correlated significantly with urinary levels of dopamine ($r = -.336$, $p < .05$), HIAA ($r = -.310$, $p < .05$), and there was a trend for HVA ($r = .258$, $p < .10$). However, no significant correlations were observed between AUDIT scores and these neurochemicals.

DRUGS OF ABUSE: AMPHETAMINES III

457.1

REVERSE DIALYSIS APPLICATION OF AMPHETAMINE PRODUCES A DELAYED, SUSTAINED INCREASE IN EXTRACELLULAR GLUTAMATE LEVELS IN RAT VENTRAL TEGMENTAL AREA. M.E. Wolf* and C.J. Xue. Department of Neuroscience, Finch Univ. of Health Sciences/The Chicago Medical School, North Chicago, IL 60064.

Considerable evidence supports the idea that excitatory amino acid (EAA)-containing projections from the prefrontal cortex (PFC) to the ventral tegmental area (VTA) are important in the development of behavioral sensitization to amphetamine (AMPH). We have shown previously that systemic AMPH produces a delayed, sustained increase in extracellular glutamate levels in the VTA that reaches statistical significance 2-3 hrs after AMPH injection and is prevented by haloperidol (Xue et al., J. Neurochem., in press). We speculated that repeated elevation of VTA glutamate levels upon repeated AMPH injection elicits compensatory changes in EAA receptors in the VTA which contribute to the development of behavioral sensitization. In the present study, reverse dialysis application of AMPH in the VTA (1-100 µM for 1 hr) was similarly found to elicit a delayed, sustained increase in extracellular glutamate levels which commenced after return to normal perfusion solution. This effect was mimicked by SKF 38393 but not quinpirole, prevented by SCH 23390, and observed in both water- and AMPH-pretreated rats. Both systemic and intra-VTA AMPH elicit sensitization when administered repeatedly, so neurochemical changes associated with both routes of administration, such as the delayed increase in VTA glutamate levels, are good candidates for a role in sensitization. Interestingly, some rats exhibited a decrease in glutamate levels *during* reverse dialysis application of AMPH in the VTA, an effect not noted after systemic AMPH. Experiments are in progress to further characterize this phenomenon. Supported by USPHS grant DA07735 from NIDA.

457.2

THE EFFECT OF REPEATED AMPHETAMINE ADMINISTRATION ON THE EXPRESSION OF NMDAR1 mRNA IN RAT VENTRAL TEGMENTAL AREA, NUCLEUS ACCUMBENS AND MEDIAL PREFRONTAL CORTEX. W.X. Lu*, L.M. Monteggia, C.-J. Xue and M.E. Wolf. Department of Neuroscience, Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064

Behavioral sensitization to amphetamine (AMPH) is associated with alterations in glutamate transmission in perikarya (ventral tegmental area, VTA) and terminal regions (nucleus accumbens, NAc) of the mesoaccumbens dopamine system. The medial prefrontal cortex (mPFC) is an origin of glutamate projections that regulate the mesoaccumbens dopamine system. We have recently found changes in mRNAs for AMPA receptor subunits in NAc and mPFC of AMPH sensitized rats (W. Lu et al, J. Neuroscience, submitted). The present study examines the expression of mRNA for the NMDA receptor subunit NR1 in VTA, NAc and mPFC of AMPH sensitized rats. Rats are treated for 5 days with 5 mg/kg/day AMPH sulphate or vehicle (control), and perfused 3 or 14 days after last injection. A novel in situ hybridization method (Lu et al, J. Neurosci. Meth. 65:69-76,1996) with two ^{35}S-labeled oligonucleotide probes (complementary to nucleotides 375-420 and 1011-1056; DuPont, PA) was used to examine NR1 mRNAs in NAc and mPFC. NIH Image computer software was used for quantitative analysis of autoradiographs. In NAc, levels of NR1 mRNA were decreased in the repeated AMPH group after 14 days withdrawal (74.8±7.7% of control group; $P<0.05$), but not after 3 days withdrawal. In mPFC, no change in NR1 mRNA was found at 3 days withdrawal, but a significant reduction was observed in the repeated AMPH group tested after 14 days withdrawal (76.1±7.1% of control group; $P<0.05$). Because of relatively low NR1 mRNA levels in VTA, parallel studies are in progress using alternative techniques with greater sensitivity, including nuclease protection assays and in situ hybridization with an NR1 ribonucleotide probe (801 StyI-1401 XhoI). Our results, along with others reported previously, suggest that adaptations in excitatory amino acid transmission accompany AMPH sensitization. Supported by NIDA grant DA 07735.

457.3

EFFECTS OF THE AMPA RECEPTOR ANTAGONIST NBQX ON BASAL AND AMPHETAMINE-INDUCED INCREASES IN LOCOMOTION AND RAT NUCLEUS ACCUMBENS DOPAMINE OVERFLOW. P. Vezina*. Department of Psychiatry, The University of Chicago, Chicago, IL 60637.

In vivo microdialysis in the freely moving rat was used to assess the effects of the AMPA receptor antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline, 30 mg/kg, i.p.) on the nucleus accumbens (NAcc) dopamine (DA) overflow and locomotor activity observed following acute systemic injections of either saline or amphetamine (1.0 mg/kg, i.p.). Measurement of the DA recovered in 20 min dialysate samples was assayed with HPLC-EC. Locomotor activity was estimated by photocell beam interruptions. NBQX produced no effect on extracellular levels of DA in the NAcc nor did it produce locomotor effects of its own. NBQX also did not affect the increase in NAcc DA levels produced by amphetamine when the latter was injected 40 min later. However, NBQX completely blocked the increased locomotion produced by amphetamine. These findings are consistent with the view that NBQX can act at sites postsynaptic to DA neuron terminals in the NAcc to influence acute amphetamine-induced locomotion. In a separate experiment, NBQX repeatedly injected with amphetamine blocked the development of locomotor sensitization normally produced by amphetamine. These results suggest an additional role for AMPA receptors in the production of locomotor sensitization by amphetamine. AMPA receptors may be recruited by the DA that is released by amphetamine in the ventral tegmental area, where it is known to act to produce locomotor sensitization.

Supported by a grant from the Ontario Mental Health Foundation.

457.4

EFFECT OF REPEATED AMPHETAMINE TREATMENT ON mRNA LEVELS FOR AMPA GLUTAMATE RECEPTOR SUBUNITS IN VENTRAL MESENCEPHALON. M.T. Bardo*[1], B.A. Mattingly[2], P.M. Robinet[1] and J.E. Margulies[3]. [1]Dept. Psychol., Univ. Kentucky, Lexington, KY 40506, [2]Dept. Psychol., Morehead St. Univ., Morehead, KY 40351, [3]Bekesy Lab., Univ. Hawaii, Honolulu, HI 96822.

Previous work has shown that repeated amphetamine treatment decreases mRNA levels for the GluR2 AMPA glutamate receptor subunit in the nucleus accumbens. The present study examined the effect of amphetamine on GluR2 mRNA levels in the ventral mesencephalon containing the A9/A10 dopaminergic cell bodies that innervate the striatum and nucleus accumbens. Rats received amphetamine (1.25 mg/kg, i.p., once daily) for 10 consecutive days and were tested for locomotor activity after each injection. The locomotor stimulant effect of amphetamine was enhanced across repeated injections; on the tenth injection, amphetamine treated rats displayed a 5-fold higher level of activity than saline controls. Following the last locomotor test, rats were killed and the ventral mesencephalon was dissected for analysis of GluR2 mRNA levels using an RNase protection assay in which a riboprobe for GluR2 was transcribed from a 3' fragment of GluR2 cDNA. Amphetamine treated rats exhibited a decrease in GluR2 mRNA levels in the ventral mesencephalon compared to saline controls. These results suggest that regulatory processes involving AMPA receptor subunits in the ventral mesencephalon, as well as in the nucleus accumbens, may be related to the development of locomotor sensitization produced by repeated amphetamine treatment.

(Supported by USPHS grants DA-05312 and DA-09687, and by grant no. 954582 from the Hawaii Community Foundation.)

457.5

AMPHETAMINE PERFUSION INTO THE NUCLEUS ACCUMBENS INCREASES EXTRACELLULAR GLUTAMATE. A. Dalia, D.M. Weinstein, N.J. Urestky, and L.J. Wallace*. College of Pharmacy, The Ohio State University, Columbus, OH 43210.

Psychostimulant drug induced locomotor activity (LMA) and reward appear to require activation of dopaminergic receptors and glutamatergic receptors in the nucleus accumbens (NAC). Consistent with this hypothesis, systemic cocaine or amphetamine increases extracellular glutamate in the NAC as measured by in vivo dialysis. This study determines whether activation of dopaminergic receptors in the NAC increases glutamate levels in this brain area. Amphetamine administration through a dialysis probe stimulated LMA and increased extracellular glutamate. Both responses were blocked by prior systemic injection of the non-specific DA antagonist haloperidol, by the D1 antagonist SCH23390, or by the D2 antagonist eticlopride. Administration of the D1 agonist SKF38393 and the D2 agonist quinpirole together through the dialysis probe stimulated LMA and increased extracellular glutamate. The glutamate uptake inhibitor DI-threo-*B*-hydroxy-aspartate also increased extracellular glutamate but did not stimulate LMA. Systemic caffeine stimulated LMA without increasing extracellular glutamate in the NAC. These data demonstrate that the combined activation of D1 and D2 dopaminergic receptors in the NAC increases the level of extracellular glutamate in this area and that increased levels of glutamate do not correlate with stimulation of LMA. (NIH grants DA06776 and DA7722)

457.6

CHARACTERIZATION OF SINGLE-UNIT ACTIVITY IN THE SUBSTANTIA NIGRA PARS RETICULATA OF FREELY MOVING RATS. J. M. Gulley* and G. V. Rebec. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN 47405.

We have previously shown that the firing rates of motor- and nonmotor-related neurons in the striatum of ambulant rats increase and decrease, respectively, after systemic administration of amphetamine and other psychomotor stimulants (Haracz et al, Neurosci. Biobehav. Rev., 17:1, 1993; White et al., J. Neural Transm., 102:99, 1995). In the present study, we extended this line of investigation to the substantia nigra pars reticulata (SNr), which receives both direct and indirect projections from the striatum. Under conditions of quiet rest, SNr neurons discharged at relatively rapid rates (20-40 impulses/sec), but showed complex changes (increases, decreases, or no changes) during periods of movement. Amphetamine (1.0 mg/kg, sc), in contrast, routinely produced increases in firing rates, and these effects were blocked by subsequent administration of haloperidol (1.0 mg/kg, sc). These preliminary results indicate that no simple relationship exists between neuronal activity in the SNr and that seen in the striatum.

Supported by NIDA (DA-02451)

457.7

ELECTROPHYSIOLOGICAL AND CONFOCAL STUDIES ON AMPHETAMINE ELICITED POTENTIAL CHANGES IN SNAIL NEURONS. M.C.Tsai*and Y.H.Chen. Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan.

The role of the intracellular calcium ions on the bursting firing of action potentials in central neurons elicited by amphetamine was studied in the identified RP4 neuron of the African snail, *Achatina fulica* Ferussac. Confocal microscopy revealed the localization of the neuron. Bursting activity of the potentials was elicited by amphetamine if amphetamine was applied either extracellularly or intracellularly. The bursting firing of potentials was decreased following either intracellular injection with EGTA or magnesium ions or extracellularly application of lanthanum ions. Intracellular injection of calcium ions elicited bursting activity of potentials in the RP4 neuron, however, intracellular injection of calcium ions did not prolong the duration of the bursting firing of action potentials elicited by amphetamine. It is concluded that the bursting activity of potentials elicited by amphetamine is associated with intracellular calcium ions. (Supported by grants NSC-84-2331-B-002-107 and NSC-85-2331-B-002-289 from National Science Council, Taipei, Taiwan, R.O.C.).

457.8

DOPAMINE BUT NOT GABA INHIBITION IS BLUNTED IN THE NUCLEUS ACCUMBENS CORE FOLLOWING REPEATED ADMINISTRATION OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA). S. R. White*, T. Obradovic and K. M. Imel. Dept. of Veterinary and Comparative Anatomy, Pharmacology and Physiology and Program in Pharmacology and Toxicology, Washington State University, Pullman, WA 99164.

We have previously reported that repeated exposure to the euphoria-inducing drug MDMA (ecstasy) attenuates the inhibitory effects of dopamine (DA) and serotonin (5HT) on glutamate-evoked firing of neurons in the nucleus accumbens (Soc. Neurosci. Abst., 1995, 21:969). The purpose of the present study was to determine whether the MDMA effect is specific for monoamines or whether MDMA-pretreatment also blunts the inhibitory effect of GABA in the accumbens. Adult male Sprague-Dawley rats received subcutaneous injections of MDMA (20 mg/kg) twice per day for 4 successive days. Control rats received equivalent injections of saline. Either 1-4 days or 9-15 days following the last injection, rats were anesthetized with urethane and multibarrel micropipettes were employed for single unit recording combined with microiontophoresis in the core region of the nucleus accumbens. Dose-response curves were generated for the effects of DA, 5HT and GABA on glutamate-evoked firing of the nucleus accumbens cells. At both early and late test periods following the last injection of MDMA, the inhibitory effects of DA and 5HT on glutamate-evoked firing were significantly reduced compared to effects in the saline control group. However, the doses of glutamate required to fire the cells at equivalent baseline firing rates, and the inhibitory effects of GABA on the glutamate-evoked firing did not differ for the MDMA-pretreated and the control rats. These results suggest that MDMA pretreatment specifically alters DA and 5HT neurotransmission in the nucleus accumbens rather than changing the general level of excitability of the accumbens cells. This work was supported by NIH grant DA08116.

457.9

METHYLENEDIOXYMETHAMPHETAMINE-INDUCED INHIBITION OF NEURONAL FIRING IN THE NUCLEUS ACCUMBENS IS MEDIATED BY BOTH SEROTONIN AND DOPAMINE. T. Obradovic*, K. M. Imel and S. R. White. Dept. of Veterinary and Comparative Anatomy, Pharmacology and Physiology and Program in Pharmacology and Toxicology, Washington State University, Pullman, WA 99164.

Methylenedioxymethamphetamine (MDMA) is a mood elevating drug that causes an increase in extracellular concentrations of dopamine (DA) and serotonin (5HT) and depresses glutamate-driven firing of neurons in nucleus accumbens (White et al., Neurosci. 62: 41, 1994). The purpose of this study was to investigate whether the inhibitory effect of MDMA on neuronal activity in the accumbens may be mediated by DA, 5HT or both monoamines. Multibarrel micropipettes were used for extracellular recording combined with microiontophoresis in the core region of the nucleus accumbens of urethane-anesthetized rats. Fluoxetine, which prevents MDMA-induced 5HT release, significantly attenuated MDMA-induced inhibition of glutamate-evoked firing in the accumbens. The 5HT antagonists ketanserin, methysergide and WAY 100135 also significantly attenuated the MDMA-induced inhibition and the inhibition was mimicked by 5HT agonists that are relatively selective for $5HT_{1A}$, $5HT_{1B}$, $5HT_{2A/2C}$ and $5HT_3$ receptor subtypes. These observations suggest that MDMA-induced inhibition in the accumbens is mediated at least in part by 5HT receptors. However, depletion of DA by pretreatment with the neurotoxin 6-hydroxydopamine and the synthesis inhibitor α-methyl-p-tyrosine significantly attenuated the MDMA-induced inhibition without altering 5HT-induced inhibition of cells in accumbens. These data suggest that MDMA depression of neuronal excitability in the nucleus accumbens core is mediated by both 5HT and DA receptors. Furthermore, multiple 5HT receptor subtypes appear to be involved in the inhibitory effect of MDMA. This work was supported by NIH grant DA08116.

457.10

AMPHETAMINE (AMPH) INCREASES PHOSPHORYLATION OF NEUROMODULIN (NM) AND SYNAPSIN I (S1) IN STRIATAL SYNAPTOSOMES, S Michelhaugh*, S Iwata, GHK Hewlett, A Czernik#, K Meiri+ and ME Gnegy. #Rockefeller Univ., New York, NY 10021; +SUNY Health Science Ctr., Syracuse, NY 13210; Univ. of MI Med. Sch., Ann Arbor, MI 48109.

AMPH increases dopamine release in striatal neurons increasing locomotion and stereotyped behaviors in animals. We found that acute AMPH increased phosphorylation of the presynaptic protein NM in rat striatum. Phosphorylation of NM at ser^{41} by protein kinase C (PKC) leads to dissociation of the Ca^{2+}-binding protein calmodulin (CaM). In this study we examined the phosphorylation of NM by AMPH, using a state specific antibody for $phosphoser^{41}$-NM, and its binding to CaM in Percoll purified rat striatal synaptosomes. Phosphorylation of NM was maximal at 2 min at 37°C using 1 μM AMPH. To test the effect of AMPH on CaM binding to target proteins, CaM was immunoprecipitated from synaptosomes treated with buffer or 1 μM AMPH for 2 min. Immunodetectable CaM increased in the AMPH-treated synaptosomes with no change in total. NM co-immunoprecipitated with CaM but was unchanged by AMPH. There was, however, a decrease in the amount of co-immunoprecipitated MARCKS (myristolated alanine rich C-kinase substrate) after AMPH. Phosphorylation of MARCKS by PKC also leads to CaM dissociation. CaM dependent protein kinase II (CKII) also co-immunoprecipitated with CaM and the amount increased after AMPH. To determine whether AMPH can activate CKII, we measured S1 phosphorylated at site 3, the CKII substrate site, using a state specific antibody. S1 phosphorylation at site 3 responded dose-and time-dependently to AMPH. To determine whether PKC activation and release of CaM led to S1 phosphorylation, synaptosomes were pretreated with 10 μM Ro 31-8220, a specific PKC inhibitor, and then treated with or without AMPH. The PKC inhibitor prevented the AMPH-induced increase in $phosphoser^{41}$-NM and site 3 phospho-S1. These results suggest that AMPH treatment leads to an activation of PKC. Phosphorylation of MARCKS and NM would dissociate CaM, which could bind to and activate CKII, increasing phosphorylation of S1. Funded by NIDA grant DA 05066.

457.11

EFFECTS OF (-)EPHEDRINE HYDROCHLORIDE ON EXTRACELLULAR DOPAMINE LEVELS WITHIN RAT NUCLEUS ACCUMBENS. P.J. Wellman*, C.L. Livermore, D.K. Miller, T. Green, and J.R. Nation. Texas A&M University, College Station, TX, 77843-4235.

Systemic injections of the sympathomimetic agent (-)ephedrine hydrochloride [(-)EPH] produce an internal cue in rats resembling that of cocaine HCl (Gauvin, D.V., et al., *Psychopharmacology, 110(3)*, 309-19). The present study examined the effects of systemic (-)EPH injections on extracellular dopamine levels within rat nucleus accumbens (NAC). Adult male Sprague-Dawley rats were surgically prepared with indwelling bilateral guide cannulae through which a 3 mm concentric microdialysis probe was subsequently positioned within the medial aspects of the NAC. After establishing basal levels of dopamine and it's metabolites (DOPAC and HVA) within 20 minute fractions, rats were injected (i.p.) with either 5, 10, or 20 mg/kg (-)EPH, with additional samples collected every 20 minutes for 100 minutes post-injection. Dopamine levels in the NAC were elevated after (-)EPH administration, whereas metabolite levels were reduced relative to basal levels. These data suggest that (-)EPH may produce neurochemical cues similar to that of cocaine HCl.

R01DA07932-02

457.12

CHARACTERIZATION OF THE EFFECTS OF METHCATHINONE INDUCED DOPAMINE RELEASE. M. P. Gygi*, J. W. Gibb, A. E. Fleckenstein and G. R. Hanson. Department of Pharmacology & Toxicology, University of Utah, Salt Lake City, UT 84112.

Methcathinone ("CAT") is an increasingly popular stimulant that was classified a Schedule I drug in 1993. The mechanism by which CAT elicits its stimulating effects is unknown, however, users compare CAT to methamphetamine and cocaine, both of which cause an increase in synaptic dopamine. The rise in extracellular dopamine is necessary for these stimulants to elicit their behavioral and toxic effects. With microdialysis we determined that in awake rats CAT (1.0 and 10 mg/kg, s.c.) induced dopamine release in a dose-dependent manner. Further, we observed that CAT produced increasing locomotor activity in rats using doses of 0.3 - 10 mg/kg, s.c., and that the highest dose also elicited stereotypy. Our laboratory previously demonstrated the neurotoxic potential of CAT in animals receiving higher doses (20 and 30 mg/kg, s.c.). Using neurotensin tissue levels to assess the downstream consequences of CAT-induced dopamine release, the striatal content of neurotensin was analyzed in animals treated with these higher drug doses and sacrificed 18 h later. We found that CAT increased neurotensin tissue levels in a dose-dependent fashion, suggesting that dopamine released by CAT has subsequent functional consequences. (This work was supported by USPHS grants DA 04222 and DA 00869, NRSA predoctoral grant DA 05722, and a fellowship from the American Foundation for Pharmaceutical Education.)

457.13

EXTRACELLULAR NOREPINEPHRINE, SEROTONIN, AND DOPAMINE RESPONSES TO METHYLPHENIDATE: COMPARISON TO AMPHETAMINE AND COCAINE, R. Kuczenski* and D. S. Segal, Dept. of Psychiatry, University of California, San Diego, La Jolla, CA 92093-0603

Although dopamine (DA) plays a critical role in the behavioral effects of amphetamine-like stimulants, the dissociation between the quantitative effects of the drug on extracellular (EC) DA and behavior (particularly stereotypies) suggests that other transmitters, including serotonin (HT) and norepinephrine (NE), likely contribute to the behavioral response. To further examine the role of HT and NE in the stimulant response, we compared the effects of behaviorally similar doses of amphetamine (AMPH), cocaine (COC) and methylphenidate (MP) on regional biogenic amines using microdialysis. These drugs were chosen because of their different mechanisms of enhancing monoamine function. As we had previously shown for AMPH and COC, MP induced a dose-dependent increase in hippocampal NE. The maximal NE response was similar to comparable doses of COC, but substantially less than comparable doses of AMPH. In contrast to the effects of all three drugs on NE, whereas stereotypy-inducing doses of both AMPH and COC increased EC HT in caudate-putamen, even the highest doses of MP tested failed to affect CP HT. With respect to DA, MP increased both CP and nucleus accumbens (NAC) DA in a dose-dependent manner. At low doses (5 - 10 mg/kg), the magnitude of the response was comparable to COC and consistent with other DA uptake blockers; i.e., the NAC response was relatively greater than the CP response. However, as the dose of MP was further increased, the DA response become more AMPH-like, both in terms of magnitude and relative regional efficacy. These data support our contention that stimulant-induced behaviors are not strictly dependent on the quantitative features of the CP and NAC EC DA responses, but are likely modulated by other transmitter systems. In addition, the data suggest first, that a stimulant-induced increase in HT is not a prerequisite for the appearance of perseverative behaviors, and, second, that higher doses of MP may release DA in addition to blocking its uptake. (Supported by NIDA)

457.14

AN ESCALATING DOSE "BINGE" MODEL OF AMPHETAMINE PSYCHOSIS: BEHAVIORAL AND NEUROCHEMICAL FEATURES, D.S. Segal* and R. Kuczenski, Psychiatry Department, UC San Diego, La Jolla, CA 92093-0603

Stimulant-induced psychosis most frequently occurs during a high dose, multiple daily ("binge") exposure pattern of stimulant abuse. Thus, an appropriate animal behavior model should include exposure to escalating doses of the stimulant, followed by characterization of the behavioral and neurochemical responses during multiple runs. This approach should provide the greatest potential for understanding the mechanisms and processes most frequently associated with the induction of psychosis. To simulate the high dose abuse pattern, rats were exposed to escalating doses of amphetamine (Escalating Dose phase: 1.0 to 8.0 mg/kg) prior to multiple daily injections of relatively high doses of the drug (Run phase: 8.0 mg/kg/2 hrs x 4 injections). The behavioral response was monitored continuously during the course of these treatments as well as to subsequent amphetamine challenges at various times after discontinuation of drug treatment. During this Escalating Dose-Run pattern of amphetamine administration, a unique behavioral profile emerged which was qualitatively and quantitatively different from other treatment regimens. The altered profile exhibits both sensitization and tolerance of some components, including a profound increase in the expression of locomotor activation relative to the time spent engaged in continuous focused stereotypy. In parallel studies, in vivo microdialysis revealed progressively declining regional extracellular dopamine and serotonin responses, both within and between successive runs. In contrast, the norepinephrine response remained relatively unaltered. The possible role of these neurochemical changes in the altered behavioral profile, and the manner in which these effects may be implicated in the induction of amphetamine psychosis will be discussed. [Supported by NIDA]

457.15

A ROLE FOR D1 AND D2 RECEPTORS IN THE MEDIATION OF LOCAL AMPHETAMINE-INDUCED ACETYLCHOLINE RELEASE IN THE NUCLEUS ACCUMBENS. A. S. Keys* & G. P. Mark. Dept. of Behavioral Neuroscience, Oregon Health Sciences University, Portland, OR 97201.

Nucleus accumbens (NAc) dopamine activity is a putative mechanism that mediates the rewarding properties of both natural reinforcers and drugs of abuse. Recent studies have demonstrated the modulation of ingestive behavior by acetylcholine (ACh) interneurons of the NAc and suggest that the ACh/dopamine interaction in this area may also be involved in drug reward processes. The present experiment examined the effect of local perfusion of amphetamine on ACh release in the NAc as measured by *in vivo* microdialysis and the possible dopamine D_1 and D_2 modulation of this treatment. A biphasic dose response curve was observed in that a moderate dose of amphetamine (50uM) produced a 140% increase in ACh release that persisted for 15 min following cessation of drug perfusion ($F=5.74$, $p<.0001$) while a high dose (1mM) produced trends toward decreased ACh release followed by a significant 135% increase during recovery ($F=57.6$, $p<.0001$). Both the increase due to the moderate dose and the rebound from the high dose were blocked by concurrent treatment with the D_1 antagonist, SCH 23390. The high dose-decrease was attenuated by the D_2 antagonist, sulpiride (10uM). Also, a tonic D_1 inhibition of ACh release in the NAc, which was not dose-responsive, was also observed. These data suggest that amphetamine's biphasic effect on NAc ACh release is at least partially mediated via selective D_1 and D_2 mechanisms.

This research was supported by Oregon Health Sciences Foundation.

457.16

CHARACTERIZATION OF β-PHENYLETHYLAMINE-INDUCED MONOAMINE RELEASE IN RAT NUCLEUS ACCUMBENS: A MICRODIALYSIS STUDY. D. Nakahara* and M. Nakamura. Department of Psychology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan

In vivo microdialysis was used to investigate the effect of β-phenylethylamine on extracellular levels of monoamines and their metabolites in the nucleus accumbens of conscious rats. At all doses tested (1, 10 and 100 μM), infusion of β-phenylethylamine through the microdialysis probe significantly increased extracellular levels of dopamine in the nucleus accumbens. These increases were dose-related. The potency of 100 μM β-phenylethylamine to increase the extracellular level of dopamine was comparable to that of the same dose of methamphetamine. On the other hand, β-phenylethylamine had a much less potent stimulating effect on serotonin than dopamine levels. The only highest dose (100 μM) caused a statistically significant action to serotonin. Over the dose range tested (1, 10 and 100 μM), β-phenylethylamine had no effect on extracellular metabolite levels of dopamine and serotonin. The results suggest that β-phenylethylamine stimulates a release of monoamines, preferentially dopamine, without affecting monoamine metabolism. Supported by a scientific research grant from the Ministry of Education, Science and Culture of Japan.

458.1

ROLE OF MESOLIMBIC δ-OPIOID RECEPTORS IN MEDIATING THE AFFECTIVE COMPONENT OF MORPHINE-WITHDRAWAL. M. Funada, A. Pani and T.S. Shippenberg* Division of Intramural Research, NIDA, NIH, Baltimore, MD 21224

δ-Opioid receptor antagonists produce aversive states in morphine-dependent, but not drug-naive, animals. The neural substrates mediating these effects were assessed using place preference conditioning and microdialysis. Male SD rats, with indwelling intracerebral guide cannula, were implanted s.c. with two pellets containing morphine (75 mg) or placebo. Place conditioning produced by the δ-opioid receptor antagonists naltrindole (NLTR) and naltriben was examined 4 days later. Systemic or intracerebroventricular (ICV) infusion of either antagonist produced place aversions in morphine-treated animals. These effects occurred in the absence of physical withdrawal signs. Place aversions were also observed after antagonist infusion into the nucleus accumbens (NAC) and the doses producing this effect were significantly lower than those effective ICV. Neither antagonist produced a conditioned response in placebo-treated animals. Preliminary dialysis studies revealed no effect of NLTR on NAC dopamine (DA) overflow in placebo-pelleted animals. However, in morphine-treated animals a ca. 40% decrease in DA was seen. These data indicated an important role of NAC δ-opioid receptors in mediating the aversive stimulus effects of morphine withdrawal. An involvement of mesolimbic DA neurons in mediating the affective component of morphine withdrawal is also suggested.

458.2

PRECIPITATED MORPHINE WITHDRAWAL FOLLOWING MICROINJECTION OF METHYLNALTREXONIUM INTO THE PAG. K.L. Jones* and G.A. Barr, Biopsychology Doctoral Program, Dept. of Psychology, Hunter College-CUNY, NY, NY 10021 and New York State Psychiatric Institute, 722 W. 168th St., NY, NY 10032.

Considerable progress has been made in understanding the anatomical substrates that underlie the opiate withdrawal syndrome in the adult animal. However, at the present time there are no available data regarding the anatomical substrates of the opiate withdrawal syndrome in the infant animal. In this study, we examined the behavioral effects of morphine withdrawal by microinjection of methylnaltrexonium into the ventral region of the periaqueductal gray (PAG). Morphine was injected s.c. (10.0 mg/kg) at the nape of the neck twice daily from the first to the seventh day of life. Controls were saline treated animals. On day 6 all animals were fully anesthetized with Metafane, placed in a stereotaxic device adapted for infant animals, and implanted with a cannula aimed at the PAG. Following surgery pups were kept in a warm, moist incubator until fully recovered, and then returned to the dam. On the afternoon of day 7, a target pup was microinjected with saline or methlynaltrexonium to precipitate withdrawal. Pups were observed in a warm chamber with the remainder of the litter for a total of 20 minutes, and behaviors were recorded every 15 seconds on a checklist. When that observation period ended, the pup was anesthetized and placed back into the litter. A total of 5 pups were injected and tested in this manner. The results show that morphine-exposed rat pups that received microinjections of methylnaltrexonium into the PAG demonstrated alterations in their behavior including increased rolling, walking, head and paw movements as compared to control animals. These alterations in behavior patterns are similar to those observed in infant rats when withdrawal is precipitated peripherally. The PAG is therefore an anatomical substrate involved in the mediation of the opiate withdrawal syndrome in the infant rat.

Supported by RO1-DA06600.

458.3

NEUROPEPTIDE SF (NPSF) PRECIPITATES MORPHINE ABSTINENCE SYNDROME IN THE RAT. J.R. Lake*, W.D. Moon, B.P. Corbit, S.L. Webb, J.K. Hall, D. Haynes, H.-Y.T. Yang and D.H. Malin. Univ. of Houston-Clear Lake, Houston, TX 77058, & NIMH Neurosci. Ctr. at St. Elizabeth's, Washington, D.C. 20032.

NPSF, recently discovered in the rat CNS, shares an identical C-terminal sequence with Neuropeptide FF (NPFF). NPFF has antiopiate activity; 2 μg precipitated a morphine abstinence syndrome in dependent rats, while 10 μg induced a quasi-morphine abstinence syndrome (QMAS) in naive rats. This study tested whether NPSF would have similar actions. Thirty-six rats were cannulated in the third ventricle and implanted s.c. with an Alzet osmotic minipump. Eighteen rats were infused for 7 days with 0.27 mg/kg/hr morphine sulfate, and 18 were infused with saline alone. Three groups of 6 morphine dependent rats were injected i.c.v. with 1.64 (equimolar to 2 μg NPFF) or 6.10 μg NPSF or with bestatin:saline vehicle alone. Three groups of 6 opiate naive rats were similarly injected. The high dose precipitated an average of 51.8* signs in 20 min, the low dose 36.3* signs, and vehicle alone 10.5 signs (*p<.05 vs. vehicle). Only the high dose induced a significant QMAS in opiate naive rats. ANOVA revealed significant effects of dependence and NPSF dose. Thus NPSF has anti-opiate activity and is similar in potency to NPFF. (NIDA DA06554, K. Burgess, Texas A&M Univ., P.I.)

458.4

A SELECTIVE AMPA ANTAGONIST, LY293558, BLOCKS MORPHINE-WITHDRAWAL-INDUCED ACTIVATION OF LOCUS COERULEUS NEURONS AND BEHAVIORAL SIGNS OF MORPHINE WITHDRAWAL. W.T. Kendrick[1], K. Rasmussen[1], J. H. Kogan [2], F.P. Bymaster[1]* and G. K. Aghajanian[3]. [1]Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285; Departments of Pharmacology[2] and Psychiatry[3], Yale University School of Medicine, 34 Park St., New Haven, CT 06508.

The glutamate receptor subtype that mediates the morphine-withdrawal-induced activation of locus coeruleus (LC) neurons was examined in this study using *in vitro* and *in vivo* single-unit electrophysiological recordings. For LC neurons recorded *in vitro* in rat brain slices, the selective AMPA antagonist, LY293558, showed a greater than 10-fold selectivity for inhibiting the excitatory effects of AMPA vs. kainate, and a greater than 30-fold selectivity for inhibiting the excitatory effects of AMPA vs. NMDA. LY293558 also greatly reduced the response of LC neurons to glutamate in a concentration dependent manner. In *in vivo* recordings in anesthetized rat, pretreatment with LY293558 (0.1-10 mg/kg, s.c.) dose-dependently suppressed the morphine-withdrawal-induced activation of LC neurons. In unanesthetized, morphine-dependent animals, pretreatment with LY293558 (1-30 mg/kg, s.c.) dose-dependently suppressed naltrexone-precipitated morphine-withdrawal signs. These results indicate: 1) AMPA receptors mediate a large component of the excitatory effects of glutamate on LC neurons; 2) activation of AMPA receptors plays an important role in the morphine-withdrawal-induced activation of LC neurons; 3) AMPA antagonists can suppress many signs of morphine withdrawal in awake animals; and, 4) AMPA antagonists may have therapeutic effects in man for the treatment of opiate withdrawal.

This work was supported in part by US Public Health Service grants MH17871 and MH14276, and the State of Connecticuit.

458.5

REGULATION OF THYROTROPIN-RELEASING HORMONE IN MORPHINE WITHDRAWAL. L.G. Gahn* and K.A. Sevarino. Division of Molecular Psychiatry, Dept. of Psychiatry, Yale U. School of Medicine, New Haven, CT 06508

Thyrotropin-releasing hormone (TRH), administered systemically, ameliorates some of the physical symptoms of opiate withdrawal in rats. In this study we investigated the role of TRH in opiate withdrawal by examining the regulation of ppTRH mRNA, TRH peptide, and TRH receptor binding. Rats were implanted with one morphine pellet (75 mg) daily for 4 days. On day 5, rats were given naltrexone to induce withdrawal and sacrificed at various times. ppTRH mRNA was quantitated by *in situ* hybridization and RNase protection, receptor binding was measured by receptor autoradiography, and TRH peptide levels were determined by radioimmunoassay. During withdrawal, levels of ppTRH mRNA in the central gray increased to twice control by three hours and to three times control for 6-36 hours, returning to normal 3 days later. Morphine or naltrexone alone did not produce the increase in message levels. At three hours of withdrawal, smaller increases were observed in the amygdala (50%) and medulla (15%). Also at three hours, TRH peptide levels were unchanged in the central gray, but decreased 30% in the amygdala, and TRH receptor binding was unchanged in the central gray but decreased 30% in the amygdala. These data demonstrate that the TRH pathway, at the levels of mRNA expression, peptide levels, and receptor binding, is regulated during morphine withdrawal and suggest a role for TRH in mediating opiate withdrawal symptoms. (Supported by N.I.D.A DA07290)

458.6

MONOAMINE METABOLISM IN THE RAT LOCUS COERULEUS AND BEHAVIOR DURING MORPHINE WITHDRAWAL : EFFECT OF A NO-SYNTHASE INHIBITOR. N. Javelle, B. Renaud, L. Denoroy* and L. Lambás-Señas. INSERM CJF 95-06, Univ. C. Bernard, 69373 Lyon, France.

Electrophysiological studies have reported an hyperactivity of noradrenergic neurons of the locus coeruleus (LC) during opiate withdrawal. To further examine the changes in the LC during withdrawal, we monitored catecholaminergic and serotonergic metabolisms using microdialysis concomitantly with behavior. Moreover, since behavioral studies have suggested a role for nitric oxide (NO) in the morphine dependence, we have examined the effect of a NO-synthase inhibitor on behavioral and biochemical changes observed during morphine withdrawal. In morphine-treated rats (2 mg/kg/h, s.c., 5 days), naltrexone (100 mg/kg, s.c.) induced a marked increase (+ 280%) in concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), an index of the functional activity of the LC noradrenergic neurons. The concentrations in the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) were also increased (+ 70%). All morphine-treated rats exhibited classic behavioral symptoms of naltrexone-precipitated opiate withdrawal. These results i) confirm the hypothesis of an activation of LC noradrenergic neurons induced by opiate-withdrawal and ii) document further the involvement of serotonergic input to the LC during morphine withdrawal.

Pretreatment of morphine-treated rats with an inhibitor of NO-synthase, L-N-nitro arginine p-nitroanilide (L-NAPNA, 100 mg/kg, i.p.), reduced the naltrexone-induced increase in DOPAC (+ 40% vs + 280%) and attenuated behavioral symptoms of morphine withdrawal. These results suggest that L-NAPNA may attenuate the morphine withdrawal syndrome by decreasing the noradrenergic hyperactivity of LC neurons.

458.7

INHIBITION OF CONSTITUTIVE BUT NOT INDUCIBLE NITRIC OXIDE SYNTHASE ATTENUATES SIGNS OF MORPHINE WITHDRAWAL. D.B. Vaupel*, A.S. Kimes and E.D. London, Brain Imaging Section, Intramural Research Program, National Institute on Drug Abuse, P.O. Box 5180, Baltimore, MD 21224

The NMDA-receptor-mediated nitric oxide pathway has been linked to opiate tolerance and dependence. Inhibitors of nitric oxide synthase (NOS), having different degrees of selectivity for constitutive (c) or inducible (i) isoforms of the enzyme, were evaluated for their ability reduce signs of naloxone-precipitated morphine withdrawal and to affect blood pressure in rats. Inhibitors specific for cNOS included 7-nitroindazole (7-NI), 3-bromo-7-nitroindazole (3-Br-7-NI) and S-methyl-L-thiocitrulline (Me-TC). These drugs characteristically produced dose-dependent reductions in weight loss, diarrhea, wet dog shakes, grooming, and mastication; and increased exploratory activity. These drugs3-Br-7-NI selectively inhibits the neuronal isoform of cNOS, whereas 7-NI exhibits no selectivity between the neuronal and endothelial isoforms. Me-TC, which is selective for neuronal cNOS, produced a strong vasopresssor response, but the indazoles did not. By comparison, aminoguanidine, which is a selective inhibitor of iNOS, reduced fewer signs of opioid withdrawal and had a lower relative potency; it also lacked vasopressor activity. These results suggest that iNOS does not have a primary role in nitric oxide-mediated processes that modulate the opioid withdrawal syndrome. If NOS inhibitors are considered for development as treatment medications, 3-Br-7-NI and 7-NI would be preferable because they do not elevate mean arterial pressure. Because 7-NI significantly increased abnormal posturing and escape jumps and 3-Br-7-NI did not, 3-Br-7-NI emerged as the most effective NOS inhibitor in reducing opiate withdrawal signs in the rat.

458.8

Microinjections of alpha-flupentixol into the nucleus accumbens block morphine reward in rats that are opiate dependent and in withdrawal, but not in animals that have recovered from dependence. K. Nader*, T. Luxton and D. van der Kooy, Neurobiology Research Group, Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario, M5S 1A8.

We have previously reported that alpha-flupentixol pretreatment, but not lesions of the tegmental pedunculopontine nucleus (TPP), blocked the acquisition of conditioned place preferences for environments paired with intra-VTA morphine (500 ng/0.5µl/side) in rats that were trained while in a state of heroin dependence and withdrawal. Training animals while in non-withdrawal states (previously drug naive or after having recovered from dependence) produced the opposite pattern of results. These findings suggest that separate dopamine dependent and independent motivational systems exist within the VTA. Furthermore, the dopamine sensitive system is activated only when animals are in motivational states of withdrawal. We tested the effects of microinjections of alpha-flupentixol directly into the nucleus accumbens on systemic morphine reward in animals that are either opiate dependent and in withdrawal or have recovered from dependence. Bilateral microinjections of either 3µg or 30 µg/µl/side of alpha-flupentixol into the nucleus accumbens, blocked the acquisition of conditioned place preferences for environments paired with 10 mg/kg (ip) morphine in animals that were trained while in a state of heroin withdrawal. These animals were given a 2 week period to recover from dependence and were subsequently conditioned using the identical procedure. Pretreatment with intra-accumbens administration of 3 µg/µl/side of alpha-flupentixol had no effect on the acquisition of conditioned place preferences for environments paired with 10 mg/kg (ip) morphine. These data suggest that the mesolimbic dopaminergic pathway is only necessary for mediating morphine's rewarding properties when animals are in states of dependence and withdrawal. (supported by MRC Canada)

458.9

ENHANCEMENT OF TAIL-BITING IN MORPHINE WITHDRAWN RATS FOLLOWING EXPOSURE TO CONTEXTUAL CONDITIONED FEAR STIMULI. B.J. Caldarone*, G.C. Abrahamsen, D.L. Mongeluzi, H.S. Stock, P. Balan, & R.A. Rosellini, Department of Psychology, University at Albany:SUNY, Albany, NY, 12222.

Previous studies from our laboratory have shown that exposure to contextual conditioned fear stimuli can enhance naloxone-precipitated withdrawal behaviors in morphine-naive rats. The present study examined the effects of conditioned fear stress on abrupt withdrawal behaviors in rats that were previously dependent on morphine. Male Sprague-Dawley rats were implanted with either two 75 mg morphine pellets or 2 placebo pellets. After 6 days, pellets were removed and abrupt withdrawal behaviors (teeth chattering, mastication, forelimb tremors, wet dog shakes, backward ambulation, and tail-biting) and weight loss were measured. Peak withdrawal effects were observed 2 days following pellet removal. Five days following pellet removal, when withdrawal behaviors returned to control levels, rats were either administered footshock to condition fear to a context, or received no fear conditioning. On the day following fear conditioning, rats were reexposed to the conditioned fear context and withdrawal behaviors were recorded immediately following removal from the context. Morphine withdrawn rats exposed to the conditioned fear context exhibited a higher frequency of tail-biting compared to control groups. No group differences were observed for any other of the measured withdrawal behaviors. These results suggest that exposure to conditioned fear stimuli can reinstate at least one behavioral sign of abrupt withdrawal in rats that were previously withdrawn from morphine. (Supported by a SUNY Graduate Student Organization grant to BJC and a SUNY Faculty Research award to RAR.)

458.10

INHIBITORY EFFECTS OF NEFIRACETAM ON THE DEVELOPMENT OF DEPENDENCE AND TOLERANCE TO MORPHINE. T. Nabeshima*, A. Itoh, S. Nakayama, Y. Noda, T. Mamiya, T. Shiotani and T. Hasegawa, Dept. of Neuropsychopharmacol. and Hospital Pharm., Nagoya Univ. Sch. of Med., Nagoya 466, Japan

Morphine (MOR), a most potent opioid analgesic, induces dependence and tolerance, and it hampered the use in clinical. Therefore, it is important to attenuate the development of dependence and tolerance to MOR. It has been reported an involvement of the changes in second messengers such as cAMP and Ca^{2+} in the action of MOR. In this study, therefore, we investigated the effects of nefiracetam on the development of dependence and tolerance to MOR, since nefiracetam affects the cAMP level and Ca^{2+} influx into the neuronal cells.

When mice were administered MOR (6 or 20 mg/kg, s.c.) twice daily for 5 days and then challenged by naloxone (NAL, 5 mg/kg, i.p.) 2 hr after the last MOR injection on the 6th day, they showed the signs of withdrawal such as jumping, diarrhea and body weight loss. Co-administration of nefiracetam (5 or 10 mg/kg, p.o.) with MOR during the subacute treatment period, significantly reduced these signs precipitated by NAL. However, acute administration of nefiracetam did not affect these symptoms in the MOR-dependent mice. Subacute administration of MOR also induced the tolerance to its analgesic effect. This tolerance was also significantly attenuated by the administration of nefiracetam in combination with MOR. However, acute administration of nefiracetam failed to produce analgesic effect and had no effects on the analgesic effect of MOR in the MOR-naive mice.

These findings suggest that co-administration of nefiracetam with MOR attenuates the development of dependence and tolerance to MOR. Although the mechanisms underlying these phenomena should be further investigated, co-administration of nefiracetam may be useful strategy to attenuate the development of dependence and tolerance of MOR in the clinic. (Patent No. 95147M)

This work was partly supported by an SRF Grant for Biomedical Research.

458.11

ONTOGENY OF MORPHINE TOLERANCE IN RATS. W. Jin, G. A. Jacob, and C. P. Cramer*. Dept. of Psychology, Dartmouth College, Hanover, NH 03755-3549

Unlike adults, neonatal rats given single daily injections of morphine do not develop tolerance (Fanselow & Cramer, 1988; Burton & Cramer, 1994). In the studies reported here, we first identified more precisely the age at which tolerance first appears. In the first two experiments, pups where given 0, 2.5, or 5 mg/kg morphine sulfate once daily for 5 days, and then tested for tolerance on the sixth day by measuring shifts in dose-dependent response to morphine-induced analgesia. When injections were begun at Day 10 postpartum, pups tested on Day 15 did not show tolerance. However, when injections were begun at Day 13, pups tested on Day 18 showed a significant shift in the dose-response curve indicative of tolerance. The third experiment explored one possible explanation for this relatively late onset, namely that the pups' behavioral repertoire did not allow expression of tolerance that had nonetheless been acquired. Pups were given the injection regime as in the first experiment, from Days 10-14, but they were instead tested on Day 18, at which age they did in fact manifest tolerance. These results suggest that there is a developmental transition between Day 15 and Day 18, specifically in the ability to express tolerance, which occurs at least several days later than the ability to acquire it.

458.12

CHRONIC TREATMENT WITH MORPHINE IS ASSOCIATED WITH HETEROLOGOUS TOLERANCE OF GUINEA PIG LOCUS CERULEUS (LC) NEURONS. J. Meng, W.W. Fleming and D.A. Taylor*. Dept. of Pharmacol. and Toxicol., R.C. Byrd Hlth. Sci. Ctr., West Virginia University, Morgantown, WV 26506-9223.

Chronic exposure to morphine leads to the development of a heterologous subsensitivity of guinea pig nTS and myenteric neurons as well as supersensitivity to several different excitatory agonists. In contrast, rat LC neurons develop a highly specific reduction in the responsiveness confined to opioids following exposure to morphine. The current studies were conducted to characterize the nature of the change in responsiveness induced by chronic exposure to morphine in guinea pig LC neurons. Morphine treatment was accomplished by pellet implantation (75 mg/pellet) 7 days prior to the experiment. Extracellular electrophysiological recordings were obtained from LC neurons in pontine slices from control and morphine-treated guinea pigs. Agonists were added to the physiological saline and administered by superfusion as either single concentrations or in a concentration-response curve of cumulatively increasing concentrations. The results indicate that LC neurons from guinea pigs treated with morphine exhibit a nearly 10-fold rightward shift of the concentration response curve to morphine with no change in maximum. The heterologous nature of the change in responsiveness was demonstrated by the similar reduction in magnitude of responses to muscimol following chronic treatment. Responses of LC neurons to muscimol (1.0 µM) were reduced from 93 ± 4 % inhibition in control neurons to 21 ± 15% inhibition in neurons from animals chronically exposed to morphine. These data are qualitatively and quantitatively similar to previous studies from both the guinea pig brainstem nTS and myenteric plexus/longitudinal muscle which indicate that chronic exposure of guinea pigs to morphine leads to a change in responsiveness which is exhibited as subsensitivity to a variety of inhibitory substances. The data provide the first demonstration of nonspecific reductions in sensitivity of guinea pig LC neurons following morphine treatment and suggest that the cellular mechanisms underlying the development of tolerance to morphine in the guinea pig LC may be similar to those previously described in either neurons of the guinea pig myenteric plexus or nTS. Supported in part by NIH Grant DA 03773.

458.13

Tolerance to Morphine is Mediated by Nutritive and Non-Nutritive Dietary Variables. KRISTEN E. D'ANCI*, ROBIN B. KANAREK, AND ROBIN MARKS-KAUFMAN. Dept. of Psychology, Tufts Univ., Medford, MA 02155.

Recent research showed that following repeated administration of morphine, morphine-induced analgesia (MIA) decreased in rats drinking a saccharin solution in comparison to rats drinking a sucrose solution. This difference in MIA may be due to a difference in the development of tolerance to morphine's analgesic effects. The present experiment evaluates the differing effects between a palatable, nutritive sucrose solution and a palatable, non-nutritive saccharin solution on the development of tolerance to MIA.

Forty-five adult male Long-Evans VAF rats were used. All rats had ad lib access to chow and water. Additionally, 15 rats were given a 0.15% w/v saccharin solution and 14 rats were given a 32% w/v sucrose solution to drink for three weeks. MIA was assessed via the radiant heat tail-flick method. To induce tolerance, half of the rats in each dietary condition were given a priming injection of 7.5 mg/kg morphine sulfate. The remaining rats received saline injections. All rats were given a tail-flick test 30 minutes post-injection. One week after the priming session, tolerance was measured according to a cumulative dose paradigm. Rats received injections of 2.5 mg/kg of morphine sulfate every 30 min. until a final dose of 12.5 mg/kg was achieved. MIA was measured immediately prior to and 30 min. following each injection.

Rats drinking the sucrose solution had significantly higher analgesic responses than rats drinking saccharin or water alone, regardless of priming condition. MIA was suppressed in rats drinking either saccharin or water one week following a priming dose of morphine but was not suppressed in rats drinking sucrose. The results suggest that intake of palatable, nutritive fluids curbs tolerance to MIA. The present study supports previous results demonstrating differences between saccharin and sucrose intake on MIA. Funded by NIDA Grant No. DA04132 to R.B.K and R.M-K.

458.14

ETORPHINE TOLERANCE STUDIES DIFFERENTIATE μ OPIOIDS IN AN ANALGESIC ASSAY. M.J.Tiano, E.A. Walker, L.A. Dykstra*. Department of Psychology, University of North Carolina at Chapel Hill. Chapel Hill, NC 27599

Opioids are differentially sensitive to receptor alteration by repeated agonist treatment. The hypothesis that these differences are related to efficacy was tested using higher efficacy agonists etorphine and *l*-methadone, high efficacy agonist morphine, and lower efficacy agonists buprenorphine and dezocine in an analgesic assay. Rats were placed in restrainers and the latencies for tail withdrawal from 55°C water were measured. Repeated treatment of 0.0064 mg/kg per day etorphine for 7 days increased the doses required for 100% maximum possible effect for morphine, buprenorphine, and dezocine. The ED_{50} values for morphine, buprenorphine and dezocine were increased by 3, 6, and 9-fold, respectively. Additionally, after repeated etorphine treatment, no dose of dezocine produced 100% maximum possible effect. Repeated treatment with 0.0064 mg/kg per day etorphine failed to alter the etorphine or *l*-methadone dose-response curves. The present finding that the greatest degree of tolerance was observed for buprenorphine and dezocine and the least amount of tolerance was observed for etorphine and *l*-methadone support the hypothesis that the magnitude of tolerance is inversely related to the relative efficacy of the agonist tested. (Supported by DA03796, DA02749, DA00033)

458.15

SPINAL AMINO ACID RELEASE AND MORPHINE TOLERANCE IN RATS CHRONICALLY INFUSED WITH SPINAL MORPHINE AND THE EFFECT OF REPETITIVE NALOXONE ANTAGONISM. **T. Ibuki*, M. Marsala, T. L. Yaksh,** Anesthesiology Res. Lab., Univ. of California, San Diego, CA 92093-0818.

Recent studies in our laboratory showed that chronic spinal infusion of morphine had no significant effect on the resting release of spinal amino acids, but naloxone injection in these morphine tolerant animals evoked an immediate increase in the release of glutamate and taurine [1]. In the present study we examined the effect of daily transient naloxone antagonism on the development of tolerance and the spinal release of several amino acids (glutamate, taurine, serine, glycine). Rats, implanted with spinal loop dialysis catheter and lumbar intrathecal catheter were continuously (3 days) infused with morphine (20 nmol/μl/hr) or saline (1μl/hr) and received daily saline (s.c.) or naloxone injections (0.6mg/kg). The degree of tolerance was evaluated daily by measuring hot plate escape latency prior to s.c. injection and withdrawal signs immediately after. In the same animals microdialysate samples were collected before and after naloxone or saline injection. Rats infused with chronic spinal morphine and transiently antagonized with naloxone developed the highest degree of tolerance. These animals also showed a significant and progressive increase in the concentration of glutamate, aspartate and taurine after repetitive naloxone injections. In control animals chronic infusion of morphine combined with saline (i.p.) injection had no effect on resting spinal amino acid release. Our results indicate that periodic antagonism yields a progressive enhancement in spinal glutamate release and an exacerbated degree of tolerance. We hypothesize that these events are causally related. (Supported by NS16541-TLY; NS32794-M.M.)

[1] Jhamandas KH et al., J Neuroscience 1996 (in press).

458.16

BETA-FUNALTREXAMINE ANTAGONISM OF THE DISCRIMINATIVE STIMULUS AND ANTINOCICETIVE EFFECTS OF MORPHINE AND ETONITAZENE. J.A. Moretti* and A.M. Young, Department of Psychology, Wayne State University., Detroit, MI 48202.

The present experiments investigated effects of the selective, irreversible μ opiate antagonist beta-funaltrexamine (β-FNA) on discriminative stimulus, rate-altering, and antinociceptive effects of morphine sulfate (MS), and antinociceptive effects of etonitazene HCL (ETZ). In all experiments, agonist potencies were determined using cumulative dosing procedures. In discriminative stimulus experiments, male Sprague-Dawley rats were trained to discriminate 3.2 mg/kg MS and saline under FR15 schedules of food reinforcement. Rats were then administered various doses of β-FNA (5-40 μg i.c.v.), and tested with MS 24 h later. β-FNA dose-dependently reduced the potency of morphine's discriminative stimulus and rate-altering effects. Higher doses of β-FNA (20 & 40 μg) suppressed the maximal discriminative stimulus effects of MS. β-FNA was also used to antagonize the antinociceptive effects of MS and ETZ in a warm-water tail withdrawal assay. β-FNA was more potent in antagonizing morphine's antinociceptive effects than its discriminative effects, suggesting that fewer receptors are needed to produce a full effect in a drug discrimination assay. Furthermore, doses of β-FNA (10 & 20 μg) that prevented MS from producing a maximal effect in the antinoniception assay merely shifted the ETZ dose-effect curve to the right. Indeed, doses of β-FNA up to 40 μg did not prevent ETZ from producing maximal antinociceptive effects, suggesting that ETZ is a higher efficacy agonist than MS.(Supported by R01 DA03796 and F31 DA05701)

458.17

EFFECTS OF PROTEIN KINASE INHIBITOR H-7 ON THE ANTINOCICEPTIVE ACTIONS OF MORPHINE IN RATS. P. Sacchetti*, D.C. Jewett, R.R. Witte II and A.M. Young. Department of Psychology, Wayne State University, Detroit MI 48202

It has been suggested that tolerance to functional effects of μ opioids may result from agonist-induced constitutive activation of μ opioid receptors *via* a phosphorylation mechanism (*Wang et al.* Life Sci. 54:PL339-350, 1994). The present study examined the ability of the general protein kinase inhibitor H-7 (1-(5-Isoquinolinylsulfonyl)-2-methylpiperazine) to reverse and/or prevent tolerance produced by cumulative or chronic exposure to morphine (MS) in rats. Male Sprague-Dawley rats were tested in a warm-water tail withdrawal assay, and ED_{50} values for MS were determined by cumulative dosing procedures. Acute pretreatment with H-7 (50-150 nmol, *i.c.v.*), 30 min prior to MS, did not change control latencies or the ED_{50} for the antinociceptive effects of MS. Furthermore, H-7 (50-150 nmol) did not alter the normal time course of MS (10 mg/kg) effects. Tolerance was established by administering doses of 17.8 mg/kg MS, b.i.d., for 2 days, followed by 32 mg/kg MS , b.i.d., for 4 days. The magnitude of tolerance was assessed by re-determination of the MS dose-response curve. Acute pretreatment with H-7 (150-1500 nmol) did not reverse MS tolerance, but did produce short-term increases in locomotor activity and vocalization. In a second experiment, doses of H-7 (50-150 nmol) were administered 30 min before each MS injection during chronic MS treatment. Co-administration of H-7 did not prevent the development of MS tolerance. These data suggest that *i.c.v.* injections of H-7 may not prevent or reverse the tolerance produced by chronic MS treatment in rats. [Supported by DA03796 and K02 DA00132]

458.18

Effect of morphine-3-glucuronide (M3G) on the analgesic actions of δ_1- and δ_2- and κ- opioid receptor agonists in mice. Jing-Tan Bian, Peter T. Thomas*and Hemendra N. Bhargava, Dept. Pharmaceutics/Pharmacodynamics, Univ. Ill., Chicago, IL 60612

M3G is a major metabolite of morphine. It has been shown to be a physiological antagonists of morphine as well as its metabolite, morphine-6-glucuronide. Binding studies have yielded contradictory results for the interaction of M3G with multiple opioid receptors. The present studies examined the in vivo interactions between M3G and δ- and κ-opioid receptor subtypes. The effects of M3G administered i.c.v (0.01, 0.1 or 1.0 μg per mouse) or i.p. (3 to 100 mg/kg) on the analgesia induced by i.c.v. injections of [D-Pen^2, D-Pen^5] enkephalin (DPDPE), a δ_1- opioid receptor agonists or [D-Ala^2, Glu ^{4}] deltorphin II (deltorphin II), a δ_2-opioid receptor agonist, were determined in male Swiss Webster mice. DPDPE or deltorphin II (15 μg per mouse) produced robust analgesic effect 15 min after the drug administration. The analgesic action of DPDPE or deltorphin II was not modified by either i.c.v. or i.p. injections of M3G. U-50,488H, a κ-opioid receptor agonist at a dose (25 mg/kg, i.p.) produced analgesia. The analgesic response to U-50,488H was also not altered by M3G (10, 20 or 40 mg/kg, i.p.). It is concluded that in vivo, M3G does not interact with δ_1- δ_2- or κ-opioid receptors (Supported by grant DA-08867 and a Research Scientist Development Award, K02-DA-00130 from the National Institute on Drug Abuse).

459.1

COMPARISON OF TESTS USED TO EVALUATE THE BEHAVIOR OF AGED ANIMALS. R.N.McLay, R.E. Harlan, S.M.Freeman, A.J. Kastin, J.E. Zadina*
Neuroscience Training Program and Depts. of Anatomy, Pathology and Medicine, Tulane Univ. Sch. of Med., and VA Med. Ctr. New Orleans, LA 70112

Young (2 mo.) and aged (24 mo.) healthy Fisher344xBrown Norway rats were evaluated in the eight arm radial maze, Barns circular platform maze, and Morris Water Maze, all of which have been used previously to demonstrate learning and memory deficits in aged animals. Rats also were tested for motor performance on a rotorod and by measurement of locomotor activity. As expected, aged animals showed significantly impaired performance ($p<.05$) in the Barns, Morris, and eight-arm radial mazes. Important differences were noted, however, among the results in the three mazes. Ten out of 10 aged animals were designated cognitively impaired (those outside the 95% confidence interval for young animals) in the eight-arm radial maze. Eight animals of 10 in the Morris maze, and only 6 of 10 in the Barns maze were designated as impaired. Evaluation of performance of aged rats in the eight-arm radial maze was judged to be problematic since aged rats had a significantly ($p < 0.01$) higher percentage of errors of omission than young animals. For aged animals, a significant ($p<0.05$) correlation also was found between motor performance on the rotorod and performance in the Morris maze, indicating that physical or motor functions could contribute to performance in this maze. No such correlation was seen in young animals or in glucocorticoid-induced models of accelerated hippocampal senescence. No significant correlations were found among motor tests and measures of learning and memory in the Barns maze. The lower percentage of animals judged to be cognitively impaired by assessment in the Barns maze may therefore reflect a smaller bias against aged animals with motor impairments.

459.2

FACILITATION OF COGNITIVE FUNCTIONS IN AGED RATS BY PAST EXPERIENCE DEPENDS ON THE TYPE OF INFORMATION PROCESSING INVOLVED. A COMBINED CROSS-SECTIONAL AND LONGITUDINAL STUDY

F. Dellu, W. Mayo, M. Vallée, M. Le Moal and H. Simon*. Lab. de Psychobiologie des Comportements Adaptatifs, INSERM U.259, Domaine de Carreire, rue Camille Saint-Saëns, 33077 Bordeaux Cedex, France.

The origin of the impact of individual history on behavior across the life-span, which is commonly observed clinically, has not been studied. This is why the role of previous training in a cognitive behavioral task in subsequent memory performances was studied in a combined longitudinal (animals repeatedly tested when young, at 2-3 months, middle-aged at 12-14 months, and old at 23-26 months) and cross-sectional (animals tested once at those ages) study, in male Sprague-Dawley rats. Different types of memory (reference and working memory) and/or information processing (route or place learning) were assessed in three different tasks (T-maze, Morris water-maze and 8 arm radial-maze). Our results indicate that experience prevents age-related impairments in the learning phase of the T-maze and the Morris water-maze, in both middle-aged and old rats. Non-experienced animals of the same age were found to present age-related memory deficits. However, previous experience did not have any effect on the learning of the radial-maze or on the transfer performance. It is suggested that controlled processes (intentional and attentional) are impaired by aging and cannot be improved by training, whereas automated processes appear to benefit from it. These data underline the heterogeneity of cognitive aging and indicate that aging is not inevitably accompanied by a decline in performance.

This work was supported by INSERM, Université de Bordeaux II, Conseil Régional d'Aquitaine, Fondation pour la Recherche Médicale and Institut Lilly (France).

459.3

DIMINISHED HIPPOCAMPAL CELLULAR RESPONSIVITY DURING CLASSICAL CONDITIONING IN AGED RABBITS. S.D. Berry*, M.A. Seager & R.L. Borgnis. Center for Neuroscience and Department of Psychology, Miami Univ., Oxford, OH 45056.

Aged rabbits as well as those with hippocampal lesions show behavioral learning deficits in a trace NM paradigm. It is also widely known that the hippocampus is adversely affected early in the aging process.

Taken together, these findings suggest that the behavioral deficits observed in old animals may be the result of a malfunctioning hippocampus. Studies done *in vitro* have demonstrated that hippocampal neurons taken from old animals after trace conditioning show reduced responsively to stimulation. However, only one study to date has recorded from the hippocampus while old animals were performing in a trace conditioning task.

The present study involved training old (40-49 mos.) and young (3-7 mos.) rabbits in a trace jaw movement paradigm with a 300 ms tone CS, 450 ms trace period, and a 200 ms water US. Animals were trained to a criterion of 8 CRs on any 9 consecutive trials on two consecutive days. Animals were then transferred to a delay paradigm where the CS and US overlapped.

Results indicate that old animals took significantly more trials to reach criterion than young animals. This learning deficit in the old group was accompanied by less responsive CA1 neurons. Both groups showed significant increases in hippocampal activity, but the young animals' increase was much greater. This was true on the first three days of training as well as on criterion day. Time series analyses are being performed to correlate unit firing with the rhythmic behavioral responses elicited in this paradigm. Preliminary results indicate that, in this trace paradigm, the cells' activity may be more closely related to the periodicity of the slow waves than to the behavior.

459.4

AGE-RELATED NONNEURAL TISSUE PATHOLOGIES IN MALE RATS: LACK OF CORRELATION WITH MNEMONIC AND MOTORIC ABILITIES. M.V. Wagster[1,5,6], I.K. Berzins[4], J.A. Kotzuk[3], K.M. Frick[6], M.E. Barra[6], M.E. Mooney[6], A.L. Markowska[6], D.L. Price[1,3,5]. Dept. of Pathol.[1], Neurol.[2], Neurosci.[3], Compar. Med.[4], and Neuropathol. Lab.[5], Johns Hopkins Univ. Sch. of Med. & Dept. of Psychol.[6], Johns Hopkins Univ., Balt., MD 21205.

Aged primates and rodents can display varied behavioral performance on a number of tasks, particularly those in cognitive and motor domains. We determined the extent to which nonneural tissue pathologies influence capabilities on tasks designed to assess these behaviors. Thirty Fisher 344 male rats aged 23 months and twelve animals divided equally among the age groups, 4, 11, and 17 months, were tested for reference memory and working memory in the Morris Water Maze and on several sensorimotor tasks. After final testing, the animals were sacrificed and organs were removed including heart, lung, liver, kidneys, pancreas, thyroid, and eyes. The paraffin-embedded tissues were stained with hematoxylin & eosin, and rated on a 0-5 scale by one of the investigators who was blind to the animals' age and behavioral status. Numerous pathologies, particularly in heart, liver, testis, kidney, and hemic system were found in the oldest animals. Moderate to severe pathology existed in the oldest animals as follows: heart, 20%; liver, 13%; kidney, 63%; and testis, 100%. Despite these findings, there was no consistent relationship determined between memory or motor abilities and extent of pathology. In summary, we conclude that age-related degenerative changes in multiple organ systems do not account for the variability seen in memory and motoric ability with age.

This work was supported by a grant from the NINDS.

459.5

SHORT-TERM BUT NOT LONG-TERM ESTRADIOL REPLACEMENT IMPROVES RADIAL-ARM MAZE PERFORMANCE OF YOUNG AND AGING RATS. C.L. Williams* Dept. of Psychology: Experimental, Duke University, Durham, NC 27708.

Circulating steroid hormones have been shown to influence hippocampal physiology and morphology. Because the hippocampal formation is known to be involved in learning and memory, the current study investigated whether short- or long-term estradiol replacement to ovariectomized rats would influence performance on a radial arm maze task.

In Experiment 1, female Sprague Dawley CD strain rats were ovariectomized at 2 months of age and were implanted with silastic capsules containing no estradiol or one of 2 doses of estradiol. Serum levels of estradiol in these groups were monitored and females were divided into 3 treatment conditions: No Estradiol (serum levels < 20 pg/ml), Low Estradiol (mean serum level = 40 pg/ml), and High Estradiol (mean serum level = 200 pg/ml). Silastic capsules were replaced regularly to maintain serum estradiol within these ranges. Rats were trained daily on a 12-arm radial maze task with 8 baited and 4 unbaited arms immediately after estradiol replacement, and retrained at 14 months of age after 12 months of chronic estradiol replacement. Estradiol at both High and Low doses improved working memory performance of rats after short-term estradiol replacement, but not after long-term replacement.

To determine if rats' responsiveness to estradiol decreases with age, or whether chronic replacement is an ineffective treatment, a second group of Sprague Dawley rats was ovariectomized at 15 months of age and given estradiol replacement via silastic capsules as described above. Rats were trained daily on a 12-arm radial maze task. Results revealed that after short-term estradiol replacement, 16 month old rats showed more accurate overall choice performance than rats with no estradiol.

These data suggest that estrogen improves spatial memory in young and aging rats if treatment is short-term. However, chronic estrogen administration may not be effective as a cognitive enhancer. (Supported by AG09525)

459.6

ACUTE BUT NOT CHRONIC ADMINISTRATION OF ESTRADIOL INCREASES SPINE DENSITY OF DENTATE GRANULE CELLS IN THE AGED RAT. P.Henderson[1], C.L. Williams[2], and G. Einstein[1]*. Dept. of Neurobiology[1] & Dept. of Psychology: Experimental[2], Duke University, Durham, NC 27710.

Estrogens have been shown to have widespread effects on many brain regions in developing and young animals. Little, however, is known about the effects of estrogens on the aging brain and associated changes in cognition. For this reason, we looked at changes in the morphology of neurons in the hippocampus, a brain region involved in learning and memory, in both aging and young rats. Female Sprague Dawley CD strain rats were ovariectomized at 2 months of age and were treated with no estradiol, chronic estradiol for over one year (either low or high dose), acute estradiol (at 24 and 48 hours prior to sacrifice), or chronic and acute estradiol. As a control for any changes due to aging alone, young adult rats (4-5 months old) were ovariectomized and treated with either no estradiol or acute estradiol. In brain slices from these animals, dentate granule cells were intracellularly filled with Lucifer Yellow, filled neurons were traced using a drawing tube, and dendritic spine density was calculated.

By doing this, we found: (1) aged rats have a significantly lower dendritic spine density than young adults; (2) chronic estradiol does not ameliorate the loss of spines with normal aging; (3) in contrast, acute estradiol does ameliorate the loss of spines with normal aging, increasing spine density to the level seen in young adults. These results suggest that the aging female hippocampus is still responsive to estrogens and that their action on this brain region depends on the temporal pattern of their administration.

(Work supported by: AFAR to P.H., AG09525 to C.L.W., and the Alzheimer's Association (IIRG-95136) to G.E.)

459.7

AGE-RELATED DECLINE IN MEMORY-RELATED BEHAVIOR IN FEMALE FISCHER 344 X BROWN NORWAY F1 RATS. G. Rajakumar*, N. C. de Fiebre, G. Simpkins and J.W. Simpkins, Department of Pharmacodynamics & Center for Neurobiology of Aging. College of Pharmacy, University of Florida, Gainesville, Florida 31610.

To determine the effects of aging on memory-related behavior in female rats, we monitored estrous cycles through vaginal lavages for one month in F344 X BN F1 rats of ages of 6 mo (young), 12-14 mo (middle -aged) and 24-28 mo (aged), then evaluated behavior in a spatial task using the Morris Water maze. Young and middle-aged animals show normal 4-5 day estrous cycles, while aged rats exhibited extended periods of diestrus, evidence of repeated pseudopregnancy. Rats were trained for three days (12 trials /day) in a paradigm using a submerged platform. Acquisition of the behavior was not different among the three age groups. Following a 24 h delay, rats were given a single learning trial with the submerged platform, then were immediately tested for behavior with the platform removed. Memory-related performance for specific age groups showed the following: Young rats spent more time in the target quadrant than the remaining three quadrants ($P<0.05$). Middle aged rats crossed the target quadrant more often ($P<0.0002$) and spent more time in the target quadrant ($P<0.004$). Aged rats showed no preference for the target quadrant by either measure assessed. When the quadrant preference across age groups was analyzed, middle aged rats crossed the target quadrant more often than old rats ($P<0.03$). Collectively these studies suggest an age related deficit in spatial learning, but not the ability to non-spatially learn the task as measured by training latencies. This deficit is associated with the age-related occurrences of repeated pseudopregnancy. (Supported by NIA 10485).

459.8

AGE ASSOCIATED BEHAVIORAL DEFICITS IN F344/BN RATS. J.P. Kesslak*, T. J. Zomorodian, J. Chia, C.W. Cotman, Institute for Brain Aging, Dept Neurology, Univ. Calif., Irvine, CA 92697.

In the normal population there is an increase in behavioral impairment associated with age. Rats of the F344/BN strain are long lived, with a mean life expectancy of 36 months. A battery of behavioral tests was used to characterize functional ability for F344/BN rats 3, 22, 32 and 36 months of age. Animals were tested on the 8-arm radial maze, water maze, spontaneous alternation and open-field. Repeated measures analysis of variance (ANOVA) indicated that after 21 days of testing on the 8 arm maze there was a significant difference between the young, 3 month old rats, and the older groups, which did not statistically differ from each other. Older rats made fewer choices and had longer latency to complete the task, however, they also made fewer errors than the young group. The water maze was more sensitive than the 8-arm maze for the detection of age related deficits, with the youngest group having the best performance and the oldest group the longest latency to locate a submerged platform after 10 days of testing. Hypoactivity and reduced spontaneous alternations was also observed with increased age. In the very aged group there was frequent physical limitations, such as reduced mobility, tumors and a high mortality rate. Results indicating a behavioral deficit for the aged rats may be due in part to performance deficits, rather than cognitive deficits. Supported by the MacArthur Foundation and NIA.

459.9

IMPAIRED SPATIAL LEARNING AND MEMORY IN MATURE AND OLD SPRAGUE DAWLEY RATS.
J.M. Wyss*, J.A. Franklin, X.H. Feng, and Th. van Groen, Dept. of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294.

Studies in our laboratory indicate that in old Sprague Dawley (SD) rats there is a selective breakdown in the limbic cortex, this reorganization should disrupt the transfer of information between the hippocampal formation and many areas of neocortex. Our previous studies demonstrated that lesions of this circuit lead to impaired spatial learning and memory. In the present study we employed a water maze task to test that learning and memory are disturbed in these old rats. At the end of the five day training period, young (i.e., three month old SD) displayed the best performance and had the shortest escape latency, i.e., 15±3 sec, 12 month old SD were somewhat slower to learn, i.e., 17±5 sec, 18 month old SD were slower to learn, i.e., 53±15 sec, and most 24 month old SD did not learn, i.e., an escape latency of 65±58 sec. Also, only young SD displayed a clear preference for the correct quadrant in the probe trial, whereas the other groups of older rats did not spent most of their time searching in the correct quadrant. However, both the 12 month old and the 18 month old SD did display a spatial bias in their learning curves. Furthermore, it should be noted that the young animals were very similar in their learning capability, but the older rats displayed a large variability in their performance, i.e., some were good learners and others did not learn at all. These data demonstrate that in old rats, the breakdown of the connections between the retrosplenial cortices and the hippocampal formation lead to an impairment of spatial learning and memory. NIH: R01 AG 11958

459.10

SPATIAL MEMORY-IMPAIRED AGED F344 RATS SHOW A REGION SPECIFIC DEFICIT IN G-PROTEIN COUPLING OF THE M1 MUSCARINIC CHOLINERGIC RECEPTOR. M.A. Rossi*, L. deToledo-Morrell, D.C. Mash*, F. Morrell, Dept. Neurological Sciences, Rush Med. College, Chgo, IL 60612; *Dept. Pharmacology & Neurology, U. of Miami School of Medicine, Miami, FL.

The ability of acetylcholine and other full agonists to stimulate postsynaptic M1 muscarinic receptor-mediated second messenger signaling is important for memory function. However, in Alzheimer's disease, as well as aged rodents, the density of this receptor subtype appears to remain unchanged relative to controls. Physiologically, in mammals, including humans, G-protein coupled high agonist affinity (K_H) and G-protein uncoupled low agonist affinity (K_L) M1 receptor states concurrently exist. The K_L/K_H ratio for agonist, an index of G-protein coupling, has been shown to directly correlate with agonist efficacy of M1 muscarinic cholinergic receptors. In the present study, we demonstrate a relationship between age-dependent spatial memory impairment and alterations in K_L/K_H. Following testing in the eight-arm radial maze task, the high and low agonist affinity states of the M1 muscarinic receptor were measured using *in situ* receptor binding autoradiography in the HF of 5 young (5 mos. old) and 12 old (27 mos. old) F344 rats. A competition binding study was performed using the M1 muscarinic cholinergic receptor antagonist [^{3}H]pirenzepine (2.5 nM), with increasing concentrations of carbachol ($1x10^{-8}$-$1x10^{-2}$ M), the competing agonist. Two major findings were observed. First, a significant age effect (young vs. old) of the K_L/K_H ratio was seen only in CA3 ($F=22.4$, $p<0.001$). Secondly, when young (n=5), old memory-intact (n=6) and old memory-impaired (n=6) animals were compared using a two-way ANOVA followed by multiple comparison tests, K_L/K_H was found to be significantly decreased ($p<0.05$) in old-impaired rats compared to the old-intact and young animals in dentate gyrus, CA3 and CA1. Moreover, no such memory-related effect was seen in neocortex, the internal control. There was no significant difference in the density (B_{max}) or affinity (K_d) of [^{3}H]pirenzepine binding between the groups. The presence of a memory-dependent effect in the HF and lack of a similar change in neocortex suggests that there is a need for a functional high affinity state of the M1 receptor-G protein complex for its agonist in a region important for spatial working memory. Supported by grant AG08794 from NIA & IBN8912372 from NSF.

459.11

A DEFICIT IN THE REDISTRIBUTION OF PROTEIN KINASE C (PKC)-γ IN AGED F344 RATS IS RELATED TO AN IMPAIRMENT IN SPATIAL WORKING MEMORY. F. Morrell*, M.A. Rossi, L. deToledo-Morrell, Dept. of Neurological Sciences, Rush Medical College, Chicago, IL 60612.

A significant association between spatial working memory and the hippocampal distribution of PKC-γ was identified in aged male F344 rats using the eight-arm radial maze task. Twelve aged (27 mos. old) rats were behaviorally separated into 6 memory-intact and 6 memory-impaired animals. As controls, we used 7 young (5 mos. old), memory-intact rats. All animals were sacrificed and brains rapidly dissected and frozen at -80°C following the last day of behavioral testing. Ten micron thick coronal tissue sections were taken beginning 1mm caudal to the septal pole of the dorsal hippocampal formation. The tissue was immediately transferred onto nitrocellulose (NC) film-coated microscope slides (Grace Bio-Oncology, MI). Avidin-biotin peroxidase immunohistochemistry was then carried out using affinity purified anti-PKC-γ (GIBCO-BRL) specific for the hinge region of this PKC isoform. Purified PKC-γ protein calibration standard ladders were prepared and processed on NC coated slides along with the tissue. All PKC immunostaining on NC film-coated microscope slides was then quantified using image analysis software. In both dentate gyrus (DG) and CA1 subfields of the hippocampal formation, the data demonstrate a significant inverse correlation between choice accuracy and the ratio of soma to proximal dendrite immunostaining. The extent of spatial memory impairment correlates with a higher cell body to dendrite ratio for DG ($r=0.814$, $p<0.001$) and CA1 ($r=0.825$, $p<0.001$) immunostaining. Absolute hippocampal levels of PKC-γ did not vary with chronological age (young vs. old). Consequently, there appears to be a deficit in a hippocampal soma-to-dendrite redistribution of PKC-γ in aged rats with poor spatial working memory. Supported by grants AG08794 from NIA & IBN8912372 from NSF.

459.12

THE SUBCELLULAR DISTRIBUTION OF HIPPOCAMPAL PKC-γ IS RELATED TO SPATIAL LEARNING IN INDIVIDUAL AGED RATS. P.J. Colombo*[1], W.C. Wetsel[2], & M. Gallagher[1] [1]Department of Psychology, University of North Carolina, Chapel Hill, NC 27599, and [2]Lab. of Cell. and Mol. Pharm., NIEHS, Research Triangle Park, NC 27709.

The protein kinase C (PKC) isoforms comprise a family of enzymes important for signal transduction and implicated in activity-dependent neural plasticity and memory formation. In the current study, long- and short-term training protocols were used to determine whether age-related spatial memory impairment is associated with age-related alterations in hippocampal PKC-γ immunoreactivity. Six- and 24-month-old male Long-Evans rats were trained using our standard spatial learning protocol in a water maze (3 trials/day for 8 days). Two weeks later, all rats were given 6 trials in a new spatial environment to acquire a new escape location. Rats were decapitated immediately after behavioral testing, the hippocampi were homogenized, and soluble and particulate fractions were prepared from individual subjects. Proteins were separated by 10% SDS-PAGE, transferred electrophoretically to PVDF membranes, and immunostained for PKC-γ. ANOVA revealed a significant increase in PKC-γ immunoreactivity in the soluble fraction of aged-impaired rats ($N=15$) in comparisons with aged-unimpaired ($N=12$) and young rats ($N=13$). The concentration of PKC-γ in the soluble fraction was correlated significantly with place-learning. These results indicate that the subcellular concentrations of PKC-γ in the hippocampus of aged rats may be related to spatial memory formation.

Supported by F32-MH11337 to PJC, K05-MH01149 and P0-AG00973to MG, and NIEHS intramural research program to WCW.

459.13

EFFECTS OF AGE AND OPEN FIELD EXPOSURE ON PHORBOL ESTER BINDING IN THE RAT HIPPOCAMPUS AND CORTEX. A.H. Nagahara, I.A. Dowd, and R.J. Handa*. Department of Cell Biology, Neurobiology, and Anatomy. Loyola Medical Center, Maywood, IL 60153.

Previous findings in our laboratory showed age-related changes in the induction of c-fos m-RNA in the prefrontal cortex and caudate of aged rats following exposure to an open field. The present study examined age-related changes in phorbol ester binding, a measure of membrane-associated protein kinase C. Three age groups (5, 14, & 21 mo) of male Fischer 344 rats were used in the present experiment. Animals were exposed to a novel square open field for 20 min or remained in their home cages. Animals were sacrificed immediately after exposure to the open field or directly from their home cage. The level of phorbol ester binding was measured by quantitative autoradiography using [3H]phorbol 12, 13-dibutyrate. As previously reported, no significant age-related difference was observed in the number of box entries or rearing in these animals. Age-related increases in phorbol ester binding were observed in the CA1, CA3, and dentate gyrus of hippocampus, the caudate, and the anterior cingulate in the 21-mo rats (vs. 5-, 14-mo); however, no age-related changes were observed in retrosplenial and somatosensory cortex. In addition, exposure to the open field resulted in a decrease in phorbol ester binding in the hippocampus, caudate, and several cortical areas across age groups. No significant Age x Open Field interaction effects were observed. These findings suggest age-related alteration in the levels of membrane-bound protein kinase C in certain brain regions of aged rats. Supported by USPHS AA08696 (RH) and K21-AA00192 (AHN).

459.14

DIFFERENTIAL GENE EXPRESSION IN AGING MICE RELATED TO SPATIAL LEARNING PERFORMANCE. L.L. Bellush*, J.P. Walker, A.M. Wright, and R.A. Colvin. Program in Neurobiology, College of Osteopathic Medicine, and Dept. of Psychology, Ohio Univ., Athens, OH 45701.

We are exploring the hypothesis that decline in spatial learning ability in aging rodents is related to changes in gene expression. Using a hidden platform version of the Morris water maze, we have consistently found about 30% of 24 mo. old mice to have no learning impairment (unimpaired), while another 30 to 40% show significant impairment relative to young controls (impaired). Motor and sensory impairments were experimentally ruled out. Poly-A$^+$ mRNA was isolated from the cholinergic forebrain nuclei of the impaired and unimpaired mice and bound to Biomag beads. Using AMV reverse transcriptase, a single stranded cDNA library was synthesized on Biomag beads using the Poly-A$^+$ mRNA as a template. Biomag-cDNA from unimpaired mice was used to synthesize a complimentary or sense strand cDNA. Subtraction hybridization was performed by adding the unimpaired sense strand to the impaired Biomag-cDNA beads. All complimentary sequences were hybridized leaving only differentially expressed sense strand cDNA (subtracted cDNA) in solution. The resulting subtracted cDNA library was amplified by PCR. Southern blot analysis revealed 3 distinct bands of 282, 331, and 532 base pairs. These results, indicate the differential expression of 3 possible genes in the unimpaired cholinergic forebrain nuclei. Subcloning and sequencing of the subtracted cDNA may reveal genes whose protein products are vital for the proper function and support of the cholinergic system. Supported by the Ohio University College of Osteopathic Medicine.

DEVELOPMENTAL DISORDERS III

460.1

Examining MCI Mechanisms Using Fractionated Reaction Time
C.A. Lewis, P.R. Surburg & R.D.Devoe*. Indiana University, Bloomington, IN 47405.
One method of assessing neural development is to look at the acquisition of motor milestones. One milestone that appears at the age of eight is cross lateral integration; inability to attain this milestone is called midline crossing inhibition (MCI). Individuals with mental retardation (MR) manifest developmental delays and MCI persists. Eason and Surburg (1993) developed a new method to assess MCI which is predicated upon an information processing model. Crossing the midline is considered a more complex task and requires more processing time than ipsilateral movements as evidenced by slower reaction times (RT). The purpose of this study was to investigate the processing that occurs during midline crossing of individuals with MR by measuring premotor and motor times. By fractionating RT into premotor and motor time, a determination can be made whether MCI is the result of information processing by the central nervous system or peripheral processing by the peripheral nervous system. Seven adults with MR were tested with the same midline crossing apparatus and protocol (Eason and Surburg, 1993) implementing a choice RT and movement time task of the lower limb. Subjects performed nine ipsilateral, nine contralateral and nine midline motions completely randomized on four different days. Surface EMG recorded muscle activity of the tibialis anterior in order to measure premotor and motor times. All data was collected online using a sample rate of 1KHz. Results of a 3 x 4 (direction by day) repeated measures ANOVA for premotor time were a significant main effect for direction and an interaction effect of day by direction. Post hoc analysis determined the premotor time in the contralateral movements were significantly slower than premotor time in the midline and ipsilateral directions. There were no significant differences for motor or movement times but total reaction time (premotor + motor) results demonstrated a significant MCI effect. In conclusion, MCI was present in these individuals with MR and longer premotor times in the contralateral direction implies that this difficulty in crossing the midline is due to information processing in the central nervous system.

460.2

QUANTITATIVE ANALYSIS OF GRAY MATTER VOLUMES IN CHILDHOOD-ONSET SCHIZOPHRENIA AND ATTENTION DEFICIT/HYPERACTIVITY DISORDER.
J.N. Giedd, F.X. Castellanos*, J.C. Rajapakse, L.K. Jacobsen, J.A. Frazier, S.D. Hamburger, J.L. Rapoport. Child Psychiatry Branch, National Institute of Mental Health, Bethesda, MD 20892-1600.

We have previously found decreases in total brain volume in children with childhood-onset schizophrenia (COS) (Frazier et al., 1996) and in boys with attention deficit/hyperactivity disorder (ADHD) (Castellanos et al., 1996). We now report gray matter volumes for 28 COS subjects and 52 age-, sex-, height-, weight-, and handedness-matched controls and 59 ADHD boys and their 64 matched controls. Gray matter volumes were obtained using an innovative automated segmentation algorithm based on probabilistic modelling of gray and white matter distributions. Subjects with COS had a 10% smaller total cerebral volume with a 15% reduction in gray matter and 6% reduction in white matter. The decrease in gray matter in COS (but not white matter) remained significant ($p=.005$) after covariance for total brain volume. In contrast, subjects with ADHD had a 4% decrease in total brain volume with a 4% decrease in gray matter and a 5% decrease in white matter. Neither decrease remained significant after covariance for total brain volume. For controls and the two patient groups gray matter decreased and white matter increased significantly with age, but none of the slopes differed between groups. These data indicate different developmental processes specific to these two disorders.

460.3

7B2 AND PC_2 ARE ABSENT IN SOME PRADER-WILLI PATIENTS THAT ARE ALSO UNABLE TO PROCESS THE VASOPRESSIN PRECURSOR CORRECTLY.
B.A.Th.F. Gabreëls, D.F. Swaab and F.W. van Leeuwen*. Neth. Inst. Brain Res., 1105 AZ Amsterdam, The Netherlands.

Prader-(Labhart-)Willi syndrome (PWS), with an incidence of 0.5 (per 10^4 births), is characterized by infantile hypotonia, early childhood obesity, mental deficiency, short stature, small hands and feet and hypogonadism. This syndrome is associated with a defect at chromosome 15q11-q13, close to the location of the 7B2 gene (15q13-q14). In 70% of the cases small or large deletions are present. The remaining PWS patients display maternal uniparental disomy (22%) or other abnormalities of chromosome 15. 7B2 is considered to be a neuroendocrine chaperone of the processing enzyme PC_2. Since 7B2 gene products are expressed in neuroendocrine cells that are probably affected in PWS (e.g. by a pleiotrophic influence of the neighboring deletion), the presence of 7B2 and PC_2 was studied immunocytochemically in the supraoptic and paraventricular nucleus of the hypothalamus of six subjects clinically diagnosed as PWS patients. Molecular genetical diagnosis of this fixed PWS material was only partially possible.
Four of the six PWS patients studied showed no reaction to the 7B2 antibody MON-102, whereas all 30 control patients did. However, two of the four MON-102 non-reacting PWS patients reacted to other 7B2 antibodies. Surprisingly, the two 7B2 immunonegative PWS patients did not react with various vasopressin antibodies either, whereas they did react normally with five antibodies recognizing the vasopressin precursor. In these two patients no PC_2 immunoreactivity was found. Thus, it is possible that the symptoms of diabetes in these two patients are not merely attributable to poorly regulated diabetes mellitus. In the other patients 7B2 and PC_2 were expressed. In conclusion, the vanishing of 7B2 gene products is not obligatory for PWS, possibly due to the variable genetic background of PWS patients. The absence of 7B2 in two patients may result in a premature processing of proPC_2 and in the subsequent inability to process the vasopressin precursor accurately. Brain material was obtained via the Netherlands Brain Bank (coordinator Dr. R. Ravid).

460.4

$GABA_A$ RECEPTOR EXPRESSION IS REDUCED IN ANGELMAN SYNDROME BRAIN. T. M. DeLorey, B. Minassian, A. Datt, A. V. Delgado-Escueta*, R. W. Olsen. Depts. of Pharmacology and Neurology, UCLA, and West Los Angeles VA Medical Ctr., Los Angeles, CA 90095

$GABA_A$ receptor expression appears to be reduced in post-mortem brain of a patient with Angelman syndrome, measured by autoradiography and mRNA levels. Angelman syndrome is a severe form of mental retardation with epileptic episodes being present in ~90% of the probands. This syndrome has a prevalence rate of 1 in 10,000 births. Approximately 70% of Angelman syndrome probands have a large (~4 Mb)deletion in chromosome 15q11-13. Within this deletion region is found a cluster of three $GABA_A$ receptor subunit genes (GABRB3, GABRA5, and GABRG3). The postmortem brain of a 3-year old female Angelman syndrome proband with epilepsy was studied for altered $GABA_A$ receptor expression as compared to an age and gender matched normal brain. Radioligand binding using the benzodiazepine antagonist flumazenil revealed a reduction of approximately 50% in the temporal cortex of Angelman syndrome brain as compared to control temporal cortex. In addition, mRNA levels as determined by RT-PCR using primer sets for the $GABA_A$ receptor subunits β_3, were found to be reduced. Other mRNAs, including β-actin and $GABA_A$ receptor subunits β_2 and α_5 were present in AS in normal amounts. Supported by NS22071 and NS 21908.

460.5

INHIBITION OF BEHAVIOR IN A MOUSE MODEL OF DOWN SYNDROME. M.E. Coussons-Read and L.S. Crnic*, Dept. of Psychology, Univ. Colorado Denver, Denver, CO 80217 and Depts. Pediatrics and Psychiatry, Univ. Colorado School of Medicine, Denver, CO 80262.

The Ts(17^{16})65Dn (Ts65Dn) mouse carries a translocation chromosome containing most of the human Down syndrome (DS) homologous region of mouse chromosome 16 and has shown promise as a model for understanding the mechanisms behind the neural, behavioral and immune phenotype of human DS. Hyperactivity, poor Morris maze learning and lack of avoidance of the open arms of the plus maze have been demonstrated. Mice were tested for spontaneous alternation and laterality in a T maze, shock motivated spatial learning and reversal in a Y maze, shock sensitivity, and auditory startle and shock potentiated startle habituation. Trisomic (TS) mice alternated significantly more than non-trisomic littermate controls (44 vs 23%, $p < .01$), but showed no difference in laterality or shock sensitivity. Simple left-right spatial learning was not impaired, however the TS mice required significantly more trials to reach criterion in reversal training (42 vs 17 trials, $p < .002$). Habituation of startle was retarded in the TS mice, with no habituation evident in the first 50 trials, in contrast to the normal habituation in controls ($p < .025$ for the interaction between genotype and trial block). This trend was not significant for the shock potentiated startle, where neither group habituated. Together with preliminary data indicating delayed reversal in a non-spatial black-white discrimination task, these data support the interpretation that inhibition of behavior is impaired in these mice. Supported by HD04024, HD17449, MH11090 and MH17449.

460.6

CNS NORADRENERGIC TRANSMISSION AND AGE-RELATED BEHAVIORAL CHANGES IN TS65-DN MICE, A PUTATIVE DOWN SYNDROME MODEL. J. Flórez*[1], R.M. Escorihuela[2], I.F. Vallina[1], M. Megías[2], C. Baamonde[1], C. Martínez-Cue[1], M.A. Lumbreras[1], A. Fernández-Teruel[2], F. Mayor[4], D. Crespo[3], A. Tobeña[2], M. Dierssen[1]. Dept. [1]Physiology & Pharmacology and [3]Anatomy & Cel. Biology, Univ. Cantabria, Santander; [2]Unit Med. Psychology, Univ. Auton. Barcelona; [4]Dept. Molec. Biology, Univ. Auton. Madrid, Spain.

Further characterization of Ts65Dn mice has included: a) the biochemical analysis of β-adrenergic transmission in hippocampus (HC), cerebral cortex (CX) and cerebelar cortex (CB), and b) the study of age-related behavioral and cognitive changes. In 4-month-old male Ts65Dn mice, binding studies performed with [^{3}H]CGP-12177 showed no change in β-adrenoceptor densities and slightly reduced affinity in CX, and reduced density in HC, compared to control (non-trisomic litter mates). Adenylate cyclase activity, measured by cAMP accumulation, was also depressed in HP and CX, but not in CB of Ts65Dn mice in basal conditions and after stimulation with both isoprenaline and forskolin. β-ARK immunoreactivity, a regulatory protein of β-adrenoceptors, was not modified in the CB of Ts65Dn mice. Ts65Dn males (9-month-old) were tested in the plus maze for the evaluation of memory (latency to move from the open to the enclosed arm), in the open field to study their habituation pattern, and in the Morris water maze for measuring spatial working memory (by using the repeated acquisition paradigm). In all experiments three groups were included: Ts65Dn, control (non-trisomic litter-mates) and swiss mice. Ts65Dn showed alterations in the plus maze and in the open field, as well as impaired learning performance. The results showed that: a) young adult Ts65Dn males have impaired β-adrenergic transmission in specific brain areas; and b) in 9-month-old Ts65Dn, behavioral and cognitive parameters remain disturbed as previously found in 4-month-old. (Supported by Ramón Areces and Marcelino Botín Foundations, DGICYT PC94-1063 and FISS 95/1779).

460.7

PELIZAEUS-MERZBACHER DISEASE (PMD) - CLINICAL, RADIOLOGICAL AND MOLECULAR BIOLOGICAL STUDY, INCLUDING A SYMPTOMATIC CAREER K Murayama, +S Murayama*, #H Kosaka, H Watanabe, M Kodama, +I Kanazawa & K Kodama Dpt. Pediatrics, National Rehabilitation Center for Disabled Children, Tokyo, Japan, +Dpt. Neurology, the University of Tokyo & #Dpt. Pharmacology, the University of California, San Diego

PMD is a sex-linked-recessive progressive neurological disorder characterized by developmental disturbance in the formation of myelin in the central nervous system. The abnormality in the gene for proteolipid protein (PLP) was reported in about 10-30% of PMD. In the middle-sized urban center for handicapped children (200 beds in the ward), ten male patients matched the clinical diagnosis of PMD. The age ranged from one year to 32 years. All cases showed psychomotor retardation associated with 1) the reversal of signal intensity in T2-weighted images (T2WI) of the gray and white matter of the brain MRI; and 2) the lack of response after the first wave in auditory brain stem response. The analysis of PLP gene proved that four out of ten cases (two out of three cases with positive family history) had a point mutation of the gene. One case with a point mutation had a concomitant heterodupulication of the gene. Clinical picture of the cases with abnormal PLP gene was relatively homogeneous and usually severer than the case with normal PLP gene. The mother of one case (32 years of age) with heterozygote of a point mutation (Exon 2, 134A-G, Leu45Arg) first presented with slowly progressive spastic paraparesis in the kindergarten. Cerebellar scanning speech was noticed in the junior high school, and intention tremor at the age of 22 years. She was now in almost wheel-chair-bound state with prominent nystagmus, dysmetria and dysarthria. Her brain MRI was typical in the reversal intensity in T2WI of the gray and white matter, but was different from male PMD in relatively normal Flair images. Since symptomatic career of PMD was very rare, the study of this case may provide new insights into the biology of PLP.

460.8

THE RELATIONSHIP BETWEEN MUTATION SIZE AND COGNITIVE AND VISUAL PROCESSING SKILLS IN FEMALE FRAGILE X CARRIERS. W. Pizzi*[1], S. Block[2], R. Brusca-Vega[1], E. Berry-Kravis[3], D. Maino[2]. Northeastern IL. Univ.[1], Illinois Coll. Optometry[2], Rush Medical Coll.[3]. Chicago, IL.

The Fragile X gene contains a trinucleotide (CGG) repeat which is unstable and expands as it is passed from carrier to the affected offspring. There are two mutated forms of the gene in female carriers; the premutation, with 43-200 CGG repeats, and the full mutation with >200 repeats. This gene is thought to produce a protein necessary for normal brain development which is reduced in the mutated state. In this study, fragile X premutation and full mutation female carriers were compared on three tasks of visual processing and cognitive skills. In each case, there were significant differences between premutation and full mutation carriers on several of the subtests or the full test. Regression analyses showed a significant negative correlation between the number of trinucleotide repeats and scores on these tests. These data suggest that as the number of CGG repeats increases there is a corresponding decrease in cognitive ability.

460.9

Subcellular Localization Supports a Role for the Fragile X Mental Retardation Protein in Transporting mRNAs from the Nucleus to Somatodendritic Ribosomes SM Hersch*, C-A Gutekunst*, Y. Feng, ST. Warren. Departments of Neurology* and Biochemistry, Emory Univ. School of Medicine, Atlanta, GA 30322

Accumulating evidence points to a role for the fragile X mental retardation protein (FMRP) in transporting specific mRNAs from the cell nucleus to cytoplasmic ribosomes; however, FMRP has not yet been visualized in the cellular compartments that this model would predict. We have used specific monoclonal antibodies and immunoelectron microscopy (EM), with DAB and immunogold as labels, to study the subcellular localization of FMRP in rat brain. Immunoreactive neurons occurred throughout the brain, containing label primarily in the perikaryon and proximal dendrites. EM revealed FMRP immunoreactivity in somata, large and small dendrites, dendritic spines and some axon terminals. Unlabeled dendrites and spines suggest that FMRP localization may be selective in particular dendrites and spines. EM, using immunogold, allowed exquisite resolution of FMRP location. Immunogold particles were found in neuronal, but not glial, nuclei. Some were visualized within nuclear pores. In neuronal cytoplasm, immunogold particles were primarily found in association with free ribosomes. Occasional immunogold particles were found on the cytoplasmic surface of rough endoplasmic reticulum. In dendrites and dendritic spines, immunogold particles were cytoplasmic and were found in the submembranous regions in which ribosomes commonly occur. These findings are consistent with the hypothesized role for FMRP as a translocating RNA binding protein. Supported by the Howard Hughes Medical Institute (SW) and NS01624 (SH).

460.10

MULTIPLANE MORPHOMETRY OF CINGULATE GYRUS IS CORRELATED WITH NEUROPSYCHOLOGICAL AND ERP MEASURES OF DIVIDED ATTENTION IN AUTISM. P. G. Lesnik, K. T. Ciesielski*, A. Caswell, J. E. Knight, E. G. Benzel, B.E. Hart, and K.Yaksich. Clinical Neuroscience Laboratory, Department of Psychology, University of New Mexico, Albuquerque, NM 87131.

Since recent PET studies showed the significant role of the anterior cingulate gyrus (CG) and consistent frontal dorsolateral co-activation in divided attention tasks in healthy humans (Corbetta, et al.,1993; Devinsky, et al.,1995), and since ERP and neuropsychological observations revealed a specific inhibitory deficit in divided attention in autism (Ciesielski,et al.,1995) we suggest that morphological abnormalities in CG may underlie deficits in divided attention and frontal brain dysfunctions reported in autism. High-functioning subjects with autism and (age, gender) matched controls were submitted to 1.5 Tesla brain MRI and to frontal lobe assesment using The Wisconsin Card Sorting Test and the Stroop Test. A new protocol for multi-plane quantitative MRI analysis of CG was developed. Results in autism in contrast to controls, revealed significantly reduced morphometric measures of anterior CG ($p<.05$). These findings were correlated with poorer performance on frontal lobe tests ($p<.001$) and with decreased amplitude of negative waves to targets recorded during an ERP divided attention task ($p<.05$) and increased enhanced negative waves to task irrelevant stimuli ($p< .05$). The above converging evidence is discussed in the context of mesial-frontal and anterior limbic involvement in autism.

460.11

PLATELET SEROTONIN IN AUTISM, ASPERGERS SYNDROME, AND PDD-NOS: GROUP COMPARISONS AND THE EFFECTS OF FAMILIAL LOADING.

G. M. Anderson*, P. Szatmari, C. J. McDougle, P. A. McBride, L. M. Hall, L. A.Hoult, and D.J. Cohen, Yale Child Study Center, New Haven, CT 06510, and the Depts. of Psychiatry, McMaster University, Hamilton, Ontario L8N 3Z5 and Cornell University Medical College, New York, NY 10021.

Studies of platelet serotonin (5-hydroxytryptamine,5HT) in autism have found substantial group mean elevations in autistic individuals compared to normal controls. Recently, we have reported that the elevation is not associated with the lower intelligence often seen in autism. In order to further examine the specificity of the increased platelet 5HT, we have studied relatively large groups of Autistic, Aspergers Syndrome, and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS) patients. In addition, we have compared patients with and without an affected sibling in an attempt to replicate the report that autistic patients with higher familial loading have even higher platelet 5HT . Such studies, in large well-characterized patient groups, should lead to a better understanding of the behavioral correlates and genetic determinants of elevated platelet 5HT.

Support: NIH grants MH30929 & 44177, MRC of Canada, & the Korczak Stichting.

460.12

EXCITATORY AND INHIBITORY NEUROTRANSMITTER RECEPTOR EXPRESSION IS ALTERED IN RETT SYNDROME AND IN A MOUSE MODEL FOR RETT SYNDROME.

M.E. Blue*, C.F. Hohmann, S. A. Wallace, S. Naidu and M.V. Johnston. Kennedy Krieger Res. Institute, Johns Hopkins Univ. Sch. of Med. and Morgan State Univ. Baltimore, MD 21205

The role of glutamate receptors in Rett Syndrome (RS), a neurological disorder almost exclusively found in females, which is characterized by profound impairments in motor and cognitive function, was examined by receptor autoradiography for NMDA, AMPA, kainate (KA) and metabotropic (mGluR) receptors in the basal ganglia of 8 cases (ages 2-30 years) and in 8 normal cases (ages 4-20 years). The distribution of EAA receptors also was studied in a mouse model for RS, in which neonatal basal forebrain (nBM) lesions lead to altered maturation of cerebral cortical neurons and connections and cognitive impairments. Mice were studied at postnatal day 10 (P10), P14 and P30. In human RS cases, the densities of AMPA ($p<0.05$) and GABA ($p<0.0001$) receptors were reduced in the putamen compared with controls, but the caudate was less affected. NMDA, KA and mGluR receptors were not significantly reduced in the caudate or putamen. The globus pallidus exhibited visible densities of mGluR and GABA sites, but neither site was significantly affected in RS. The density of AMPA, KA and NMDA receptors in the basal ganglia decreased with age in both groups. NMDA receptors were decreased significantly in all layers except layer VI of parietal cortex on the nBM lesioned side versus both sides of control mice. AMPA and KA receptors were not significantly affected. mGluR receptors were increased significantly in layers I-III on the nBM lesioned side at P10; but not at P14 and P30. The findings suggest that both excitatory and inhibitory transmitter function is profoundly but selectively altered in the basal ganglia of RS patients. The preferential decline of binding in the putamen, which serves a primary motor function, is consistent with the prominent motor deficits observed in RS. Preservation of the mGluR, NMDA and KA sites suggests that specific neurotransmitter systems are affected in RS. The selective changes in glutamate receptors observed in the nBM lesioned mice, with some similarities to those observed for RS, support its use as an animal model for RS. Supported by N.I.H. grants # HD-24448 and # HD24061.

460.13

INDICES OF AMINO ACID TRANSMISSION IN HUMAN INFANTS VULNERABLE TO HYPOXIA

D. L. Andersen and P. R. Dodd* *Clinical Research Laboratory, Royal Brisbane Hospital Research Foundation, Brisbane, Q 4029, AUSTRALIA.*

Selective neurotoxicity may underlie vulnerability to asphyxia at birth, as well as SIDS. Term (39 weeks' gestation) control cases have more GLU-R_{NMDA} sites (labelled with [^{3}H]MK801) in frontal cortex than do preterm (~28 weeks') control cases, but vulnerable cases fail to show this increase. Cerebral cortex samples were obtained at autopsy and slow-frozen for storage at –80°C. Homogenate binding assays were carried out on rapidly thawed tissue pieces; data were processed with the EBDA and LIGAND programs. There were no significant differences in GLU-R_{NMDA} binding-site affinity (K_D) or density (B_{MAX}) between SIDS cases and controls. However, these sites continued to accumulate postnatally, as evidenced by a rise in B_{MAX}. There were no differences between SIDS cases and controls in the maximal glutamate enhancement of [^{3}H]MK801 binding, although this parameter had still not reached adult levels, suggesting that GLU-R_{NMDA} sites continue to undergo structural reorganization postnatally. $GABA_A$ receptor sites were invariant in K_D and B_{MAX} for [^{3}H]diazepam or [^{3}H]flunitrazepam across all regions during infant development, but values differed from those found in adults. [^{3}H]Diazepam B_{MAX} was significantly higher in infant than in adult controls, particularly in temporal and occipital cortices, whereas the B_{MAX} for [^{3}H]flunitrazepam was lower. GABA enhancement of [^{3}H]diazepam binding in temporal cortex differed significantly in infants and adults. Taken together, these data suggest that the amounts and the structures of the human $GABA_A$ receptor site differ markedly in infants and adults.

Supported by the National SIDS Council of Australia and the NHMRC

DEGENERATIVE DISEASE: ALZHEIMER'S-BETA-AMYLOID—ACCUMULATION AND AGGREGATION

461.1

PERMEABILITY AND RESIDUAL PLASMA VOLUME OF HUMAN, DUTCH VARIANT, AND RAT AMYLOID β-PROTEIN 1-40 AT THE BBB

Geoffry L. Curran*, Jill J. Haggard, Dennis J. Selkoe, and Joseph F. Poduslo
Mayo Clinic/Foundation and Harvard Medical School

Amyloid β-protein (Aβ) has been identified as a primary component of both parenchymal and vascular amyloid deposits in Alzheimer's disease (AD). While Aβ is produced continuously by cells in the nervous system as well as in peripheral tissues, the extent to which Aβ in brain parenchyma of AD is derived from peripheral locations is not known. The ability of Aβ to cross the BBB may lead to localized toxic effects in specific neuronal populations with subsequent increased production of parenchymal Aβ. To begin to address this issue, the permeability of normal human, the Dutch variant, and rat Aβ 1-40 at the BBB was determined in the normal adult rat. The permeability coefficient-surface area (PS) product was quantified for each protein after correction for the residual plasma volume (V_p) occupied by the protein in the capillary bed of different brain regions using the i.v. bolus injection technique with the same protein radioiodinated with a second isotope of iodine to determine separately PS and V_p values. The PS for normal and Dutch Aβ ranged from 13-22 x 10^{-6} ml/g/s in different brain regions, which is 130 to 220 times greater than albumin. These high PS values compare to that of insulin, whose uptake is decidedly by a receptor-mediated transport process, and suggest a similar mechanism for Aβ. Remarkably, the PS for rat Aβ was 4 times higher and ranged from 54-82 x 10^{-6} ml/g/s for different brain regions, suggesting a distinctive species specificity. While the V_p values of human and rat Aβ were comparable, the Dutch variant was 2 to 3 times higher, indicating adherence to the capillary walls in different brain regions, consistent with the pathology that has been described in brain capillaries with this variant. SDS-PAGE of ^{125}I-human Aβ after 60 min uptake revealed intact protein in plasma and in different brain regions. The high PS values observed for Aβ at the BBB suggest sources outside the nervous system may contribute, at least in part, to the cerebral Aβ deposits seen in AD (Mayo Foundation).

461.2

ACTIVATED ALZHEIMER'S DISEASE PLATELETS RETAIN MORE MEMBRANE-BOUND AMYLOID PRECURSOR PROTEIN.

Davies TA, Long HJ, Sgro K, Seetoo KF, Tibbles H, Billingslea AM, Fine RE, Levesque CA, Smith SJ, Berkowitz, EM*, Wells JM, Simons ER. Boston U. Sch. of Med. and Boston City Hospital, Boston, MA and V.A. Hosp, Bedford, MA

Activated platelet α-granules' soluble contents are secreted and membrane-bound contents translocated to the plasma membrane. Membrane-bound proteins include the ß-amyloid precursor protein (APP) from which the ß-amyloid (Aß) deposits found in the cerebrovasculature of patients with Alzheimer's Disease (AD) may originate. We found that activated platelets from AD patients exhibit less APP processing, retaining more of the protein on their surface and secreting less as soluble fragments, than do controls. Surface labeling demonstrated that there is little APP or p-selectin on the surface of resting platelets. Upon activation, control platelets exhibited both proteins on their surface while advanced AD patients exhibited similar amounts of p-selectin as controls, but retained significantly more surface APP. AD platelets secreted similar amounts of most soluble α-granule contents as controls, but less APP fragments. Platelets from non-demented age-matched donors, Pick Disease or Down Syndrome patients, or patients in early to mid-stage AD, resembled controls. Together these results suggest an APP processing defect specific to platelets from patients with advanced AD. Since activated platelets adhere to the endothelium, the excess APP carried on the membrane of activated AD platelets may account for greater deposition of Aß-containing products in the cerebral vasculature of these patients. Supported by NIH AG11526, AG10684, HL07501 and VA Merit grant.

461.3

INTERFERON-γ INDUCES ACCUMULATION OF THE AMYLOID ß PRECURSOR PROTEIN IN MULTIVESICULAR BODY-LIKE ORGANELLES IN HUMAN BRAIN PERICYTES AND SMOOTH MUSCLE CELLS. M.M. Verbeek[1], I.Otte-Höller[1], D.J. Ruiter[1], R.M.W. de Waal[1], A.R. Cools[2]*. Depts. of [1]Pathology and [2]PsychoNeuroPharmacology, University Hospital, Nijmegen, The Netherlands.

Accumulation of the amyloid ß protein (Aß) in leptomeningeal and cortical vessels is one of the pathological characteristics of the brains from patients with Alzheimer's disease (AD). Previously it was suggested that excessive Aß production in AD brains is related to abnormal intracellular trafficking of the amyloid ß precursor protein (APP). We studied the intracellular localization and secretion of APP in cultured smooth muscle cells from human leptomeningeal vessels as well as human brain pericytes from cerebrocortical capillaries. Both these cell types are candidate for producing vascular Aß in AD brains. Microvascular endothelial cells were studied as a control celltype. By using immunofluorescence and immunoelectron microscopy we demonstrated that APP is found in both lysosomal and non-lysosomal organelles in these cells, but in the ER and Golgi compartments of endothelial cells. Treatment with the cytokine interferon (IFN)-γ or with chloroquine caused an accumulation of APP in multivesicular body (MVB)-like organelles, accompanied by a reduction of APP-secretion. FITC-labeled dextran or 6 nm-gold particles coupled to BSA, allowed to be ingested by these cells, accumulated in these MVB-like organelles, confirming their endosomal nature. Our studies show that inflammatory mediators, such as IFN-γ, may contribute to the process of aberrant APP routing by concentrating APP in a MVB-like compartment, possibly by enhancing the rate of endocytosis of cell surface molecules, such as APP.

Supported by the Netherlands Organization for Scientific Research (NWO).

461.4

REMOVAL OF ALZHEIMER AMYLOID BETA PEPTIDE BY LEPTOMENINGEAL MEMBRANES. G. E. Wihl, G. Pavlakovic, D. D'Urso, R. Frank, I. Prikulis, H. Koeller* and R. Prior. Department of Neurology, University of Düsseldorf, 40225 Düsseldorf, Germany.

Soluble amyloid beta peptide (Aß) is produced by cortical neurons and released to the cortical extracellular space and the cerebrospinal fluid, where Aß species of different length are detected in nanomolar amounts. Progressive precipitation of soluble Aß into senile plaques and the amyloid deposits of cerebral amyloid angiopathy (CAA) is a central event during the pathogenesis of Alzheimer's disease. The precipitation of soluble Aß is dependent on the concentration of the hydrophobic Aß peptide. An efficient removal of Aß from the cortical interstitial fluid may therefore be important to prevent excessive Aß-amyloidogenesis.

Cultured rat microglial cells have been shown to remove Aß added to the cell culture medium (Shaffer et al., Neurobiol Aging 16: 737, 1995). We have established organ cultures of canine leptomeninges as a model to study the pathogenesis of CAA (Prior et al., Neuroreport 6: 1747, 1995). Leptomeninges cover the surface of the cerebral cortex and are interposed between the cortical extracellular space and the cerebrospinal fluid. Therefore, we investigated whether leptomeningeal membranes have an Aß-removing capability. Organ cultures of canine leptomeninges were incubated with 1 µM of biotinylated Aß1-40 in the culture medium. ELISA-analysis of culture supernatants was performed at 6, 24, 48 and 72 h and revealed a progressive decline of Aß to 200 nM at 72 h. Control experiments with dead leptomeninges or with medium in the absence of leptomeninges showed only minor decreases of Aß concentrations. Preliminary immunofluorescence data suggest that the uptake of Aß is accomplished by macrophages or other phagocytosing cells present in the leptomeninges. Our results demonstrate the ability of leptomeningeal membranes to remove soluble Aß from cerebral fluids indicating their potential function in the prevention of Alzheimer's disease neuropathology. Supported by DFG (Mu630/5-3 to R.P.)

461.5

FOCAL UPTAKE OF ALZHEIMER AMYLOID BETA PEPTIDE BY MECHANICALLY INJURED LEPTOMENINGEAL VESSEL WALLS R. Prior*, G.E. Wihl, D. D'Urso, R. Frank, I. Prikulis and G. Pavlakovic. Department of Neurology, University of Düsseldorf, 40225 Düsseldorf, Germany.

The abnormal deposition of amyloid beta peptide (Aß) is a major neuropathological feature of Alzheimer's disease (AD) and of cerebral amyloid angiopathy (CAA). In CAA, Aß is deposited within the wall of small cortical and leptomeningeal vessels. CAA is associated to AD but may occur also without dementia. CAA is an important cause of intracerebral hemorrhage in the elderly. We have previously established organ cultures of canine leptomeninges as a model to study the pathogenesis of CAA (Prior et al., J Neurosci Meth, in press). Focal Aß deposits were observed in living canine leptomeningeal vessels when leptomeninges of old dogs with CAA were incubated in culture medium containing nanomolar concentrations of Aß (Prior et al., Neuroreport 6: 1747-51, 1995). This experimental setup resembles the situation in vivo, where leptomeningeal vessels are surrounded by cerebrospinal fluid (CSF) containing soluble Aß. Leptomeningeal vessels without CAA or vessels of young dogs did not show any Aß deposits.

Here we report that deposition of soluble Aß can be observed in normal vessels of young dogs after mechanical damage to the vessel wall. Mechanically injured or cut living vessels were incubated in culture medium containing 100 nM to 10 µM of fluorescein-coniugated Aß(1-40) or Aß(1-42). After two days, cryosections were analyzed by fluorescence microscopy. Sections were immunostained for different vessel wall antigens (smooth muscle actin, desmin, collagen III, fibronectin) and confocal microscopy analysis showed that Aß1-40 and Aß1-42 deposits were localized within the tunica media of small arterioles as usually observed in CAA. Our results suggest that CAA may be experimentally induced in normal vessels and that soluble Aß derived from the CSF may bind to an extracellular matrix component of the vessel wall in vivo. Extracellular matrix binding and progressive accumulation of CSF-derived Aß may be an important pathogenetic mechanism during the development of CAA. Supported by DFG (Mu630/5-3 to R.P.).

461.6

DISTRIBUTION OF AMYLOID $ß_{42}$ SPECIES AND INTRA-CEREBRAL HEMORRHAGE IN ALZHEIMER'S DISEASE

R.N. Kalaria*, H. Mori, D. L. Cohen and D.R.D. Premkumar. Departments of Neurology and Pathology, and the UH Alzheimer Center, Case Western Reserve University, Cleveland, Ohio 44106, USA; Tokyo Institute of Psychiatry, Setagaya-ku, Tokyo 154, Japan.

Recent advances have demonstrated that amyloid ß (Aß) protein exhibits carboxyl- and amino-terminal heterogeneity in cortical and cerebrovascular amyloid deposits of Alzheimer's disease (AD). Using carboxyl end-terminal specific antibodies to Aß peptides, both $Aß_{40}$ and the longer more pathogenic $Aß_{42}$ species have been localized in cerebral vessels of AD subjects. Here, we assessed the distribution of Aß species in cerebral vessel fractions from AD subjects with and without cerebral amyloid angiopathy related intracerebral hemorrhage. Using quantitative immunochemical and ELISA methods, we found that the ratios of $Aß_{42}/Aß_{40}$ were greater in samples derived from AD subjects with hemorrhages compared to those without. While it is not definitively known whether vascular cells also produce the $Aß_{42}$ form, further immunocytochemical assessment of the vascular abnormalities and amyloid infiltrated cerebral vessels indicated that the longer $Aß_{42}$ appears associated with the degenerative process, particularly in subjects with intracerebral hemorrhage. Our studies suggest that the $Aß_{42}$ species is also involved in cerebrovascular degeneration leading to intracerebral hemorrhage that is found in a population of AD subjects. Supported by grants from WHO-NINDS, NIA (NIH) and ADRDA.

461.7

PHARMACOLOGICAL MODULATION OF ALZHEIMER'S β-AMYLOID PRECURSOR PROTEIN LEVELS IN THE CSF OF RATS WITH FOREBRAIN CHOLINERGIC SYSTEM LESIONS. V. Haroutunian*, N. Greig, X-F. Pei, T. Utsuki, L. D. Acevedo, R. Gluck, K.L. Davis and W.C. Wallace Dep. Psychiatry, The Mount Sinai Sch. of Med., New York, NY. and Lab. Cell. and Mol. Biol., Gerontology Res. Cent., NIA, Baltimore, MD.

Abnormal deposition and accumulation of Alzheimer's amyloid β-protein (Aβ) and degeneration of forebrain cholinergic neurons are principal features of Alzheimer's disease. In rat model systems, lesion-induced forebrain cholinergic deficits (nbM) are accompanied by significant induction of cortical β-amyloid precursor protein (β-APP) mRNAs and increased levels of secreted β-APP in the CSF. We studied whether β-APP secretion from cholinergic targets could be altered pharmacologically. Scopolamine, a muscarinic receptor antagonist, was administered to determine whether APP could be induced by direct disruption of muscarinic receptor function. One week of scopolamine administration (0.5mg/kg/hr, N=7) led to a significant ($p<0.01$) increase in the CSF levels of secreted β-APP in sham lesioned rats indicating that antagonism of muscarinic receptors is sufficient for induction and secretion of β-APP. However, scopolamine did not upregulate further β-APP secretion in nbM lesioned rats. Opposite results were seen when cholinergic function was augmented by cholinesterase inhibition. One week of treatment with phenserine (2.5mg/kg/bid, N=7), a novel, reversible acetyl-selective cholinesterase inhibitor, significantly decreased the levels of secreted β-APP in the CSF of forebrain cholinergic system-lesioned (nbM) rats ($p<0.01$), whereas DFP, a relatively non-specific cholinesterase inhibitor, failed to affect CSF levels of secreted β-APP. These results suggest that the secretion of β-APP into the CSF can be pharmacologically modulated, but that this modulation is dependent upon the status of the forebrain cholinergic system and the pharmacological properties of the drugs used to influence it. Support: R01-AG10138 to V.H.

461.8

CEREBROSPINAL FLUID TAU AND BETA-AMYLOID 1-40/42 IN ALZHEIMER'S DISEASE M. Kanai*, E. Matsubara, Y. Igeta, Y. Tomidokoro, K. Ishiguro, T. Kawarabayashi, M. Watanabe, M. Shizuka, T. Nakamura, K. Okamoto, S. Hirai and M. Shoji. Department of Neurology, Gunma University School of Medicine, 3-39-15 Showa, Maebashi, Gunma 371, Japan. Tokyo Metropolitan Neurological Hospital, 2-6-1 Fuchu, Tokyo 183, Japan.

The 4-kd amyloid beta protein (A β) deposited as amyloid in Alzheimer's disease (AD) is produced and released by normal proteolytic processing of the amyloid beta protein precursor (β APP) and is readily detected in cerebrospinal fluid (CSF). Here, we present the levels of A β in CSF from a total of 77 subjects, including 45 patients with AD, 16 with early-onset AD and 29 with late-onset AD, 24 normal control subjects, and 8 patients with other neurological diseases. The CSF level of a novel form of the β -amyloid peptide (A β) extending to position 42 (A β 1-42) was determined in patients with AD as well as controls. In addition to measurement of CSF A β 1-42 levels, A β 1-40 peptides, microtubles-associated protein tau, and were also assessed. The CSF tau levels were 219 $\pm$ 89pg/ml in early onset AD, 377 $\pm$ 220pg/ml in late onset AD, 124 $\pm$ 96pg/ml in control, and 105 $\pm$ 69pg/ml in other neurological diseases. The CSF A β 1-40 were 1448 $\pm$ 734fmol/ml in early onset AD, 1479 $\pm$ 582fmol/ml in late onset AD, 1434 $\pm$ 726fmol/ml in control and 1416 $\pm$ 391fmol/ml in other neurological diseases. The CSF A β 1-42 were 115 $\pm$ 50fmol/ml in early onset AD, 113 $\pm$ 45fmol/ml in late onset AD, 212 $\pm$ 125fmol/ml in control and 159 $\pm$ 91fmol/ml in other neurological diseases. The tau level was significantly increased in late onset AD patients as compared with that in patients with other diseases and control subjects. And it is interesting that CSF A β 1-42 levels were found to be significantly lower in AD patients relative to controls, whereas deposit in the brain tissue of patients with AD, suggesting that diminished in clearance may account for its reduction in CSF. Tau levels were increased in AD patients. Taken together, our results suggest that the enzyme-linked immunosorbent assay of cerebrospinal fluid tau and A β 1-42 may prove to be a reliable and early diagnostic test for AD.

461.9

AMYLOID β PROTEIN SPECIES IN CEREBROSPINAL FLUID OF PATIENTS WITH ALZHEIMER'S DISEASE. A.Tamaoka*(1), N.Sawamura(1), S.Shoji(1), M.Shoji(2), S.Hirai(2), Y.Furiya(3) and H.Mori(3). Department of Neurology, University of Tsukuba, Tsukuba, Ibaraki 305(1); Department of Neurology, Gunma University, Maebashi, Gunma 371(2); Department Of Molecular Biology, Tokyo Institute for Psychiatry, Setagaya, Tokyo 156, Japan(3).

To search for a diagnostic laboratory test for Alzheimer's disease (AD), cerebrospinal fluid (CSF) levels of amyloid β protein (Aβ) species (CSF-Aβ) with different carboxy termini, i.e., AβX-40 and AβX-42(43) as well as Aβ1-40 and Aβ1-42(43), were measured in patients with AD and age-matched controls without dementia (CTR) using sandwich enzyme-linked immunosorbent assays (ELISAs). The present study revealed that both CSF-AβX-42(43) and Aβ1-42(43) levels were significantly lower in the AD patients ($p<0.005$) than in the CTR group, whereas neither CSF-AβX-40 nor CSF-Aβ1-40 levels showed any differences between the two groups. In addition, although there was no difference between the ratios of AβX-40 to Aβ1-40 in the AD and CTR groups, the ratios of AβX-42(43) to Aβ1-42(43) were increased in the AD group compared with those in the CTR group ($p<0.05$). Recently, Aβ42(43) with carboxy termini ending at residue 42(43), the amino terminal of which is often truncated and/or modified, has been shown to play an important role in the formation of amyloid fibrils, and to preferentially deposit as diffuse plaques, the earliest neuropathological changes in AD brains. Taking these facts into consideration, increased adsorption of Aβ42(43) to Aβ deposition in AD brains, decreased secretion of Aβ42(43) to CSF and/or increased clearance of Aβ42(43) from CSF might explain the diminished levels of Aβ42(43) in the CSF of AD patients. Moreover, CSF-Aβ42(43) could reflect increased amino terminal truncations and/or modifications of Aβ42(43) in AD brains.

461.10

Quantification of Alzheimer's β-amyloid peptide levels in the plasma, CSF and brain tissue from guinea pig following administration of classical proteases inhibitors. D.M. Tummolo, S.C. Sun, Q. Xiang, H. Zhou, J.S. Jacobsen, and J. Sonnenberg-Reines* Central Nervous System Disorders, Wyeth-Ayerst Research, CN-8000, Princeton, New Jersey 08543.

Alzheimer's disease is characterized by the progressive formation of insoluble amyloid plaques in the brain which consist of the 4 kDa β-amyloid peptide (βAP). βAP is derived by proteolysis from the amyloid precursor protein, βAPP. Using a highly sensitive and selective sandwich ELISA, developed to analyze βAP levels in transfected cells, we have found inhibitors which alter the levels of βAP in the conditioned media. To further assess these inhibitors *in vivo*, we have modified a guinea pig model first reported by C. Eckman and S.Younkin in 1995 (Neurosci.Abstract, # 208.5). Similar to their initial reports, we find the plasma levels of βAP to increase 2 to 4 fold in animals treated with phosphoramidon, a metalloprotease inhibitor. Levels of βAP in the cerebrospinal fluid are similarly elevated in these animals. This phenomenon is dose dependent and transient with peak values observed at 2 to 4 h and return to basal values by 24 h post treatment. Analysis of brain extracts indicate that βAP levels in these animals are not altered by this treatment. One interpetation of the data is that phosphoramidon is inhibiting a catalytic protease that is responsible for the turnover and degradation of βAP. The effect of pepstatin, an aspartyl protease inhibitor, reported to inhibit βAP in tissue culture cells was also tested in this paradigm and will be discussed. Since βAP formation leads to amyloid deposition, the guinea pig model may be useful in developing therapeutic stratagies to inhibit βAP associated with Alzheimer's disease.

461.11

ANALYSIS OF PLASMA Aß CONCENTRATION IN NON-DEMENTED SUBJECTS OF ALL AGES.

C.B. Eckman , D. Yager, N. Graff-Rafford, and S.G. Younkin*
Mayo Clinic Jacksonville, Jacksonville, FL. 32224

Using sandwich ELISAs that specifically detect Aß1-40 and Aß1-42(43), we have recently found that the various FAD-linked PS1 mutations, one FAD-linked PS2 mutation, and the FAD-linked mutations at both sites in the ßAPP gene significantly increase the concentration of Aß1-42(43) in human plasma. During the course of this experimentation an apparent correlation was observed between age and Aß concentration in a small group of young controls. To pursue these observations further we utilized sandwich ELISAs specific for Aß1-40 (BAN-50/BA-27), Aß1-42(43) (BAN-50/BC-05), and Aß42(43) (BNT77/BC-05) to analyze approximately 170 non-demented subjects ranging in age from 21-97 years old. Our analysis of plasma samples from these subjects (i) confirms that Aß1-40 is the major Aß peptide in plasma, (ii) shows that in a small percentage of individuals Aß42(43) is elevated into the range associated with FAD, and (iii) indicates that the concentration of plasma Aß1-40 and Aß42(43) increases with age. Collectively these results suggest that AD may develop in some patients as a consequence of elevated extracellular Aß42(43) that is demonstrable in plasma long before disease develops. Additional results from our ongoing as well as prospective studies are clearly needed, however, before drawing any conclusion about the risk for AD that is conferred by elevation of plasma Aß42(43).

(Supported by NIH AG6656 and the American Health Assistance Foundation)

461.12

BIOCHEMICAL ANALYSIS OF AMYLOID β PROTEIN WITH AMINO-TERMINAL PYROGLUTAMATE 3 IN ALZHEIMER'S DISEASE BRAINS. Y. Harigaya*[1], C. B. Eckman[1], C-M. Prada[1], T. C. Saido[2] and S. G. Younkin[1]. [1]Mayo Clinic Jacksonville, Jacksonville, FL 32224, [2]Department of Molecular Biology, Tokyo Metropolitan Institute of Medical Science, Tokyo 113, Japan

The amyloid deposited in senile plaques of the Alzheimer's disease (AD) brain is composed of a 39-43 amino acid protein referred to as the amyloid β protein (Aβ). In previous work, we employed specific sandwich ELISAs to analyze the total Aβ in AD cerebral cortex after direct extraction into formic acid, and showed that most of the Aβ in AD brain is amino-terminally truncated or modified (Gravina et al. JBC 270:7013,1995). Recent immunocytochemical studies have shown that Aβ species with amino-terminal pyroglutamate3(3pE) are deposited in senile plaques (Saido et al. Neuron 14: 457,1995). To quantitate these Aβ species beginning at 3pE biochemically, we developed specific sandwich ELISAs to detect Aβ3pE-40 (BA-27/anti-N3pE) and Aβ3pE-42(BC-05/anti-N3pE). Using these ELISAs, we have analyzed direct formic acid extracts of temporal cortex from AD patients and normal controls. Using specific sandwich ELISAs employed in our previous work, the Aβ1-42(BAN-50/BC-05), Aβ1-40(BAN-50/BA-27), AβX-42(BC-05/4G8) and AβX-40(BA-27/4G8) in these extracts was analyzed in parallel. Cryostat sections cut from blocks adjacent to the regions analyzed biochemically were immunostained with 4G8 antibody. Both Aβ3pE-42 and Aβ3pE-40 were invariably detected in AD brains, although the amounts varied from case to case. In all AD cases, a substantial fraction of the total Aβ ending at Aβ42 was Aβ3pE-42 and a substantial fraction of the total Aβ ending at Aβ40 was Aβ3pE-40. Thus the results of our biochemical analysis suggest that species beginning at Aβ3pE constitute a substantial fraction of the Aβ deposited in the AD brain. This suggests that Aβ species beginning at 3pE may play an important role in amyloidogenesis and plaque formation.
Supported by Uehara Memorial Foundation, Japan (Y.H.)

461.13

ALZHEIMER'S SOLUBLE AMYLOID β IS A NORMAL COMPONENT OF URINE. E. Matsubara[1], S. Governale, M. Calero, T. Wisniewski, B. Frangione and J. Ghiso*. Dept of Pathology, New York University, New York, NY 10016 and [1]Dept of Neurology, Gunma University, Gunma, Japan.

Amyloid beta (Aβ) is a constituent of the fibrils deposited into senile plaques and cerebral blood vessels of patients with AD, Down's syndrome and HCHWA-Dutch type.Although originally thought to be an aberrant degradation product of its precursor βPP, it has been found as a soluble component (sAβ) in plasma and CSF where it is normally present at a very low concentration (1 - 5 ng/ml). sAβ circulates in both biological fluids associated to high density lipoprotein (HDL) particles. The biochemical basis for this relationship is not fully understood; however, several studies indicate that apolipoprotein J, present in a subset of HDL particles plays a role as a carrier molecule for sAβ in biological fluids and in the transport of sAβ across the blood-brain barrier.

In order to understand the factors involved in the regulation of the plasmatic levels of sAβ, we investigated whether the peptide was present in urine and how alterations in the glomerular filtration and/or tubular resorption may affect the urinary levels of sAβ. Immunoprecipitation experiments were carried out with anti-Aβ antibodies and detection of sAβ was achieved via immunoblot analysis. Soluble Aβ (4.4 kDa) immunoreactivity was present in normal urine, and was more evident when specimens from glomerular and/or mixed proteinuria were examined. The identity of sAβ was corroborated by N-terminal sequence. Edman degradation of the 4.4 kDa band obtained from immunoprecipitation of 20 mg of urinary lyophilized protein from 2 cases exhibiting mixed proteinuria yielded the sequences DAEFRxDxGxEV and DAEFRhDsGYEV respectively corresponding to the intact N-terminus of the Aβ peptide. The data indicate that sAβ is a normal component of the urine, and entities affecting the glomerular filtration and/or tubular resorption will reflect the amount of sAβ present in the urine.
Supported by NIH grants AG05891, AG10953 and AR02594.

461.14

STRUCTURE ACTIVITY ANALYSIS OF Aβ DEPOSITION

WP Esler, ER Stimson, JR Ghilardi, AM Felix, Y-A Lu, BP Tseng, N Casey, HV Vinters, ML Karnovsky*, JP Lee, PW Mantyh and JE Maggio

BCMP Dept., Harvard Med. Sch., Boston, MA 02115; Molec. Neurobiol. Lab (151), VA Med. Ctr., Mpls, MN 55417; Peptide Research, Hoffmann-La Roche, Nutley NJ 07110; Chem. Dept., Boston Univ., Boston, MA 02215; Dept. Pathol. & Lab. Med., UCLA Med. Ctr. LA, CA 90024.

Deposition of the β-amyloid peptide (Aβ) in the form of amyloid plaques in the cerebral cortex of patients is widely considered to play a causative role in the development of Alzheimer's disease (AD). The process of Aβ deposition in AD can be modeled *in vitro* by measuring the deposition of physiological concentrations of radiolabeled Aβ peptides onto pre-existing plaques in preparations of unfixed AD human cerebral cortex. While *in vitro* deposition of Aβ peptides onto amyloid in AD cortex is very favorable under physiological conditions, essentially no binding is observed with age-matched non-AD tissue. To elucidate conformational features in the Aβ peptide important to plaque growth, Aβ congeners with a series of sequence alterations within the central hydrophobic cluster (residues 13 to 23) were synthesized and examined in deposition assays. Minor sequence alterations (point mutations) in this region of Aβ were found to have significant effects on the ability of the peptide to deposit. While some point mutations abolished activity, others accelerated *in vitro* deposition. Using biophysical methods, effects of sequence alterations on activity were found to correlate with particular changes in Aβ analog conformation in water (which is neither α-helix nor β-sheet). Together, these results suggest that certain primary, secondary and tertiary structural features in Aβ are important for amyloid deposition and may serve as targets for therapeutic intervention. Supported by NIH (AG11852, AG12853, AG10123, AG12435), VA (Merit Review), and the American Health Assistance Foundation (AD94071).

461.15

TEMPLATE SPECIFIC DEPOSITION OF Aß$^{1-40}$ ONTO CEREBROVASCULAR AMYLOID AND Aß$^{1-42}$ ONTO PARENCHYMAL AMYLOID IN THE HUMAN ALZHEIMER DISEASE BRAIN

J.R. Ghilardi, M.P. Finke, S.D. Rogers, E.R. Stimson, W.P. Esler, J. E. Maggio, H.V. Vinters, M.W. Dysken*, P.W. Mantyh. Mol. Neurobio. Lab (151) and GRECC, VA Med. Cntr., Mpls., MN 55417; BCMP Dept., Harvard Medical School, Boston, MA 02115; UCLA Med. Cntr., LA, CA 90024; Dept. of Psychiatry, Univ. of Minnesota, Mpls., MN 55455

Although cerebral deposition of the ß-amyloid peptide (Aß) is an invariant feature of Alzheimer disease (AD), there are marked differences in the forms of Aß that comprise parenchymal and cerebrovascular amyloid (CVA) deposits. Whereas parenchymal amyloid is primarily composed of Aß$^{1-42}$, CVA is primarily composed of Aß$^{1-40}$. To determine if template specificity plays a role in the differential composition of CVA and parenchymal amyloid deposits, we examined the localization and kinetics of physiological concentrations of ^{125}I-Aß$^{1-40}$ and ^{125}I-Aß$^{1-42}$ deposited onto aggregates of Aß$^{1-40}$ or Aß$^{1-42}$ and onto sections of human AD brain. In aggregates composed of either Aß$^{1-40}$ or Aß$^{1-42}$, ^{125}I-Aß$^{1-40}$ preferentially deposits onto aggregates of Aß$^{1-40}$ whereas ^{125}I-Aß$^{1-42}$ deposits preferentially onto aggregates of Aß$^{1-42}$. Similarly, in sections of AD brain ^{125}I-Aß$^{1-40}$ deposits more avidly onto CVA than ^{125}I-Aß$^{1-42}$, whereas ^{125}I-Aß$^{1-42}$ deposits more avidly onto parenchymal plaques than ^{125}I-Aß$^{1-40}$. Additionally, ^{125}I- Aß$^{1-42}$ appears to deposit onto a greater number of parenchymal plaques in sections of human AD brain than does ^{125}I-Aß$^{1-40}$. These data suggest that different forms of Aß (Aß$^{1-40}$ or Aß$^{1-42}$) have an intrinsic template specificity in that they preferentially deposit onto self vs. non-self aggregates. This intrinsic template specificity of different forms of Aß may contribute to the differential localization, composition and toxicity of CVA and parenchymal amyloid deposits. Blockade of Aß deposition onto CVA or plaques may therefore require distinct therapeutic agents. Supported by NIH AG11852, AG12853 and a VA Merit Review.

461.16

A MODEL FOR STRUCTURE DEPENDENT BINDING OF CONGO RED TO ALZHEIMER B-AMYLOID FIBRILS.

D.B.Carter* and K.C.Chou. CNS Research, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001.

The cytotoxic β-amyloid fibril is a logical candidate for the entity causing the initiating damage to neurons in AD and Down's syndrome. We have derived a model of binding for the dye molecule, Congo Red (CR), to a gb-sheet structure of β-amyloid (1-42). This model is based on the crystal coordinates of CR binding to porcine insulin fibrils from Turnell and Finch (JMB 227:1205-1223, 1992). The stretch of 9 amino acids (**ERGFFYTPK**) of the pseudo β-sheet in the insulin dimer has a pattern of residue types common to other congophilic amyloidogenic peptides such as Alzheimer β-amyloid (15-24) (**QKLVFFAED**), transthyretin (**PLMVKVLDA**), and Islet amyloid peptide (**NNFGAILSS**). The common pattern of residue types suggests the possibility that the amyloid-like fibers of amyloidogenic peptides are congophilic because the corresponding cross segments can also form an anti-parallel β-sheet similar to the one in the insulin dimer. The CR molecule has been aligned along the homologous stretch of amino acids in Alzheimer β peptide (2 molecules in anti-parallel distorted or pseudo β-sheet conformation) using the crystal coordinates from the Turnell-Finch paper to arrive at a putative structure for CR binding to Alzheimer's amyloid fibrils. Funded by Pharmacia and Upjohn Inc.

461.17

CONGO RED BLOCKS THE INHIBITORY EFFECT OF ALZHEIMER'S Aß PEPTIDE ON Na^+/Ca^{2+} EXCHANGE.

R.A. Colvin*, C.A. Derrico and A. Wu. Program in Neurobiology, Ohio University College of Osteopathic Medicine, Athens, OH 45701.

The inhibition of Na^+/Ca^{2+} exchange by synthetic Alzheimer's amyloid-β peptides (Aβ) was studied using plasma membrane vesicles isolated from whole rat brain. Only pre-aggregated $A\beta_{1-40}$ and $A\beta_{25-35}$ peptides were shown to reduce vesicular Ca content. Both peptides produced a maximal reduction in Ca content of approximately 50%. The peptides reduced Ca content with similar potency and half maximal effects were seen at less than 10 µM for $A\beta_{25-35}$. Calcium loaded vesicles mediate a rapid Ca^{2+}/Ca^{2+} exchange, which also was inhibited by aggregated $A\beta_{25-35}$. Aggregated $A\beta_{25-35}$ did not affect the passive Ca^{2+} permeability of the vesicles. Aggregated $A\beta_{25-35}$ reduced Ca content in plasma membrane vesicles isolated from normal and AD frontal cortex with less potency but the same efficacy as seen in rat brain. Aggregated $A\beta_{25-35}$ did not produce nonspecific effects on vesicle morphology such as clumping or loss of intact vesicles. When placed in the buffer used to measure Ca content, Congo red at molar ratios of less than one blocked the inhibitory effect of preaggregated $A\beta_{25-35}$. When added in equimolar concentrations to freshly dissolved and unaggregated $A\beta_{25-35}$, Congo red also was effective at blocking the inhibitory effect on Ca content. Vitamin E (antioxidant) and N-tert-butyl-α-phenylnitrone (spin trapping agent) failed to block the inhibitory action of $A\beta_{25-35}$. These data are consistent with a proposed mechanism where aggregated Aβ peptides interact with the exchanger protein and inhibit Ca^{2+} transport. Supported by a grant from the Alzheimer's Association.

461.18

Aβ AGGREGATION IN CELL CULTURE: SIMILAR AGGREGATION OF ^{125}I-Aβ AND ENDOGENOUS Aβ AND INHIBITION BY CONGO RED.

M.B. Podlisny*, P. Amarante, D. Walsh, E. Stimson, J. Maggio, D. Teplow & D. Selkoe. Harvard Med. School, Boston, MA

Aggregation of soluble 4 kD Aβ monomers into HMW insoluble fibrils is a critical process in the development of AD. We previously described a cell culture model of aggregation of secreted Aβ under physiological concentrations and conditions. Immunoprecipitation (IP) of conditioned media (CM) of metabolically labeled APP-transfected CHO cells revealed SDS-stable oligomers of Aβ of 6, 8, 12 and 16 kD, in addition to Aβ and p3. Oligomer:monomer ratios were increased when media were conditioned without serum. Congo red (CR) prevented this enhanced oligomer formation. To study these low MW oligomers without IP, we have performed experiments using ^{125}I-$A\beta_{1-40}$ ($IA\beta_{40}$) as a tracer in the CM and analyzed the results by SDS-PAGE and size exclusion chromatography (SEC). Whereas $IA\beta_{40}$ incubated at 37° in serum-free unconditioned medium (DMEM) migrated at 4 kD, $IA\beta_{40}$ incubated in serum-free medium of APP-CHO cells migrated at ~4, 6, 8, 16 and 25 kD. $IA\beta_{40}$ incubated with these cells in the presence of 10 µM CR migrated only at 4 kD. Addition of 10% serum to the cultures decreased the rate of $IA\beta_{40}$ aggregation. Incubation of $IA\beta_{40}$ with APP-CHO CM alone (without cells) produced similar aggregation of the peptide. Consistent with our gel analyses, SEC showed an extra peak of $IA\beta_{40}$ in samples incubated with cells vs in DMEM alone. We conclude that both endogenous and synthetic Aβ spontaneously undergo aggregation into SDS-stable oligomers in CHO cultures, and the process is inhibited by CR. This system can be used to examine initial events in physiologic Aβ aggregation and to screen anti-aggregation compounds. Supp:AG 06173.

461.19

ELECTROSPRAY IONIZATION MASS SPECTROMETRY STUDY OF β-AMYLOID PEPTIDE: CONFORMATIONAL DEPENDENT AGGREGATION

[1]X. G. Chen, [2]S. K. Brining, [1]V. Nguyen , [2]O.P. Rabin*, [1]A. Yergey* [1]Section on Metabolism and Mass Spectrometry, The National Institute of Child Health and Development, [2]The National Institute of Aging, NIH, Bethesda, MD 20892-1580.

Electrospray ionization mass spectrometry (ESI-MS) was used to study the conformation and aggregation of the synthetic β-amyloid peptide, residue 1-40 (βA4) as a function of concentration (250, 125, 57 and 6 µM) and sample aging. All mass spectra showed a major envelope of peaks at m/z 619.4, 722.8, 866.8, 1083.3, and 1444.2 corresponding to charge states of 7-3 respectively of a monomeric form of βA4. We found that the average charge state of the envelope associated with the monomeric form decreased by ca. 0.35 as samples were aged. In the 250µM samples, additional, weaker envelopes of peaks corresponding to charge states of βA4 dimeric, trimeric and tetrameric βA4 species were also seen. We found that these aggregates became weaker and ultimately were absent in both the more dilute solutions and in aged aliquots of the concentrated sample. It is known that βA4 solutions exhibit a random coil <-> β-sheet equilibrium which shifts toward the β-sheet form with solution aging. We interpret our mass spectra in a manner that is consistent with these models of β-amyloid behavior. First, the decreasing charge state of the ESI-MS envelope of βA4 is taken to show that the relatively open form of the peptide associated with a random coil form is capable of supporting a higher charge state than the more compact β-sheet form. The higher charge states indicating the presence of aggregated peptide are taken to be associated with random coil aggregates since they are seen to disappear as amyloid sample is aged. We interpret our inability to see an ion envelope which can be associated with aggregates of the β-sheet form to be a consequence of the presumed very compact nature of this form which is likely to be present in a charge state that would place it at a m/z beyond the range of our instrument. We have shown that ESI-MS can be used to monitor conformational changes and aggregations of β-amyloid proteins simultaneously, an ability that is a novel tool for the study of these molecules.

461.20

THE TOXICITY OF THE ALZHEIMER'S ß-AMYLOID PEPTIDE CORRELATES WITH A DISTINCT FIBRE MORPHOLOGY.

B. Seilheimer*, B. Bohrmann, D. Stüber, F. Müller, and H. Döbeli.
Hoffmann-La Roche Ltd, CH-4002 Basel, Switzerland.

In an attempt to understand better the relationship between physical properties, morphology and cellular toxicity we have prepared several ß-amyloid (Aß) peptides according to a highly standardized biotechnical method. Peptides generated were either mutated inside the membrane anchor segment around amino acid position 35 or their carboxyl terminus was shortened from 42 to 41, 40 or 39 amino acids. The time-dependent self assembly of monomeric Aß into fibres was simultaneously monitored by electron microscopy, circular dichroism spectroscopy, analytical ultracentrifugation, and Aß-mediated cellular toxicity was quantified in PC12 cells using inhibition of MTT reduction. The transition of Aß monomers into fibres was analyzed by more than 600 electron micrographs. It revealed morphological changes from seed-like structures to premature and mature fibres. Seeds were of spherical appearance, premature fibres presented as elongated structures with a rough surface and with varying thickness depending on the Aß's sequence. Mature fibres were characterized by a periodic variation of their thickness along the length axis. The proportion of these different structures and the total amount of aggregated Aß was amino acid sequence-depended. We could demonstrate that wild-type Aß1-42, and its oxidized version carrying a methionine sulfoxide residue at position 35 have the highest fibre formation rate observed and exert toxic activity in the MTT-assay at very low nanomolar concentrations. The carboxyl-terminus shortened Aß peptides 1-41, 1-40, 1-39, and most of the mutated Aß variants tended to aggregate at a much slower rate, and are non-toxic at naturally occurring concentrations. Thus, correlations can be drawn between physical properties, fibre morphology and cellular toxicity.

461.21

PERLECAN BINDING TO ALZHEIMER'S DISEASE BETA-AMYLOID PROTEIN (Aß) 1-40 REGARDLESS OF THE EXTENT OF FIBRILLOGENESIS

G.C. Castillo, C.Ngo, and A.D. Snow*. Dept. of Pathology, Neuropathology Labs, Box 356480, University of Washington, Seattle, WA 98195

Perlecan is a specific heparan sulfate proteoglycan implicated in Alzheimer's disease (AD) amyloidosis. In the present study, we assessed perlecan's postulated effects on beta-amyloid protein (Aß) fibrillogenesis and determined the binding interactions of perlecan with Aß during increasing stages of amyloid fibril formation. The effects of perlecan on Aß fibrillogenesis were first evaluated using a Thioflavin T fluorometry assay. Aß 1-40 or 1-42 at 25 µM in TBS (pH 7.0) was incubated at 37°C for 2 weeks either alone or in the presence of 0.1 µM perlecan. Aliquots were taken for analysis at 1 hr, 3 days, 1 week and 2 weeks. Perlecan was found to be a potent and early accelerator of Aß 1-40 fibrillogenesis, causing a significant 4.8-fold increase at 1 hour, and a 2.4-fold increase at 3 days. By 1 and 2 weeks, perlecan had no further effect on Aß 1-40 fibrillogenesis. Perlecan also enhanced Aß 1-42 fibrillogenesis, with a significant 1.8-fold increase observed at 3 days, and a significant 2.0-fold increase maintained at both 1 and 2 weeks. In a second study, using a solid phase binding immunoassay, we determined whether Aß 1-40 binds perlecan regardless of the extent of Aß fibrillogenesis. Aß-perlecan interactions were evaluated following incubation of Aß at 37°C for 1 day (minimal Aß fibril formation), 3 days and 1 week (maximal Aß fibril formation). The extent of Aß fibril formation was assessed by Thioflavin T fluorometry and found to increase 2.7-fold from 1 day (97 fluorescent units) to 1 week (262 fluorescent units). Aß 1-40 bound perlecan at all time points with both high affinity and low affinity binding sites observed at 1 day (K_d = ~ 1.5 X 10^{-13} M and K_d = 8.8 X 10^{-9} M), 3 days (K_d = ~ 5.0 X 10^{-13} M and K_d =2.4 X 10^{-8} M) and 1 week (K_d = ~ 4.5 X 10^{-13} M and K_d = 1.1 X 10^{-7} M). These results demonstrate that Aß interacts with perlecan regardless of the extent of Aß fibrillogenesis and further implicates an important role for perlecan even in early stages of Aß amyloidosis.

Partially supported by NIH AG12953-02 and AG05136.

461.22

BUTYRYLCHOLINESTERASE (BCHE) ALTERS THE AGGREGATION STATE OF Aß AMYLOID

K.L. Barber[1], M.M. Mesulam[2]*, G.A. Krafft[3] and W.L. Klein[1] [1]Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208; [2]Neurology & [3]Mol. Pharmacology, Northwestern Univ. Sch. of Med., Chicago, IL 60611

Amyloid B peptide (Aß) is the principal component of senile plaques in Alzheimer's disease (AD). Recent observations suggest that the putative neurotoxicity of Aß may be influenced by the supramolecular state of the Aß aggregates. The state of Aß aggregation may be determined by numerous factors, including the effects of additional proteins that are also present within senile plaques. One of these proteins, BChE, is found in significantly higher quantities in AD plaques than in plaques of age-matched non-demented brains. The current study was designed to explore the effect of BChE on the *in vitro* aggregation of Aß. The Aß aggregates, formed with or without the addition of BChE, were adsorbed to formvar coated gold grids and processed for examination by whole mount transmission electron microscopy (WMTEM). Aggregates of Aß comprised structurally heterogeneous forms, including flocculant, amorphous and fibrillar species. Co-incubation of Aß 1-42 with low doses of BChE for 24 hours blocked the formation of fibrils and resulted in the aggregation mostly of amorphous precipitates. At 72 hours, numerous fibrillar structures were detected. Co-incubation with higher concentrations of BChE for 24 hours inhibited even amorphous aggregates and yielded clouds of tiny speckles. At these higher concentrations of BChE, no fibrillar deposits were seen even after 72 hours. These preliminary observations indicate that BChE promotes a more amorphous, flocculent, and less fibrillar state of Aß aggregation. The mechanisms underlying this effect and its potential influence on Aß-induced neurotoxicity remain to be determined.

Supported by grants to WLK from NIH & Alzheimer's Association

DEGENERATIVE DISEASE: ALZHEIMER'S-BETA-AMYLOID—NEUROPATHOLOGY

462.1

TOWARDS AN ANIMAL MODEL FOR ALZHEIMER'S DISEASE

C Czech, S Dreisler, N Touchet, N Clavel, B Schombert, G Ret, F Revah*, L Pradier, and GL Tremp Rhône-Poulenc Rorer S.A., GENE & MEDICINE Department, Centre de Recherche Vitry-Alfortville, 94403 Vitry sur Seine, France

The causative factors in Alzheimers disease (AD) aetiology implicate both genetic and environmental factors. A small percentage of early-onset familial AD cases are due to mutations in the APP gene. The large majority of these cases is linked to mutations in two genes, named presenilin 1 and 2 (PS 1, 2). The mechanism(s) by which the mutations lead to neurodegeneration remains to be elucidated. The modified proteins might interfere with the normal production and clearance of beta-APP.

In order to get insight into this process and to create a rodent model for AD, we decided to express human wildtype and mutated PS1 in transgenic mice and rats. The transgenes consist of an housekeeping gene promoter and the corresponding human PS1 cDNA. We obtained several independent transgenic mouse and rat lines harbouring 2-20 copies of the hybridgene. A robust overexpression of transgene-specific RNA was observed in the brains of mice from several independent lines. To study the interaction between PS 1 and APP *in vivo*, we created mice expressing both, mutated human APP and human PS 1. Since AD is a multigenic disease, we intend to combine several AD linked genes and risk factors in the same animal. A better understanding of the genetic and extra-genetic events; their interaction and timing, leading to AD is crucial for the development of an innovative therapeutic approach.

462.2

Gene-Targeted Mice For APP and Cu-Zn SOD-1 for Studying Aß Metabolism and Amyloid-Related Neuropathologies

D. Howland , A. Reaume, M. Savage*, D. Flood, S. Trusko, Y.G. Lin, L. Pinsker, D. Lang, B. Greenberg, R. Siman, and R. Scott Cephalon, 145 Brandywine Pkwy., West Chester, PA. USA, 19380.

We have used gene-targeting to introduce the Swedish familial AD (FAD) mutations and convert mouse Aß to the human sequence in mouse. Swedish mutant "humanized" APP is expressed at normal levels in brain and cleavage at the mutant ß-secretase site is both accurate and enhanced resulting in elevated Aß production. In brains from mice homozygous for the mutant APP allele, human Aß levels are approximately one order of magnitude greater than those measured in normal human brain. Correct developmental and spatial mutant APP expression in conjunction with high levels of human Aß production in the absence of APP overexpression make this animal model ideal to screen for pharmacological modulators of Aß production. We are currently administering compounds, predicted to affect Aß metabolism, in these animals to measure effects on Aß (by ELISA) and associated APP processing fragments. In addition, the neuropathological consequences of chronic high level Aß production in brain is being analyzed in a longitudinal study. To complement these experiments we have generated mice double homozygous for the mutant APP allele and a cytoplasmic Cu-Zn SOD-1 knockout allele (see D.G. Flood et al. Soc. Neurosci. Abstr., 1996) to analyze the combined effects of Aß overproduction and increased oxidative stress on brain neuropathology.

462.3

ACCUMULATION OF AMYLOID β PROTEIN IN TRANSGENIC MICE.

T. Kawarabayashi[1], M. Shoji[1]*, M. Sato[2], A. Sasaki[3], E. Matsubara[1], T. Iizuka[1], Y. Igeta[1], M. Kanai[1], K. Ishiguro[1], Y. Tomidokoro[1], T. Kobayashi[2], N. Tada[2], K. Okamoto[1] and S. Hirai[4]. [1]Department of Neurology and [3]Department of Pathology, Gunma University School of Medicine, Maebashi, Gunma 371, Japan, [2]Laboratory for Animal Center, Pharma Research and Development Division, Hoechist Japan Limited, Kawagoe, Saitama, 350-11, Japan, [4]Tokyo Metropolitan Neurological Hospital, Futyu, Tokyo 183, Japan.

The deposition of amyloid β protein (Aβ) is an important early event in the development of Alzheimer's disease. To produce animal model which overexpresses Aβ, we generated three sets of mice expressing the transgene of carboxyl-terminal fragments (CTF) of amyloid β protein precursor (βAPP) (βANORβ: signal peptide and 99 amino acid residues of CTF, βAΔNORβ: methionine and 103 amino acid residues of CTF, and βAΔNLβ: methionine and 103 amino acid residues of CTF with substitution of KM-NL type mutation) under control of the cytomegalovirus enhancer / chicken β-actin promoter. The transgenic mRNA was detected by RT-PCR. The transgenic protein was detected as 11.4 kD CTF in βANORβ, and as 11.8 kD CTF in βAΔNORβ and βAΔNLβ. The transgenic CTF was detected from many organs, but the amount of mRNA, CTF, and Aβ did not correlate well. In βANORβ, Aβ deposition was detected in pancreas with severe degeneration of acinar cells and infiltration of macrophages. In βAΔNORβ and βAΔNLβ, Aβ deposition was not obvious in pancreas, in spite of much expression of the transgenic CTF in pancreas. In brain, Aβ deposition was detected only in βAΔNLβ. In the brain of βAΔNLβ, there was intracellular deposition of dense substances that were stained by anti-Aβ antibodies in the neurons of cerebral cortex and hippocampus. Our findings indicate that tissue-specific posttranslational processing may play a pivotal role in Aβ production, and that the KM-NL type mutation may accelerate intracellular Aβ deposition in neurons *in vivo*.

462.4

PREDICTORS OF β-AMYLOID DEPOSITION IN THE CANINE.

M. J. Russell*, Y. Hou, M. Bobik, J. S. Marks, and R. G. White. Comparative Aging Lab., Dept. of Anesthesiology, University of California, Davis, CA 95616.

Canines spontaneously develop β-amyloid (Aβ) plaques in their brains. The deposition of diffuse Aβ is viewed as an early stage of plaque development and is the most common form of plaque in dogs. We have examined a number of factors as predictors of Aβ deposition and found a positive relationship between several. Our findings suggest that four indicators are strong positive predictors. These are: 1) The cerebral spinal fluid (CSF) levels of soluble amyloid precursor proteins (sAPP). There is a high correlation of diffuse amyloid plaques with sAPP CSF levels. 2) The existence of littermates with plaques in their brains. Animals who have littermates with amyloid plaque deposition also show plaques in their brains. 3) The breed of dog. Breeds differ in the onset and distribution of amyloid plaques. 4) The age of the animal. In a colony of dogs raised under controlled environmental conditions age of onset within the colony is very specific. These data indicate that Aβ deposition can be predicted with accuracy in the canine by a combination of factors. In environmentally restricted dogs a regression of age and sAPP levels provides R value of 0.92 with amyloid deposition in brain. Supported by NIA grant RO1AG 11350.

462.5

DISTRIBUTION OF β-AMYLOID IN THE CANINE BRAIN.

Y. Hou*, M. Bobik, R. G. White, J. S. Marks, and M. J. Russell.
Canine Aging Lab., Dept. Anesthesiology, Univ. California, Davis., CA 95616.

The distribution of amyloid plaque deposition in the canine brain was studied by employing β-amyloid (Aβ) immunohistochemistry (10D5 antibody from Athena), in the brains of ten randomly selected aged dogs. Formalin fixed tissues were embedded in paraffin and serial sectioned. Aβ plaques were counted in eight sections from each dog, analyzed by brain region and combined by quantitative image analysis to form an average distribution in the canine brain. Positive plaques were widely distributed. The highest densities were found, in descending order of concentration in the: 1) dentate gyrus of the hippocampus, 2) medial and posterior ectosylvius gyrus, 3) medial and posterior sylvius gyrus, and 4) proreus gyral cortex in the frontal lobe.

There are significant differences between dog and human Aβ distribution. Plaques were found in the olfactory bulb in only one dog, and the entorhinal cortex was one of cortical areas least affected. No amyloid deposits were detected in the brain stem or cerebellum. This study reveals a general distribution of β-Amyloid in canine brain and better defines the canine Alzheimer's disease-like Aβ deposition in this AD animal model.

Supported by NIA grant RO1 AG 11350.

462.6

AMYLOID ß PROTEIN DEPOSITION IN BRAINS OF NON-DEMENTED EAST AFRICANS

J.A. O'gengo , D. L. Cohen, J.G. Sayi, W.B. Matuja, J. N. Kitinya, J.K. Kimani, R.P. Friedland and R.N. Kalaria. (SPON: Society of Neuroscientists of Africa) Departments of Neurology and Pathology, Case Western Reserve University, Cleveland, Ohio 44106, USA; Department of Human Anatomy, University of Nairobi, Kenya; Neurology Unit and Pathology Institute, Muhimbili Medical Centre, Dar es Salaam, Tanzania.

Environmental and genetic factors have been invariably implicated in the etiopathogenesis of Alzheimer's disease (AD). The investigation of prevalence rates of AD in different populations could provide clues to the identification of these factors. Previous studies have suggested that AD and Aß deposition is uncommon in Nigerian Africans. We assessed the deposition of amyloid ß (Aß) protein and other lesions associated with typical AD in brains of aging East Africans. Brain tissue was obtained from routine or coroner cases that came to autopsy at two academic institutions in East Africa. Here, we found that 17% of the (5/30) brains from subjects with an age range 50 to 83 years exhibited parenchymal as well as vascular amyloid ß deposits. Plaques were predominantly positive for the longer more pathogenic $Aß_{42}$ form. Our observations suggest the occurrence of Aß deposits in brains of non-demented East Africans seems similar to age-matched normal controls from our institutions in Cleveland.

Supported by grants from WHO-NINDS, NIA (NIH) and ADRDA.

462.7

DEVELOPMENT OF Aß IMMUNOREACTIVE PLAQUE-LIKE DEPOSITS IN HIPPOCAMPAL SLICE CULTURES

M.E. Harris*, J.J. Sigel and S.A. Frautschy UCLA Depts. Medicine and Neurology, Los Angeles, CA 90095 and GRECC, Sepulveda VAMC, Sepulveda, CA 91343

Hippocampal slice cultures provide an excellent *in vitro* model to study the development of Aß deposits and the resulting multi-cellular changes associated with Aß plaque development. Using monoclonal antibodies anti-ß(42) [2G9] and anti-ß(40) [10G4], Aß immunoreactive plaque-like deposits were observed in slices following treatment with Aß (1-40) + (1-42). These deposits are 31-42 um in diameter and are characterized as diffuse. Addition of transforming growth factor beta, which orchestrates the brain's response to injury, results in deposits that are larger (36-54 um) and stain more intensely with 2G9. These deposits are more compact and contain numerous glial processes running throughout the deposits. Using imaging techniques, we can study the time course of plaque development in these slices. These slices also provide a model to rapidly screen therapeutics designed to reduce Aß deposition and plaque formation. This work is supported by grants NS30195 and AG10685.

462.8

PROGRESSION OF REGIONAL NEUROPATHOLOGY IN ALZHEIMER'S DISEASE (AD) AND NORMAL ELDERLY: FINDINGS FROM THE NUN STUDY.

DS Wolf, M Gearing, DA Snowdon, H Mori, WR Markesbery, SS Mirra*. Dept of Pathology & Lab Medicine, VA Medical Center & Emory Univ School of Medicine, Atlanta, GA; Sanders-Brown Center on Aging, Univ of Kentucky, Lexington, KY; & Tokyo Inst of Psychiatry, Tokyo, Japan.

While diffuse plaques in the neocortex may represent an early stage in the evolution of neuritic plaques, plaques in striatum and cerebellum retain their predominantly diffuse nature in AD, regardless of disease duration. Exploring these regional differences may provide clues to factors influencing the progression of AD neuropathology. We had the opportunity to study autopsy brain tissue derived from 15 cognitively normal and 5 AD subjects. All participants were enrolled in The Nun Study, a longitudinal study on aging and AD (Snowdon et al, JAMA 1996;275:528-532); subjects were elderly Catholic sisters in whom the CERAD neuropsychology battery had been performed within one year of death. Diffuse and neuritic plaques, neurofibrillary tangles (NFT), and distribution of beta-amyloid (Aß) and apolipoprotein E (ApoE) were assessed in temporal cortex, striatum, and cerebellum without knowledge of clinical status. Examination revealed diffuse plaques in striatum in 6 (40%) and in cerebellum in 4 (27%) of the 15 non-demented sisters and in all 5 AD cases. These subcortical plaques labelled with antibody to $Aß_{42}$ but not $Aß_{40}$. In the 20 cases overall, the frequency of striatal and cerebellar plaques generally paralleled the frequency of neuritic plaques and NFT and correlated with decline in some neuropsychological measures, e.g. delayed word recall, word recognition, and MMSE. Our findings suggest that subcortical diffuse plaques occur relatively early in the progression of AD pathology and coincide with neocortical pathology and cognitive changes. Thus, it is unlikely that temporal factors alone account for regional differences in progression of AD neuropathology. Supported by R01AG09862, AG05144, and AG10130.

462.9

ENTORHINAL CORTEX PATHOLOGY AND COGNITIVE PERFORMANCE IN AGING AND ALZHEIMER'S DISEASE: FINDINGS FROM THE NUN STUDY

Tina L. Tekirian*, D.A. Snowdon, D. Racke, K.P. Riley, D.G. Davis, I.W. Ashford, N.S. Soultanian, W.R. Markesbery, J.W. Geddes. *Sanders-Brown Alzheimer's Disease Research Center & Department of Anatomy & Neurobiology, Lexington, Kentucky.*

Whether ß-amyloid (Aß) plaques, neurofibrillary (NF) pathology, or a combination of these neuropathological hallmarks are associated with cognitive and memory dysfunction in Alzheimer's disease (AD) or normal aging has been debated. Our studies specifically focus upon these issues and the entorhinal cortex, as this area is critical for learning and memory. Neuropathological data was correlated with neuropsychological data from the Nun Study, a longitudinal study of aging and Alzheimer's disease in 678 Catholic Sisters; the Sisters are annually examined using the CERAD cognitive test battery. Aß load (% of entorhinal cortex occupied by Aß), was measured in 47 cases. NF pathology (59 subjects) was assessed with Gallyas and PHF-1 immunostaining and further characterized by Braak staging (*Acta Neuropathol.* 82: 239, 1991). Participants were evaluated as a total group and were also subdivided into cognitively impaired and unimpaired groups. Regression analyses indicate that Aß load is associated with lower scores on the Mini-Mental State Examination (MMSE) in both the total and impaired groups but not in the unimpaired group [$MMSE_{total}$ ($F=10.06$, $p=.003$, $r^2=.18$), $MMSE_{impaired}$ ($F=9.09$, $p=.007$, $r^2=.32$), $MMSE_{unimpaired}$ ($p>.05$). This trend was observed with other cognitive tests (i.e. Boston Naming, Verbal Fluency, Delayed Word Recall, and Constructional Praxis). Additionally, each 1% increase of Aß load (range: 0-14%) was associated with a 1.7 point decrement on the MMSE. With regard to NF pathology, the shift from the Braak transentorhinal/entorhinal stages (I & II) to the limbic stages (III & IV) was associated with a small, yet significant ($p<.05$) decrement on memory tests (i.e. Delayed Word Recall) but no clear difference in performance on other cognitive tests. Transition from the limbic to isocortical stages (V & VI) was significantly associated with poorer performance on all cognitive tests ($p<.001$). The results indicate that both extent of NF pathology and entorhinal cortical Aß deposition correlate with decrements in performance on cognitive tests. Supported by NIH grants to T.L.T., D.A.S., W.R.M. & J.W.G.

462.10

AMYLOID PLAQUE DISTRIBUTION ALONG THE LONGITUDINAL AXIS OF THE HIPPOCAMPUS IN ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE.

F.-L.F. Chang*, X. Figueroa and A. Wagner UCSF-Fresno Dept. of Neurology, and Alzheimer's Dis. Ctr., and VA Med. Ctr., Fresno, CA 93703

Both Alzheimer's disease (AD) and Parkinson's disease (PD) are neuro-degenerative disorder. While the pathophysiology and clinical presentation of the two diseases are quite different, cognitive impairment frequently develops as PD progresses. Questions were raised regarding the neural substrates of the dementia associated with PD.

We studied the distribution of amyloid plaques within the hippocampus along its entire longitudinal axis in AD or PD patients using Bielschowsky silver and thioflavin-S stains. We found a significant positive linear correlation between the extent of cognitive impairment (estimated from scores on Mini-Mental State Exam within one year of death) and the density of amyloid plaques in the CA1 subfield of the hippocampus for AD. We found a similar correlation in our PD cases using regression analysis. There was a trend of higher plaque density in the rostral, head segment (pes hippocampus) along the longitudinal axis in both AD and PD. A gradient of increased amyloid plaque density was also noted from the subfield CA2, through CA1, to the subiculum in both AD and PD.

Our findings suggest comparable distribution of pathology within the hippocampus as dementia progresses in both AD and PD. Studies are needed to document more comprehensively the distribution of pathology for PD patients developing dementia in later years. The results can benefit diagnostic classification and staging, and provide a basis for researches into treatment modalities.

(Supported by the State of California Department of Health Services, the Fresno VA Medical Center, and the UCSF Fresno Foundation.)

462.11

BOTH β-AMYLOID 1-40 AND 1-42/43 ARE DEPOSITED IN THE EARLIEST FORMED PLAQUES DURING AGING AND EARLY DEMENTIA. J.L. Price*, C.D. Myers and D.W. Wavek, Dept. of Anatomy & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110

Previous work in other labs has shown that β-amyloid (Aβ) occurs in a shorter, 40 amino acid form and a longer, 42-43 amino acid form. Aβ42/43 is more insoluble, and in early Down syndrome cases is preferentially deposited in the earliest formed plaques, suggesting that it is responsible for initial plaques formation. Down syndrome is advantageous for the study of early plaque formation, because all cases show substantial plaques in their early 20's, but the cellular processes that produce excess Aβ in Down syndrome are different from those in aging or sporadic Alzheimer's Disease (AD).

In this study, early plaque formation was studied in 9 non-demented aging cases (CDR=0) and 7 very mildly demented subjects (CDR=0.5). The subjects were assessed in the ADRC of Washington University, either before death, or by a retrospective dementia interview. Sections through the orbital, insular and temporal cortex were stained with the 10D5 antibody (against both forms of Aβ), or with the end-specific antibodies BA27 (against Aβ40) or BC05 (against Aβ42/43). All of the CDR=0.5 cases met standard criteria for AD, and had more plaques than the CDR=0 cases, which were selected to ensure the presence of at least a few plaques. Plaques were mapped with the aid of a computerized microscope digitizer, and areas of maximum plaque deposition were selected for determination of the density of plaques stained with each antibody.

In all of the brains, plaques were found that were positive for both Aβ40 and Aβ42/43. Although precise quantitative comparisons between immunohistochemical stains with different antibodies are limited, in half of the brains more Aβ40 positive plaques were found than Aβ42/43 positive plaques, while in the other brains there were more Aβ42/43 than Aβ40 plaques. The ratio of Aβ40 to Aβ42/43 plaques showed no relation to age, dementia rating, or to total plaques stained with the 10D5 antibody. It appears, therefore, that there may be no preference for either form of Aβ in aging and early sporadic AD. (Supported by NIH grant AG-03991).

462.12

Novel 100kD protein associated with amyloid plaques in AD brains. M.L.Schmidt*, M. Forman, V. M.-Y. Lee, and J.Q. Trojanowski. Path. and Lab. Med., U.of Penn. Sch. of Med., Philadelphia, Pa 19104-4283.

Monoclonal antibodies (MAB) were raised to a PHFtau enriched preparation obtained from pooled brains of 2 Alzheimer's disease (AD)patients and 1 Downs Syndrome (DS) patient with AD. 59 MABs recognized the PHFtau preparation by ELISA and 55 of these MABs immunostained neurofibrillary lesions in AD brain sections. The other 4 MABs (i.e. AMY 117, 135,144,155), however, immunostained plaques in AD tissue and immunoblotted a protein of about 100 kD. Double immunolabeling with an AMY MAB and a polyclonal antibody to AD amyloid ß protein (Aß) showed that AMY immunoreactivity was frequently located at the periphery or adjacent to the Aβ positive plaques. AMY positive plaques were found in the frontal cortices of the majority of AD patients and all DS/AD patients. No AMY positive plaques were observed in cerebella of AD and DS/AD patients that had Aβ deposits. AMY positive plaques were extremely rare or not detectable in elderly normal and schizophrenic patients as well as in patients with other neurodegenerative diseases. These results indicate that a hitherto unrecognized 100 kD protein is closely associated with a large majority of senile plaques in AD and DS/AD. Further studies are necessary to clarify whether this protein contributes to the toxicity of amyloid deposits or the formation of dystrophic neurites and loss of neurons and synapses in AD.
Supported by grants of the National Institute on Aging.

462.13

LOCALIZATION OF RNA WITHIN NEUROFIBRILLARY TANGLES AND SENILE PLAQUES IN ALZHEIMER'S DISEASE. S.D. Ginsberg*[1], P.B. Crino[2], V.M-Y. Lee[1], J.H. Eberwine[2], and J.Q. Trojanowski[1]. Depts. of [1]Pathology and [2]Pharmacology, Univ. Pennsylvania Sch. of Med., Philadelphia, PA 19104.

The distribution and composition of neurofibrillary tangles (NFTs) and senile plaques (SPs) have been characterized within the Alzheimer's disease (AD) brain, although little data exists on their non-proteinaceous components. We tested the hypothesis that NFTs, neuropil threads (NTs), and SPs contain cytoplasmic RNA species. Acridine orange (AO) histofluorescence was employed, by itself or in combination with thioflavine-S (TS) staining and immunohistochemistry for astroglial and microglial epitopes, to identify RNA in paraffin-embedded tissue sections of the hippocampal formation and entorhinal cortex from 33 postmortem cases of AD, Down's syndrome (DS), and age-matched normal controls. AO stained the cytoplasm of hippocampal and entorhinal neurons in normal controls, and labeled NFTs, NTs, and SPs within the brains of AD and DS patients. AO histofluorescence was abolished with RNase, but not DNase or proteinase K, indicating the relative specificity for RNA species. Quantitative analysis demonstrated approximately 80% of NFTs identified with TS colocalized AO, whereas approximately 55% of TS-stained SPs contained AO labeling. AO-stained SP's colocalized with microglial cells, and were apposed to, but not colocalized with astrocytes. The characterization of the RNAs in NFTs and SPs may lead to the identification of gene transcripts that contribute to their deposition. Supported by grants from the NIH.

462.14

NEURONAL ENERGY METABOLISM IS NOT REDUCED BY SENILE PLAQUES IN ALZHEIMER'S DISEASE.

K. Hatanpää*, D.R. Brady, K. Chandrasekaran and S.I. Rapoport.
Lab. of Neurosciences, NIA, Bethesda, MD 20892.

A decrease in cerebral energy metabolism precedes clinical dementia in patients with Alzheimer disease (AD). In the post mortem AD brain, mRNAs for subunits of cytochrome oxidase (COX) are decreased. Because COX expression is regulated by functional needs for energy, these decreases likely reflect synaptic degeneration, which leads to reduced synaptic firing and downregulation of oxidative phosphorylation enzymes, including COX. Our recent results indicate that a decrease in COX mRNA precedes formation of neurofibrillary tangles (NFTs) in AD pyramidal neurons in midtemporal cortex, and that COX mRNA further decreases as NFT formation progresses [Hatanpää et al. 1996, Ann. Neurol., in press]. In this study, we focused on senile plaques, that with NFTs are the major pathologic features in AD. Frozen sections from midtemporal cortices of 4 AD cases were subjected to in situ hybridization for COX subunit III mRNA (COX mRNA), followed by immunohistochemistry with PHF-1 antibody to detect neuritic plaques. Additional sections were used for COX histochemistry to detect COX enzyme activity, followed by Thioflavin S staining to detect plaques. There was no statistically significant decrease in COX mRNA in tangle-free neurons within 15 µm from a plaque, compared with tangle-free neurons that were not close to any plaque. Plaques also were not associated with decreased COX activity, with the exception of plaque cores, that were devoid of COX activity. Therefore, plaques likely do not contribute to decreased energy metabolism in AD and may not be a causal factor in neurodegeneration.

462.15

NEOCORTICAL NEURITIC PLAQUES ARE LESS REACTIVE WITH ANTI-PHF ANTIBODY IN LEWY BODY DEMENTIA THAN IN ALZHEIMER DISEASE. W. Samuel*, R. Crowder, L. Hansen. Dept. of Neurosciences; Univ. of Calif., San Diego; La Jolla, CA 92093. **BACKGROUND:** Besides having neocortical plaques and entorhinal neurofibrillary tangles (NFT) with paired helical filaments (PHF), the brains of about 25% of patients meeting criteria for Alzheimer disease (AD), also have cortical and subcortical Lewy bodies (LB). Designated the Lewy body variant (LBV), they differ from pure AD cases in having a predominance of diffuse and neuritic plaques (NP) and very few neocortical NFT. Using different techniques, we sought to replicate a recent Western blot analysis of brain homogenates which found that the neocortex of LBV patients reacted positively with anti-PHF antibody only in the relatively few cases in which neocortical NFT were seen. **METHODS:** Routine histopathological examination classified cases as AD (n=14) or LBV (n=12) and quantified midfrontal neocortical NP per microscopic field using a 10X objective and NFT using a 40X objective. Midfrontal 10um paraffin sections were processed by the immunoperoxidase method for reactivity with a monoclonal antibody which binds phosphorylated PHF. After washing, the sections were counterstained with thioflavin-S. NP were counted in five randomly-selected 10X fields, following which we switched from fluorescent to light microscopy so as to assess each plaque for anti-PHF reactivity. **RESULTS:** The LBV and AD did not differ significantly in age (79.5-81.2 yrs), male/female ratio, or terminal neuropsychological test scores. On routine histopathology, the LBV (39.7) and AD (42.1) did not appreciably differ in numbers of NP per 10X field, but the LBV (0.33) had significantly ($p<.05$) fewer NFT per 40X field than did the AD (1.29). Among subjects observed to have midfrontal NFT, which comprised most of the AD cases but only a third of the LBV, 81-84% of NP reacted positively with the anti-PHF antibody. Among subjects without NFT, 52% of the NP of AD cases but only 12% of the NP of LBV cases were anti-PHF positive ($p<.05$). **CONCLUSION:** LBV differs from AD in that its plaque neurites do not, for the most part, contain PHF. **SUPPORT:** National Institute on Aging; Veteran's Administration Central Office.

462.16

BUTYRYLCHOLINESTERASE (BCHE) IN THE LIFE CYCLE OF ALZHEIMER'S DISEASE (AD) PLAQUES. A.L. Guillozet[1]*, J.F. Smiley[1], D.J. Selkoe[2], D.C. Mash[3], M.-M. Mesulam[1]. [1]Northwestern University, Chicago, IL 60611. [2]Harvard University, Boston, MA 02115. [3]University of Miami, Miami, FL 33136.

The normal human cerebral cortex contains very little BChE, expressed mostly by neurons of deep layers and subcortical neuroglia. In AD, the core, halo and neurites of senile plaques express intense BChE enzyme activity. Whether BChE expression is an early or late event in the process of Aß plaque maturation is unknown. Our assumption concerning the life-cycle of plaques is that the Aß peptide is initially deposited in a diffuse (thioflavin S-negative) form which subsequently undergoes transformation first into a compact (thioflavin S-positive) but non-neuritic form, and then into a compact-neuritic form. This transformation may take many years and only the final neuritic form is significantly correlated with clinical dementia. In 8 brains from control (non-demented) and AD subjects, matching sections were processed for the demonstration of Aß, thioflavin-S, and BChE. In 2 control specimens where all Aß plaque deposits were thioflavin-S negative (i.e., diffuse), the plaques were also BChE-negative. In the other 3 control specimens, regions and layers that contained only diffuse plaques had no plaque-bound BChE whereas regions and layers with compact plaques contained plaque-bound BChE. In 3 AD specimens where Aß deposits were almost invariably of the compact or neuritic type, plaques were almost always associated with intense BChE activity. We had previously shown that plaque-bound BChE is likely to originate from neuroglia and that its enzymatic activity is selectively inhibited by protease inhibitors (Wright, Geula, Mesulam, PNAS, '93). The present observations indicate that BChE activity appears at the intermediate stage of plaque formation and that it may therefore constitute one of the factors involved in the transformation of an initially benign Aß deposit into a compact and neuritic form associated with neural degeneration and dementia.

Supported by the Alzheimer's Association

462.17

LOCALIZATION OF PRESENILIN-2 RNA IN RAT BRAIN BY IN SITU HYBRIDIZATION. S.A. Benkovic*, D.G. Morgan, and M.N. Gordon. Department of Pharmacology and Therapeutics, University of South Florida, Tampa, FL 33612-4799.

We have generated templates for in situ hybridization probes by RT-PCR using rat brain RNA. The nucleotide sequence for PS-2 was obtained from Genbank (accession number L44577) and primers were selected from the region of least homology between PS-1 and PS-2 (hydrophilic loop VI-VII). Probe templates were sequenced and confirmed to be PS-2. Densitometric analysis of in situ hybridization revealed that PS-2 is expressed in the rat brain in virtually all brain regions examined. Levels of PS-2 in superficial cortex were higher than deeper cortex. Levels in the caudate nucleus and superior colliculus were moderate and similar to deep cortical expression. The strongest signal for PS-2 was seen in the neuronal layers of the dentate gyrus and hippocampal CA fields. Expression of PS-2 in the cerebellar granule cell layer was high, while levels in the molecular layer and pons were low. Liver and spleen displayed strong hybridization signals, while the level in kidney was lower. Emulsion dipped slides indicated a purely neuronal localization of PS-2 mRNA. The molecular layers of cortex and hippocampus were devoid of signal. White matter regions had grain densities similar to sense strand control signals. These data indicate a distribution of PS-2 RNA with a strong, if not exclusive localization to neurons. Supported by Alzheimer's Association IIRG 93-083 and Pfizer Inc.

462.18

A PS1 MUTATION LEADS TO AD PATHOLOGY AND INCREASED $A\beta_{42}$ BURDEN IN BRAIN BY MIDDLE AGE IN 4 FAD PATIENTS. C.A. Lemere*, F. Lopera, K.S. Kosik, J. Ossa, T.C. Saido, H. Yamaguchi, D.J. Selkoe, J.C. Arango V. Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

Overproduction of $A\beta_{42}$ appears to play a role in FAD due to certain missense mutations (e.g, βAPP). Here, we examined C- and N-terminal Aß immunoreactivity (IR) and other markers of AD pathology in the brains of 4 Colombian FAD patients (47-62 yrs) bearing the E280A PS1 mutation. All 4 cases contained massive amounts of $A\beta_{42}$ in plaques in cortex, hippocampus, midbrain and cerebellum, and in blood vessels. Less $A\beta_{40}$ IR was seen. FAD patients had a significantly greater $A\beta_{42}$ plaque burden in 4 brain regions compared with those of 12 sporadic AD cases. In addition to $A\beta_{40}$ vascular staining, many blood vessels also contained $A\beta_{42}$ IR. N-terminal Aß staining revealed a subset of plaques (mostly cored) and blood vessels throughout the brain. Many tau- and ubiquitin-positive NFTs were observed in cortex and hippocampus. Neurofilament-positive neurites were seen in a subset of plaques, many of which were tau-negative. The cerebellum contained abundant $A\beta_{42}$ plaques, a subset of which contained ubiquitin-positive, tau-negative neurites. Severe gliosis in the cerebellum and cerebral cortex was observed. PS1 IR was seen in a minority of neuritic plaques. Our results are consistent with earlier findings of increased $A\beta_{42}$ production in fibroblasts and plasma from patients with PS1 mutations. The increased levels of $A\beta_{42}$ in brain provide insight into the mechanism by which mutant PS1 leads to amyloidogenesis in AD. We are now examining these tissues for Aß-associated proteins. Supported by NIH grants to KSK and DJS.

462.19

PRESENCE OF INSULIN-LIKE GROWTH FACTOR II/ MANNOSE-6-PHOSPHATE RECEPTORS IN THE NEURITIC PLAQUES OF ALZHEIMER'S DISEASE BRAIN. S. Kar*, U. Beffert, S. Doré, [1]S. Gentleman, [2]Y. Robitaille, [3]J.H. Grubb, [3]W.S. Sly, D. Dea, J. Poirier and R. Quirion. Douglas Hosp. Res. Center, Dept. Psychiat., McGill Univ., Montreal, Canada, [1]Dept. of Anat., Charing Cross and Westminster Med. Sch., London, U.K., [2]Dept. Pathol., Hopital Ste-Justine, Montreal, Canada, [3]Edward A. Doisy Dept. Biochem. Mol. Biol., Saint Louis Univ., St. Louis, USA.

The insulin-like growth factor-II/Mannose-6-phosphate (IGF-II/Man-6-P) receptor is a multifunctional protein which binds different classes of ligands including IGF-II and Man-6-P bearing lysosomal enzymes. This receptor, besides participating in transmembrane signal transduction and endocytosis also functions in the targeting of newly synthesized lysosomal enzymes. In Alzheimer's disease (AD) brain, the marked over expression of certain lysosomal enzymes in vulnerable neuronal populations and their association to ß-amyloid containing neuritic plaques have been correlated to altered metabolic functions. These data when analysed with reference to functions of IGF-II/Man-6-P receptors in sorting of lysosomal enzymes may suggest a role for these receptors in the pathogenesis of AD. In the present study, to investigate the possible involvement of IGF-II/Man-6-P receptors in AD, we studied the anatomical distribution and measured the levels of these receptors in selected regions of AD (n=6) and age-matched normal (n=6) postmortem brains. Our immuno-histochemical results showed that IGF II/Man-6-P receptors are widely but selectively distributed in pyramidal neurones and ß-amyloid containing neuritic plaques of frontal and hippocampal regions of AD brains. Western blot analysis also revealed a parallel increase in the level of these receptors in the frontal cortex and hippocampal regions of certain AD brains. These results together with the altered levels of lysosomal enzymes provide an anatomical basis to suggest a role for IGF-II/Man-6-P receptors in the pathogenesis of AD. (Supported by MRCC and Alzheimer Soc. Canada)

462.20

ARE INSULIN-LIKE GROWTH FACTOR II/ MANNOSE-6-PHOSPHATE RECEPTORS INVOLVED IN THE UPTAKE OF ß AMYLOID PEPTIDE INTO RAT PRIMARY HIPPOCAMPAL CULTURED NEURONS ? D.S. Auld*, U. Beffert, S. Kar, N. Aumont, S. Doré, J. Poirier and R. Quirion. Douglas Hospital Research Centre, Depts. of Psychiatry, Neurol. & Neurosurg., McGill Univ., Montreal, Quebec, Canada H4H 1R3.

Excessive extracellular deposition of ß-amyloid (Aß), a polypeptide of 39-43 amino acids, in brain parenchyma and cerebral blood vessels is a pathological hallmark of Alzheimer's disease (AD). It is further suggested that Aß accumulation may initiate and/or contribute to the process of neurodegeneration in AD. However, at present, it is not clear how the effects of Aß peptides are mediated in either physiological or pathological conditions. In this context, the recent localization of insulin-like growth factor II/ Mannose-6-phosphate (IGF II/Man-6-P) receptor in the Aß-containing neuritic plaques of the AD brain (S. Kar et al., this meeting) raises the possibility that these receptors may be involved in mediating the effects of Aß peptides. Indeed, evidence accumulated over the last decade indicates that IGF II/Man-6-P receptor is a multifunctional protein which is distributed widely but selectively throughout the brain and binds different classes of ligands including IGF II, Man-6-P bearing lysosomal enzymes, proliferin, transforming growth factor ß and thyroglobulin. In the present study, to evaluate the functional interrelationship, if any, between Aß peptides and IGF II/Man-6-P receptors, we have used Western blot analysis to measure the effects of IGF II on the cellular uptake of Aß peptides in rat primary hippocampal cultured neurones. Our results indicate that IGF II (10nM - 1µM) significantly decreases cellular uptake of Aß1-40 into cultured hippocampal neurones. Furthermore, apolipoprotein E-induced cellular uptake of Aß1-40 is also decreased by IGF II (10nM - 1µM). These results indicate that IGF II/Man-6-P receptors may modulate the cellular uptake of Aß peptides and therefore may have implications for AD pathology. (Supported by MRCC, FRSQ and Alzheimer Soc. Canada)

462.21

RAT SOLEUS MUSCLE IN CHLOROQUINE-INDUCED MYOPATHY SHARES IMMUNOHISTOCHEMICAL CHARACTERISTICS WITH ALZHEIMER'S DISEASE. K. Tsuzuki[1)]*, R. Fukatsu[2)], Y. Takamaru[3)], T. Yoshida[2)], Y. Hayashi[2)], N. Sasaki[2)], H. Yamaguchi[4)], N. Fujii[1)] and N. Takahata[2)] Depts of [1)]Microbiology and [2)]Neuropsychiatry, Sapporo Med. Univ., South 1, West 17, Sapporo 060, Japan. [3)]Sapporo City General Hospital, [4)]College of Medical Care and Technology, Gunma Univ., Gunma 371, Japan

Alzheimer's disease (AD) affected brain is characterized by the deposition of amyloid β protein (Aβ). Aβ is proteolytic cleavage product of amyloid precursor protein (APP) that vary slightly at the carboxy-terminus, yielding species of 40, and 42(43) amino acids. These species have been reported to play different role in AD pathogenesis.

Chloroquine-induces myopathy in rats similar to rimmed vacuole (RV) myopathy in humans. We demonstrated immunohistochemically that Aβ, APP N-, C-terminal regions and cathepsin D accumulate in RVs in chloroquine-induced myopathy in rats. Moreover, Aβ immunoreactivity co-localizes with apolipoprotein E, SP-40,40, α_1-antichymotrypsin, and ubiquitin, so-called amyloid-associated proteins.

The purpose of this study, is to elucidate whether Aβ is cleaved from APP, or APP fragments containing Aβ accumulate in the RVs, which species of Aβ is present in the RVs. Two end-specific for the C- terminus of Aβ, BC40 and BC42, and Aβ90/12, a monoclonal antibody were used, which reacts with the N-terminus of Aβ, reacting both with APP containing Aβ and cleaved Aβ. Immunohistochemical studies were carried out with denervated soleus muscle 14 days after the initial injection of chloroquine. Transverse paraffin sections were stained according to the streptavidin-biotin peroxidase technique. Muscle fibers suffering from chloroquine-induced myopathy contained both cleaved Aβ40 and Aβ42. Aβ42 appeared to predominate over Aβ40.

In conclusion, our results provide further evidence that chloroquine-induced myopathy in rats is a useful model for understanding APP processing into Aβ in terms of the pathogenesis of AD.

463.1

EFFECTS OF GENETIC RISKS ON CEREBRAL GLUCOSE METABOLISM IN ALZHEIMER'S DISEASE.

M. Higuchi[1)2)], H. Arai[1)]*, M. Itoh[2)], S. Higuchi[3)], T. Nakagawa[1)], Y. Kosaka[1)], H. Sasaki[1)].

[1)]Department of Geriatric Medicine, Tohoku University School of Medicine, Sendai 980, JAPAN, [2)]Cyclotron and Radioisotope Center, Tohoku University School of Medicine, Sendai 980, JAPAN, [3)]Department of Psychiatry, National Institute on Alcoholism, Kurihama National Hospital, Kanagawa 239, JAPAN.

Studies with positron emission tomography (PET) and ^{18}F-2-fluoro-2-deoxy-D-glucose (FDG) for patients with Alzheimer's disease (AD) have indicated a typical metabolic pattern with reductions in the temporo-parietal junction and considerable heterogeneity in the metabolic alterations. On the other hand, polymorphisms of several genes and increased levels of certain molecules have been associated with the onset or the clinical progression of AD, while the biological mechanisms underlying these changes remain unsettled. In the present study, FDG-PET scans were performed on patients with AD following determination of the apolipoprotein E (APOE), α1-antichymotrypsin (ACT), and presenilin-1 (PS-1) genotypes and levels of cerebrospinal fluid tau (CSF-tau). We sought to investigate the effects of these indices as well as the Mini-Mental State Examination (MMSE) scores and the age of onset, on cerebral glucose metabolism. Significant and positive relationships between the regional metabolic rate of glucose (rCMRglc) and gene dose of APOE*4 was observed particularly in the frontal areas, and gene dose of ACT*A was greatly and negatively associated with rCMRglc in multiple regions, especially in the temporal and parietal areas by simple correlational and multiple regression analyses. The levels of CSF-tau were negatively related to rCMRglc in the medial frontal areas for mildly-demented patients, suggesting that patients with high levels of CSF-tau show reduced frontal metabolism from the earliest stage of AD. The effects of gene dose of PS-1*1 on rCMRglc were relatively weak in all the cerebral regions. Finally, these relationships were confirmed using statistical parametric mapping (SPM) analysis on a pixel-by-pixel basis.

463.2

REDUCED CYTOCHROME OXIDASE ACTIVITY IN CA1 NEURONS AND POSTERIOR PARIETAL CORTEX AFTER CHRONIC BRAIN ISCHEMIA

J.C. de la Torre*, A. Cada, N. Nelson, G. Adams, R.J. Sutherland, F. Gonzalez-Lima. Departments of Neurosurgery and Psychology. Univ. of New Mexico, Albuquerque, NM 87131 and Univ. Texas, Austin, TX 78712.

We have reported that chronic 2-vessel occlusion (bilateral carotid artery ligation) induces a mild cerebral ischemic insult that mimics Alzheimer's dementia in rats.[1] Chronic 2-VO in 10 month old rats, results in selective damage to CA1 neurons accompanied by hippocampal reactive astrocytosis and visuo-spatial memory deficits. In the present study, male Sprague-Dawley rats age 19 months were subjected to 2-VO and tested in the Morris water maze (MWM) weekly for 4 weeks after surgery. Following MWM testing, rats were decapitated and brains were flash-frozen for cytochrome oxidase (CO) activity. Immunoreaction for GFAP and MAP-2 and for Palmgren silver stains were done a separate group of similarly treated rats.

Results show a significant effect by 2-VO on visuo-spatial memory impairment as compared to controls. CO analysis revealed that only the posterior parietal cortex and posterior CA1 neurons had a marked reduction of activity. Other regions of the brain showed no CO reductions after 2-VO with respect to controls. Increased GFAP density and reduced MAP-2 activity were noted in 2-VO rats but no CA1 neuronal damage or loss was apparent.

These findings suggest that reduced mitochondrial oxidative phosphorylation in posterior parietal cortex and CA1 neurons may impair visuo-spatial memory function. Ischemia-induced metabolic decline of the cell's energy supply may be a key element in triggering the structural neuronal damage that eventually develops in this model.

1. de la Torre JC, et al: Progressive neurodegeneration in rat brain after chronic 3-VO or 2-VO. In: Neurodegenerative Diseases, Plenum , N.Y., 1996.

463.3

EVALUATION OF STEREOTACTIC NORMALIZATION OF PET IMAGES IN ALZHEIMER'S DISEASE (AD) USING NONLINEAR KERNEL ANALYSIS

H.I. Litt[1,2], B. Horwitz*[2]. [1]Department of Biophysics, SUNY at Buffalo School of Medicine, Buffalo, NY 14214, [2]Laboratory for Neurosciences, NIA, NIH, Bethesda, MD, 20892.

Transformation of images into a standard stereotactic space is often used in intersubject PET studies. The normalization process may be less successful if the image being normalized is significantly different from the stereotactic template, e.g. as a result of atrophy or functional change in AD. We have developed a method that allows quantitative evaluation of a stereotactic normalization using the Volterra model of nonlinear systems. This model considers the output at a point to be dependent upon interactions among points in a region of the input known as the system memory. We calculated Volterra kernels for the transformation between a PET template in stereotactic space and FDG-PET images from 6 AD patients (Mini-Mental Status (MMS) score 0-26) and 9 matched controls. A 7*7 pixel system memory modelled the transformation for controls better than AD patients, as assessed by the difference between the model's predicted output and the template image. There was also a significant correlation between the model error and disease severity as measured by MMS score. Areas of greater error in the model fit included parietal, temporal, and dorsal occipital cortex, as well as parts of the cingulate gyrus, regions significantly affected in AD. These results demonstrate that spatial normalization algorithms may not perform as well in patients with AD, especially for those with severe disease.

Support: SUNYAB MSTP fellowship and NIH intramural funding

463.4

REGIONAL CEREBRAL GLUCOSE METABOLISM DURING STIMULATION PREDICTS DEMENTIA SEVERITY IN ALZHEIMER DISEASE PATIENTS.

A. Dani*, P. Pietrini, M.L. Furey, U. Freo, M.J. Mentis, G.E. Alexander, S.I. Rapoport, and M.B. Schapiro Lab. Neurosci., Natl. Inst. Aging, NIH, Bethesda MD 20892, USA

In patients with Alzheimer disease (AD), rCMRglc at rest (eyes/ears covered) is abnormally reduced in most neocortical areas and reductions become more severe with worsening of dementia. To test the hypothesis that brain metabolism during functional stimulation would be a better predictor of dementia severity than metabolism at rest, due to a progressive loss in the ability of the AD neurons to fully respond to stimulation, we studied 15 patients with probable AD and different degrees of dementia (9M/6F; mean age 70±10 yr, Mattis dementia rating scale score 91±33, range 23-128) at rest and during a passive, broad audiovisual stimulation (AS) paradigm (watching a movie). By using PET and the double FDG injection procedure (Brooks, 1987), rCMRglc was measured at rest and during AS. The two experimental conditions were counterbalanced. As compared to rest, absolute rCMRglc (mg/100 g/min) during AS was significantly higher in regions primarily involved in the task, including temporal auditory and in parietal and occipital visual cortical areas (up to 21% over rest). Global gray matter CMRglc significantly predicted dementia severity (as measured by the Mattis DRS) during AS (r= .69; p=.004) but not at rest (r=.47; ns). Similarly, rCMRglc in primary and association visual cortical areas was significantly correlated with dementia severity during AS (e.g., Left occipital association and L calcarine r=.61, p=.016; L auditory r=.52, p=.047) but not at rest (r=.32, .25, and .47 respectively, ns). These results demonstrate that rCMRglc measures of neuronal function during activation of visual cortical regions closely reflect dementia severity whereas the same regions at rest do not. At rest, functional reserve mechanisms may minimize differences, whereas stimulation may function as a stressor that maximizes severity related differences in neuronal activity. This research was supported by the NIA intramural program.

463.5

FUNCTIONAL RESPONSE TO AUDIOVISUAL STIMULATION IN ALZHEIMER PATIENTS: POTENTIAL FOR THERAPEUTIC INTERVENTIONS.

P. Pietrini*, M.L. Furey, A. Dani, U. Freo, M.J. Mentis, G.E. Alexander, S.I. Rapoport, and M.B. Schapiro. Lab. Neurosci., Natl. Institute on Aging, NIH, Bethesda MD 20892, USA

Positron emission tomography (PET) studies in patients with Alzheimer disease (AD) at rest (eyes/ears covered) have shown reductions in regional cerebral metabolic rates for glucose (rCMRglc) in neocortical association areas that worsen with increasing dementia severity. This study assessed the ability of AD patients of different dementia severity to respond to stimulation. High-resolution PET and the double FDG injection procedure were used to measure rCMRglc at rest and during a passive audiovisual stimulation (AS) paradigm (watching a movie) in 15 patients with probable AD (9M/6F; mean age 70±10 yr, Mattis dementia rating scale score 91±33, range 23-128) and in 14 matched healthy controls (6M/8F; mean age 71±8 yr; Mattis score 141±3). As compared to controls, at rest AD patients showed decreased rCMRglc in most cortical areas with a sparing of the subcortical regions. During AS, controls showed significant rCMRglc increases in auditory temporal and in occipital and parietal visual cortical regions (up to 28% in calcarine cortex). Despite the metabolic reductions at rest, during AS the AD patients also showed significant rCMRglc increases in the same areas (up to 21% in occipital-parietal visual cortex), although to a lesser extent than controls in some parietal and occipital visual areas. The magnitude of rCMRglc responses in primary and association visual cortex in AD patients was directly correlated with the Mattis score (L calcarine r=.79; p=.0005; L occipital association r=.74; p=.002). The 6 mildly demented AD patients (Mattis score 120±9) had rCMRglc responses in visual regions similar to controls, although they had significantly lower rCMRglc in parietal areas in both experimental conditions. In contrast, the 3 severely demented AD patients (Mattis 36±11) showed minimal or no response to stimulation in visual areas. These results indicate that the brain of mild AD patients retains an almost normal capability to be stimulated in at least some cortical regions and suggest that neuronal function in areas affected by the pathological AD process in mildly/moderately demented patients may benefit from timely therapeutic intervention once appropriate agents become available. (Supported by intramural NIA).

463.6

OLFACTORY EVOKED REGIONAL CEREBRAL BLOOD FLOW IN HEALTHY AGING AND ALZHEIMER DISEASE.

D.A. Kareken, G. D. Hutchins, R.L. Doty*, P.J. Moberg, K. Caldemeyer, & M.R. Farlow. Indiana Alzheimer's Disease Cntr/Indiana Univ. Schl. of Med. & VA Med. Cntr., Indianapolis, IN 46202.

Olfaction deteriorates in normal aging, but in Alzheimer Disease (AD) the deterioration is greater, and begins early in the disease. As a prelude to study of the regional cerebral physiology of olfaction in AD, healthy elderly subjects were studied with positron emission tomography (PET) while inhaling a set of odorants. The sample consisted of 5 right handed, elderly subjects without history of cerebral disease or dementia (4 female, 1male; mean age=72.2±7.35; all non-smokers). All scored normally on olfactory tests (identification, threshold). Subjects were imaged in a Siemens 951/31R PET scanner after injecting 50 mCi of H_2O^{15} into a venous catheter in the subject's dominant arm for each of four scans. Two scans were acquired while subjects sniffed 16 consecutive odors (scans 1 & 3), and two while subjects sniffed odor blanks (scans 2 & 4). Image subtractions were (A) 1-2, and a replication set (B) 3-4. In subtraction A, a significant increase (8.40%) in regional cerebral blood flow (rCBF) occurred near the right frontotemporal junction, corresponding to piriform (olfactory) cortex. In the replication (B), an rCBF increase (7.29%) at near identical coordinates fell just below the significance threshold. Areas of increased rCBF that missed statistical significance, but were replicated across both sets of scans, included the left piriform area, and left and right orbitofrontal areas. The results agree with prior studies showing similar regional activation during olfactory stimulation. Contrasted with other studies, there is greater right than left activity in the piriform area. This may be due to gender or aging effects. The findings demonstrate the potential of PET for characterizing olfactory related changes in the regional cerebral physiology of aging. Future comparable studies are planned in patients with AD. *Supported by: Physiologic Imaging Research Center & the Alzheimer Disease Center (P30 AG10133), Indiana University; VA Medical Center, Indianapolis; Smell and Taste Center, University of Pennsylvania.*

463.7

INSULIN-INDUCED ENHANCEMENT OF MEMORY IN ALZHEIMER'S DISEASE IS INDEPENDENT OF GLUCOSE S. Craft, S. Asthana, J. Newcomer, I. Tio-Matos, E. Hunter, C. Lofgreen, M. Raskind*, C. W. Wilkinson, R. Veith, S. Plymate, K. Brodkin, L. Gibson, S. Latendresse. GRECC, VA Puget Sound Health Care System and University of Washington, Box 358280 (182B), Seattle, WA, 98195.

Alzheimer's disease (AD) is accompanied by disruption in glucose metabolism that may contribute to its characteristic memory impairment. Increasing glucose availability by raising plasma glucose improves memory in patients with AD (Craft et al. 1992, 1994; Manning et al., 1993). Such memory improvement is associated with glucose-stimulated elevation in endogenous insulin levels, raising the question of whether improvement is due to changes in insulin, independent of hyperglycemia. To address this question, 12 patients with AD and 10 control subjects participated in four metabolic conditions on separate days: 1) Elevated insulin (75μU/ml)/ baseline glucose (95 mg/dl); 2) Elevated glucose (225 mg/dl)/baseline insulin (8 μU/ml; 3) Elevated insulin/glucose; and 4) Baseline insulin/glucose. Intravenous infusions of insulin, dextrose, saline, and somatostatin were used to achieve steady metabolic states, during which memory (prose recall) and selective attention (Stroop interference test) were measured. Patients with AD showed improved delayed memory [$F(1,11)=5.45$, $p<.04$] and reduced selective attention errors [$F(1,11)=5.16$, $p<.04$] in the elevated insulin/baseline glucose condition relative to the baseline insulin/glucose condition. No changes were noted in the elevated glucose/baseline insulin condition. These results suggest that glucose-improved memory in AD is secondary to elevations in endogenous insulin in response to hyperglycemia. These results also suggest that insulin activates mechanisms affecting the medial temporal memory system. Documented insulin receptors in the hippocampus, insulin-mediated increases in glucose utilization in entorhinal cortex, and insulin modulation of cholinergic and noradrenergic systems are potential mechanisms for such effects. Finally, our findings suggest that neuroendocrine factors play an important role in the pathophysiology of AD.

This study was supported by RO1-AG-10880 from the National Institute on Aging.

ISCHEMIA: APOPTOSIS

464.1

NEURONAL DEATH IN THE RAT (SHRSP) CEREBRAL CORTEX FOLLOWING PERMANENT FOCAL ISCHEMIA. T. Nitatori[1]*, Y. Karasawa[2], H. Komiyama[2], H. Araki[2], E. Kominami[3] and Y. Uchiyama[4]. Dept. of Cell Biol. & Neuroanat.[1], Sch. of Med. Iwate Med. Univ., Morioka 020, Japan, Pharmacol. Lab.[2], Taisho Pharmaceutical Co. Ltd., Ohmiya 330, Japan, Dept. of Biochem.[3], Juntendo Univ., Sch. of Med., Tokyo 113, Japan, and Dept. of Anat.[4], Osaka Univ. Med. Sch., Osaka 565, Japan.

The cortical neurons are known to be vulnerable to anoxic insults, which result in neuropathological changes within the infarcted area. The present study examined the early histopathological changes in the cortical neurons after focal cerebral ischemia, induced by permanent unilateral occlusion of the middle cerebral artery (MCA). Cortical neurons within the focal ischemic area at the territory of MCA rapidly underwent cell death, whereas within ischemic penumbra, neuronal death required a certain time period after ischemic onset. It remains, however, undefined whether death of these neurons are necrosis or apoptosis. We therefore analyzed the degenerating process of the cortical neurons within the focal area and ischemic penumbra of the cerebral cortex after focal ischemic onset. At 2 hrs after ischemic onset neurons within the focal area were already swollen, while their plasma membrane were broken into pieces and fragments of disintegrated organelles scattered within the cytoplasm. Expanded Nuclei of these neurons showed positive staining of TUNEL. On the other hand, neurons with typical necrotic swelling and those with a high electron density were detected within the ischemic penumbra 6 hrs after the onset of ischemia. The latter neurons exhibited cell shrinkage accompanied with an increase in immunoreactivity for lysosomal cysteine proteinases. Nuclei of these neurons showed positive staining of TUNEL from 1 to 3 days after ischemic onset. Degenerated neurons were heterophagocytosed by microglial cells invaded into the area. These results suggest that acute death of the cortical neurons within the focal area after the onset of ischemia by MCA occlusion is necrotic, whereas neuronal death within the ischemic penumbra consisted of necrosis and apoptosis.

464.2

APOPTOSIS AND NECROSIS FOLLOWING SPINAL CORD ISCHEMIA IN THE RAT. H. Kato*[1], G. Kanellopoulos[2], M. Mackey[2], Y. J. Wu[1], C. Y. Hsu[1], N. T. Kouchoukos[2] and D. W. Choi[1]. Depts. of Neurology[1] and Surgery[2], and Center for the Study of Nervous System Injury. Washington University School of Medicine, St. Louis, MO 63110.

We examined the nature of neuronal death induced by ischemia in the rat spinal cord. Transient (15-20 min) spinal cord ischemia was induced in male adult Long-Evans rats using a 2F Fogarty catheter, passed through the left carotid artery to occlude the aortic arch, combined with blood volume reduction. Histopathological changes were assessed between 6 hr and 7 d later.

Changes were most marked in the lumbar cord. Two major types of neuronal death were observed. The first type, prominent in intermediate grey matter, was characterized by typical acute "ischemic cell change" by 6 hr: in H&E sections, neurons became darker with pyknotic nuclei. Between 1-7 days, these cells gradually underwent acidophillic degeneration and lost nuclear staining ("ghost cells"). Diffuse nuclear TUNEL-positivity developed after 1 day. The other type of neuronal death was seen only between 1-2 days in small neurons in the dorsal horn. These neurons exhibited nuclear condensation, apoptotic bodies, and intense nuclear TUNEL staining. DNA extracted from whole lumbar cord showed a ladder pattern on gel electrophoresis. Injection of the NMDA antagonist, dextrorphan (30 mg/kg) i.p. 15 min before a 15 min period of spinal cord ischemia attenuated both types of neuronal death and resulted in improved functional outcome at 1-2 days after ischemia.

Study was supported by NIH NINDS grant NS 32636 (DWC) and APA (DWC and CYH).

464.3

Delayed Neuronal Death Following Transient Forebrain Ischemia in Rat Hippocampus: A Comprehensive Temporal Analysis

M. Guglielmo*, P. Chan, C. Doberstein, D. Demers, M. Primiano, D. Palm, N. Knuckey, C. Johanson and M. Epstein; Dept. of Clin. Neurosci., Program in Neurosurg., Brown Univ. & RI Hospital, Providence, RI 02903

The pyramidal cells of the hippocampal CA1 region are particularly susceptible to delayed neuronal death (DND) following transient forebrain ischemia (TFI). Although necrotic cell death does occur after ischemia, evidence for a gene-regulated active process in DND has been demonstrated in rodent models of global ischemia (Kirino, *et al*, 1984, Heron, *et al*, 1993), as well as in focal ischemia (Li, *et al* 1995). To investigate the mechanisms of DND in CA1 following TFI, we comprehensively examined the temporal distribution of TUNEL immunostaining, neuronal ultrastructure, and DNA fragmentation.

TFI was induced in normothermic adult rats by bilateral carotid artery occlusion with hypotension. Animals were perfused with 10% neutral buffered formaldehyde 6, 12, 18, 24, 48, 72 hours and 7, 14, and 28 days following the induction of TFI (n=4, each time point). Paraffin 8μm sections were stained using a TUNEL staining kit (Oncor) and DAB chromagen. Similar recovery animals were used for CA1 electron microscopy and for DNA gel laddering.

Cresyl violet stains of rat hippocampus demonstrated early ischemic changes in the CA1 region by 24 h and clear evidence of cell death by 48h. Later time points confirmed extensive but selective cell loss throughout CA1 alone. Faint TUNEL staining was detected within the nuclei of CA-1 neurons as early as 12 hours following TFI. Dense staining at 48 and 72h post ischemia corresponded with the appearance of characteristic 200bp DNA fragments detected by electrophoresis at the same time points. Ultrastructural findings at these time points demonstrated chromatin condensation and nuclear indentation with relative preservation of cellular organelles. These results suggest that ischemia-induced hippocampal neuronal death is an active process resembling apoptosis.

Supported by NIH grant NS27601 and funds from Rhode Island Hospital.

464.4

DELAYED NEURONAL DEATH OF THE CA1 PYRAMIDAL CELL OF THE RAT HIPPOCAMPUS FOLLOWING TRNSIENT FOREBRAIN ISCHEMIA IS APOPTOTIC. C. S. Bae, J. K. Kim[1], M. S. Suh, Y. Y. Chung, K. H. Lee and B. Y. Kim*. Dept. of Anatomy and [1]Pharmacology, Chonnam University Medical School, Kwangju 501-190, Korea

The CA1 pyramidal cells in the hippocampus are selectively vulnerable and undergo cell death several days after brief forebrain ischemia. It is controversial whether this delayed neuronal death is necrotic or apoptotic. To delineate the morphological characteristics of the delayed neuronal death, the structural changes of the CA1 pyramidal neurons were examined using in situ nick-end labeling and electron microscopy. Ischemia was induced for 20 min using four vessel occlusion in Sprague-Dawley rat. Nuclei of the CA1 neurons were nick-end labeled by biotinylated dUTP mediated by terminal deoxytransferase 3 and 4 days after ischemic insult, but not in 1 and 2 days and not in CA2-4 sectors. Strong reaction was noted along the margin of nucleus. In electron microscopy two types of degenerating neurons in CA1 pyramidal layer were observed 2 days after ischemic insult; one type, the majority of degenerating neurons, showed increased cellular electron density, the other lost most cytoplasm. Many degenerating pyramidal neurons with dense cytoplasm showed irregular in shape and were shrunken, and contained well-developed cytoplasmic organelles such as Golgi complex and endoplasmic reticulum. Some dense cells contained dense bodies, vacuoles and fragmentary membranous organelles. Chromatin condensation was often noted along the nuclear membrane. A microglia-like cell fused with various degenerating neurons. These data suggest that delayed death of the CA1 pyramidal neurons showing increased cellular density after brief ischemia is mainly apoptotic.

464.5

REVELATION OF AN APOPTOTIC COMPONENT OF NEURONAL CELL DEATH AFTER OXYGEN DEPRIVATION USING ANTINECROTIC AGENTS.
J.-C. Copin, Y. Li, L.F. Reola, and P.H. Chan*. Departments of Neurosurgery and Neurology. University of California. San Francisco CA 94143.

We have previously shown that 3-day-old mouse cortical neurons in primary culture subjected to 9 h of oxygen deprivation died by necrosis but showed some signs of apoptosis (DNA laddering and decreased of DNA degradation by cycloheximide treatment) which may indicate that necrosis masks apoptosis under these experimental conditions (Copin et al., J. Neurotrauma, in press). In order to further study this phenomenon we have treated neurons in primary culture with different pharmacological agents known to interfere with the necrotic pathway elicited by the sequence of oxygen deprivation and reoxygenation.

In the non-treated group, 50% of the cells died 24 h after 9 h of oxygen deprivation. Inhibitors of nitric oxide synthase (L-NAME and 7-nitroindazole) and a hydroxyl radical scavenger (alpha-pheny-tert-butylnitrone, PBN) were totally ineffective to prevent this cell death; verapamil and nimodipine (L-type calcium channel blockers), alpha-tocopherol (an antioxidant involved in membrane protection) and MK-801 were partially effective; trolox (a water-soluble analog of alpha-tocopherol) and DNQX (a kainate/quisqualate-receptor antagonist) were totally effective. These two latter agents were also able to reduced free radical generation detected in the non-treated group by flow cytometry in the presence of 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate. However, TUNEL staining as well as Hoechst staining revealed much more apoptotic cells in DNQX treated cells as compared to the non-treated hypoxic group. Apoptotic cells were also detected in the trolox-treated group. These results were confirmed by TUNEL staining coupled with flow cytometry. Furthermore, two days after oxygen deprivation, 30 % of the trolox-treated neurons degenerated, though inhibition of protein synthesis dramatically prevented this post-hypoxic degeneration.

Our data indicate that DNQX and trolox treatment can prevent cell necrosis induced by oxygen deprivation and reoxygenation, and that apoptotic process can be unmask after hypoxia using neuronal protective agents. (supported by NIH grants NS14543, NS25372, AG08938)

464.6

NUCLEAR CONDENSATION AND FRAGMENTATION FOLLOWING CEREBRAL HYPOXIA-ISCHEMIA OCCURS MORE FREQUENTLY IN IMMATURE THAN OLDER RATS.
U I. Tuor*, R.Sidhu and M.R.Del Bigio. Inst. for Biodiagnostics, NRC, R3B 1Y6 and Univ. of Manitoba, Winnipeg, Manitoba.

One feature of apoptotic cell death is condensation and fragmentation of the nuclear chromatin into apoptotic bodies. Nuclei with punctate chromatin, indistinguishable light microscopically from those observed during apoptosis, were observed frequently following cerebral hypoxia-ischemia in 1 week old rats whereas they appeared less common in older animals. In the present study we investigate quantitatively the age dependence of this type of cell death.

Cerebral hypoxia-ischemia was produced in 18 rats by occluding the carotid artery under anesthesia and then exposing the animal to 8% oxygen for 2, 1 or 0.5 hr in 1,2 and 4 wk old animals, respectively. The animals were perfusion fixed with 10% buffered formalin 1-2 days after hypoxia-ischemia. Paraffin embedded sections were stained with hematoxylin and eosin and the number of pyknotic nuclei or nuclei with fragmented chromatin within damaged areas of the cerebral cortex were counted with the aid of image analysis.

There was a pronounced difference between the incidence of pyknosis or fragmented chromatin in 1 week old compared to 2 and 4 week old animals. In 1 week old animals the majority of injured neurons in the cortex had nuclei consisting of punctate condensed chromatin whereas relatively few (18.0±2.2%) were pyknotic (663±97 vs.139±24 cells/mm^2, respectively; ±SEM). In the 2 and 4 week old animals the majority (73±9% and 92.9±1.8%, respectively) of the injured cells were pyknotic ($p<0.002$, compared to 1 week). A few scattered cells with punctate chromatin were observed in the contralateral cerebral cortex.

Similar morphological differences in the appearance of the nucleus of hypoxic-ischemic cells have also been observed in human infants compared to adults. Because DNA laddering studies support an apoptotic type of cell death in 1 week old rats, we suggest that the immature brain retains a part of the cell death "program" that is reactivated following a hypoxic-ischemic insult.
(Supported by the Heart and Stroke Foundation of Manitoba).

464.7

FUNCTIONAL RELATIONSHIP OF CHANGES IN MICROTUBULE ASSOCIATED PROTEIN 2 (MAP-2) WITHIN DENDRITES AND EXPRESSION OF PROTO-ONCOGENES BCL-2 AND BAX IN GERBIL HIPPOCAMPAL NEURONS AFTER ISCHEMIA.
R.C.S. Lin*. Dept. of Neurobiology and Anatomy, Medical College of Penn. and Hahnemann Univ., Philadelphia, PA 19102

We previously reported that dendritic beading and subsequent loss of MAP-2 immunoreactivity in CA1 hippocampal pyramidal cells occurs after a 5-min. ischemic insult, assessed with immunocytochemical and immunoblotting methods. In particular, a beaded MAP-2 immunostaining pattern in the apical dendrites of CA1 pyramidal neurons was noted within 1 day after ischemic insult. To further assess the cellular mechanisms and potential significance of functional changes in dendrites and somata of CA1 neurons, we compared a 5-min. ischemic insult which results in hippocampal neuronal death vs. a 2-min. insult which is sublethal to hippocampal neurons, to examine possible differences in dendritic morphology using immunocytochemical techniques. We found that a 2-min. sublethal ischemic insult also induced dendritic beading of MAP-2 immunoreactivity in the apical dendrites of CA1 hippocampal pyramidal cells. However, in contrast to the 5-min. insult, there was no obvious loss of MAP-2 immunoreactivity or cell loss in the hippocampus up to 7 days post-ischemia. Dendritic beading of MAP-2 was particularly obvious in CA1 pyramidal cells 3-4 days post-ischemia. Dendritic beading of MAP-2 appeared less obvious after longer survival (up to 7 days), suggesting reversibility of MAP-2 changes in dendrites after a 2-min. injury. Upregulation of bcl-2, a presumed neuronal survival marker and oncoprotein, was found in the hippocampal CA1 sector after a 2-min. ischemic insult. In addition, increased immunoreactivity of bax, a presumed programmed cell death marker and oncoprotein, was also noted in the hippocampal CA1 sector. Co-expression of beaded MAP-2 immunoreactivity in dendrites with proto-oncogenes bcl-2 and/or bax at the single cellular level in hippocampal neurons is being investigated. Supported by Alzheimer Foundation to RCSL.

464.8

EFFECTS OF BCL-2 ON MITOCHONDRIAL Ca^{2+} UPTAKE AND PROTEOLYTIC ACTIVITY.
A.N. Murphy*, Vamsi Mootha[1], D.E. Bredesen[2], and G. Fiskum. Dept. of Biochem., Geo. Washington Univ. Med. Ctr, Wash., D.C. 20037, [1]HHMI-NIH Research Scholar, Lab. of Cardiac Energetics, NHLBI, NIH, Bethesda, MD 20892, and [2]Burnham Institute, La Jolla, CA 92037.

bcl-2 overexpression 1) significantly potentiates the maximal Ca^{2+} uptake capacity of mitochondria oxidizing NAD^+-linked substrates by 1.7 fold using digitonin-permeabilized GT1-7 neural cells and 3.9 fold using isolated mitochondria, and 2) inhibits Ca^{2+}-induced mitochondrial respiratory inhibition. Measurements of pyridine nucleotide redox state during uptake of sequential Ca^{2+} pulses indicate that Bcl-2 enhances the rate of recovery of NAD(P)H following Ca^{2+}-induced oxidation, and inhibits the persistent net oxidation associated with high Ca^{2+} loads in control mitochondria. In correlation, TPP^+ electrode-based measurement of mitochondrial membrane potential ($\Delta\Psi$) and simultaneous measurement of Ca^{2+} uptake indicate that a persistent complete loss of $\Delta\Psi$ is associated with lower Ca^{2+} loads in control vs. *bcl-2* overexpressors. In addition, the presence of 3 mM ATP or 20 μM cyclosporin A (an inhibitor of the mitochondrial membrane permeability transition) significantly enhances uptake and decreases, although does not eliminate, the difference (1.3 fold) in maximal uptake between control and *bcl-2*-expressing digitonin-permeabilized cells. Preliminary evidence also indicates that proteolytic ICE-like activity (measured using the fluorogenic substrate Z-D-E-V-D-AFC) is 2 fold higher in digitonin-permeabilized control than *bcl*-expressing cells, and is detectable in the mitochondrial fraction of control cells. These data implicate a role for Bcl-2-enhanced resistance to loss in $\Delta\Psi$ and protease activation in protection from apoptotic death. (ONR #N00014-96-0284, NS34152)

464.9

BAX GENE KNOCKOUT DELAYS CEREBRAL INFARCTION IN MICE SUBJECTED TO PERMANENT FOCAL ISCHEMIA.
C. Du[1]*, C.M. Knudson[2], R. Hu[1], X.Z. Liu[1], S. J. Korsmeyer[2], C.Y. Hsu[1] and D.W. Choi[1]. [1]Center for the Study of Nervous System Injury and Dept. of Neurology, [2]Howard Hughes Medical Institute, Washington Univ. School of Medicine, St. Louis, MO 63110.

Growing evidence suggests that ischemic brain injury may in part reflect the occurrence of programmed cell death. Since programmed cell death in many systems is reduced by elimination of the *bax* gene, we tested the hypothesis that mice deficient for the *bax* gene would sustain reduced cerebral infarction after a focal ischemia.

Wild type and *bax*-null littermates (back-crossed extensively into a C57/B6 background) were subjected to bilateral common carotid occlusion for 90 min, combined with permanent ligation of the right middle cerebral artery. After 1 day, increased Bax immunostaining was present in neurons in the ischemic cortex. Gross infarct volume at 1 day assessed by TTC staining was dramatically decreased in *bax* knockout animals; histological examination showed less neuronal degeneration and TUNEL staining. However, by 3 days, infarct volume in *bax* knockout animals was not significantly less that control.

These results suggest that the *bax* gene system importantly influences brain cell death occurring over the first day after a permanent focal ischemic insult, but that other factors can compensate for *bax* gene absence in the ultimate pathogenesis of brain infarction after such insults.

Supported by NIH NINDS grant NS 32636 (DWC).

464.10

BOTH BCL-2 AND BCL-X_L PROTECT PRIMARY ASTROCYTE CULTURES FROM H_2O_2 AND SUBSTRATE DEPRIVATION INJURY, BUT NOT COMBINED OXYGEN-SUBSTRATE DEPRIVATION.
R.G. Giffard*, I.L. Koumenis, X. Sun, L. Xu and M.C. Papadopoulos. Dept. of Anesthesia, Stanford University, Stanford, CA 94305

Retroviral vectors were used to overexpress bcl-2, bcl-x_L, or β-galactosidase in primary astrocyte cultures. More than 95% of the cells in the cultures expressed the gene of interest, as determined by immunocytochemistry. Cells were subjected to injury by exposure to hydrogen peroxide, substrate deprivation or combined oxygen substrate deprivation (OSD). Injury was assessed morphologically and quantitated by release of lactate dehydrogenase. Both bcl-2 and bcl-x_L reduced 200-400 μM hydrogen peroxide injury by 40-50%, and 24 h substrate deprivation injury 40-50%. Neither was able to provide significant protection from OSD. In an attempt to define why these genes are protective in oxidative stress injuries, we measured antioxidant enzymes. Both bcl-2 and bcl-x_L led to higher superoxide dismutase activity, bcl-2 increased glutathione peroxidase 40% compared to uninfected controls, while catalase was unchanged with either. Glutathione levels were increased 35% over β-gal controls by bcl-2. The level of lipid peroxidation measured with cis-parinaric acid was less in bcl-2 overexpressing cells subjected to substrate deprivation than control cells. Bcl-2 overexpression appears to increase antioxidant defenses and protects against hydrogen peroxide and substrate deprivation but not combined oxygen substrate deprivation.

464.11

NEUROPROTECTION OFFERED BY OVEREXPRESSION OF BCL-2 IS DEPENDENT ON THE DURATION OF REPERFUSION. N.Panahian*[1], M.Halterman[1,2], H.Gelbard[3], H.J.James[3], D.Howard[1,2], H.Federoff[1,2], Division of Molecular Medicine&Gene Therapy, Dept. of Neurology[1]; Dept. of Microbiology and Immunology[2], and Pediatric Neurology[3] Univ. of Rochester School of Medicine, Rochester, NY 14642.

In the ischemic cascade leading to programmed cell death, protooncogene bcl-2 blocks apoptosis at its distal step. This study examines the role of postischemic reperfusion and its duration on the outcome of neuroprotection offered by cortical bcl-2 overexpression. Five hr prior to induction of ischemia, 2 µl of viral suspension containing 10^5 infectious particles of either HSVlac or HSVbcl2 was stereotaxically injected into the border zone distribution of the ischemic penumbra. Focal ischemia was induced for 1 hr under halothane anesthesia (filament model) followed by either 23 or 30 hrs of reperfusion. Consecutive sections were stained with H&E, internucleosomal DNA fragmentation (ONCOR) and for presense of antigen expression (PGP polyclonal AB against bcl-2;Krajewski et al.). HSVbcl2 treated rats sacrificed at 24 hr showed less ischemic changes ($p<.01$) as well as reduced cerebral edema ($p<0.05$) in the cortex compared to HSVbcl2 treated rats sacrificed at 31 hr [(26.7+/-14% (n=6) vs 65.3+/-7% (n=4)]. No significant neuroprotection was observed in both HSVlac treated groups sacrificed at the same time intervals. HSVlac and HSVbcl2 treated groups failed to show any statistical difference in the cumulative behavioral score and stroke volume. It was not possible to salvage the neurons of the caudate (ischemic core) in any of the treated animals. Ongoing studies are needed for evaluation of extent of TUNEL labeling of HSVbcl2 and HSVLac injected animals to determine whether regional neuroprotection was achieved. (supported by the Markey Foundation to NP and NIH HD31300 to HF)

464.12

ISCHEMIC CA_1 HIPPOCAMPAL NEURONS EXPRESS *bax* AND CAN BE RESCUED BY *bcl-2* INDUCTION WITH A HERPES SIMPLEX VIRUS (HSV) AMPLICON. F.J. Antonawich*, H.J. Federoff[1], S. Krajewski[2], J.C. Reed[2] and J.N. Davis. SUNY at Stony Brook, Stony Brook, NY 11794, [1]University of Rochester, Rochester, NY 14627, [2]LaJolla Cancer Research Center, LaJolla, CA 92037.

5 min. of bilateral common carotid artery occlusion in the Mongolian gerbil results in a selective, delayed death of CA_1 pyramidal neurons. *bcl-2* appears to protect a variety of cells from cell death *in vitro*. We used Western blots to estimate levels of *bcl-2, bax,* and *bcl-x* at 6 and 72 hr after carotid occlusion. *Bcl-2* and *bcl-x* remained the same following transient ischemia, however, *bax* levels dramatically increased at 6 hr of ischemia compared to sham operated animals. At 72 hr *bax* levels were still elevated but were less than at 6 hr.

500 nl of an HSV amplicon encoding human *bcl-2* was injected into the CA_1 region 24 hr before carotid occlusion and the animals sacrificed 72 hr after occlusion. Immunohistochemistry demonstrated both glial and neuronal *bcl-2* expression around the injection site with apparent rescue of neurons demonstrating *bcl-2* immunoreactivity. By contrast, silver degeneration stains demonstrated ischemic cell death in areas remote from the site. Neurons in the immediate center of the injection site also showed cell death. This cytopathic effect may be due to either cell debris or helper virus in the amplicon preparations. These experiments suggest that 1) *bax* overexpression may be important in ischemic cell death and 2) *bcl-2* overexpression can prevent ischemic cell death *in vivo*. (Supported by NS 30559 from the NIH and the VA)

464.13

TRANSGENIC MICE DEFICIENT IN THE p53 PROTEIN ARE RESISTANT TO TRANSIENT RETINAL ISCHEMIA. D. R. Rosenbaum*, P. S. Rosenbaum, A. Aggarwal and J. A. Kessler. Albert Einstein College of Medicine, Bronx, NY 10461.

There is mounting evidence that apoptosis plays an important role in the delayed cell death that occurs following transient ischemia. Furthermore, the p53 protein is an important factor that leads to cell death in circumstances where apoptotic cell death occurs. The purpose of this study was to determine if transgenic mice that have attenuated expression of p53 are resistant to transient retinal ischemia. We have previously demonstrated that cell death in this model is in part secondary to apoptosis. Wild type and two groups of transgenic mice, one homozygous and the other heterozygous for a p53 null gene were subjected to transient retinal ischemia by raising IOP to greater than systemic pressure for 60 minutes. Seven days later the eyes were examined histologically. In addition, electrophysiologic (ERG) studies were performed at baseline and at 7 days post reperfusion. There was preservation of the retinal histoarchitecture as determined histopathologically in both groups of transgenic mice as compared to wild type controls. There was also preservation of the ERG b-wave in the transgenic groups as well. These data further support the hypothesis that the delayed cell death that occurs following transient retinal ischemia is in part apoptotic and that p53 plays a significant role in this process.

NIH NS20778, 20013 (JAK) and Research to Prevent Blindness (PSR).

464.14

DELAYED NEURONAL DEATH IN POST-ISCHEMIC RAT HIPPOCAMPUS IS NOT ASSOCIATED WITH TRANSCRIPTION OR TRANSLATION OF THE APOPTOTIC GENES p53 AND INTERLEUKIN CONVERTING ENZYME-1(ICE) J.Torres-Munoz [1], B.Roberts [1], C.Wood [1], T.S.Nowak,Jr. [2], W.A. Pulsinelli [2], C.K.Petito [1]*. [1]Dept. of Path., Univ. of Miami Sch. of Med., Miami, FL, 33101 and [2]Dept. Of Neurology, Univ. of Tennessee College of Medicine, Memphis, TN 38163.

Mechanisms of neuronal death following cerebral ischemia are controversial since morphology studies, and recent TUNEL studies in our laboratory, indicate that these cells die by necrosis yet gel electrophoresis of extracted DNA shows the characteristic ladders of the oligonucleosomes of apoptosis. To more fully evaluate cell death following ischemia, we looked at two apoptotic modulators, p53 and ICE. The p53 transcription factor encodes a protein that has been implicated in programmed cell death or apoptosis (Selter H and Montenarh M, Int J Biochem 1994; 26:154). ICE encodes a putative cysteine protease and induces apoptosis when overexpressed in cultured cells (Kumar et al., Gene Dev 1994; 8:1613). Adult Wistar rats were subjected to 10 min of global ischemia and sacrificed after 1,2,3,5,7, and 14 days. Paraffin embedded sections were examined by in situ end-labeling by the TUNEL procedure; wt p53 and ICE proteins and mRNA by immunohistochemistry, in situ hybridization and northern blot. Results showed necrosis of CA1 neurons between 2 and 3 d. after ischemia with TUNEL-positive cells appearing with, but not preceding, necrosis. Neither p53 nor ICE protein and message were present in the CA1 neurons. Apoptotic bodies, that were TUNEL positive and ICE immunoreactive, were rare and co-localized with oligodendrocyte or microglial-specific markers as previously described (Petito et al., J Neuropath Exp Neurol, in press). These results show that post-ischemic death in CA1 hippocampal neurons is not regulated by p53 or ICE. Rather, they support those studies that indicate that neuronal cell death after global ischemia is mediated by non-apoptotic mechanisms. Supported in part by RO1-NS27416-08, CKP.

464.15

OCCURRENCE OF NEURONAL NECROSIS AND APOPTOSIS FOLLOWING THE RETINAL ISCHEMIA: INDUCTION OF p53 AND bcl-2 mRNA C.K. Joo[1]* K.-Y. Park[2], M.S. Park[1], B.J. Gwag[2], and M.H. Choun[1], [1]Depts. of Opthalmology and Anatomy, Catholic Univ. School of Medicine, Seoul 137-040, Korea; [2]Dept. of Pharmacology, Ajou Univ. School of Medicine, Suwon 44 1-749, Korea.

We investigated patterns and molecular events of neuronal degeneration following ischemic injury in retina. Elevation of intraocular pressure(IOP) to 160-180 mmHg for 90 minutes produced a widespread neuronal degeneration over 1 - 5d. Interneurons in the inner nuclear layer appeared to undergo the initial degeneration over 1 - 3d. Retinal ganglion cells slowly degenerated over 5d following the ischemia. The electron microscopy study revealed concurrent induction of neuronal necrosis (characterized by dispersed chromatin condensation and mitochondria swelling) and apoptosis (characterized by chromatin aggregation and condensation).

To study whether cell death or survival genes are involved in the process of neuronal degeneration (e.g. apoptosis), expression pattern of p53, interleukin-1 beta converting enzyme(ICE), or bcl-2 mRNA was analyzed using a reverse transcriptase-polymerase chain reaction(RT-PCR). Levels of p53 mRNA were not changed 4 hrs after initial injury, and gradually elevated during 24-72 hrs. The mRNA expression of ICE remained constant through 3 d following the ischemia. Expression of bcl-2 mRNA was increased in 24 hrs and returned to the control level in 72 hrs. Levels of actin mRNA were not changed through the degeneration process. The present study suggests that retinal ischemia induces neuronal necrosis and apoptosis through mechanisms involving upregulation of p53 and Bcl-2 mRNA. Supported by Biotech. 2000

464.16

INVOLVEMENT OF INTERLEUKIN CONVERTING ENZYME AND ITS FAMILY PROTEASE IN THE CELL DEATH FOLLOWING TRANSIENT GLOBAL ISCHEMIA. T. Himi*, Y. Ishizaki, H. Wakisaka, M. Ikeda and S. Murota. Department of Physiological Chemistry, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, JAPAN.

Recent evidence suggest that interleukin converting enzyme (ICE) and its family protease have a key role for apoptosis to occur in wide variety of cell types. In order to know the molecular mechanism of delayed neuronal death following transient global ischemia, we have investigated the involvement of ICE family protease.

Nine-week-old gerbils were subjected to 10 min of bilateral common carotid artery occlusion followed by reperfusion. Western blot analysis showed cleaved fragment (85 kDa) of poly(ADP-ribose) polymerase in hippocampus in post-ischemic day 4, but not in day 8. Immediate post-ischemic administration of ICE inhibitor, benzyloxycarbonyl-Asp-fluoromethylketone (zD, 25 nmol *i.c.v.*; 3 times at intervals of 12 hr), significantly ameliorated the number of TUNEL-stained neurons (90 %, day 4) and decrease in the number of CA1 pyramidal cells (80 %, day 8). Ultrastructural study showed these protected neurons sustained normal morphology (day 8). This neuroprotective effect of zD was, however, not observed when administration was started 24 hr after the ischemic insult. In both cases, the number of hypertrophic astrocytes expressing glial fibrillary acidic protein markedly increased in the hippocampal CA1 region (day 8). These findings indicate that ICE family protease plays important roles in early stage of delayed neuronal death following transient global ischemia and that delayed neuronal death can be suppressed by blocking ICE family protease. (Work supported by Research Grant for Young Scientists from the Ministry of Education, Science and Culture of Japan and by Japan Foundation for Health Sciences)

464.17

APOPTOSIS AFTER ISCHEMIA AND REPERFUSION IN RAT BRAIN: ROLE OF INTERLEUKIN 1-BETA CLEAVAGE ENZYME (ICE). L. Denner*, C. Munsch, C. Kagan, L. Sturgis and R. Tilton. Dept. of Cell Biology, Texas Biotechnology Corporation, Houston, TX 77030

Reversible occlusion of the middle cerebral artery in the rat brain is an established model of stroke in man. Following transient ischemia in the rat, subsequent reperfusion results in the development of an infarct containing cells that eventually die by necrosis and/or apoptosis. One feature of apoptotic death is fragmentation of DNA which can be detected by TUNEL. Using the rat model we showed by TTC staining that 1 hr of ischemia and 4 hr of reperfusion induced a substantial infarct along the rostral-caudal axis that was also positive for TUNEL. Since the ICE family of proteases plays an important, potentially requisite, role in apoptotic death, RT-PCR was used to investigate ICE family member gene expression in apoptosis induced by ischemia/reperfusion. With primers to the known rat sequence, rat ICE mRNA was detected in control neocortex and in neocortex exposed to 1 hr of ischemia followed by reperfusion for 2 hr, 4 hr, 1 day, 2 days or 3 days. Under these same conditions, degenerate primers to the conserved pentapeptide sequence (QACRG) in the ICE family were used with specific primers to the known human sequences. In normal and stroke brains, rat mRNA was detected for Mch2a and Ich1-L but not apopain, TX or Ich1-S. Western blotting indicated that ICE protein was expressed in control brains as well as brains from the various times of reperfusion. ICE catalytic activity was demonstrated by cleavage of the natural substrate, the 33 kDa proIL1-beta precursor, to the 17 kDa mature, active IL1-beta. Catalytic activity was also shown in a fluorogenic assay using a peptide containing the cleavage site in proIL1-beta. These results suggest that ischemia and reperfusion lead to rapid and extensive development of apoptosis which may be mediated by ICE.

464.18

ADENOVIRUS-MEDIATED NAIP OVEREXPRESSION CONFERS PROTECTION AGAINST GLOBAL ISCHEMIA. S.J. Crocker[1]*, D.G. Xu[1], A.M. Hakim[2], J.-E. Ikeda[3], N. Roy[4], R.G. Korneluk[4], A. MacKenzie[4], and G.S. Robertson[1]. Department of Pharmacology[1], Neuroscience Research Institute[2], University of Ottawa, Ottawa, Canada K1H 8M5, The Institute of Medical Science[3], Tokai University, Kanagawa 259-11, Japan, Molecular Genetics Laboratory[4], Children's Hospital of Eastern Ontario, Ottawa, Canada K1H 8L1.

We have demonstrated that motor neuron death in spinal muscular atrophy is associated with deletions of a gene which encodes the *n*euronal *a*poptosis *i*nhibitory *p*rotein (NAIP). More recently we have shown that transient global ischemia selectively increases NAIP levels in neuronal populations which are resistant to the damaging effects of this treatment (Robertson *et al.* abstract). This observation suggests that resistance to ischemic injury may be conferred by elevated NAIP expression. Consequently, we investigated whether NAIP overexpression would protect CA1 hippocampal neurons from delayed neuronal death following transient global ischemia. Recombinant adenoviral constructs (Ad5d1xDNA-TPC) containing either *myc*-tagged NAIP (3 μ1; 1 X 10^6 particles μl^{-1}) or *lacZ* (3 μ1; 1 X 10^6 particles μl^{-1}) were stereotaxically injected into the right and left dorsal hippocampus, respectively. Seven days later, all animals were subjected to 10 min of four-vessel occlusion (4-VO). Animals were perfused with 4% paraformaldehyde 5 days following recirculation. Tissue sections from the hippocampus were processed for immunohistochemical detection of NAIP-, Myc-, β-gal- and the neuron specific marker, NeuN. Cell counts of NeuN immunoreactivity revealed that the NAIP construct significantly attenuated the loss of hippocampal CA1 neuron after 4-VO. These results suggest that manipulations which elevate NAIP expression may be useful in the treatment of stoke.

This work was supported by a grant from the Heart and Stroke Foundation of Canada.

464.19

DOWNSTREAM TARGETS OF NF-kB ARE INDUCED FOLLOWING TRANSIENT GLOBAL FOREBRAIN ISCHEMIA. E. P. Dixon, D. Stephenson, J. Clemens*, S. P. Little. CNS/Molecular Biology Division, Eli Lilly and Company, Indianapolis, IN 46285

Ischemic injury initiates a vast array of events including the generation of free radicals. We have previously reported in a rat model of transient forebrain global occlusion that activation of NF-kB occurs in areas of ischemic damage. Events following NF-kB activation ultimately determine whether cells will undergo necrosis or programmed cell death (apoptosis).

We report the induction of Cu/Zn superoxide dismutase (SOD) protein following 20 minutes of 4-vessel global occlusion in brain regions where ischemic damage and activated NF-kB is apparent. SOD was induced in a time-dependent fashion, with maximal induction occurring at 72 hours post ischemia. SOD levels then returned back to basal levels at 120 hours post ischemia. This increase in SOD may act as an elaborate defense system in neuronal cells to detoxify and protect O_2 or free radical formation during anoxia and reperfusion damage.

We previously reported that typical DNA fragmentation during apoptosis occurs in conjunction with activation of NF-kB in ischemic damaged cells from the CA1 hippocampal region. Sinced apoptosis is tightly regulated by several redox-sensitive transcription factors, we looked at the expression of Bc1-X. Bc1-X has two splice variants and is known for its regulation of apoptosis. In damaged CA1 regions of our ischemic rat model, dramatic increases in Bc1-X_S protein expression occurred at 72 hours post ischemia. The observed increase in Bc1-X_S supports the idea that neurons in the CA1 hippocampal subsector die by the process of apoptosis.

In conclusion, since both the pro-apoptotic gene Bc1-X_S and the neuroprotective gene SOD contain elements in their promotors that are responsive to active NF-kB, it is difficult to clearly define the role of NF-kB in ischemic neuronal damage at this time.

TRAUMA III

465.1

RECOVERY OF FUNCTION AFTER TRAUMATIC BRAIN INJURY (TBI): IS ULTIMATE RECOVERY DEPENDENT UPON TESTING FREQUENCY? J.S. Sobolsky*, L.L. Colgin, D.A. Chorney, June F. Davidson, and M.E. Carey., Neurotrauma Res. Lab., Dept. Neurosurgery, LSU Medical Cntr., New Orleans, LA 70112

We have developed an animal model of TBI in which the hind- and forelimb sensorimotor cortices are injured unilaterally in anesthetized rats by a piston impact depressing the dura 1mm @ 5 m/sec. A battery of tests previously indicated that motor recovery occurs in 28-35 days. Two important questions are: 1) whether the recovery rate is dependent on testing frequency and 2) whether the recovery is test specific. To help answer these questions we injured 2 groups of rats. The first group was tested using our usual tests and testing frequency over 35 days. The second group was left undisturbed for 35 days prior to the start of testing. The results of 3 motor tests (narrow flat beam, pegged beam and paw preference) indicated that rats which were not tested until 35 days after TBI showed the same amount of recovery at the beginning of testing that the first group of rats showed at the end of 35 days of frequent testing. These results suggest that the ultimate motor recovery or behavioral adaptation is not dependent upon the frequency of testing and that a general neuronal plasticity occurred over 35 days which did not require test-specific experience. Supported by U.S. Army contract DAMD17-93-C-3008

465.2

SCOPOLAMINE BLUNTS BRAIN LACTATE ACCUMULATION AND THE ACTIVATION OF THE PIP_2 SIGNAL TRANSDUCTION PATHWAY AFTER TRAUMATIC BRAIN INJURY. Q-Z. Gong* B.G. Lyeth, H.S. Dhillon and M.R. Prasad. Divisions of Neurosurgery, Medical College of Virginia, Richmond, VA and University of Kentucky, Lexington, KY.

Hippocampal levels of lactate, free fatty acids (FFA), and fatty acids (FA) extracted from phosphatidylinositol 4,5-bisphosphate (PIP_2) were measured after central fluid percussion traumatic brain injury (TBI) in rats. Anesthetized rats were frozen *in situ* with liquid nitrogen at 5 or 20 min after injury. Hippocampi decreased in PIP_2 (by 11% & 19% respectively) while these same acids were dissected and fatty acids extracted and separated from PIP_2 and FFA. Lactate in the suspension was measured with a spectrophotometer. At 5 min after injury, levels of stearic and arachidonic acid fractions were significantly increased in the FFA fraction by 180% & 143% respectively. These alterations were blunted (not significantly different from control) by pre-injury administration of the muscarinic antagonist, scopolamine (1.0 mg/kg i.p.). No significant changes were observed in FA at the 20 min time point in saline-treated TBI rats. Lactate was increased by 232% at 5 min and 165% at 20 min in saline treated rats. In scopolamine-treated rats, lactate increased by only 86% at 5 min and 74% at 20 min. These results suggest that the PIP_2 signal transduction pathway is rapidly activated in the hippocampus after TBI and that the enhanced phospholipase C-catalyzed phosphodiestric breakdown of PIP_2 is a mechanism of liberation of FFA after TBI. The blunting of PIP_2 and FFA alterations in animals treated with scopolamine suggests that activation of muscarinic receptors significantly contributes to the phospholipase C signal transduction pathophysiology in TBI. The reduction in lactate by scopolamine suggests that muscarinic activation also contributes to increased glycolytic metabolism and/or ionic imbalances.

Funding from NIH grants NS 12587, NS 29995 (BGL) and NS 31816 (MRP).

465.3

EXPRESSION OF CHEMOKINES AND NITRIC OXIDE SYNTHASE-2 FOLLOWING CEREBRAL TRAUMA.

D. Grzybicki*, A. Loihl, S. Kardos, R. Schelper, S.A. Moore, A.R. Glabinski, R.M. Ransohoff and S. Murphy. Depts. Pathology and Pharmacology, Univ. of Iowa College of Medicine, Iowa City, IA 52242, and Dept. Neuroscience, Cleveland Clinic Foundation, Cleveland, OH 44195.

Traumatic injury to the brain initiates multiple interrelated processes (angiogenesis and reactive gliosis) involving resident parenchymal and vascular cells and also infiltrating inflammatory cells. Nitric oxide (NO) and chemokines have been implicated as regulators of the CNS injury response. Following a cryogenic lesion of the cerebral cortex in mice, mRNA for NO synthase (NOS)-2 was detected by RT-PCR ipsilaterally 12 h after injury and persisted for two weeks. While mRNA was also detected contralaterally the time course was shorter (one week). By immunocytochemistry, NOS-2 was seen ipsilaterally in infiltrating macrophages and in glial cells in both hemispheres at 72 h. The expression of monocyte chemoattractant protein (MCP-1) preceeded NOS-2. On the lesion side MCP-1 peaked at 6h, was sustained to 24h and had declined by 48h. On the unlesioned side MCP-1 was expressed to a lesser extent (50%) and declined by 24h. MCP-1 has been shown to block the inudction of NOS-2. The expression of NOS-2 in glia could be initiated either by signals from infiltrating cells or by declining MCP-1 levels.

Supported by NS29226.

465.4

Immunocytochemistry of tumor necrosis factor in spinal cord following a weight-drop injury in rat.

CX Wang* and JR Wrathall. Department of Cell Biology, Georgetown University, Washington DC 20007, USA

Previously, we have demonstrated that tumor necrosis factor alpha (TNF) is produced in spinal cord following a static load injury (Wang et al., Eur. Cyt. Net. 5:208, 1994). In the current study, we investigated the presence of TNF in the spinal cord after a standardized weight-drop (10g X 2.5cm) injury at T8 (Wrathall et al., Exp. Neurol. 88:108-122, 1985). The spinal cord at the lesion site was removed for immunocytochemistry at 1, 8, 24 and 72 h after the injury and was also collected from uninjured controls (n=3 per group). TNF staining was absent in the spinal cord from the controls, but was present in all samples of the injured spinal cords. The number of TNF positive cells appeared to increase between 1 and 8 h, and remained at this level through 72 h after the injury. Most of the TNF staining was in the lesion site and in the gray matter adjacent to it. Moreover, the number of TNF positive cells appeared greater at the border of the lesion than in the center. Many neurons in the preserved gray matter adjacent to the lesion site developed cytoplasmic staining for TNF after the injury. In addition, TNF staining was also observed in swollen axon terminals at the edge of the lesion site. Double labeling studies showed macrophages and endothelial cells were not TNF positive. In summary, TNF is present in the spinal cord by 1 h after the injury, and cells stained positively for TNF meet the morphological criteria of neurons. These data indicate TNF production is an acute reaction and may play a role in the death of neurons and degeneration of axons in the injured spinal cord.

(Supported by PVA No:1451-01)

465.5

DESCENDING SPINAL CORD EVOKED POTENTIALS EVOKED BY STIMULATION AT ACUPOINT.

Qing-Yin Zheng, Zhen-Feng Cheng, Lu Guo-Wei, S.S. Haghighi*. Capital University of Medicine, Beijing P.R. China, Dept. of Neurology, & MCPHU, Dept. of Neurosurg. Phila., PA.

Segmental cord potential (Y-sSCEP) evoked by stimulation at the Y acupoint (the center of posterior paw) of Sprague-Dawley rats vanished gradually when the recording electrode was placed more rostrally, for example at C6, another positive slow potential Y-dSCEP could be recorded. Y-dSCEP disappeared completely when the spinal cord had been sectioned at a higher cervical level. Electrolytic lesions of ventral periaqueductal gray (PAG) could also decrease remarkably the amplitude of Y-dSCEP induced by stimulation at the Y acupoint. Similarly, another descending SCEP, N-dSCEP could be induced from L3 by stimulation at the N acupoint (2/3 down the anterior limb). It also proved to originate from supraspinal structures. Acupoint stimulation induced dSCEPs had properties of spatial and temporal summation. Local administration of picrotoxin (specific antagonist of presynaptic inhibition) could significantly attenuate the P wave of the dSCEP. These results suggest that the P wave of the dSCEP. These results suggest that the P wave of the dSCEP is the reflection of the primary afferents by activating brainstem strucures.

465.6

LEFT BUT NOT RIGHT DORSAL NORADRENERGIC BUNDLE (DNB) LESIONS IMPAIR MOTOR RECOVERY AFTER RIGHT SENSORIMOTOR CORTEX (SMCTX) INJURY IN THE RAT.

L.B.Goldstein*, S. Bullman.
Durham V.A. & Duke Univ., Durham, NC 27705

The ability of rats to traverse a narrow elevated beam has been used to quantitate recovery of hindlimb motor function after unilateral SMCTX injury. A left but not right locus coeruleus(LC) lesion 2 weeks prior to a right SMCTX lesion impairs beam-walking (BW) recovery (Goldstein et al.,1993). We now compared the effects of left and right DNB lesions. Rats underwent either left (LDNB), right (RDNB) or sham (SDNB) 6-OHDA lesions and one week later were trained at the BW task. Two weeks after DNB surgery, rats underwent right SMCTX suction-ablation (CORLES) or sham cortex surgery. BW was measured over the next 12days. Rats were sacrificed and the extent of the cortex lesion, norepinephrine (NE) levels in cerebral and cerebellar hemispheres, and LC cell counts were measured. DNB lesions did not affect BW training or BW after sham SMCTX lesions. CORLES rats with prior LDNB lesions (n=7) had impaired BW recoveries compared to CORLES rats with prior SDNB lesions (n=8) (ANOVA $F_{3,16} = 11.6$, $p<.0003$; Fisher LSD, $p<0.02$). In contrast, the recoveries of CORLES rats with prior RDNB lesions (n=7) did not differ from CORLES rats with prior SDNB lesions (n=7) (Fisher LSD. p=0.81). There were no differences between the groups in the extents of cortex lesions or in LC cell counts. DNB lesions resulted in significant decreases in NE levels in the ipsilateral cerebral cortex, but not in the contralateral cerebral cortex or either cerebellar hemisphere. These data show that NE mediates motor recovery after SMCTX lesions, at least in part, through effects in the contralateral, but not ipsilateral cerebral cortex.

Supported by the Department of Veterans Affairs

465.7

KDN--A NEW DRUG FOR TRAUMA AND VASCULAR DISEASE OF NERVOUS SYSTEM.

Lu Shoulong*, Liu Xin, Wang Jianlong and Ying qi Department of Neurosurgery No.411 Naval Hospital. Shanghai,200081.China

The authors developed a new drug (KDN)which was prepared from medicinal herbs. It's main indications are: (1)convalescence of various kinds of brain trauma;(2) recovry of cerebrovascular diseases; and (3) encephalatrophy. Animal experiments (with Mongolian rats) showed that KDN could increase the toleratace for hypoxia and ischemia ($p<0.01$). It has been applied clinically in 5,000 patients with a total effective rate of 96%. The drug was taken twice daily, with 10ml each time. One course of treatment was 30 days. Usually, 1 to 2 courses were needed. CT, TCD and r-CBF examinations were made in part of the patients before and after taking the medicine and the results were compared. No toxicity and side effect of this drug were found.

465.8

HYPOXIA DELAYS RECOVERY OF FOOD INTAKE AND WHEEL RUNNING AFTER FLUID PERCUSSION INJURY.

R. Bauman*, J. Widholm, J. Dave, and J. Long. Div. of Neurosci., Walter Reed Army Inst. of Research, Washington, D.C. 20307.

The purpose of the present study was twofold: (1) to characterize behavioral deficits resulting from lateral fluid percussion injury (FPI) using measures of activity and ingestion, and (2) to evaluate whether these functional deficits would be intensified by post-traumatic hypoxia (PTH). All rats were exposed to a 12 hour light:dark cycle and were housed in individual feeding cages that were attached to standard running wheels. The wheel was freely available and within each feeding cage, the only available food was delivered for leverpressing; 5 leverpresses were required for the delivery of a single 45 mg food pellet. After food intake and wheel running stabilized, rats were surgically prepared. Five days later, in the early hours of the light period, they were anesthetized with halothane and subjected to either parasagittal FPI (4.5±0.1 atm, 20 msec duration) or sham injury (SI). For 30 min after injury, a gas mixture containing 13% O_2 and halothane was used to induce hypoxia in half the FPI and SI rats; in normoxic rats, halothane anesthesia was maintained with a 100% O_2 source. Recovery of wheel running and food intake was monitored during light and dark periods for one week after FPI or SI. On the day of injury, mean food intakes in the dark, but not the light, for both hypoxic and normoxic FPI groups were reduced below both SI group means, which were themselves not significantly different. Neither mean intakes nor wheel turns of the hypoxic or normoxic FPI rats differed significantly in the dark on the day of injury. However, on the second and third recovery days, mean intakes and mean wheel turns in the dark were significantly less for the FPI hypoxic rats than for the SI normoxic rats. Neither intake nor turns were significantly reduced by either FPI or hypoxia alone on these recovery days. Therefore, hypoxia delayed recovery from FPI. These results indicate that PTH is an important pathophysiological factor influencing recovery from brain trauma.

Research funded by the U. S. Army Medical Research and Materiel Command

465.9

CEREBRAL EFFLUX OF INTERLEUKIN-1ß IN CHILDREN WITH TRAUMATIC AND ANOXIC BRAIN INJURY, AA Pinto, JW Kuluz, CL Schleien*, Pediatric Crit Care Med, Univ of Miami Sch of Med, Miami, FL 33101

Introduction: IL-1ß is produced in the brain in response to ischemia, trauma and infection and has been shown to worsen ischemic brain damage. Several studies have shown that the brain concentration of IL-1ß increases after these insults, however, the net flux of IL-1ß across the blood brain barrier (BBB) has not been documented. In this study, we measure the flux of IL-1ß accross the brain in children with severe traumatic and anoxic brain injury.

Methods: Global CBF was measured (N_2O, Kety - Schmidt) in children with severe traumatic (n=4) and anoxic (n=4) brain injury within 24 hr of admission and again daily. Jugular (JV) and arterial (Art) blood samples were drawn within the first 24 hr of admission and then daily. Blood samples were centrifuged, plasma stored at -20°C and Il-1β measured by ELISA (Genzyme). Cerebral efflux of Il-1β was calculated by CBF x JV-Art [IL-1β]. Values are mean ± SEM.

Results: IL-1β JV was greater than Art in 12 of 14 time points in the first 48 hours and in only 5 of 12 time points after 48 hr. Over the first day, efflux tended to be greater in the trauma group, with decreasing levels between 24-48 hr in that group. IL-1β efflux approached zero after 48 hr in both groups.

	0-24h	24-48h	>48h
Trauma	621±444	403±391	63±180
Anoxia	359±253	483±256	47±99

Conclusion: Il-1ß efflux from brain to blood occurs in children during the first 48 h after severe brain injury. Possible explanations for this efflux include transport of IL-1 across a disrupted BBB as well as increased intraluminal production of IL-1 by endothelium or leukocytes. The peak efflux occurs sooner after trauma then after anoxia which may be due to the usual delay in BBB disruption after anoxia.

465.10

INHIBITION OF NEURAL NETWORK ACTIVITY WITH GAMMA-AMINOBUTYRIC ACID (GABA) DOES NOT INCREASE SURVIVAL OF SPINAL CORD (SC) NEURONS SUBJECTED TO PHYSICAL (MEMBRANE DISRUPTION) INJURY. J.H. Lucas* and G.W. Gross. *Dept. of Physiology, Ohio State Univ., Columbus OH 43210 and Center for Network Neuroscience, Univ. of North Texas, Denton TX 76203.

Previous investigations showed that posttraumatic cooling (2h at 17°C followed by rewarming) or barbiturates (thiopental or pentobarbital) increase survival of mouse SC neurons subjected to a defined, physical injury (UV laser microbeam transection of a primary dendrite; J. Neurotrauma 11: 35, 1994). A possible protective mechanism for both treatments is reduction of activity-dependent metabolism.

Barbiturates decrease activity by potentiating GABA-induced inhibitory postsynaptic potentials. We investigated the effect of GABA on neuronal survival after dendrotomy. In mouse SC cultures (21-28 DIV) neurons were selected for surgery or to be unlesioned controls. In the experimental group 30 µM GABA was added to the medium just after cell surgery. This concentration stops spontaneous burst activity of mouse SC cultures grown on multimicroelectrode plates. A primary dendrite was transected from each neuron selected for surgery (distance from soma = 100 µm). Survival was tested at 24h by erythrosin B. In the experimental group survival was 42% ± 8 compared to 45% ± 12 in the untreated control group (5 cultures/50 lesioned neurons per group). Survival of the 50 unlesioned control neurons in each group was 100%. These data indicate that reduction of activity-dependent metabolism is probably not the mechanism of protection by hypothermia and barbiturates, and agree with earlier studies showing lack of protection by other inhibitors of synaptic and/or spike activity (10-12 mM Mg^{+2} and 2 µM tetrodotoxin). Supported by NINDS 29683 to JHL.

465.11

MECHANISM AND PREVENTION OF LETHAL COLD STRESS TO SPINAL CORD (SC) NEURONS. G. Craenen*, S. Jeftinija[@], J. Grants, and J.H. Lucas. Dept. of Physiol., The Ohio State Univ., Columbus, OH 43210; [@]Dept. of Veterinary Anat., Iowa State Univ., Ames, IA 50011.

Hypothermia has been found to be beneficial in CNS trauma. The parameters for treatment by cooling are still being defined. We previously reported that posttraumatic cooling to 17°C for 2h increased survival of mouse SC neurons subjected to physical (dendrite transection) injury, but that cooling below 17°C caused a lethal NMDA receptor-linked stress to both lesioned and uninjured neurons (J. Neurotrauma 11: 35, 1994).

The present study tested whether cooling below 17°C increases extracellular levels of excitatory amino acids. SC cultures were placed at 10°C or 37°C. HPLC showed that Glu and Asp levels were much higher in the medium of the cooled cultures after 0.5 h (23 ± 4 nM/µg vs 4 ± 1 nM/µg and 4 ± 1 nM/µg vs 1 ± 0 nM/µg respectively). The concentration of each amino acid then declined and reached a plateau at 1-4h that was still several fold higher than control levels (3 cultures/group; p<.0001, two-factor ANOVA). Other amino acids (Gly, Asn, Ser, Gln) showed an opposite pattern with higher levels in the 37°C group.

Both NMDA and non-NMDA antagonists prevented lethal cold injury. Death (tested by erythrosin B) of SC neurons cooled at 10°C for 2h and rewarmed for 22h was 32 ± 10% in the control group, 6 ± 5% with a non-NMDA receptor blocker (50 µM CNQX), 3 ± 5% with a NMDA receptor blocker (100 µM D-APV), and 1 ± 2% with CNQX + D-APV (each group = >120 neurons in 5 cultures; p < .0006, one-factor ANOVA). These results show that death of SC neurons by cooling below 17°C is caused by elevated extracellular Glu and Asp and requires activation of both NMDA and non-NMDA receptors. Supported byNINDS grant 29683 to JHL.

465.12

CHARACTERIZATION OF A MODIFIED NERVE INJURY MODEL OF NEUROPATHIC PAIN IN RATS. J.Rémillard[1], R. Quirion[1], A. Dray, P .Birch, C. Wahlestedt, D.P. Ménard. Astra Pain Research Unit, 275 Armand-Frappier, Laval, Québec, CANADA, H7V 4A7, [1] Douglas Hospital Res. Ctr., Dept. Pharmacol. and Therap., McGill University, 6875 Lasalle, Verdun, Québec, CANADA, H3H 1R3

A standardized constriction nerve injury model was recently proposed by Mosconi and Kruger (Pain 64: 37-57, 1996). The application of fixed-diameter polyethylene cuffs to the rat sciatic nerve produces behavioral responses rather similar to those observed in human peripheral neuropathies. The aim of this study was to quantify the nociceptive behavior of these animals. Surgeries were performed on male Sprague-Dawley rats under ketamine/xylazine anesthesia. An incision of 1 cm was made at the mid-thigh level and the left sciatic nerve was exposed. A 2 mm long cuff of PE-60 polyethylene tubing (ID 0.03") was cut longitudinally and applied to encapsulate the nerve. Animals showed pain-related behaviors such as abnormal gait and limb position. Paw-flick latencies and paw-pressure thresholds were recorded to assess thermal and mechanical hyperalgesia. To test for mechanical allodynia, a series of 10 von Frey hairs of incremental stiffness (0.4 - 20.9g) was presented 6 times to each paw 10 days following surgery and the percent response was calculated. Paw-flick latencies (ipsilateral paws: 5.5±0.5s, contralateral paws: 8.5±0.4s, control rats: 7.7±0.4s) and paw-pressure thresholds (ipsilateral paws: 138±17g, contralateral paws: 174 ±18g, control rats: 228±49g) ipsilateral to the cuffed nerve were decreased compared to control and contralateral paw values 6 days post-surgery. The injured paws showed mechanical allodynia i.e. 53±10% compared to 5±2% for controls in response to the 0.98g hair. However, the contralateral paws of operated animals displayed a decreased response (23±13% compared to 92±5% for controls using the 12.5 g hair). These animals hence display mechanical and thermal hyperalgesia along with mechanical allodynia providing a good model to study analgesics and chronic pain mechanisms. Supported in part by MRC to R.Q.

465.13

MODE OF SPINAL MOTONEURON DEATH FOLLOWING INJURY IN ADULT MICE: AN EM STUDY L. Li[1], L.J. Houenou[1], W. Wu[2], M. Lei[1], D.M. Prevette[1] and R.W. Oppenheim[1] [1]Neurobiology & Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27157, [2]Neurosurgery, Eastern Virginia Medical School, Norfolk, VA 23501.

Experimental lesions have been widely used to induce neuronal degeneration and to test the ability of trophic molecules to prevent lesion-induced alterations. However, the morphological mechanisms of spinal motoneuron death (i.e., apoptosis or necrosis) following different lesion modes is not clear at the present time. At the light microscopic level, we have found that spinal motoneurons are atrophied following sciatic nerve axotomy in neonatal mouse. By contrast, motoneurons in adult mouse increase in size following spinal root avulsion. The objective of this study is to characterize injury-induced cell death using electron microscopy (EM).

Our results show that one day following spinal root avulsion, motoneuron size and morphology on the lesion side was similar to that of control, untreated animals except for some apparent cytoplasmic condensation. From 3 days to 2 weeks after the root avulsion, the motoneurons on the lesion side increased in size and the cytoplasm became hyperchromatic, the nuclear envelope was indistinct, and the nucleoli and nuclei were condensed and irregular. The cytoplasm contained fragmented organelles and numerous small granular profiles. Mitochondria were absent, and the cell membrane was disrupted indicating, that the injured neurons were not viable. In addition, the motoneurons on the lesion side appear to have lost contact with adjacent glial cells and neurons. Collectively, these observations indicate that adult mouse motoneurons die by necrosis following injury by root avulsion. We are also examining the mode of cell death in the neonatal mouse following axotomy and comparisons will be made for the two lesion modes (i.e., distal axonal axotomy vs root avulsion) in mice. In addition, studies are in progress to examine whether DNA fragmentation detected by the TUNEL method occurs in these injury models. Supported by MDA and NIH.

465.14

INVOLVEMENT OF TUMOR NECROSIS FACTOR-ALPHA IN THE PATHOPHYSIOLOGY OF EXPERIMENTAL BRAIN INJURY. L. Fan*, S.M. Knoblach, A.G. Yakovlev and A.I. Faden, Georgetown Institute for Cognitive and Computational Sciences, Georgetown Univ. Medical Center, Washington DC 20007.

Tumor necrosis factor-alpha (TNF-α) is a pleiotropic cytokine implicated in the immune and inflammatory cascades initiated by a variety of CNS insults, including brain trauma. To examine the role of TNF-α in the pathophysiology of brain trauma, we studied the modulation of this cytokine in a widely used, clinically relevant model of traumatic brain injury (TBI).

Anesthetized, male Sprague-Dawley rats were subjected to craniotomy followed by sham (0 atm) or moderately-severe levels (2.5-2.8 atm) of lateral fluid percussion injury. The level of TNF-α mRNA in the ipsilateral hippocampus was determined via semi-quantitative RT-PCR in samples taken from 0.5 h to 3 wks after TBI. Post-traumatic increases in TNF-α mRNA peaked 0.5 h after injury, and remained significantly elevated at 24 h. In addition, at 4 and 8 h after TBI, TNF-α was present in CNS cells visualized with double-label immunostaining.

To examine the effect of injury-induced elevations in TNF-α, chimeric soluble TNF receptors (sTNFR:Fc) were used to specifically antagonize TNF-α in separate studies that evaluated behavioral and histological outcome measures. Animals treated with intracerebroventricular (37.5 µg/10 µl) injections of sTNFR:Fc 15 min. before and 1 h after TBI performed significantly better in a series of standardized motor tasks than did IgG-treated, injured controls. In addition, histological analysis of the hippocampus ipsilateral to the injury revealed that sTNFR:Fc treatment significantly preserved CA1 cells of the pyramidal layer, compared to injured controls. However, when administered intravenously (0.2-5 mg/kg) after trauma, sTNFR:Fc did not improve behavioral outcome.

These results indicate that enhanced posttraumatic expression of TNF-α within the CNS contributes to the behavioral deficits and histological changes associated with TBI. The effects of treatment with sTNFR:Fc appear to depend upon route of administration and/or timing. (Supported by CDC CCR306634 and NCMRR T32HD07459).

465.15

UPREGULATION OF RETINOIC ACID (RA) SYNTHESIS FOLLOWING SPINAL CORD INJURY. D.J. Morassutti*, P. Zhang, U.C. Drager and P. McCaffery. Dept of Neurological Surgery, U. of Louisville, Louisville, KY 40292 and E.K. Shriver Center for Mental Retardation, Waltham, Mass 02254.

Endogenously synthesized RA in the embryonic mouse spinal cord may serve to rescue motoneurons from morphogenetic cell death, and also stimulate neurite outgrowth into developing limbs. This proposal is supported by *in vitro* reports which demonstrated that RA substantially improved spinal motor neuron explant survival and enhanced axonal growth. By postnatal day 13, RA falls to very low and evenly distributed levels throughout the adult SC. These data, and the concept that regenerative efforts likely involve the recapitulation of developmental events, prompted us to examine the expression of retinaldehyde dehydrogenase (RalD), a RA synthetic enzyme, in adult SC injury.

Control Sprague-Dawley rats had a laminectomy but no SC injury; the experimental group received a mild contusive injury(12.5cm; NYU impactor). There were 30 rats per group, and animals were sacrificed begining on the day of surgery, and then every 2d until day 10. The SC's were removed in 3mm long sections at the surgery/injury site, and immediately rostral and distal to it. RalD activity was analysed, and the results revealed a 3-4 fold increase in activity over the first 4 days, in all groups; with a return to normal levels by D-8 to10 in all except the lesion site. This significant upregulation of RalD activity suggests that RA may be playing a role in SC injury. This work was supported by NIH grants HD05515 and EY01938.

465.16

INFLUENCE OF C4/C5 CHRONIC SPINAL CONTUSION ON INSPIRATORY MOTOR DRIVE DURING AUGMENTED BREATHS IN ANESTHETIZED RATS. G.W. Schrimsher[2], D.C. Bolser[1]*, B. O'Steen[2], J. McKenzie[1], and P.J. Reier[2]. Dept. Of Physiological Sciences[1], and Depts of Neuroscience and Neurosurgery[2], University of Florida, Gainesville, Fl, 32610.

Patients with cervical spinal injuries have impaired pulmonary defensive reflexes due to loss of descending motor drive to spinal motoneurons. We speculated that rats with chronic cervical spinal contusions would have reduced motor drive to diaphragm (DIA) and pectoralis (P) muscle motoneurons during an inspiratory defensive reflex, the augmented breath (sigh). Wistar rats were subjected to spinal contusion at the C4/C5 spinal level. Two months later, lesioned animals (n=7) and age-matched controls (n=10) were anesthetized and EMGs were recorded from the right hemidiaphragm and right pectoralis major muscles. Augmented breaths (AB) occurred spontaneously or were evoked by mechanical stimulation of the intrathoracic trachea. DIA and P EMGs during augmented breaths were normalized to the largest burst observed during asphyxia (asphyxic maximum), performed at the end of the experiment. In intact animals, both DIA and P EMGs during ABs were 58±3% of the asphyxic maximum. In animals with cervical contusions, DIA ABs were 103±21% ($p<0.02$) and P ABs were 29±2% ($p<0.001$) of the asphyxic maximum. There also was a positive linear relationship between DIA and P burst amplitudes during ABs in intact animals ($r=0.44$, $p<0.01$), that was not present in animals with cervical contusions ($r=0.02$, $p<0.9$). We conclude that C4/C5 spinal contusion eliminates phrenic motoneurons that provide inspiratory reserve and reduces excitatory inspiratory motor drive during ABs to cervical spinal motoneurons caudal to the lesion. (Supported by the State of Florida BSCIRTF and the M.F. Overstreet Chair for SCI Research).

465.17

INFLUENCE OF C4/C5 CHRONIC SPINAL CONTUSION ON EXPIRATORY MOTOR DRIVE DURING THE EXPIRATION REFLEX IN ANESTHETIZED RATS. D.C. Bolser[1], G.W. Schrimsher[2], B. O'Steen[2], J. McKenzie[1], and P.J. Reier[2]*. Dept. Of Physiological Sciences[1], and Depts of Neuroscience and Neurosurgery[2], University of Florida, Gainesville, Fl, 32610.

Patients with cervical spinal injuries have impaired pulmonary defensive reflexes due to loss of descending motor drive to spinal motoneurons. We speculated that rats with chronic cervical spinal contusions would have reduced motor drive to rectus abdominis (RA) and pectoralis (P) muscle motoneurons during an expiratory defensive reflex, the expiration reflex (ER). Wistar rats were subjected to spinal contusion at the C4/C5 spinal level. Two months later, lesioned animals (n=7) and age-matched controls (n=8) were anesthetized and EMGs were recorded from the left rectus abdominis and right pectoralis muscles. Expiration reflexes were evoked by mechanical stimulation of the larynx. RA and P EMGs during expiration reflexes were normalized to the largest burst in each EMG observed during asphyxia (asphyxic maximum), performed at the end of the experiment. In intact animals, ER bursts for the RA and P were 18±3% and 47±7% of the asphyxic maximum, respectively. In animals with cervical contusions, ER bursts for the RA were 28±5% ($p<0.05$) and P were 37±5% ($p<0.2$) of the asphyxic maximum. In intact animals, there was no significant relationship between RA and P burst amplitudes during the ER ($r=0.23$, $p<0.3$). However, in animals with cervical contusions a significant positive linear relationship existed between RA and P burst amplitudes during the ER ($r=0.7$, $p<0.001$). These results suggest that cervical spinal contusions produce highly specific and sometimes subtle effects on motor drive to cervical and thoracolumbar spinal motoneurons during pulmonary defensive reflexes. (Supported by the State of Florida BSCIRTF and the M.F. Overstreet Chair for SCI Research).

465.18

GENDER SPECIFIC ALTERATION IN NITRIC OXIDE SYNTHASE ISOFORMS AND ACTIVITY FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT. L.K. Gorman*, M.J. Williams, P.D. Hurn and R.J. Traystman. Department of Anesthesiology and Critical Care Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21287.

Nitric oxide (NO) has been implicated as a neurotoxin in numerous animal models of focal and global cerebral ischemia although its role in traumatic brain injury (TBI) has not been well-studied. Many isoforms of nitric oxide synthase (NOS), the enzyme that synthesizes NO, exist. Some may be deleterious to the brain (e.g., neuronal NOS - nNOS) following TBI and some may be beneficial (e.g., endothelial NOS - eNOS). We determined if the activity and the levels of NOS were altered in a gender specific manner following a midline fluid percussion injury. Three month old male, female, and female ovariectomized Sprague Dawley rats were subjected to a moderate (2.8 atm) fluid percussion injury. Rats were decapitated, and their brains were rapidly removed and dissected into various cortical areas, medial septal area (MSA), dorsal and ventral hippocampus, and cerebellum. NOS activity was measured by assessing the conversion of ^{14}C-arginine to ^{14}C-citrulline. Both nNOS and eNOS levels were measured using a human brain and endothelial synthase ELIZA. Immediately following TBI, overall NOS activity was decreased in the male rats (30-66%), increased in the female rats (33-97%), and showed no change in ovariectomized rats. However, nNOS levels for all of the groups revealed increases in the cortical regions (20-45%) and in the MSA (57-59%). One hour following TBI, eNOS levels were increased in the MSA of male and ovariectomized rat, and decreased in female rats. These results demonstrate that while NOS is altered following TBI, specific isoforms of the enzyme may be affected in a time dependent and gender specific manner. (Supported by NS20020)

465.19

INCREASED NITRIC OXIDE SYNTHASE (NOS) ACTIVITY IN SENSORY AND MOTOR PATHWAYS DURING LOCOMOTOR RECOVERY AFTER SPINAL CORD INJURY: A POSSIBLE ROLE FOR NITRIC OXIDE (NO) IN NEURAL PLASTICITY, Z. Zhang* and L. Guth. Dept. Biology, College of William and Mary, Williamsburg, VA 23187.

Spinal hemisection in rats produces immediate ipsilateral hemiplegia; this is followed by restoration of locomotor function during the ensuing 7-10 d. Nitric oxide (NO) may play a role in this phenomenon, since injection of the i-NOS inhibitor, aminoguanidine, transiently restores hindlimb movements in paraplegic mice, and since n-NOS is upregulated after injury (Wu, 1993). The present report describes increased n-NOS in sensory and motor pathways concomitant with restoration of locomotor function. Spinal hemicrush at T10. was performed on adult rats. At intervals of 3 d to 4 wks postoperatively, cryostat sections were incubated for NOS activity by the NADPH-diaphorase reaction. In normal rat, NOS was expressed in discrete sensory and autonomic neuronal populations of the gray matter, but not in white matter. After injury: (a) Sensory system. Many ascending tracts became lightly-stained rostral to the lesion at 2 wks. This reaction was initially ipsilateral and subsequently bilateral. No change occurred caudal to the lesion. (b) Motor system. by 2-4 wks, axons of many descending tracts caudal to the lesion became intensely stained ipsilaterally. In the ventral horns caudal to the lesion, the motor neurons stained bilaterally with increasing intensity from one to four wks post-operatively. This staining was especially pronounced in the lumbar cord; it did not occur rostral to the lesion. Conclusion: The correlation between locomotor recovery and enhanced expression of neuronal NOS prior to nerve regeneration, suggests a possible physiological role for NO in this aspect of neuronal plasticity. (Supported by NIH grant NS-21460).

465.20

AMPHETAMINE TREATMENT DOES NOT ALTER EDEMA FORMATION AFTER CORTICAL CONTUSION IN THE RAT. R.L. Sutton*, Department of Surgery, Hennepin County Medical Center and Department of Neurosurgery, University of Minnesota, Minneapolis, MN 55415

Delayed treatment with D-amphetamine (D-AMPH) has been previously reported to improve sensorimotor recovery after traumatic brain injury (TBI). To determine if these behavioral effects are related to actions of D-AMPH on edema formation, rats with sham injury (N=12) or TBI (N=12; 2 mm compression of right sensorimotor cortex at 3.3 m/s velocity) were treated (IP) with saline or D-AMPH (2 mg/kg) at 18 h postsurgery. The percent water content (Mean ± SEM) in frontal (FCx), parietal (PCx) and visual cortex (VCx) and hippocampus (Hp) was determined at 24 h postsurgery using the wet-dry weight method [$\%H_2O$ = ((wet tissue weight-dry tissue weight)/wet tissue weight) X 100].

Saline-treated rats with TBI had significant (p's <0.05 vs sham) increases in percent water content in the right PCx (84.38±0.74 vs 78.00±0.48), VCx (81.39±0.83 vs 78.81±0.30) and Hp (80.59±0.47 vs 78.48±0.55). D-AMPH treatment did not alter the extent of edema formation in PCx (83.69±1.35), VCx (80.77±0.63) or Hp (80.61±0.18) ipsilateral to TBI. No significant edema was found in the right FCx or in any regions contralateral to TBI. Support: Minneapolis Medical Research Foundation.

466.1

RAPID ASTROGLIAL ACTIVATION FOLLOWING C2 SPINAL CORD HEMISECTION

Scott D. Hadley, Robert P. Skoff*, and Harry G. Goshgarian. Department of Anatomy and Cell Biology, Wayne State Univ., Sch. of Med., Detroit, MI 48201

The retraction of astroglial processes in the phrenic nucleus within two hours following C2 spinal cord hemisection in the rat has been suggested to be related to the unmasking of the latent crossed phrenic pathway which mediates recovery of the ipsilateral hemidiaphragm (Exp. Neurol. 120:233-44). In the present LM study, left C2 spinal hemisection causes a very rapid (within 1h) ipsilateral increase in glial fibrillary acidic protein (GFAP) immunoreactivity (IR) 1-2 spinal cord segments rostral and caudal to the C2 lesion. GFAP-IR is significantly enhanced in the ventral horn of spinal cord segments C3-4 ipsilateral to the lesion as compared to the contralateral ventral horn. Astrocytes in this region are characterized by thickened, highly branched immunoreactive processes. The dorsal horn and white matter ipsilateral to the lesion also demonstrate increased GFAP-IR when compared to the contralateral side. The early time course of these observations suggest that the increased GFAP-IR may not be due to an increase in GFAP biosynthesis as has been observed with fiber tract degeneration. Rather, early detection of enhanced GFAP-IR is evidence for rapid astroglial activation in response to spinal cord injury and may represent a depolymerization and redistribution of GFAP from astroglial processes to soma. Depolymerization and redistribution of GFAP may be necessary prerequisites to astrocytic process retraction during neuronal-glial plasticity in the phrenic nucleus and may be induced by extracellular ionic changes, alterations in neurotransmitter signals, or by some other diffusible factor released upon injury.

Supported by NIH grant HD 31550.

466.2

POLYAMINE METABOLISM FOLLOWING TRAUMATIC BRAIN INJURY.

C. Henley, C. Mills, K. Wey, D. Himes, J. Goodman*, C. Robertson. Dept. of Otorhinolaryngology and Communicative Sciences and Neurosurgery Baylor College of Medicine, Houston, TX 77030, USA.

The inital step in polyamine synthesis, the decarboxylation of ornithine to form putrescine, is catalyzed by ornithine decarboxylase (ODC). Putrescine accumulation, resulting from increased ODC activity following injury, has been hypothesized to mediate blood brain barrier breakdown, vasogenic edema, and neuronal death. The purpose of this study was to quantify changes in ODC and polyamines after traumatic brain injury (TBI). Adult, male anesthetized rats underwent a right parietal craniectomy and were subjected to pneumatic impact injury (2.5mm deformation; 5m/sec). Tissue samples were obtained 4,8,12,24,72 hr after TBI from 4 bilateral areas: superomedial "A", lateral "B", and inferomedial "C" parietal cortex and the hippocampus. Controls received SHAM surgery. ODC activity was determined by measuring decarboxylation of [^{14}C]-ornithine and polyamines were quantified by high performance liquid chromatography-fluorometic detection. ODC increased after TBI in the ipsilateral cortex and hippocampus in a bi- or tri-phasic fashion: the first peak 8 hr (up to 40-fold), the second 24 hr in "B" and "C" and 72 hr in "A", "B", and hippocampus. A biphasic increase in putrescine was also observed. In the ipsilateral cortex, putrescine increased up to 10-fold 4 hr post TBI, followed by a decrease at 8 and 12 hr. A secondary increase occured 24 or 72 hr post TBI. ODC activity and putrescine levels followed the same temporal pattern in the contralateral cortex. In general, the magnitude of increase did not exceed that in the ipsilateral cortex. We have shown that ODC and putrescine increase significantly in the traumatized brain. Biochemical and molecular studies of the ODC/polyamine system after TBI should help identify the role of polyamines in CNS injury-recovery and provide rationale for pharmacological studies to identify neuroprotective agents useful in management of head injury. (Work supported by NINDS - #NS27616-05A1 and The Coker Memorial Research Foundation)

466.3

EFFECTS OF INFANTILE HYDROCEPHALUS ON ASTROCYTOSIS AND AXONAL OR SYNAPTIC GROWTH.

J.P. McAllister, II*,R.W. Connelly, W.E. Bingaman, N. Boonswang, M.B. Slivka, and M.G. Luciano. Department of Neurosurgery, The Cleveland Clinic Foundation, Cleveland, OH 44195.

Although hydrocephalus occurs frequently, relatively little is known about both the onset of neuronal damage and the potential for recovery in this disorder. To investigate the temporal changes in astrogliosis and axonal or synaptic growth that occur during the progression of hydrocephalus, GFAP and GAP-43 levels were analyzed in a feline model of kaolin-induced infantile hydrocephalus. Hydrocephalus was induced by intracisternal injection of kaolin in 10 day old kittens. Animals were subsequently prepared as acute untreated, shunt treated, and chronic hydrocephalic groups, and compared to saline injected age-matched control animals. Progression and severity of ventriculomegaly was monitored by ultrasonography at various times before and after shunting and at the time of sacrifice. RNA and total protein were isolated from primary visual cortex (area 17) and analyzed by Northern and Western blotting techniques. Acute untreated hydrocephalic animals exhibited GFAP RNA and protein levels that were 100% and 159% higher than controls, respectively. GFAP levels returned to normal in the shunt treated animals, except when shunt malfunction occurred. Chronic hydrocephalic animals exhibited GFAP RNA and protein levels that were 209% and 764% higher than controls, respectively. In contrast, GAP-43 RNA and protein levels remained unchanged in all experimental groups compared to controls. These results suggest that an astrocytic response occurs during the progression of hydrocephalus, this response can be reversed by successful shunt treatment, and untreated chronic hydrocephalus continues to produce an even more pronounced astrocytic response. The fact that GAP-43 levels do not decline may indicate either that axonal and synaptic growth is not impaired, or that plasticity and regeneration occur as hydrocephalus becomes more severe.

Support: The Cleveland Clinic Foundation Department of Neurosurgery and Research Foundation

466.4

DEVELOPMENT OF A LARGE ANIMAL MODEL OF ADULT ACQUIRED OBSTRUCTIVE HYDROCEPHALUS

M.J. Johnson*, M.G. Luciano, I. Ayzman, A.S. Wood, J.P. McAllister, II. Dept. of Neurological Surgery, The Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195

Hydrocephalus is a condition characterized by cerebral ventricular enlargement and neurological deficit. While neuropathological charges due to hydrocephalus have been assessed in patient populations, these studies lack stringent control of variables such as the location of the obstruction which produces hydrocephalus or the time course of the disease. Most of the animal models currently used in hydrocephalus research are too small to enable implementation of clinical hardware or to provide optimal resolution with diagnostic imaging. Thus, we have developed a large canine model of acquired obstructive hydrocephalus in which both the duration and cause of hydrocephalus are controlled. This model will allow us to study the anatomical and physiological progression of the disease with high resolution imaging techniques such as MRI, PET, and SPECT, and to correlate these images with histological and immunohistochemical changes. The size of this model also facilitates the measurement of the reversibility of functional changes by surgical intervention. Acute procedures on 13 animals allowed examination of the location and size of the obstruction immediately after surgery. In 14 chronic animals, hydrocephalus was induced and the progression of ventriculomegaly was monitored using MRI for 5-6 weeks. During model development, several materials were tested. Focal obstruction of the fourth ventricle could be produced using either a cotton pledget or cyanoacrylic glue in combination with a balloon catheter. Immunohistochemical analysis of hydrocephalic tissue compared to controls has revealed increases in GFAP as a function of the severity of hydrocephalus. Thus, GFAP appears to be a useful marker of damage in the hydrocephalic brain. In summary, this large animal model of obstructive hydrocephalus will allow examination of the normal progression of this disease and the efficacy of clinical intervention.

Support: Johnson & Johnson Professional, Inc. and the Cleveland Clinic Foundation

466.5

THE EFFECTS OF ISOPROSTANES AND $PGF_{2\alpha}$ ON RAT CEREBRAL ARTERIOLAR DIAMETER.

S. W. Hoffman* and E. F. Ellis. Dept. Pharmacol/Tox, Medical College of Virginia, Richmond, VA 23298-0613.

Isoprostanes are prostaglandins generated by cyclooxygenase-independent, free radical attack of membrane arachidonate. Others have reported that isoprostanes are potent vasoconstrictors of the peripheral vasculature, however their effect on cerebral blood flow (CBF) is unknown. Previous research has shown that traumatic brain injury (TBI) causes brain lipid peroxidation (LP) and CBF reduction. Although free radical scavengers have been shown to be therapeutically effective at ameliorating these pathological processes, no causal link has been made between LP and vasoconstriction. Therefore, the investigation of isoprostanes may be relevant in understanding ischemia that follows TBI. We investigated the activity of 10^{-9} - 10^{-5} M E_2- and F_2-isoprostanes (8-epi-PGE_2 and 8-epi-$PGF_{2\alpha}$) and $PGF_{2\alpha}$ on pial arteriolar diameter in anesthetized rats using the closed cranial window technique and *in vivo* microscopy. All three prostanoids produced significant vasoconstriction. Of these prostanoids, 8-epi-$PGF_{2\alpha}$ produced the greatest vasoconstriction (34%±2), followed by 8-epi-PGE_2 (25%±4), then $PGF_{2\alpha}$ (20%±2). After six washouts over a 30 min. period, both 8-epi-$PGF_{2\alpha}$ and 8-epi-PGE_2 treated vessels remained slightly constricted, whereas the $PGF_{2\alpha}$ treated vessels regained normal vasomotor tone. The co-infusion of a thromboxane A_2/PGH_2 receptor antagonist (SQ29548) completely blocked the vasoconstriction by 8-epi-$PGF_{2\alpha}$ and 8-epi-PGE_2, indicating that these prostanoids mediate their effects through activation of this receptor. These results show that by-products of LP can affect the cerebral vasculature and may play an important role in secondary events after TBI. Supported by NS27214.

466.6

HO-1 ACTIVATION IN BRAIN FOLLOWING SUBARACHNOID INJECTIONS OF HEMOGLOBIN, HEME AND HEME ANALOGS.

Christopher P. Turner, Frank R. Sharp & S. Scott Panter. Dept. Neurology 127, VA Medical Center, 4150 Clement St., San Francisco, CA 94121, USA.*

In the brain, the pro-oxidant heme is metabolized into biliverdin, iron and carbon monoxide (CO) by the enzyme hemeoxygenase (HO). The potentially toxic free iron is bound by ferritin, and CO may be a critical player in a number of important neuronal processes. Expression of HO is controlled by two separate genes, HO-1, found mainly in glia, and HO-2 found mostly in neurons. Using immunohistochemical techniques, we have previously shown that injection of a purified oxy-hemoglobin (oxy-Hb) solution into the subarachnoid space of adult female rats leads to a global induction of the stress gene protein, HO-1, throughout the brain. This induction was observed principally in microglia. We have recently observed a similar induction in microglia following subarachnoid injection of methemoglobin. We hypothesized that the heme component in Hb is responsible for the HO-1 induction, and subarachnoid injection of heme promoted an increase in microglial expression of HO-1. The same result was observed following injection of heme analogs such as Zn- or Sn-protoporphyrin. These observations support the hypothesis that the protoporphyrin-IX metal complex of Hb is the principal agent in promoting HO-1 induction. Heme analogs are known to be inhibitors of HO activity but were still able to induce HO-1 expression. It is likely that these compounds act directly through the heme/metal binding site of the HO-1 gene, or that they promote heme accumulation which acts through the same regulatory site. Since HO-1 is expressed in very small amounts in glia, activation of this gene would be necessary to promote *de novo* expression of HO-1 protein and, thus, control potentially toxic elevations of intracellular heme. Direct activation of the HO-1 gene by heme would be one way of promoting rapid induction. Such a mechanism would suggest a protective role for the HO-1 stress gene. Supported by NIH Grant HL53040 and DVA/DOD Cooperative Research Program.

466.7

REGIONAL PATTERN OF 72 kD HEAT SHOCK PROTEIN (HSP72) AND IN SITU DNA FRAGMENTATION IN NEURONS AFTER SEVERE CONTUSIVE BRAIN INJURY IN RATS. M. Chen*, R.S.B. Clark, P.M. Kochanek, J. Chen, R.A. Stetler, K. Basta, D.W. Marion, S.T. DeKosky, R.P. Simon, and S.H. Graham. Depts. of Anesthesiology/Critical Care Medicine, Neurology, and Neurosurgery, and the Safar Center for Resuscitation Research, University of Pittsburgh, PA 15213.

Hsp72 is induced in neurons after ischemic and traumatic brain inury (TBI), is a sensitive marker of cellular stress, and may serve a neuroprotective role. We examined the regional and cellular pattern of hsp72 mRNA and protein expression and compared it to the pattern of *in situ* DNA fragmentation after severe TBI in rats.

Anesthetized adult rats were subjected to severe controlled cortical impact injury to the left parietal cortex immediately followed by 30 min of hypoxia (PaO_2=35-45 torr). Rats (n=27) were killed at 6, 8, 24, 72, or 168 h after trauma. Naive rats were used as controls. Brains were removed and *in situ* hybridization, immunocytochemistry, and Western analysis for hsp72, or terminal deoxynucleotidyl transferase-mediated biotin-dUTP nick-end labeling (TUNEL) was performed. Hsp72 mRNA was expressed in neurons in the ipsilateral cortex, CA3 hippocampus, hilus, and dentate at 6 h. Hsp72 mRNA was expressed primarily in ipsilateral cortical neurons at 24 h and by 72 h hsp72 mRNA expression returned to basal levels. Hsp72 protein was seen in ipsilateral cortical neurons, and few hilar and CA3 neurons at 24 and 72 h. Hsp72 protein was also detected in endothelium and glia. Western analysis detected an increase in hsp72 protein in ipsilateral cortex at 8 and 24 h. Induction of hsp72 mRNA or protein was not detected in the contralateral hemisphere or controls. TUNEL-positive neurons were seen in ipsilateral cortex and dentate at 6 h, and in ipsilateral cortex, dentate, hilus, and CA3 at 24 and 72 h, but not in the contralateral hemisphere or in controls.

The regional pattern of hsp72 mRNA and protein expression in neurons is similar to the pattern of *in situ* DNA fragmentation after severe TBI in rats. Further study is required to determine if this represents a neuroprotective response, or is merely a marker of cellular injury. Supported by NIH/NINDS 2P50 NS30318-04A1.

466.8

SUCROSE GAP RECORDING OF MEMBRANE RESEALING IN CUT MAMMALIAN SPINAL CORD AXONS R. Shi*, T. Asano and A.R. Blight, Div.Neurosurg., Univ. NC, Chapel Hill, NC 27599.

Membrane sealing is an important part of the neuronal response to injury. Sealing of axons was studied in isolated adult guinea pig spinal cord, using sucrose gap recording to monitor membrane potential changes, and uptake of horseradish peroxidase (HRP) to examine permeability to large molecules. A 35 mm strip of white matter was placed in a recording chamber, the central 12.5 mm superfused with Krebs' solution and the ends carried through sucrose gaps to compartments filled with isotonic KCl. The negative gap potential stabilized over 30-60 min., and the evoked compound potential similarly increased in amplitude to a stable plateau. When the strip was cut at the face of the sucrose gap, the gap potential decreased immediately towards 0 mV, then began to repolarize. Recovery of 90% of the initial potential occurred in c.40 min. at 37°C, with a single exponential time course. At 25°C, the rate of recovery was slowed by a factor of 2. HRP histochemistry showed an equivalent rate of sealing of the severed axons. At 37°C, almost all axons were stained when transferred to 0.015% HRP solution at 1 and 15 minutes after transection, less than half were stained when transferred at 30 minutes, and few were stained at 1 hr. At 25°C, approximately 50% of axons stained when transferred at 1 hr post injury. The correlation between gap potential and HRP exclusion indicates the potential can be used as a continuous indicator of membrane integrity. Removal of Ca^{2+} from the medium arrested resealing of axons within the duration of the experiment. Supported by the Canadian Spinal Research Organization and grant NS-33687 from NIH-NINDS.

466.9

EVIDENCE FOR APOPTOSIS OF OLIGODENDROGLIA IN LONG TRACTS UNDERGOING WALLERIAN DEGENERATION AFTER SPINAL CORD INJURY (SCI) IN MONKEYS. J.C. Bresnahan*, S.L. Shuman, M.S. Beattie, Dept. of Cell Biol., Neurobiol., and Anat., and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

We have recently shown that cells in the white matter exhibit several features of apoptosis over a considerable period following spinal cord contusion lesions in the rat (Crowe et al. 1995; Shuman et al., 1996, this meeting). We found that nuclear condensation typical of apoptosis was detectable using either nuclear stains (e.g. Hoechst 33342), TUNEL staining, toluidine blue staining of semi-thin plastic sections, or standard Nissl stains. We therefore examined material from a series of monkey (*macaca mulatta)* spinal cord contusion cases (Bresnahan et al, 1976, J. Neurol. Sci., 28:521) to determine whether apoptotic nuclei were present in this material. Alternate sections were stained using cresyl echt violet and for degenerating fibers, using the Fink-Heimer silver stain. This allowed for a comparison between the locations of apoptotic cells and degenerating fiber tracts. Four contusion lesions (200-500 g-cm) and one spinal hemisection (7-10 da post-SCI) were studied , with apoptotic profiles and fiber degeneration plotted independently.

In tracts both rostral and caudal to lesions in every case, there was precise correspondence betweeen the presence of apoptotic profiles and degenerating fibers. Electron microscopy showed that some of the apoptotic cells were oligodendrocytes. It is suggested that demyelination of long tracts after SCI is due at least in part to apoptotic death of oligodendrocytes associated with dying axons. (Supported by NS-10165).

466.10

MORPHOLOGICAL EVIDENCE OF GLIAL INVOLVEMENT IN APOPTOTIC CELL DEATH FOLLOWING SPINAL CORD CONTUSION. S.L. Shuman*, J.C. Bresnahan, and M.S. Beattie. Neuroscience Program, Dept. of Cell Biology, Neurobiology & Anatomy, The Ohio State University, Columbus OH 43210

Apoptosis is a form of active cell death characterized by morphological features such as condensed chromatin, reduced cytoplasmic volume and membrane blebbing. Apoptotic cell death has recently been observed following contusion injury to the rat spinal cord (Crowe, et al.) and is present up to three weeks post-injury, particularly in white matter tracts. We used immunocytochemical techniques in an effort to identify the cell types involved in this model of apoptotic cell death. Seven rats received a 25g-cm contusion injury at T10 using the NYU weight drop device. Animals were sacrificed at 24 hours (n=2), 8 days (n=3) or 3 weeks (n=2) post-injury. Sham injuries were performed on two additional animals which survived for 24 hours or 8 days. Spinal cord tissue was sampled up to 17mm rostral and caudal to the lesion center. Tissue sections were stained with the monoclonal antibody OX-42 to identify microglia and counterstained with cresyl violet. Cresyl violet staining revealed clusters of condensed material that were identical in morphology to apoptotic cells stained with the nuclear dye Hoechst 33342 (HO342). The spatial and temporal distribution of apoptotic profiles observed with cresyl violet was also similar to previous studies using HO342. A wave of apoptosis began at the contusion site, and over a period of three weeks spread both rostrally and caudally in the white matter. OX-42 (+) profiles ranged from ramified resting microglia to round, darkly stained cells containing one or more clumps of condensed Nissl material. Some apoptotic profiles were not immunoreactive for OX-42; many of these appeared to be contacted by microglia, especially at greater distances from the lesion center (70.0% at 7mm; 83.3% at 13mm). Only a small fraction (11.2%) of normal cells in the white matter exhibited such close apposition, suggesting a role for microglial activity in apoptotic cell death following spinal cord injury. (Supported by NS-32000 and NS-10165).

466.11

EFFECT OF CYCLOHEXIMIDE ON DNA BREAKDOWN, TISSUE LOSS, AND BEHAVIORAL OUTCOME AFTER SPINAL CORD IMPACT INJURY. X. Z. Liu[1]*, X. M. Xu[2], R. Hu[1], C. Du[1], G. S. Fan[1], C. Y. Hsu[1], D. W. Choi[1]. [1]Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110; [2]Dept Anat. & Neurobiol, St. Louis Univ Sch Med, St. Louis, MO 63104.

In the preceding companion abstracts, we show morphological and biochemical evidence suggestive of apoptosis cell death in the rat spinal cord after mild impact injury. To test this idea further, we subjected the rat spinal cord to moderate (12.5 g-cm) impact using the NYU device, and treated the rats with cycloheximide (1 mg/kg immediately post injury followed by 1 mg/kg q 3d for 4 wks), or saline.

Cycloheximide treatment reduced the amount of DNA breakdown in injured tissue (ELISA assay) by 50% at 24 hr post injury. Weekly behavioral assessment using the Basso -Beattie-Bresnahan (BBB) OSU open field grading system showed that this cycloheximide treatment produced an improvement in hindlimb function detectable as early as 2 wks post injury and persisting up to 4 wks post injury (median BBB score: cycloheximide, 16; saline, 12, n=12, $P < 0.05$ by Mann-Whitney 2-sample test). Morphological assessment of tissue loss showed that the cycloheximide treatment reduced tissue loss in the epicenter (cycloheximide, 0.932; saline, 0.446 mm^3, n=12, $P < 0.05$ by Student's t-test) as well the adjacent rostral and caudal segments. These findings corroborate the accompanying morphological and biochemical studies suggesting that apoptosis contributes importantly to cell death and loss of function after submaximal impact injury, and support the possibility that acute pharmacological blockade of apoptosis may achieve therapeutic benefits.

Supported by the American Paralysis Association (CYH and DWC) and Daniel Heumann Fund for Spinal Cord Research (XMX).

466.12

COMBINATION TREATMENTS OF CHRONIC SPINAL CORD INJURIES WITH SCAR DISRUPTER, NEUROTROPHIC FACTOR-COATED BEADS AND FETAL CELLS, MATRIX OR MYELIN ANTIBODY. J.D. Peduzzi* and F.R. Fischer, Dept. Physiol. Optics, University of Alabama at Birmingham, Birmingham, AL 35294.

Modification of the spinal cord structure after chronic injury was attempted using combination treatments. Adult Sprague-Dawley rats received a contusive spinal cord injury (10 g wt. dropped 2.5 cm) at T8 vertebral level. Treatment was given at 2 months post injury to animals exhibiting similar behavioral deficits using the combined behavioral score (Gale, Kerasidis & Wrathall, 1985). The 3 treatment groups received scar disrupter (EDTA) then NT-3 coupled to red fluorescent beads and BDNF coupled to green fluorescent beads. These microspheres label cells binding the neurotrophic factors and permit longer availability of the factors (Riddle, Lo & Katz, 1995). In addition, group 1 received laminin-collagen matrix, group 2 - E14 fetal spinal cord cells and group 3 - Gal-C antibody. The control group received buffer and plain fluorescent beads only. Four days after treatment, animals were sacrificed and the brains and spinal cords were examined. Numerous double-labeled cells were present in spinal cord-projecting regions of the brain. Transplants had regions of good integration with spinal cord. With the Gal-C antibody or matrix treatment, cavity boundaries became irregular.

Supported by the Spinal Cord Society.

466.13

OMENTAL TRANSPOSITION IN CHRONICALLY SPINAL CORD INJURED RATS. H. Q. Yan*, G. L. Clifton, S. J. Liu, K. Yang, C. E. Dixon and R. L. Hayes. Dept. of Neurosurgery, Universityt of Texas Medical School at Houston, Houston, Texas 77225.

The omentum has been used over the years for a variety of clinical problems. Recently it has been shown that placing the omentum on the brain and spinal cord can lead to an extensive development of vascular connections at omental/CNS interface. These findings had led to increasing interest in placing the omentum on to the human spinal cord. The conflicted resent reports of clinical trials are throughout the world (United States, Europa, Asia). We investigated the effect of omental transposition (OT) on chronically spinal cord injured rats and wanted to explain the clinical findings. Four groups (spinal cord injury (SCI) with OT, SCI with sham OT, Sham SCI with OT and sham SCI with sham OT) of animals were used for the study. SCI was made by an electronically controlled apparatus. The severity of the injury was measured by open field walking (Tarlov), inclined plane (angle test) and grid walk. One month after the injury or the sham injury, the rats were undergone OT or sham OT. After another one month, the animals were sacrificed for histopathological studies. The injured segment of spinal cord becomes thinner than the control (sham SCI) and most of them (4 out of 5) have a big cavity in the center of the cord. OT produces the pressure on the spinal cord in both injured and control rats. However, in SCI with OT group, the cavity in the center of the cord is markedly reduced or disappeared in 3 out of 4 animals. Our experiment also confirms other reports that there are new vascular connections between the omentum and spinal cord. Our data suggest that the effect of OT in humans might be due to the revasolization and/or reduce the size of the cavity in the center of the spinal cord resulted from SCI.

Supported by Neurosurgery Department fund of Univ. of Texas at Houston.

466.14

SEX DIFFERENCES IN RECOVERY AFTER CORTICAL TRAUMA IN RAT DEPEND ON ESTROUS STAGE. D.L. Stibick* & D.M. Feeney Depts. of Psychology & Physiology, University of New Mexico, Albuquerque, NM 87131.

Female sex hormones reportedly lessen severity of brain injury and promote behavioral recovery; however, these studies utilized males and/or ovariectomized female rats administered hormones at very high dosages. We propose that normal levels of the multiple circulating hormones in female rats produce differences in recovery from cortical injury. To test this hypothesis, male and female rats received unilateral impact injury to the sensorimotor cortex (Weisend & Feeney, 1994). Female rats were contused during one of four estrous stages determined by vaginal smears taken the day of surgery. Functional recovery was determined using a beam walk task and rating scale described in the above reference by observers blind to condition. Testing was conducted hourly for 5 hrs. beginning 24 hrs. after injury or sham surgery, then every other day for 15 days beginning 48 hrs. post-surgery. At 25 hrs. post-injury, females recovered significantly faster than males and this depended upon their estrous stage. Female rats in proestrous, when sex hormones are most similar to males, are not significantly different from males. Only rats contused during the other 3 estrous stages with higher levels of sex hormones showed significantly facilitated beam walk recovery compared to males. This excludes other sex differences that could account for these data. Analysis of histology is underway. The results of this study indicate that estrous cycle phase at time of cortical trauma modifies functional outcome. Normal levels of female sex hormones enhance recovery and this could be exploited for therapeutic intervention. [Supported by UNM RAC grant].

466.15

EARLY EFFECTS OF TRAUMATIC BRAIN INJURY STUDIED "IN VITRO" D. O. Maris, R. D'Ambrosio, S.M. Grady, H.R. Winn* and D. Janigro. Univ. of Washington, Dept. of Neurosurgery Seattle, WA 98104

In vivo studies have demonstrated that hippocampal long-term potentiation is impaired or abolished following cortical fluid percussion injury (FPI), an animal model of traumatic brain injury (TBI). Furthermore, FPI has been shown to decrease CA1 post-synaptic excitability measured *in vivo*. We tested the hypothesis that the effects of TBI induced in juvenile rats *in vivo* persist and can be investigated in isolated hippocampal slices maintained *in vitro*. TBI was induced by medial FPI. After 24 to 48 hours both hippocampi were extracted and slices were obtained with standard methods. Recordings of field excitatory post-synaptic potentials (fEPSPs) and population spikes (p.s.) were performed from CA1 strata radiatum and pyramidale. fEPSPs threshold differed in control *vs.* post-FPI slices (59±3 and 82±6 μA respectively, n=9, $p<0.02$). Higher stimulus intensities were required to elicit a population spike in post-FPI animals (80±7 *vs* 185±27 μA, $p<0.01$). While half maximal activation of CA1 pyramidal cells from FPI rats was achieved with current intensities of 242 ±7. μA, 130 ±5 μA were required to elicit the same response in slices from control rats. Thus, in agreement with results from *in vivo* experiments, post-FPI responses recorded *in vitro* were characterized by a marked neuronal hypo-excitability. The opposite form of LTP, long-lasting depression of synaptic activity (LTD), could be elicited in both FPI and control slices (21±3% and 26±8% depression respectively as measured 30 minutes after LTD induction). We conclude that FPI deficits persist and can be studied *in vitro*. At least at early stages, FPI does not affect the synaptic mechanisms involved in LTD induction and maintenance. Supported by NHLB 51624, NS 30305.

466.16

ENRICHED HOUSING IMPROVES WATER MAZE PERFORMANCE IN MEDIOFRONTAL CORTEX CONTUSED AND SHAM MALE RATS. E.A. Castro*, M.E. Fritts, & R.L. Roof, Dept. of Psychology and Neuroscience Program. Texas Christian University, Ft. Worth, TX 76132.

Enriched housing conditions (EC) result in thicker cortices and increased dendritic branching in brain intact rats. EC rats also perform better on maze tasks than rats kept in impoverished housing conditions (IC). EC after brain injury may encourage new neuronal connections, thus replacing some of those lost, as well as encourage behavioral flexibility and use of multiple strategies to solve tasks. EC's effects after brain injury have been examined but primarily after damage to specific sensory and motor areas and with lesion models that do not mimic injury normally sustained by humans.

We tested EC/IC effects after injury to the mediofrontal cortex, an area important for attention and spatial orientation and also a brain area commonly damaged in humans. Male rats were given cortical contusions or sham surgery followed by 38 days in either EC (large group cage, with ladders, tunnels, toys, etc) or IC housing (small, individual cages, no objects). On the last 8 days, rats were tested in the Morris water maze (2 trials/day). The release position was changed between trials. The platform remained in the same position for the duration of testing.

A strong, overall, beneficial effect of EC on water maze performance was seen. In addition, EC lesion rats performed better than IC lesion rats and closer to sham levels. The brains of these rats are being examined for anatomical changes. It is not yet known whether the housing influenced physiological recovery, but benefits of EC partially counteracted the water maze deficit associated with this lesion. These data suggest EC after brain injury may improve recovery of function.

This research was supported by the TCU-Research Foundation.

466.17

PROGESTERONE, BUT NOT METHYLPREDNISOLONE, REDUCES EDEMA IN FEMALE RATS AFTER CORTICAL CONTUSION. M.E. Fritts*†, E.A. Castro †, D.G. Stein‡, and R.L. Roof †. †Dept of Psychology and Neuroscience Prog., Texas Christian Univ., Ft. Worth, TX 76132. ‡Dept of Neurology, Emory Univ., Atlanta, GA 30322.

Progesterone (P) has been shown to reduce brain edema, lipid peroxidation, secondary neuronal death, BBB damage and cognitive deficits after cortical contusion. Methylprednisolone (M) may also improve recovery after brain injury but is associated with negative side effects and has a limited window of opportunity. P is effective in relatively small doses (4 mg/kg compared to 30 mg/kg) and reduces edema when given up to 24h after injury[1]. We have recently found P to be more effective at reducing edema after contusion in male rats[2].

To compare the effectiveness of the two steroids in reducing edema in females, brain water content was measured 72h after mediofrontal cortical contusion in female rats treated with P, M, or the vehicle (propylene glycol). Females were given contusions (while in proestrus) using a pneumatic piston impactor. P was injected 1h(ip), 6, 24, & 48h (sc) after injury (4 mg/kg per inj.), a regimen previously shown to be effective. M was given 1h (60Mg/kg, ip), 2h and 6h (30 mg/kg, ip), the regimen shown to be most effective in the males[2].

As was seen in male rats, P was significantly more effective at reducing edema compared to vehicle ($t(13)=4.05$, $p<0.01$) and M-treated females ($t(14)=3.07$, $p<0.01$). M did not significantly reduce edema compared to the vehicle. Female data will be presented alone and in comparison to that of males. Our results support continued investigation of progesterone and demonstrate its potential as a treatment for brain injury. [1]Exp. Neur. 138:246-251 (1996). [2]Poster at SFN 1996 meeting. Supported by Centers for Disease Control (PHS) (R49-CCR412307). Methylprednisolone donated by Pharmacia & UpJohn, Inc.

466.18

PROGESTERONE IS MORE EFFECTIVE THAN METHYLPREDNISOLONE AT REDUCING EDEMA AFTER CORTICAL CONTUSION IN MALE RATS. R.L. Roof,*† M.E. Fritts,† E.A. Castro,† R.A. Powell,† & D.G. Stein‡. †Dept. of Psychology and Neuroscience Prog., Texas Christian Univ., Ft. Worth, TX 76132. ‡Dept. of Neurology, Emory Univ., Atlanta, GA 30322.

We previously reported that Progesterone (P) reduces edema after cortical contusion in rats. P also reduces lipid peroxidation, secondary neuronal death, BBB damage and cognitive deficits in this injury model. Methylprednisolone (M) is currently used to treat spinal cord injury and has been shown to improve recovery after brain injury. However, its efficacy is limited by negative side effects associated with the large doses needed and by its limited window of opportunity. P is effective in relatively small doses (4 mg/kg compared to 30 mg/kg) and reduces edema when treatment is delayed 24h after injury[1].

To compare their effectiveness in reducing edema, brain water content was measured 72h after mediofrontal cortical contusion in male rats given P, M, or the vehicle. P was injected 1h(ip), 6, 24, & 48h(sc) after injury (4 mg/kg per inj.), a regimen previously shown to be effective. M was given in one of three regimens (all ip) (A: 60 mg/kg at 1h, 30 mg/kg at 2 & 6h; B: 60 mg/kg at 1h, 30 mg/kg at 6, 24, & 48h; C: 90 mg/kg at 1h, 45 mg/kg at 2 & 6h). 60-90 mg/kg M given ip has been shown to be equivalent to 30 mg/kg given iv.

There were no significant differences among the M regimens, but a trend ($p=0.07$) suggested A was most beneficial. In no case did M significantly reduce edema compared to vehicle controls. P-treated rats had less edema than the vehicle group and all of the M-treated groups. These findings support continued investigation of progesterone and demonstrate its potential as a treatment for brain injury.

[1]Exp. Neur. 138:246-251 (1996). Supported by Centers for Disease Control (PHS) (R49-CCR412307). Methylprednisolone donated by Pharmacia & UpJohn, Inc.

466.19

MICROGLIAL CELL ACTIVATION IN THE BRAIN OF THE ADULT RAT FOLLOWING EXPOSURE TO AIR BLAST. J.K.E. Persson*[1,3], A. Säljö[2,3] and A. Suneson[3]. [1]Department of Neuroscience, Karolinska Institutet, Anatomy building, S-171 77 Stockholm, Sweden. [2]Department of Surgery, University of Göteborg, S-413 45 Göteborg, Sweden. [3]Department of Human Studies (FOA 54), National Defence Research Establishment, S-172 90 Stockholm, Sweden.

It has been controversial whether the exposure to moderate air blasts at levels not inducing any macroscopical signs of damage to the brain or skull results in structural CNS changes. The aim of this study was to elucidate the microglial cell reaction in the CNS after exposure to a moderate air blast injury.

Anaesthetized rats were exposed to a pressure characterized air blast generated by either of two different charge weights of pentaerythrittetranitrate at a distance of 1 m in an open detonation chamber. Four-six hours, 18 hours, 48 hours and one week, respectively, following blast exposure the rats were reanaesthetized, perfused and the brain removed followed by immunohistochemical processing for detection of the complement receptor CR3 which is localized at the membranes of microglial cells.

Compared to sham-exposed rats, there was an increased anti-CR3 immunoreactivity in all investigated parts of the brain following exposure to either charge at 18 hours, 48 hours and one week, but not at four-six hours post-blast. Light microscopic examination of cresyl violet stained sections from representative parts of the brains did not reveal any histological differences between the experimental and the sham groups. Immediately after the blast exposure and during the following survival period there were no behavioural differences between the experimental and the sham rats, indicating any blast-caused soft-tissue damage or fracture otherwhere.

This observed microglial cell activation could be either primary, or secondary to phenotypic changes of other cell types in the CNS, presumably neurones. Irrespectively of the origin of this activation "signal", the resulting observed microglial cell reaction could have deleterious short as well as long term effects on brain function, since reactive microglial cells have been shown to produce a number of potentially neurotoxic factors, such as cytokines, complement factors and reactive oxygen intermediates which are known to impede neuronal function and survival. Multiple reports on human cases exposed to blasts of various intensities and types suggest that such victims may suffer from both acute and chronic complex neuropsychiatric disorders. However, further studies are necessary to evaluate if there is any causality between the development of such disorders and the above reported structural CNS changes following air blast exposure.

This study was supported by grants from the Swedish National Defence Research Establishment.

466.20

THE EFFECTS OF PROTRIPTYLINE ON FUNCTIONAL RECOVERY FOLLOWING TRAUMATIC BRAIN INJURY. X. Ma*, R.G. Griffith, P. Robichaud, B. Wolfson, G. Stolz, D. Marion, and C.E. Dixon. Brain Trauma Research Center, Department of Neurosurgery, University of Pittsburgh, Pittsburgh, PA 15260.

Protriptyline, a tricyclic antidepressant with activating properties, has been reported in a series of case studies to improve function in brain injured patients [Wroblewski, et al., Brain Injury, 7(4), 353-362, 1993]. However, there are currently no published laboratory reports on the use of protriptyline to enhance functional recovery following experimental TBI. We examined the effects of daily protriptyline treatment on functional recovery (motor and spatial memory performance) in twenty rats following controlled cortical impact injury (4 m/sec, 3.0 mm tissue deformation). Sham animals (n=5) were surgically prepared, but not injured. Beginning one day after injury, animals were injected daily with either protriptyline (5 mg/kg, i.p., n=10) or saline (n=10). Rats were trained prior to injury and retested on days 1-5 for motor (beam balance and beam walking) performance. On days 14-18, animals were trained on the Morris water maze task. Results indicate that a 5 mg/kg dose of protriptyline does not attenuate motor or spatial memory deficits after TBI. However, during the water maze swim, injured rats made significantly more right turns (101.4 ± 9.4) than left turns (72.1 ± 3.9) ($p < 0.01$). This pattern of turning after protriptyline administration may be analogous to circling behavior that has been reported to unmask chronically lowered striatal dopaminergic activity on the injured side of the brain. Supported by CDC R49CCR-312296 and NIH P50NS30318.

NEUROPSYCHIATRIC DISORDERS: SCHIZOPHRENIA I

467.1

THE NOVEL, ATYPICAL ANTIPSYCHOTIC, S 16924, ENHANCES DOPAMINE (DA) AND NORADRENALINE (NAD) RELEASE, AND DECREASES SEROTONIN (5-HT) RELEASE, IN FRONTAL CORTEX OF FREELY-MOVING RATS. J.-M. Rivet*, A. Gobert, L. Cistarelli, S. Girardon, O. Muller, G. Lavielle and M.J. Millan, I.D.R.S., 125 Chemin de Ronde, 78290 Croissy, France.

S 16924 ((-)-l-(benzodioxane-5yl-3-[3-(4-fluorophenacyl)pyrrolidine]-l-oxa-propane)) has high affinity at cloned 5-HT_{1A} (K_i = 1.9 nM), D_4 (6.2) and 5-HT_{2A} (2.4) vs D_2 (28.8) receptors. Here, we examined its influence upon monoaminergic transmission in rats implanted with a cannula in frontal cortex (FCX), or both accumbens and striatum. Samples were taken every 20 min and analysed by HPLC/coulometric detection. After 3 basal values, drugs (mg/kg, base) were injected s.c. and analysed over 60 min. WAY 100,635 (N-{2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclo-hexanecarboxamide) (WAY) was given 20 min prior to S 16924. Changes (Mean ± S.E.M. in %) are relative to basal (= 100 %).

Drug	Frontal Cortex DA (1.4)	Frontal Cortex NAD (1.8)	Frontal Cortex 5-HT (1.0)	Accumbens DA (4.9)	Accumbens 5-HT (0.8)	Striatum DA (9.2)
VEH / VEH	92±3	105±5	99±7	100±2	88±4	97±2
VEH / S 16924 (2.5)	163±5	200±10	53±4	112±8	54±5	115±2
WAY / VEH	88±3	103±10	103±10	91±2	99±8	101±4
WAY (0.16) / S16924 (2.5)	123±6*	158±11*	105±9*	N.T.	N.T.	N.T.
VEH	98±3	107±6	90±6	99±2	85±4	96±2
Clozapine (2.5)	163±8	238±15	101±5	109±6	89±6	94±3
Haloperidol (0.63)	131±5	174±11	75±6	130±6	89±6	129±2

Basal (pg/20 min); VEH=vehicle; N.T. = not tested; * $P < 0.05$ vs VEH/S 16924.

Both S 16924 and clozapine selectively increased DA release in FCX. S 16924 also decreased 5-HT release and the 5-HT_{1A} antagonist, WAY, reduced its actions. This pattern of release may contribute to the distinctive antipsychotic profile of S 16924.

This study was supported by Servier Pharmaceuticals.

467.2

NEURODEVELOPMENTAL PATHOGENESIS OF SCHIZOPHRENIA. N. Rajakumar*, P.C. Williamson, J.A. Stoessl and B.A. Flumerfelt, Departments of Psychiatry, Clinical Neurol. Sci. and Anatomy and Cell Biology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

Neuroimaging and postmortem evidence implicates two candidate neural circuits in the manifestation of schizophrenic symptomatology, namely, the prefrontocortical-temporal lobe circuit and the prefrontocortical-subcortical circuit. Based on these findings, Weinberger and associates have proposed an animal model of schizophrenia employing neonatal lesions of the ventral hippocampus (*Neuropsychopharmacology* '93; **9**: 67-75). The present study suggests an alternative model in which neonatal excitotoxic lesions of the mediodorsal thalamic nuclei result in adult manifestations indicating enhanced activity of the mesolimbic dopaminergic system similar to that seen in schizophrenia. Sprague Dawley rat pups (postnatal day 4) were injected bilaterally with quinolinic acid (120 nM/µl) or saline into the mediodorsal thalamic nuclei using a stereotaxic approach. Both lesioned and sham animals were tested for behavioral manifestations following saline, d-amphetamine (2.5 mg/kg,i.p.), or apomorphine (300 µg/kg, s.c.) injection in a well habituated environment at 10 weeks. The duration of locomotion, rearing, sniffing, mouth movements and grooming were recorded in 3 min blocks of time for a period of one hour using a microcomputer with custom-designed software. The animals were sacrificed and the extent of the lesions was verified using Nissl stain. The results indicated markedly enhanced locomotion and exploratory activity in response to novel environment or external stimuli in lesioned animals 7-10 weeks postnatally. Moreover, the lesioned animals showed increased locomotion and rearing following d-amphetamine or apomorphine treatment at 10 weeks, in contrast to sham rats in which these responses were suppressed, suggesting enhanced mesolimbic dopamine activity. [Supported by MRC Canada].

467.3

INTRINSIC ACTIVITY OF THE POTENTIAL ANTIPSYCHOTIC CI-1007 AT D2, D3 AND D4 DOPAMINE RECEPTORS. L.M. Georgic*, D.H. VanLeeuwen, R.G. MacKenzie, T.A. Pugsley, Y.H. Shih, L.D. Wise, J. Wright and T.G. Heffner. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105.

Whereas current antipsychotics are antagonists at dopamine (DA) D_2 receptors, CI-1007 is a partial DA agonist with moderate intrinsic activity at DA D_2 receptors in neurochemical, electrophysiological and behavioral tests. We compared the affinity and intrinsic activity of CI-1007 at cloned human DA D_2, D_3 and D_4 receptors. Receptor binding affinity determined in CHO cell membranes with [^{3}H]-spiroperidol revealed K_i values of 25.5 nM at D_2, 16.6 nM at D_3 and 90.9 nM at D_4 receptors. CI-1007 showed agonist-like stimulation of [^{3}H]-thymidine uptake in CHOp-5 cells transfected with D_2 or D_3 receptors with maximal stimulation of 66% and 84%, respectively, and EC_{50} values of 1.3 nM and 4.5 nM, respectively. CI-1007 caused only 10% maximal stimulation of [^{3}H]-thymidine uptake in D_4-transfected cells and blocked the stimulation of [^{3}H]-thymidine uptake caused by the DA agonist quinpirole with an IC_{50} of 40 nM. These results demonstrate that CI-1007 is a partial agonist with moderate intrinsic activity at DA D_2 receptors, high intrinsic agonist activity at D_3 receptors and low, antagonist-like intrinsic activity at D_4 receptors. This spectrum of activity may contribute to an improved antipsychotic profile for CI-1007. (Supported by Warner-Lambert.)

467.4

BEHAVIORAL PROFILE OF THE D2 / D3 / 5-HT1A PARTIAL AGONIST, PD 158771: A POTENTIAL ANTIPSYCHOTIC. F. W. Ninteman*, A. E. Corbin, D. J. Johnston, J. N. Wiley, C. L. Christoffersen, L. T. Meltzer and T. G. Heffner. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI, 48105.

PD 158771, a partial agonist at brain dopamine (DA) D_2 and D_3 as well as serotonin (5-$HT)_{1A}$ receptors, produced antipsychotic-like behavioral effects in preclinical rodent and primate tests. PD 158771 inhibited spontaneous locomotion and blocked amphetamine- and apomorphine-stimulated locomotion in mice and rats, effects consistent with its D_2 partial agonist activity. PD 158771 did not cause catalepsy in rats at a dose 20-fold higher than the ED_{50} dose for locomotor inhibition. PD 158771 also produced anxiolytic-like effects in a Vogel conflict test in rats, effects consistent with its 5-HT_{1A} partial agonist activity, but did not show antidepressant-like effects in a behavioral despair test in rats. Like known antipsychotics, PD 158771 caused long-lasting and potent inhibition of conditioned avoidance responding (CAR) in squirrel monkeys but had a lower liability than haloperidol and SDZ 208-912 for inducing EPS in haloperidol sensitized squirrel and Cebus monkeys. EPS signs were absent at the CAR ED_{50} dose and were of lesser intensity than typical antipsychotics at multiples of the CAR ED_{50} dose. These results suggest that PD 158771 may provide an improved antipsychotic profile. (Supported by Warner-Lambert.)

467.5

EFFECTS OF THE $D_2/D_3/5\text{-}HT_{1A}$ PARTIAL AGONIST PD 158771, A POTENTIAL ANTIPSYCHOTIC ON MONOAMINE SYNTHESIS AND RELEASE IN RATS.

H. C. Akunne*, S. Z. Whetzel, M. D. Davis, L. W. Cooke, L. T. Meltzer, T. G. Heffner, T. A. Pugsley. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105.

PD 158771 (PD) is a novel dopamine (DA) D_2-like and serotonin ($5\text{-}HT_{1A}$) partial agonist with minimal extrapyramidal side effects being developed as a potential antipsychotic (see Ninteman et al., and Zoski et al., at this meeting). As expected from the in vitro studies indicating partial DA agonist (PDA) actions, PD (3-30 mg/kg, i.p. or p.o.) antagonized the gamma-butryolactone (GBL)-induced accumulation of DOPA in striatum and mesolimbic regions of rat brain, causing a maximal 61 % reversal in striatum. PD (2.5 mg/kg, i.p.) inhibited the relatively slow neuronal firing of substantia nigra zona compacta DA neurons in chloral hydrate anesthetized rats, an effect reversed by haloperidol. Intravenous dosing of PD indicated that it produced partial inhibition of more rapidly firing DA neurons consistent with a PDA action. In absence of GBL, PD (0.3-30 mg/kg, i.p.) decreased DA synthesis in striatum and mesolimbic region, but not in frontal cortex probably reflecting a lack of DA autoreceptors controlling DA synthesis in this region. In intracerebral microdialysis studies, PD (10 mg/kg, i.p) decreased DA overflow from both striatum and nucleus accumbens of anesthetized rats, with the effect being greater in the nucleus accumbens than in the striatum. PD (0.3-30 mg/kg, i.p) decreased 5-HT synthesis and release in various brain regions probably reflecting an agonist action at $5\text{-}HT_{1A}$ autoreceptors on the dorsal raphe nucleus based upon its high affinity (K_i = 3.5 nM) for $5\text{-}HT_{1A}$ receptors . PD (3-30 mg/kg, i.p.) did not effect DOPA accumulation in the NE-enriched brainstem arguing against potent alpha-adrenergic activity in rat brain. These findings indicate that PD exhibited agonist actions at DA autoreceptors and $5\text{-}HT_{1A}$ receptors in vivo, effects that may provide an improved antipsychotic profile. (Supported by Warner-Lambert.)

467.6

RECEPTOR BINDING AND FUNCTIONAL CHARACTERIZATION OF THE $D_2/D_3/5\text{-}HT_{1A}$ PARTIAL AGONIST PD 158771, A POTENTIAL ANTIPSYCHOTIC.

K.T. Zoski, H.C. Akunne, L.M. Georgic, R. M. MacKenzie, T.A. Pugsley, Y.H. Shih, L.D. Wise, D.L. Wustrow, T.G. Heffner*. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105.

Partial D_2 agonists (PDA) that have a preferential affinity for dopamine (DA) autoreceptors combined with serotonin ($5\text{-}HT_{1A}$) agonist action reduce DA as well as 5-HT synthesis and release, and could exert antipsychotic-like effects. PD 158771 (PD, {4-[2-(4-phenyl-piperazin-l-yl)-ethyl]-cyclohexyl}-pyrimidin-2-yl-amine trans) exhibits antipsychotic/anxiolytic effects in certain rodent and monkey models (see Ninteman et al., this meeting). *In vitro* PD was shown to have potent affinity for human D_{2L}, D_3 and $D_{4.2}$ receptors expressed in CHO K1 cells with K_i values of 5, 14 and 35 nM, respectively. PD is a PDA with about 60 % and 23 % intrinsic activity at human D_{2L} and D_3 receptor (inducing [^{3}H]thymidine uptake in CHO p-5 cells), respectively, as compared to quinpirole that exhibits 100 % intrinsic activity; at human $D_{4.2}$ receptors the compound was antagonist-like. PD had high affinity for rat hippocampal (K_i = 3.5 nM) and human (K_i = 2.6 nM) $5\text{-}HT_{1A}$ receptors. Similar to the $5\text{-}HT_{1A}$ agonist 8-OH-DPAT, its affinity for $5\text{-}HT_{1A}$ receptors was reduced in the presence of the guanyl nucleotide Gpp(NH)p and the receptor density was increased with Mg^{2+}, findings consistent with an agonist action at $5\text{-}HT_{1A}$ receptors. In addition, PD exhibited moderate affinity (K_i < 100 nM) for $5\text{-}HT_2$, $alpha_1$ histamine H_1 and sigma receptors and weak or no affinity for DA D_1, muscarinic, and a variety of other receptors. In conclusion, PD exhibited potent affinity and partial agonist actions at DA D_{2L}, D_3 and $5\text{-}HT_{1A}$ receptors, results that could provide an improved antipsychotic profile. (Supported by Warner-Lambert.)

467.7

FANANSERIN (RP 62203), A POTENT $D_4/5\text{-}HT_{2A}$ RECEPTORS ANTAGONIST IN PRECLINICAL STUDIES

Piot O, Doble* A, Heuillet E, Petitet F, Reibaud M, Betschart J, Pauchet C, Obinu M-C, Malleron J-L, Mignani S and Imperato A.
Rhône-Poulenc Rorer, CRVA, Psychopharmacology,Vitry sur Seine, France

Fananserin (RP 62203), a naphtosultam derivative, exhibited higher affinity for human dopamine D_4 receptors (Ki = 5.8 nM) than for D_2 receptors (Ki >1000 nM). RP 62203 also displayed a high affinity for serotonin $5\text{-}HT_{2A}$ receptors (Ki = 0.12 nM). Functionally, RP 62203 was an antagonist which blocked the inhibition of forskolin-stimulated adenylate cyclase activity by the dopamine agonist quinpirone and blocked the interaction of ^{3}H-GTPγS with the activated D4 receptor/G protein complex. RP 62203 did not increase the extracellular concentrations of either dopamine itself or its metabolites DOPAC and HVA in the frontal cortex in rats. RP 62203 had also no effect on the metabolism or utilization of 5-HT. RP 62203 dose-dependently reduced acetylcholine release and the enhancement of acetylcholine release induced by amphetamine in the prefrontal cortex in rats. RP 62203 was able to block apomorphine-induced climbing and to reduce some behaviours induced by relatively low doses of amphetamine. Moreover, RP 62203 has been shown to inhibit head-twitches produced by different 5-HT agonists. Interestingly, RP 62203 reduced some effects induced by phencyclidine, a drug which produces psychotomimetic effects in humans. Unlike D_2 receptor antagonists, RP 62203 did not block the conditioned avoidance responses in rats. RP 62203 neither produced an impairment of motor performance nor induced catalepsy in rodents. This could suggest a low propensity of RP 62203 to induce extrapyramidal side-effects in man. Certain atypical antipsychotics, in particular clozapine has higher affinity for dopamine D_4 receptors and serotonin $5\text{-}HT_{2A}$ receptors than for D_2 receptors. This preferential binding may be the basis of the unique clinical profile of such compounds. The preclinical profile of fananserin indicates that it may have promise as an antipsychotic agent.

467.8

Effect of fananserin, a D4/5HT2 antagonist, on DA and ACh release in the prefrontal cortex of rats. Interaction with amphetamine.

M.C Obinu., J.M. Miquet and A. Imperato* Rhone-Poulenc Rorer, Centre de Recherche de Vitry-Alfortville, 13 Quai Jules Guesde, Vitry sur Seine, France, 94403.

Classical neuroleptics are known to induce acute and long-term extrapyramidal symptoms in up to 75% of patients. In contrast, clozapine has been defined as an "atypical neuroleptic" because does not induce any significant extrapyramidal symptom and because, differently from classical neuroleptics, is active not only on positive but also on negative symptoms of schizophrenia.

Clozapine has been found to be the only antipsychotic with higher affinity for D4 than for D2 dopamine receptors. Classical neuroleptics have similar degree of affinity for both receptors. Hence, it seems that the preferentiality of clozapine for D4 compared to D2 receptors may be an important mechanism contributing to its "atypical" clinical profile.

Fananserin, a naftosultam derivate, has recently been found to be selective for D4 receptors (Ki = 5.8 nM) compared to D2 receptors (Ki > 1000 nM). We studied its effect on DA and ACh release in the prefrontal cortex of rats, where there is the highest density of D4 receptors, and we have observed that this compound at the doses of 3 and 15 mg/Kg s.c. does not modify DA release and metabolism. In contrast, haloperidol (0.25 mg/Kg s.c.) as well as clozapine (10 mg/Kg s.c.) increases DA release and metabolism. Moreover, fananserin (3, 5 and 15 mg/Kg s.c.) like haloperidol (0.25 mg/Kg s.c.) decreases ACh release and reduces the amphetamine-induced increase in ACh release.

Since, fananserin has also a high affinity for 5HT2 receptors, we have compared the effects of fananserin to those of ritanserin. We have observed that ritanserin (5 and 10 mg/Kg s.c.) does not modify DA release and metabolism but differently from fananserin does also not modify ACh release.

The lack of increase in DA release could suggest that fananserin, better than haloperidol and clozapine, can reduce DA transmission and, consequently, be more effective as antipsychotic.

467.9

CHRONIC LOW-DOSE HALOPERIDOL EFFECTS ON D3 INDUCED HYPOMOTILITY.

M.R.Lynch* and J.Loucks Ormsby. V.A. Medical Center and S.U.N.Y. Health Science Center., Syracuse, NY 13210

Mesolimbic dopamine (ML DA) D3 receptors have been implicated in pathophysiologic substrates for schizophrenia. D3 agonism *suppresses* behavioral activity and *decreases* ML DA metabolism. Thus, D3 sites may participate in generating negative symptoms of schizophrenia (anergia and amotivation). We attempted to determine if 21 days of 0.1 mg/kg haloperidol (H) would induce an alteration in this receptor population, detected by behavioral assessment. We previously reported that similar treatments induce **sub**sensitivity to low-dose apomorphine hypomotility, possibly via alteration in this D3 substrate. Matched groups of male, Sprague-Dawley rats (500-550g, n=16/group) received daily H or lactic acid (LA) and were retested on day 21 for photocell activity. Both groups then received acute 7-OHDPAT (7-OH, selective D3 agonist) or saline (S) 48 h after the last H or LA injection and were tested for 10 min in the activity chamber at 5 min post s.c. agonist or S. Acute 7-OH significantly suppressed behavioral activity, but rats 48 h withdrawn from H showed similar hypomotility. D3 agonism decreased both ML and striatal DA turnover, but these effects were also no different for chronic H *versus* LA. **Funded by the Department of Veterans Affairs Medical Research Program.**

467.10

INCREASE IN DOPAMINERGIC INNERVATION OF THE CINGULATE CORTEX INDUCED BY CHRONIC, BUT NOT ACUTE, NEUROLEPTIC ADMINISTRATION IN THE RAT.

A. J. Lança*[1,2], Manklow, C.[1], de Cabo, C.[2], Vaccarino[2], F.J. and Arifuzzaman[2], A.I.
Depts. [1]Pharmacology and [2]Psychology, University of Toronto, Toronto, ON, Canada

Pharmacological studies of antipsychotic drugs (i.e., neuroleptics) reveal a consistent correlation between their clinical efficacy and their affinity for dopamine (DA) receptors. While classical neuroleptics preferentially bind to the DA D_2 receptor, atypical neuroleptics, such as clozapine, have a higher affinity for the D_4 receptor. Previous studies have shown that, in the mesostriatal system, acute neuroleptic administration increases DA release and turnover, as well as the affinity of tyrosine hydroxylase (TH) for its pteridine cofactor. In the mesostriatal system, tolerance to these neuroleptic-induced effects develop after chronic administration. Interestingly, in the mesocortical system tolerance to these effects does not occur. However, the neuroanatomical and cellular basis of these processes remains unclear. The aim of the present study was to assess the effects of acute and chronic administration of different DA antagonists on the regionally specific pattern of DAergic innervation of the cingulate cortex, using immunocytochemistry with antibodies against TH (the rate-limiting enzyme of DA synthesis). Male rats were assigned to two different treatment groups, and received daily i.p. injections for either 3 days (acute treatment) or 30 days (chronic treatment). In each group the animals were assigned to different drug-treated sub-groups, and received either the classical neuroleptic haloperidol (HAL, 2mg/Kg), the atypical neuroleptics clozapine (CLOZ, 10mg/kg) or raclopride (RACLO, 1mg/kg), the selective D_1 antagonist SCH23390 (0.1mg/kg), or vehicle controls.

The results obtained revealed that acute (3 days) administration of the different DA antagonists, as well as vehicles, did not alter the distribution pattern of TH-immunoreactive fibers in the different regions of the DAergic system. However, after chronic (30 days) treatment a dramatic increase in the DAergic innervation was observed in the cingulate cortex of the neuroleptic-treated, but not in the vehicle-treated rats. The cingulate area showing the highest increase in TH-immunoreactive (TH-IR) fibers was Cg3. In the HAL-treated animals, a strong increase in number and intensity of the TH-IR fibers was equally seen in all the cortical layers (I-VI). In the CLOZ- and RACLO-treated rats these effects were stronger in the deeper (V-VI) than in the most superficial (I-III) layers. These neuroleptic-induced effects were not seen in adjacent cortical areas (e.g., frontal and parietal cortices), or in other terminal compartments of the DAergic system, such as the striatum and the nucleus accumbens. Supported by NARSAD to A.J.L.

467.11

SCHIZOPHRENIA-LIKE LIMBIC-CORTICAL NEUROPATHOLOGY IN RATS: LOCOMOTOR RESPONSES TO ANTIPSYCHOTIC DRUGS. J. G. Csernansky*, P. S. Jacobs, J. L. Jackson, & M. E. Bardgett Department of Psychiatry, Box 8134, Washington Univ. Sch. Med., St. Louis, MO 63110.

Spontaneous and amphetamine-elicited locomotor activity in rats has been used as a reliable preclinical indicator of antipsychotic drug action. We have recently demonstrated that rats with limbic-cortical neuropathology exhibit a heightened level of novelty- and amphetamine-induced locomotor activity relative to controls. The purpose of the present study was to determine if the suppressive effects of antipsychotics on novelty and amphetamine-elicited behavior were altered in rats with limbic-cortical neuropathology. Young adult male rats (60 days of age) received ventricular infusions of kainic acid (KA) (4.5 nmol per side) or saline. Every rat which received KA exhibited hippocampal pyramidal cell loss and, in a minority of the lesioned rats, cell loss in the amygdala, piriform cortex, and laterodorsal thalamus was observed as well. Thirty days after surgery, lesioned and control rats were injected with either haloperidol (.15 mg/kg, s.c.) or clozapine (6.3 mg/kg, s.c.), at doses which bind approximately 50% of dopamine D_2 receptors in the nucleus accumbens. In response to both antipsychotics, lesioned rats demonstrated less suppression of novelty- and amphetamine-elicited locomotor behavior. These data indicate that limbic-cortical neuropathology can disrupt normative behavioral responses to antipsychotics, thus raising the possibility that limbic-cortical neuropathology may undermine the clinical efficacy of antipsychotic drugs. Supported by MH01109 to MB.

467.12

SCHIZOPHRENIA-LIKE LIMBIC-CORTICAL NEUROPATHOLOGY IN RATS: FOS RESPONSE TO ANTIPSYCHOTICS. D. L. Roe, M. E. Bardgett, C. A. Csernansky*, & J. G. Csernansky Department of Psychiatry, Box 8134, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Fos, the protein product of the immediate early gene, *c-fos*, is proving useful in the preclinical assessment of antipsychotic drugs (APDs), since it has been found that typical and atypical APDs produce distinctive patterns of Fos induction. While both induce Fos in the nucleus accumbens shell, typical APDs also induce Fos throughout the striatum. Previous work in this lab has shown that increases in dopamine turnover following acute haloperidol are diminished in the accumbens shell in rats that have received intracerebroventricular (ICV) infusions of kainic acid (KA). ICV infusion of KA also produces graded neuronal loss in several limbic-cortical brain areas that show signs of pathology in schizophrenia. Thus, we hypothesized that similar differences should be seen in Fos induction in KA lesioned and non-lesioned animals. In the present study, rats received ICV infusions of either KA (4.5 nmol) or vehicle. At 25-28 days post-lesion, rats received either 17.8 mg/kg clozapine, 1.5 mg/kg haloperidol, or saline, and were sacrificed three hours later. These drug doses were chosen to produce a maximal Fos response. Fos production was assessed in several regions, including the nucleus accumbens core and shell, and the striatum. Though the differential patterns of Fos induction produced by clozapine and haloperidol were in agreement with previous studies, no significant differences were found between lesioned and non-lesioned animals. We are now gathering data at additional lower doses of clozapine and haloperidol. Supported by MH01109 to MB.

467.13

Schizophrenia-Like Limbic-Cortical Neuropathology in Rats: Delayed Effect of a Developmental Lesion. E-M. E. Montgomery*, M. E. Bardgett, & J. G. Csernansky. Department of Psychiatry, Box 8134, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Kainic acid (5nmol or 10 nmol/microliter concentration) or artificial CSF was delivered to post-natal 7 (P7) rats through a bilateral intracerebroventricular injection. Cell body counts were conducted at P8, P14, P45, and P75. Nissl stained coronal sections at the level of the dorsal and ventral hippocampus, striatum, and nucleus accumbens were examined.

Although the injection of kainic acid was delivered at P7, significant reductions in cell body populations were not observed until P45 and P75. In addition, kainic acid had a dose-dependent effect, with the greatest cell loss at P45 observed in the K10 group. At P75, significant cell loss was observed in both the K5 and K10 groups. Furthermore, significant cell loss was observed in the dorsal but not ventral hippocampus. In addition, the effects of kainic acid administration on Glur2/3 and GluR1 subunit expression were examined in the same areas and at the same ages.

Since the neuropathology associated with schizophrenia may stem from developmental disruption of hippocampal efferents and abnormal mesolimbic glutamate-dopamine interactions, these results shed light on the pathogenesis of schizophrenia. (Conducted under NIH Guide for the care and use of Laboratory Animals). Supported by a grant from MH01109 to MB

467.14

CLOZAPINE AS A DOPAMINE D_1 RECEPTOR AGONIST. P. Salmi[1,2] and S. Ahlenius[2]*, Division of Biological Psychology[1], Department of Psychology, Stockholm University, Stockholm, Sweden and Department of Behavioral and Biochemical Pharmacology[2], Astra Arcus AB, Södertälje, Sweden.

Clozapine is generally considered to be an antagonist at dopamine D_1 receptors. Thus, clozapine not only blocks adenylyl cyclase-activity coupled to dopamine D_1 receptors but also various dopamine D_1 receptor agonist-induced behaviors in the rat.

In the present study we have examined rat body temperature after administration of clozapine and its two major metabolites. Clozapine (0.625-10.0 mg kg^{-1}, s.c.) produced a robust hypothermia in the rat. This effect was not observed after administration of the two major clozapine metabolites, N-desmethylclozapine (0.625-10.0 mg kg^{-1}, s.c.) or clozapine-N-oxide (0.625-10.0 mg kg^{-1}, s.c.), suggesting that the two metabolites do not contribute to the hypothermia produced by clozapine administration. Furthermore, the clozapine-induced hypothermia was fully blocked by pre-treatment with the selective dopamine D_1 receptor antagonist NNC 687 (4.0 mg kg^{-1}, s.c.), providing evidence for dopamine D_1 receptor agonist properties of clozapine. Thus, the present study demonstrates the efficacy of clozapine as an agonist at dopamine D_1 receptors involved in temperature regulation in the rat. We suggest that the intrinsic efficacy of clozapine at dopamine D_1 receptors may importantly contribute to its atypical profile as an antipsychotic.

467.15

THE DISCRIMINATIVE STIMULUS EFFECTS OF CLOZAPINE IN SQUIRREL MONKEYS. J.Bagley, G.Carey and J.Bergman* Harvard Medical School, NERPRC, Southborough, MA 01772-9102.

Drug discrimination procedures were used to study the involvement of serotonergic ($5HT_2$) and muscarinic (MUSC) actions in the interoceptive-stimulus effects of clozapine (CLZ). Squirrel monkeys were trained to respond under a 10-response fixed-ratio schedule on one lever after injections of CLZ (1.0mg/kg,i.m.) and on a second lever after injection of vehicle. Cumulative dosing procedures were used to determine the effects of the $5HT_2$/MUSC antagonists cyproheptadine (CPR;0.01-3.0mg/kg) and promethazine (PRM;0.3-10.0mg/kg), the MUSC antagonists atropine (ATR;0.03-1.0mg/kg) and scopolamine (SCP;0.01-0.3mg/kg), and the $5HT_2$ antagonists ritanserin (RTN;0.1-10.0mg/kg), ketanserin (KTN;0.1-17.8mg/kg) and cinanserin (CNN;0.1-17.8mg/kg). SCP, ATR, PRM and CPR produced dose-related increases in responding on the CLZ associated lever and, at one or more doses, fully substituted in all monkeys. KTN, RTN and CNN produced responding on the CLZ-associated lever (avg $<40\%$) but did not fully reproduce the effects of CLZ in any monkey. However, combinations of moderate doses of SCP (0.01-0.03mg/kg) and KTN (0.1-0.3mg/kg) or CNN (10.0mg/kg), but not RTN, fully mimicked the effects of CLZ in all monkeys. These results suggest that the discriminative stimulus effects of clozapine in squirrel monkeys involve its actions at both $5HT_2$ and MUSC receptors. (supported by USPHS DA003774 and MH07658)

467.16

SOME BEHAVIORAL EFFECTS OF CLOZAPINE AND NOVEL ANTIPSYCHOTICS IN MONKEYS. J. Tidey*, G. Carey and J. Bergman. Harvard Medical School, NERPRC, Southborough, MA 01772.

The atypical antipsychotic drug, clozapine, has several therapeutic benefits which have prompted the search for compounds with similar behavioral actions. The present experiments were conducted to compare the behavioral effects of clozapine (CLZ), haloperidol (HAL), and the novel drugs olanzapine (OLAN), seroquel (SER), risperidone (RIS) and remoxipride (REM) in squirrel monkeys using three procedures. In a procedure related to conditioned avoidance, which may predict the potential antipsychotic activity of drugs, CLZ and OLAN were found to decrease response rates without greatly increasing the delivery of multiple shocks; the other drugs both decreased response rate and, at doses at least 2- to 3-fold higher, increased the delivery of multiple shocks. In an observational procedure, all drugs except CLZ and SER produced catalepsy-associated behavior (static, rigid and/or abnormal postures and movements) in all monkeys. In a drug discrimination procedure, in which monkeys were trained to discriminate injections of CLZ from those of saline, CLZ, SER, and OLAN dose-dependently increased responding on the CLZ-associated lever. SER fully reproduced the discriminative stimulus effects of clozapine; OLAN partially substituted for CLZ at doses up to 0.3 mg/kg but higher doses eliminated responding. These results suggest that SER and OLAN have behavioral effects similar to those of CLZ and may have promising therapeutic advantages. (supported by DA03774, MH07658, and RR00168).

467.17

A MARMOSET MODEL FOR TARDIVE DYSKINESIA

Per E. Andrén* and Lars M. Gunne. Dept. of Pharmaceutical Biosciences and Dept. of Psychiatry, Ulleråker, Uppsala University, PO Box 591, S-75124 Uppsala, Sweden

Tardive dyskinesia (TD), a potentially irreversible syndrome of involuntary movements following long-term neuroleptic administration, is characterized by orofacial dyskinesias and/or choreoathetoid movements in limbs and trunc. TD-symptoms similar to those seen in humans have been observed in experiments on monkeys (*Cebus apella, Macaca speciosa, Macaca mulatta*) following long-term treatment with neuroleptic drugs.

In the present study we have investigated the possibility of using marmosets (*Callitrix jacchus*) as a model for TD. Six marmosets were administered haloperidol-decanoate (Haldol Depot 5-15 mg/kg i.m.) once a month. After 21 months of treatment three animals showed occasional perioral twitchings and grimacing as well as tongue protrusions. Two of these animals had a frequency of masticatory movements which was less than 6 times/min, whereas one animal had masticatory movements of 6-20 times/min and also showed dyskinetic movements in the right leg and arm. All neuroleptic-treated monkeys showed somewhat rigid movements, decreased rapidity of coordinated movements, head checking and vocalizing. Our investigations demonstrate that marmoset monkeys treated for months or years with haloperidol-decanoate will develop symptoms resembling TD.

This study was supported by the Swedish MRC grant #11565.

467.18

STRIATAL LESIONS AND ORAL DYSKINESIAS IN RATS AFTER CO-TREATMENT WITH HALOPERIDOL AND 3-NITROPROPIONIC ACID.

O. A. Andreassen[1]*, R. J. Ferrante[2], M. F. Beal[3] and H. A. Jørgensen[4]. Depts. of [1]Physiology and [4]Psychiatry - Sandviken Hosp., Univ. of Bergen, N-5009 Bergen, Norway. [2]GRECC Unit, Bedford VA Medical Center, Bedford MA,[3]Massachusetts General Hosp. and Harvard Medical School, Boston, MA.

The pathophysiologic basis of tardive dyskinesia (TD) remains unclear. It has been proposed that TD may be a result of excitotoxic neurodegeneration in the striatum caused by a neuroleptic-induced increase in striatal glutamate release and impaired energy metabolism. To investigate this hypothesis, haloperidol decanoate (HAL; 38 mg/kg every 4 weeks IM) and the succinate dehydrogenase inhibitor 3-nitropropionic acid (3-NP; 8 mg/kg/day via osmotic minipumps), were administered alone or together (HAL/3NP) for 16 weeks to 4 months old female Sprague-Dawley rats (n=15-18/group). Control rats received sesame oil IM and had empty plastic tubes SC. Vacuous chewing movements (VCM), a putative analogue to human TD, were recorded during and after drug treatment. 3-NP, HAL, and HAL/3NP treatments resulted in increased VCM. VCM, however, was more pronounced and appeared earlier in the HAL/3NP rats. After drug-withdrawal, increases in VCM persisted for 20 weeks in the HAL and HAL/3NP rats. Brains from each group were analyzed for histopathological alterations. Bilateral striatal lesions were present only in rats with high levels of VCM in the HAL/3NP treated rats. Nerve cell depletion and astrogliosis were prominent histopathologic features. There was selective neuronal sparing of both large- and medium-sized aspiny striatal neurons. These results suggest that mild mitochondrial impairment in combination with neuroleptics results in striatal excitotoxic neurodegeneration which may underlie the development of persistent VCM in rats and possibly irreversible TD in humans.

Support: Norwegian Research Council, European Commission, NIH grant AG12922, Huntington's Disease Soc. of America and Dept. of Veterans Affairs.

467.19

ALTERATIONS IN NMDA RECEPTOR DENSITY IN A RAT MODEL OF TARDIVE DYSKINESIA.

M. F. Egan*, E. Hamid, S. Baca, T. M. Hyde. NIMH Neuroscience Research Center at St. Elizabeths, Washington, D.C. 20032

Long-term neuroleptic treatment in rodents induces a syndrome of vacuous chewing movements in some (+VCM group) but has little effect in others (-VCM group). The VCM syndrome, a behavioral analogue of tardive dyskinesia (TD) in humans, has been used to investigate the neurochemistry of TD. To assess the role of alterations in cortical regulation of the striatum in TD, we measured NMDA receptor density using quantitative autoradiography. Rats were treated for 36 weeks with haloperidol decanoate or vehicle and then withdrawn for an additional 28 weeks. VCM ratings were performed every 3 to 4 weeks. A +VCM group, a -VCM group and vehicle-treated controls were selected for post mortem studies (N=10 to 12 per group). Tritiated MK801 was used to label NMDA receptors in sections through the mid striatum and nucleus accumbens. An overall group effect was found ($F=18.0$, $p=.0001$). This effect was most pronounced in the ventromedial striatum and the core region of the nucleus accumbens. Post hoc comparisons demonstrated that -VCM rats had lower levels than +VCM and vehicle-treated controls. +VCM rats were intermediate between -VCM and control groups. These findings parallel changes reported previously in CCK gene expression in the medial prefrontal cortex, where -VCM rats had reduced levels compared to +VCM and vehicle groups. The data suggest that -VCM rats have greater alterations in glutamatergic corticostriatal activity compared to +VCM rats. The VCM model may be useful in elucidating the role of altered prefrontal regulation of subcortical structures in TD. Funding for this research was provided by NIMH.

467.20

MU OPIOID RECEPTORS AND TARDIVE DYSKINESIA: A STUDY ON A RAT MODEL OF TD.

E. H. Hamid*, T. M. Hyde, S. M. Baca, and M. F. Egan. National Institute of Mental Health, Neuroscience Center at St. Elizabeths Hospital, Washington, DC 20032.

Chronic neuroleptic therapy induces tardive dyskinesia (TD) in a sub group of patients on this class of medications. Similarly, in a rat model of TD, chronic haloperidol treatment induces vacuous chewing movements (VCMs) only in a sub group of treated animals. Examining the neurochemical differences between rats with (+VCM) and without (-VCM) neuroleptic-induced dyskinesia may shed light on the pathophysiology of TD. Previous studies on the TD rat model has shown an induction in enkephalin mRNA in rat striatum after chronic treatment with haloperidol, suggesting the involvement of mu-opioid system in the development of VCMs. To test this hypothesis, rats were treated with haloperidol decanoate or vehicle for 36 weeks and withdrawn for 28 weeks. +VCM, -VCM groups as well as vehicle controls were selected for post mortem receptor autoradiography studies that was performed using ^{3}H-DAMGO as a specific ligand for mu-opioid receptors. Our data show a significant reduction in mu-receptor density in dorsolateral, dorsomedial, and ventromedial striatum in both the +VCM and -VCM groups compared to vehicle treated rats. No significant differences were seen between +VCM and -VCM groups. The results indicate that chronic haloperidol treatment produces a very long term down-regulation of mu-opioid receptors. These results also suggest that endogenous mu-opioid receptors are not directly involved in the expression of VCMs, yet it does not exclude their role in eliciting motoric side effects after long term neuroleptic treatment.

This work was supported by intramural funding from the NIMH.

NEUROPSYCHIATRIC DISORDERS: IMAGING II

468.1

IN VIVO ^{31}P NMR SPECTROSCOPY SHOWED AN INCREASE IN THE GLYCEROLPHOSPHORYLCHOLINE CONCENTRATION IN THE PREFRONTAL CORTEX OF SCHIZOPHRENIC PATIENTS.

Y. Shirayama*, T. Yano, K. Takahashi and T. Ogino. Dept. Psychiatry, Kanto Teishin Hospital, Div. Biochem. Cellular Biology, National Institute of Neuroscience, NCNP and Dept. Psychiatry, National Center Hospital, NCNP, Tokyo, Japan.

Phospholipid metabolism of cell membranes and high energy phosphate metabolism in the prefrontal cortex of patients with schizophrenia (12 males) receiving neuroleptic medication were studied non-invasively with in vivo ^{31}P NMR localized (ISIS) spectroscopy operating at 2 Tesla, and compared to normal controls (age matched 16 males). Phosphodiesters (PDE) are breakdown products of phospholipids while phosphomonoesters (PME) are precursors of membrane phospholipids. The peak intensities of PDE's components, glycerolphosphoryl-ethanolamine (GPE) and glycerolphosphorylcholine (GPC), and PME's components, phosphorylethanolamine (PE) and phosphorylcholine (PC), in the ^{31}P NMR spectra were analyzed using a non-linear least squares method combined with prior knowledge of the chemical shifts. Absolute metabolite concentrations were quantified using a spectrum that was acquired using the same ISIS sequence from a phantom containing a phosphate solution of known concentrations. T_1 of each component was measured by a localized (cereberum) inversion recovery method to compensate saturation effects. This strategy provided a high accurate method for the concentration estimates. (mean error : less than 5 %). The schizophrenic patients showed an increased concentration in GPC but not other phospholipid metabolites such as GPE, PE and PC. No differences in high energy phosphate metabolism including phosphocreatine, inorganic phosphate and adenosine triphosphate were observed between controls and patients. The alteration in the GPC concentration may contribute to pathophysiology of schizophrenia with mild negative syndrome.

468.2

A MEDIAL PREFRONTAL ^{1}H MRS STUDY OF NEVER-TREATED SCHIZOPHRENICS AND NORMALS.

P.C. Williamson, R. Bartha, D.J. Drost, A. Malla, N. Rajakumar and J.P. Girvin*, University of Western Ontario, London, Canada N6A 5A5.

The objective of this study was to quantify medial prefrontal levels of N-acetyl aspartate (NAA), glutamate (Glu), glutamine (Gln), γ-aminobutyric acid (GABA), choline (Cho) and phosphocreatine and creatine (PCr) with ^{1}H magnetic resonance spectroscopy (MRS) in never-treated schizophrenic patients ($n=9$) compared to normals ($n=9$) matched for age, sex, and parental education. Single volume MR spectra (4.5cc) were acquired on a Siemens SP4000 Helicon whole body imager operating at 1.5 Tesla with a STEAM sequence (TR = 1500ms, TE = 20ms, TM = 30ms, 550 averages, 4096 points, 250 μs dwell time, total time = 14 minutes). All data were analyzed in the time domain using an automated fitting algorithm incorporating *in vitro* prior knowledge of metabolite peak characteristics. Metabolite levels were normalized to the water signal from the region of interest. No significant differences were found in levels of NAA, Glu or Cho between the groups in this region which included anterior cingulate cortex. However, schizophrenic patients had higher levels of Gln ($p<0.010$) and PCr ($p<0.090$) and lower levels of GABA ($p<0.025$) compared to normals (t-test, one tailed). As Gln is a precursor of Glu, decreased glutamatergic activity was suggested in schizophrenic patients. Decreased GABA levels may reflect a loss of interneurons which has been reported on post-mortem examination. Findings are consistent with the involvement of basal ganglia-thalamocortical pathways in schizophrenia.

Supported by the Medical Research Council of Canada and the National Institute of Mental Health (Rockville, MD).

468.3

DOSE-DEPENDENT EFFECTS OF METHYLPHENIDATE ON ACTIVITY, ATTENTION, AND MAGNETIC RESONANCE MEASURES IN CHILDREN WITH ADHD. M.H. Teicher*, A. Polcari, C.D. English, C.M. Anderson, S.L. Andersen, C.A. Glod, P. Renshaw. *Dept Psychiatry, Harvard Med. School, McLean Hospital, Belmont MA 02178.*

Twelve prepubertal children with ADHD completed a four week triple-blind placebo controlled trial of methylphenidate (MPH; 0.5, 0.8 and 1.5 mg/kg in divided dose). Activity was assessed using an infrared motion analysis system that tracked the position of 4 reflective markers 50 times per sec. to a resolution of 0.04 mm while they completed a repetitious continuous performance attention task (Teicher et al 1996). MR data were obtained at each dose using a 1.5T GE Signa scanner with an Advanced NMR Systems whole-body echo planar coil. T2* relaxation times were calculated from a 32 echo planer spin echo series using incremental increases in TE.

MPH exerted strong effects on composite measures of activity and attention. Activity measures were 61.1% worse on placebo than in normal controls. Subjects were 33%, 5.6%, and 2.4% overactive on low, intermediate, and high dose, respectively ($F_{3,33} = 3.3$, $p = 0.03$). Patients were 19.8%, 10.1% and 2.1% less attentive than normal controls on placebo, low and intermediate dose, and were 1.5% more attentive on high dose ($F_{3,33} = 9.49$, $p = 0.001$).

There were striking associations between blind MRI and quantitive behavioral measures. In 7 males with completed vol. measures there was a 0.892 correlation between right caudate vol. and number of movements (microevents) on placebo ($p = 0.01$). Similarly, in 8 subjects with complete and analyzed T2* measures, there was a 0.810 correlation between right caudate T2* and number of microevents ($p = .015$). The optimal dose of MPH for each subject exerted significant effects on frontal and caudate T2* ($p = 0.013$), which varied by hemispere ($p = 0.008$), affecting right hemisphere much more than left. MPH decreased caudate and increased frontal T2*. (Supported by NIMH RO1 48343 {MHT} & John Alden Trust {PR}).

468.4

GRAY MATTER VOLUME DEFICITS IN WEIGHT-RECOVERED ANOREXICS. E. K. Lambe, D. K. Katzman, D. J. Mikulis, and R. B. Zipursky*. Clarke Institute of Psychiatry, Toronto, Ontario, Canada.

Structural changes have been observed in the brains of low-weight anorexics, including increased cerebrospinal fluid (CSF) and decreased gray matter. We hypothesized that recovered anorexics would show reduced gray matter and elevated CSF volumes compared to controls.

We used magnetic resonance imaging (MRI) to compare the brains of 12 weight-recovered anorexics (mean age ± standard deviation: 19± 7 years; time recovered: 5±6 years, range 1-23 years) with 18 healthy controls (mean age: 20±7 years) and 13 acutely ill anorexic adolescents (mean age: 15±1 years). Axial, dual-echo scans of the whole brain were segmented into gray matter, white matter, and CSF compartments using Brain Image software developed in 1994 by Reiss. Brain measures were corrected for the significant effects of intracranial volume and age, based on regression analysis of 30 healthy female controls.

T-tests showed that the weight-recovered group had significantly smaller gray matter volumes ($p<.01$) and greater CSF volumes ($p<.01$) than normal controls. By contrast, the recovered anorexics had significantly smaller CSF volumes ($p<.01$) and greater gray ($p<.05$) and white matter volumes ($p<.05$) than low-weight anorexics. Neither the gray matter deficits nor CSF elevations in the recovered group were correlated with the length of time since recovery.

The persistent gray matter volume deficits in recovered anorexics suggest that there may be an irreversible component to the brain changes associated with anorexia nervosa. The neuropathological features of this irreversible component have yet to be explained.

(Supported by a grant from the Associates Fund of the Clarke Institute.)

468.5

MRI MEASUREMENTS OF THE AMYGDALA-HIPPOCAMPUS COMPLEX AND THE PARAHIPPOCAMPAL GYRUS IN SCHIZOPHRENIA. K. Vladar*, R.W. Buchanan, W.T. Carpenter. Maryland Psychiatric Research Center, UMAB, Md, 21228.

Measurements of the amygdala-hippocampus (AH), and the parahippocampal gyrus (PHG) are presented in a large group of patients with schizophrenia, and age, race, gender, handedness, and SES matched controls (Sch Male: 37, Healthy Male: 20, Sch Female: 22, Healthy Female: 16). Three mm contiguous coronal MR-images of the brain were used to measure the whole extent of the structures. PHG was divided into anterior and posterior part, the anterior part containing mostly the entorhinal cortex. The presence and extent of the uncal notch was also assessed on a scale of 0-2. A series of ANCOVA procedures were used to analyze the results of volume estimations, side (R or L) used as repeated measures, total cranial volume and age as covariates. Notch assessment was analyzed by Chi-square statistics. Mean volumes (adjusted for total cranial volume) were smaller for every measurements in the schizophrenia group. There was a significant main effect for the volume of AH and the total PHG by diagnostic groups ($P< 0.004$, $p< 0.04$, respectively), but no significant effect by gender or side. The group difference in PHG was accounted for by the anterior part (Tukey test $p < 0.04$), with no significant contribution from the posterior part. Volume measurements for both AH and PHG were strongly correlated in the schizophrenia group (p values <0.000), while a modest correlation was found in the healthy control group($p< 0.03$ and $p<0.06$). There was no significant difference by diagnostic group in the presence and extent of the uncal notch.

468.6

RELIABILITY AND VALIDITY OF A PROCEDURE FOR MEASURING SPECIFIC REGIONS OF THE PREFRONTAL CORTEX: A MAGNETIC RESONANCE IMAGING STUDY. R.W. Buchanan, K. Vladar, W.T. Carpenter, P. Barta, and G. Pearlson*. Md. Psych. Res. Ctr., Univ. of Md., Baltimore, Md, 21228.

Altered prefrontal cortical function has been implicated repeatedly in the pathophysiology of schizophrenia. Attempts to examine if this altered function reflects structural changes in the prefrontal cortex have been hampered by the failure to examine more functionally and structurally homogeneous subregions. We have developed reliable neuroanatomical landmarks for dividing the prefrontal cortex into superior, middle, inferior, and orbital regions. These landmarks are "drawn" on a 3-dimensional MRI representation of the brain. The four regions are then "painted" with different colors, with the colors depicted on MRI slices displayed in the coronal, sagittal, and transaxial views. The total gray matter volume of each region is estimated by the Cavalieri method, using the "paint" and the three views to guide the measurements. The validity of the procedure was examined in 19 normal controls (NC) (14 males and 5 females) and 18 schizophrenic patients (SP) (12 males and 6 females). The only regions that significantly differentiated SP from NC were the right and left inferior prefrontal cortex (which include Broca's Area) (mean±SD) (right: SP: 7.7±1.5; NC: 9.1±2.1; t=2.26, p=.03; left: SP: 8.0±1.5; NC: 9.0±1.6; t=2.39, p=.02). The relationship of these prefrontal region volumes and symptom and neuropsychological test performance data will be presented.

Supported by MH48225(RWB), MH40279(WTC), MH40391(GP), MH43775(GP), and MH50867(PB).

468.7

MRI-ASSISTED MEASUREMENTS OF EEG ASYMMETRIES IN SCHIZOPHRENIC PATIENTS F.Yang, R. Coppola,* M. Myslobodsky, and D.R. Weinberger CBDB, NIMH Neuroscience Center at St. Elisabeths, Washington, DC 20032

This exploratory study aims to investigate whether the corrections of electrode placements based on individual MRI data alter interhemispheric EEG asymmetry values. EEG was derived from 32 channel recordings in 6 pairs of MZ twins discordant for schizophrenia (relaxed wakefulness, eyes closed). An interactive algorithm was developed for projecting surface electrode locations to the 3D brain rendering based on 3D MR images acquired in the sagittal plane, 124 slices at 1.5 mm spacing. Given that no α difference between the twins was obtained α values were pooled. Spearman **r** was computed between total α (8.5 - 12.5 Hz) and lateral brain asymmetry values assessed at the top 20% of the dorso-ventral length (DVL) in the plane passing through P3-P4 and slanted at 32-39° to the AC-PC plane. Full range α values were found to correlate at statistically significant level ($r = .51$, $p < 0.05$) with lateral brain asymmetry. The significance was contributed by slow alpha (α_1) (8.5 - 10.5 Hz) ($r = .56$, $p < 0.05$). Fast alpha (α_2) (10.5 - 12.5 HZ) showed modest and statistically nonsignificant correlations with brain asymmetry index. The magnitude of **r** values for α and α_1 was reduced when electrodes were repositioned so as to maximize the anatomical homotopy of locations. The difference between the twins emerged when θ (6.0 - 8.5 Hz) and δ (0.5-6 Hz) bands were compared. Both correlated significantly with frontal asymmetry at F3 and F4. These data indicate that imbalanced EEG potentials depend to a significant extent upon anatomical brain asymmetry. It is thus imperative to use individual MRI data to provide corrections of bilateral electrode placements.

468.8

SEX DIFFERENCES IN REGIONAL PREFRONTAL ANATOMY IN PATIENTS WITH SCHIZOPHRENIA P.E. Cowell*, R.A. Adler, M.X.-H. Yan, R.I. Grossman# and R.E. Gur. Depts. of Neuropsychiatry & Neuroradiology#, University of Pennsylvania, Philadelphia, PA 19104.

A prior study from our lab showed sex differences in correlations between symptom severity and frontal lobe size in patients with schizophrenia. The present study examined subfields of the prefrontal cortex to further probe differences in cortical anatomy between men and women with schizophrenia, and compare them to normative sex differences. Subjects were 27 controls (12 men; 15 women) and 22 patients with schizophrenia (14 men; 8 women). Spoiled GRASS MRIs with 1mm slice thickness were obtained from a 1.5 Tesla scanner at UPENN. MRIs were segmented for gray matter, white matter and CSF, then aligned to a standard sagittal plane on which prefrontal regions were drawn. Prefrontal cortex was divided into 4 subfields within each hemisphere: dorsolateral; dorsomedial; orbitolateral; orbitomedial. Interrater reliability for 10 cases yielded ICCs of .81-.99. Analysis of covariance with repeated measures was used to investigate regional effects of schizophrenia and their interactions with sex. Diagnosis and Sex were classifying variables; Dorsal vs. Orbital (DO), Medial vs. Lateral (ML), Hemisphere (RL), and Tissue Type (GW) were repeated measures; Cranial Volume and Age were covariates. Examination of the DO x GW x Diagnosis effect ($p<.05$) revealed that, overall, dorsal gray subfields were smaller in patients than controls. Post-hoc analyses of the DO x ML x GW x Diagnosis x Sex effect ($p<.01$) indicated that sex differences in patients were localized to the dorsolateral prefrontal white matter - women had smaller volumes than men ($p<.01$). Sex differences in controls were nonsignificant and similar in magnitude across all subfields. These findings suggest that the neurodevelopmental processes distinguishing patients from controls may be semi-independent of those underlying sex differences within schizophrenia . Funded by MH42191 & MH43880.

468.9

EVENT-RELATED POTENTIAL AND NEUROANATOMICAL CORRELATES OF LANGUAGE COMPREHENSION DEFICITS IN SCHIZOPHRENIA. P. B. Ward*, C. Levitan, C. Loneragan, B. Liebert, J. Redenbach, E. Ganser, S. Chaturvedi, S. V. Catts, P. T. Michie, S. Andrews, N. McConaghy. School of Psychiatry, University of New South Wales, Sydney, 2052 Australia.

This study examined the relationship between ERP indices of semantic processing (N400) and auditory sensory memory (mismatch negativity: MMN), MRI-derived volumetric measures, and measures of language comprehension in schizophrenic patients. N400 was elicited by visual presentation of sentences with congruous and incongruous endings. MMN was elicted by infrequent deviant longer duration tones (100ms) presented among shorter (50ms) standards while patients undertook a simple visual distraction task. 3D volumetric MRI scans were obtained, and cortical areas visualized and measured using the BRAINS package developed at the University of Iowa (NC Andreasen et al). Language comprehension was assessed using the Relational Concepts scale of the Luria-Nebraska Neuropsychological Battery, WAIS Vocabulary and Comprehension scales and the Peabody Picture Vocabulary Test. Twenty five patients, all of whom meet DSM-III-R criteria for schizophrenia and are completely right-handed, were tested. Language comprehension deficits were associated with reduced left hemisphere N400 amplitudes, and were not correlated with MMN amplitudes. Such deficits were also correlated with reduced left superior temporal gyrus volumes, but not with measures of prefrontal cortical volume. These results are consistent with lateralised temporal cortical dysfunction as the neural basis of language comprehension deficits in schizophrenia. Supported by the National Health and Medical Research Council of Australia and the Rebecca L Cooper Medical Research Foundation

468.10

VERBAL MEMORY AND REGIONAL BRAIN VOLUME IN SCHIZOPHRENIA JM Goodman*, LJ Seidman, JM Goldstein, EA Hoge, DN Kennedy, N Makris, VS Caviness, MT Tsuang. Dept. of Psychiatry, Harvard Medical School and Center for Morphometric Analysis, MGH, Charlestown, MA 02129

Although memory impairment in schizophrenia is well documented, the nature and cause of the dysfunction remains unclear. Methods: We used the California Verbal Learning Test, a 16 item multiple trial list learning task, to assess auditory verbal episodic memory acquisition, storage and retrieval in 28 schizophrenic patients and 24 matched controls. Subjects also underwent whole brain MR imaging for calculation of regional brain volumes. We hypothesized that schizophrenics would demonstrate impaired encoding and retrieval, and would have regional brain volume deficits in areas thought to contribute to episodic memory processes. Based on neuroimaging and lesion studies, we hypothesized that encoding would correlate with left prefrontal volumes, storage with medial temporal lobe and thalamic volumes, and retrieval with prefrontal, cingulate and precuneal volumes. Results: Schizophrenics performed significantly more poorly than controls on total number of words learned and were less likely than controls to use semantic information to aid encoding. Patients also benefited disproportionately from a recognition vs a free recall test format, indicating a retrieval strategy deficit. Patients did not, however, show accelerated rates of forgetting. Significant differences in regional brain volumes were detected in the thalamus, hippocampus-amygdala complex, anterior cingulate gyrus, and the left middle frontal gyrus. Significant correlations between regional brain volumes and memory measures which were present in normals were absent in schizophrenic patients. Conclusion: Schizophrenic patients have structural abnormalities in a widely distributed system for episodic memory which contribute to disruption of memory functions.

Supported by: NIMH Grant MH-00976 and Scottish Rite Schizophrenia Research Dissertation Fellowship

468.11

PREFRONTAL CORTEX ACTIVATION IN SCHIZOPHRENIA IS RELATED TO RESPONSE ACCURACY ON A WORKING MEMORY TASK AS MEASURED BY FMRI D. S. Manoach, D.C. Goff, G. Schlaug, C. Saper, D. G. Darby*, A. Benfield, R. R. Edelman, and S. Warach. Dept. of Neurology, Beth Israel Hosp., Harvard Med. Sch., Boston, MA 02215.

Previous studies have shown altered activity in prefrontal cortex in schizophrenia ("hypofrontality"). We investigated whether a non-spatial working memory task would lead to task-related signal changes in prefrontal cortex and whether activation would be associated with performance. Task-related signal change was examined using the Sternberg Item Recognition Paradigm (SIRP) and fMRI. The SIRP is a continuous performance, choice reaction time test that requires working memory. Eight male schizophrenic subjects were visually presented with 5 target digits to remember. Following presentation of the targets, subjects responded to single digits that appeared on a screen by pressing triggers to indicate whether or not the digit was a target. In the control condition, which did not involve working memory, subjects responded to the visual display of arrows pointing right or left by pressing the corresponding trigger. Reaction time and trigger press were recorded for both conditions. Eight contiguous axial slices were acquired using a gradient-echo echo-planar imaging sequence and a 1.5T Siemens Vision scanner to detect changes in cerebral blood oxygenation (BOLD technique) related to task condition. Regions of activation were determined by cross-correlation analyses using a boxcar reference function to compare the working memory condition to the control condition. Suprathreshold prefrontal cortical activation was observed in 5 of the 8 subjects. The 3 subjects who did not show activation in this region made significantly more errors in the working memory condition (although still performing above chance level) but not in the control condition. These findings suggest that prefrontal activation on working memory tasks in schizophrenics may depend on ability to perform accurately.

468.12

TEMPORAL CORTICAL VOLUME CORRELATES WITH ILLNESS DURATION IN FIRST EPISODE SCHIZOPHRENIA G.L. Haas*, M.S. Keshavan, J.A. Sweeney, N.R. Schooler, W.W. Bagwell, and J.W. Pettegrew. Center for Neuroscience of Mental Disorders (CNMD), Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Cumulative evidence indicates volume reductions in temporal cortex in schizophrenia, particularly on the left side. Observations of an association between illness duration and temporal lobe volume reductions have also been found in schizophrenia. These findings have been taken to support a progressive neuropathological process in schizophrenia. In this study, we examined the association between indices of illness duration (time from onset of prodrome to first psychotic symptom, and time from onset of first psychotic symptom to assessment) and the volume of brain structures implicated in the pathogenesis of schizophrenia, i.e. the superior temporal cortex, the amygdala-hippocampal complex, prefrontal cortex, caudate, and lateral ventricles in a series of 18 neuroleptic-naive, first-episode DSM-III-R schizophrenic patients. All scans were conducted using a 3-D SPGR pulse-sequence and morphometric measurements were carried out by a rater blind to clinical information Duration of illness was inversely correlated with left superior temporal cortical volume ($p<.01$) and left amygdala-hippocampal complex volume ($p<.04$), controlling for age and cranial size. Caudate volume did not vary with duration of psychotic symptoms; instead, caudate volume was inversely correlated with duration of prodrome ($p<.01$) and age of first prodromal behavior change ($p<.05$). Each of these findings was more prominent in males. No such relationships were seen in prefrontal cortical or lateral ventricular volumes. These findings suggest that there may be progressive changes in temporal lobe independent of treatment. In contrast, caudate abnormalities appear to be related to the age at onset of the earlier behavioral changes. Other brain regions implicated in schizophrenia, such as PFC, may not manifest evidence of progression during the early phase of illness. Supported by NIMH awards K02 MH 01180 (MSK) MH 46614 (JWP) MH 48492 (GLH) MH 45156 (D Lewis).

468.13

CEREBRAL GLUCOSE METABOLISM IN CHILDHOOD ONSET SCHIZOPHRENIA [1]L.K. Jacobsen, [1]A.J. Zametkin, [1]A.C. King, [1]S.D. Hamburger, [2]J.D. Van Horn, [2]K. F. Berman, [1]J.A. Frazier, [1]K. McKenna, [1]C.T. Gordon, [1]J.L. Rapoport*
[1]Child Psychiatry Branch, [2]Clinical Brain Disorders Branch, NIMH, NIH, MD, 20892

Decreased frontal cortical glucose metabolism has been frequently replicated in adult schizophrenics both at rest and while engaging in tasks normally associated with increased metabolism in this brain region, such as the Continuous Performance Test (CPT). This relative "hypofrontality" may reflect abnormal development of neural circuitry subserving the frontal cortex. Cerebral glucose metabolism was examined in 16 adolescents (mean age 14.2±1.7) with onset of schizophrenia by age 12 (mean age at onset 9.9±1.8) and 26 healthy adolescents matched for age, sex, and handedness. Subjects were injected with 33.7 to 50.3 Mbq of ^{18}F-fluorodeoxyglucose shortly after beginning a 30 minute auditory CPT task with eyes patched.

Schizophrenics made fewer correct identifications (hits; $t=3.11$, $p<.01$) and more incorrect identifications (false alarms; $t=4.67$, $p<.001$) during the CPT. Region of interest analysis revealed no significant group differences in global cerebral glucose metabolism, but increased metabolism in parietal ($F=6.74$, $p<.05$) and posterior frontal regions ($F=7.09$, $p<.05$) and decreased metabolism in anterior frontal ($F=6.72$, $p<.05$) and anterior medial frontal ($t=2.04$, $p<.05$) regions in schizophrenics. After controlling for performance for the subset of 13 schizophrenics and 20 healthy subjects for which performance data were available, schizophrenics continued to show increased parietal metabolism ($F=5.27$, $p<.05$). Negative symptoms were significantly negatively correlated with anterior frontal ($r=-.68$, $p<.01$) and anterior temporal ($r=-.52$, $p<.05$) regions and positively correlated with parietal ($r=.55$, $p<.05$) regions. Positive symptoms were negatively correlated with posterior frontal regions ($r=-.52$, $p<.05$).

These findings support neurobiologic continuity between childhood and adult onset schizophrenia and suggest that events leading to emergence of schizophrenic symptoms in association with abnormal neurodevelopment can occur in early adolescence.
NIMH

468.14

KETAMINE-INDUCED EFFECTS ON BEHAVIOR AND rCBF IS ENHANCED IN SCHIZOPHRENIC COMPARED TO NORMAL INDIVIDUALS A.C. Lahti*, H.H. Holcomb, M.A. Weiler, P.K. Corey, M. Zhao, D. Medoff, C.A. Tamminga. MPRC, University of Maryland School of Medicine, Baltimore, MD 21228

We have previously shown in schizophrenia that the non-competitive NMDA antagonist ketamine in subanesthetic doses produced a dose-dependent short-lived exacerbation of psychotic symptoms (Lahti, 1995) and that regional cerebral blood flow (rCBF) measured using $H_2{}^{15}O$ and PET increased in anterior cingulate, and diminished in hippocampal and primary visual cortex (Lahti, 1995). We have now compared this response in schizophrenics with that in normal volunteers. Normal individuals (n=10) show a dose-related increase in psychotic features with ketamine. Psychotic symptoms included illusions and perceptual distortions, unusual thought content and suspiciousness. These were milder than symptoms in schizophrenic patients who experienced hallucinations, delusions and thought disorganization. The effect of ketamine lasted longer in patients than in normals (26.6 (±5.2) minutes for patients, 16.6 (±8.2) minutes for normals). The effect of ketamine on rCBF changes was evaluated by contrasting the first three post ketamine injection scans (6,16 and 26 minutes after ketamine) with the three baseline scans. Both normals and schizophrenics showed an increase in anterior cingulate rCBF, but the activation was significantly greater ($P<0.05$) in schizophrenics. Differences in the course of rCBF changes over 65 minutes in the two groups will be presented. These data suggest that already-psychotic persons are more sensitive to the effect of ketamine and implicate a dysfunctional glutamatergic system in schizophrenia.

468.15

REGIONAL BRAIN ACTIVITY ASSOCIATED WITH PURSUIT GAIN IN FIRST DEGREE RELATIVE OS SCHIZOPHRENIC PATIENTS. G.A. O'Driscoll*, C. Benkelfat, R. Joober, S. Lal, S. Mattysse, P. Holzman, P. Florencio, S. Evans, McGill University, Department of Psychology, Montreal, Canada, H3A 1B1.

Compared with normal controls, schizophrenic patients and their first-degree relatives have elevated rates of abnormal eye tracking. This familial pattern is not found in non-schizophrenic psychotic disorders. These data suggest that eye tracking deficits are a manifestation of a genetically-mediated brain pathology in schizophrenia. We used positron emission tomography (PET) and the ^{15}O water bolus subtraction method to characterize patterns of brain activation associated with pursuit eye tracking deficits in first-degree relatives and matched controls. Subjects performed smooth pursuit and fixation in the PET scanner. Eye movements were monitored with high-speed infrared oculography.

We predicted that relatives and controls would differ in activation of frontal eye fields (FEF), a hypothesis supported by correlations between pursuit quality and performance on frontal neuropsychological tasks in schizophrenic patients and relatives (Katsanis and Iacono, 1991; Park and Holzman, 1994). Preliminary results (10 first degree relatives, 7 controls) support this prediction. Activity in right FEF was significantly correlated with a measure of pursuit quality, gain (r=.58). Activity of motion area MT/MST, an area sensitive to target movement on the retina, was negatively correlated with FEF activity (r=-.69), and with pursuit quality (r=-.44). These results suggest that alterations in frontal function may underlie eye tracking deficits in first degree relatives. However, data collection is ongoing, and a larger sample size is warranted.

468.16

RESPONSE TIME (RT) VERSUS rCBF CORRELATIONS IN SCHIZOPHRENIC PATIENTS (SZ) AND NORMAL VOLUNTEERS (NV) DURING TONE RECOGNITION H. H. Holcomb*, A. C. Lahti, D. R. Medoff, P. J. Caudill, P. Corey, D. Warfel, Z. Zhao, L. W. Chen, and C. A. Tamminga. Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD 21228.

Healthy observers take longer to make recognition decisions about stimuli that are similar than about stimuli that are different. As the observer's accuracy falls the RT normally increases. In this PET study of tone recognition we have determined how healthy NV differ from unmedicated SZ, in rCBF vs. RT correlation patterns. Fourteen unmedicated schizophrenic patients and twelve healthy volunteers were scanned using the bolus water, $H_2{}^{15}O$ PET methodology. During each study subjects made 60 forced choice decisions, each during a fixed 2 second intertrial interval, regarding the frequency identity of the stimuli, high or low. Accuracy and RT were digitally recorded for each trial. The average RT for accurate trials was correlated with rCBF using SPM95 statistical methods. Whereas the NV group exhibited a positive correlation between RT and rCBF in the right frontal cortex, the SZ group's significant positive correlations were restricted to bilateral middle temporal gyri. Significant negative correlations for NV were found in the cerebellum and right primary auditory cortex; SZ subjects exhibited a significant negative correlation in the left primary motor cortex. The patient group's correlation pattern differed significantly from the NV group's. These observations are consistent with the SZ group's inability to assign greater neural activity to the sensory-motor system during fast accurate decisions, and to the frontal lobe during slow recognition choices. Research supported by NIMH (CRC MH40279).

468.17

THE METHODOLOGICAL TEST-RETEST EFFECTS OF REPEATED [15]O PET BLOOD FLOW IMAGES IN SCHIZOPHRENIC AND NORMAL VOLUNTEERS AT REST AND DURING TASK PERFORMANCE. D.R. Medoff*, H.H. Holcomb, A.C. Lahti, Z. Zhao, L. Chen, C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland School of Medicine, P.O. Box 21247, Baltimore, MD 21228

Blood flow studies allow up to 15 or more scans to be completed in the same person in the same imaging session. Therefore this technique is suitable to explore the effects of a variety of behavioral and/or pharmacologic probes in a repeated measures type design. However, time in the scanner itself may have a significant effect on metabolic activity, which may vary across subject populations or activity state. We have looked at the time effect and the variability across scans of the same condition in normal controls and schizophrenic patients during rest, a sensory motor control task, and a demanding attentional task in which subjects were fully trained. Comparisons between the four rest scans showed that both normals and schizophrenics had significantly greater blood flow in the frontal and temporal areas in the first scan compared to the following three scans. Over time, regardless of condition, there was a trend for the posterior cingulate to have decreased blood flow in normal controls. These findings are an important consideration in the design and interpretation of blood flow data. In particular it highlights the importance of having more than one scan per experimental condition and to be sure the order of the scans is counterbalanced.

469

SYMPOSIUM. NEUROBIOLOGY OF OB PROTEIN (LEPTIN): A PERIPHERAL SIGNAL ACTING ON CENTRAL NEURAL NETWORKS TO REGULATE BODY ENERGY BALANCE. L.A. Campfield, Hoffmann-La Roche (Chairperson); J. Flier, Harvard Univ.; S.C. Chua, Rockefeller Univ.; S.C. Woods, Univ. Washington.

OB protein is secreted from adipose tissue, circulates in plasma and acts on central neural networks controlling body fat through coordinated regulation of feeding, metabolism and the autonomic nervous system. Following demonstration of biological activity, OB protein binding to choroid plexus led to the cloning of a central OB-R receptor. L. A. Campfield will present an overview of OB protein neurobiology including the effects following central administration, localization of central OB-R receptors, and patterns of neuronal activation following peripheral and central administration of OB protein. J. Flier will discuss the role of OB protein in the modulation of neuroendocrine responses to fasting and obesity. S. C. Chua will review the role of mutations of the OB-R receptor in the development of the phenotypes of the obese *diabetes* (*db/db*) mouse and obese *fatty* (*fa/fa*) rat. S. C. Woods will review studies on the interaction of centrally administered OB protein with insulin and other neuropeptides on central neuronal networks and the resulting coordination of behavioral and metabolic effects. A discussion period involving the audience and the speakers will end the symposium.

470

SYMPOSIUM: GLUTAMATERGIC NEUROTRANSMISSION: A VIEW FROM THE DENDRITE. R. Weinberg, UNC, and F. Conti, Univ. Ancona (co-chairs); L. Trussell, Univ. Wisconsin; H. Monyer, Univ. Heidelberg; R. Huganir, Johns Hopkins; F. Edwards, University College (London).

Glutamate plays a central role in neurotransmission. By integrating recent data from multiple disciplines, we will provide a functionally-oriented overview of current research into glutamate, from the point of view of the postsynaptic dendrite. Trussell will review electrophysiological data and modeling studies showing how glutamate diffusion and uptake interact with different ionotropic receptors to shape the EPSC. Monyer will review data from molecular biology, biophysics, and immunocytochemistry showing the influence of subunit composition on channel conductances. Calcium entry (via NMDA receptors or calcium-permeable AMPA receptors) may activate kinases and other second messengers. Huganir will discuss how phosphorylation may influence glutamate receptors, and present recent data on proteins that may be involved in anchoring receptors at the synaptic membrane. Edwards will review the structure of simple and perforated excitatory synapses in the hippocampus, explaining how synaptic heterogeneity may account for the observed distribution of synaptic events, and raising the possibility that changes in synaptic structure may underlie long-term plasticity.

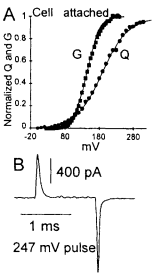
473.1

LOCALIZATION OF Ca^{2+}-BINDING DOMAINS IN HUMAN (*hslo*) Ca^{2+}-ACTIVATED K^+ CHANNELS. J.D. Krause* C.D. Foster & P.H. Reinhart, Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710, and Dept. of Pharmacology GlaxoWellcome Research Institute, RTP, NC 27709.

We have used a combination of site-directed mutagenesis and inside-out patch clamp analysis to localize Ca^{2+}-binding domains in Ca^{2+}-activated K^+ channel α-subunits cloned from human brain (*hslo*). Preliminary experiments indicated that single amino acid substitutions at a number of sites can alter the apparent Ca^{2+}-sensitivity of this channel. In order to determine whether amino acid substitutions at these sites alter the apparent Ca^{2+} sensitivity by disrupting a Ca^{2+} binding domain or by perturbing protein rearrangements subsequent to Ca^{2+}-binding, we have measured the selectivity for channel activation by calcium versus other divalent cations. This measure is expressed as the ratio of the concentration of each divalent ion required to half maximally activate the channel at zero membrane potential, normalized to the concentration of calcium required to activate the channel. For the wild-type channel the normalized ratio for $[Ca^{2+}]:[Sr^{2+}]:[Mn^{2+}]$ is approximately 1:120:2000. Thus, Ca^{2+} is three orders of magnitude more potent than Mn^{2+} and two orders of magnitude more potent than Sr^{2+} in activating the channel. For mutations which act downstream of Ca^{2+}-binding it is assumed that this ratio should remain largely unaffected, whereas, mutations which act by disrupting the Ca^{2+}-binding site are likely to perturb this ratio. Mutating asparagine 222 to serine (N222S) reduces the apparent sensitivity to these three ions approximately 100-fold, without significantly changing the potency ratio. In contrast to this effect, neutralization of aspartate residues in two separate C-terminal domains causes a large shift in the potency ratio. Mutating aspartate 358 to asparagine (D358N) gives rise to a potency ratio of 1:200:15 while mutations at a second site (D886N) give rise to a potency ratio of 1:100:10. These observations indicate that at least two distinct C-terminal domains contribute to the formation of Ca^{2+}-binding sites, and regions of the protein more proximal to the pore participate in the structural rearrangements leading to channel activation. Supported by NIH grant NS31253 to PHR.

473.2

COUPLING OF CHARGE MOVEMENT AND PORE OPENING IN A HUMAN MAXI K CHANNEL (*HSLO*): NUMBER OF CHARGES PER CHANNEL. E. Stefani* and L. Toro. Dept. of Anesthesiology, UCLA, Los Angeles, CA 90095-1778.



We studied the coupling between gating and ionic currents of a Maxi K channel (*hslo*) and determined the number of effective charges per channel. We used Cs^+ to measure ionic currents, and tetraethylammonium to isolate gating currents (**B**). **A** shows G-V and Q-V curves of *hslo* expressed in oocytes. At negative voltages, a small amount of charge moves prior to pore opening indicating the presence of closed states prior to the open states. These initial closed states agree with the "Cole-Moore" shift in the ionic current. With further depolarization, in contrast to other voltage gated ion channels, the G-V curve crosses the Q-V curve and charge movement follows pore opening. Raising internal Ca^{2+} shifts both curves. These results indicate that the channel transits through closed states before opening and that open transitions also carry charge. We normalized both the number of channels and the amount of charge per membrane capacity measured in 3-6 giant patches of the same cell; we obtained 4.4 ± 0.8 charges/channel (± SD, 3 cells). This number is consistent with less charged residues in the S4 region of *hslo* as compared to other voltage gated ion channels of the S4 superfamily. Supported by NIH. L. Toro is an EI of the AHA.

473.3

A "CALCIUMSTAT" CONTROLS BK CHANNEL GATING. M. Schreiber and L. Salkoff*. Department of Anatomy and Neurobiology, Washington University School of Medicine, Saint Louis, MO 63110.

The high conductance Ca^{2+}-activated K^+ channel (mSlo) plays a key role in regulating calcium entry in a variety of cell types. Our previous analysis of the functional architecture of this channel indicates that it has two distinct parts: a "core" resembling a voltage-dependent K^+ channel, and an appended "tail" that provides the calcium-sensing function (Wei et al., 1994). To reveal the function of the tail in gating, we created two mutants which differ in sensitivity to calcium by a factor of 10^4. We then coexpressed them to create mosaic channels. Single channel analysis detected five forms, corresponding to five stoichiometries. Each exhibited a unique calcium dependence. All were similar, however, with regard to voltage sensitivity and activation kinetics. Several lines of evidence suggest that all four tails are needed for channel activation and may form a single structure, a "calciumstat," which senses free calcium. Calcium, however, binds independently to at least one site on each tail. Binding of calcium to the calciumstat regulates the conformational free energy difference between the closed and open states of the channel, and sets the position of its g/V curve on the voltage axis. The substitution of a hypersensitive mutant tail for a hyposensitive mutant tail contributes an incremental amount of conformational free energy equivalent to a shift of -45 mV. The apparently simple opening kinetics of all mosaic forms suggests a single voltage-dependent step leading to channel opening. This could be explained by the calciumstat cross-linking the four voltage-dependent subunits, in effect creating a single voltage sensor out of four. *Supported by the NIH and the Muscular Dystrophy Association (L.S.)*

473.4

STABLE EXPRESSION OF THE HUMAN CA^{2+} ACTIVATED K^+ CHANNEL α- AND β-SUBUNITS IN HEK293 CELLS. Philip K. Ahring, Dorte Strøbæk, Palle Christophersen, Søren-Peter Olesen and Teit E. Johansen*, NeuroSearch A/S, Smedeland 26, DK-2600 Glostrup, Denmark.

The high-conductance Ca^{2+} activated K^+ channel (BK channel) is composed of an α-subunit and a β-subunit, of which the α-subunit gives rise to functional channels when expressed alone. We have stably expressed the BK α-subunit and co-expressed the α-subunit along with the β-subunit in HEK 293 cells. For co-expression we constructed a plasmid with two expression cassettes, the α-subunit was expressed under the control of the CMV promoter and the β-subunit was expressed under the control of the SV40 promoter. Cell lines expressing the α-subunit have proved stable for more than 40 passages, and cell lines co-expressing the α+β-subunits for more than 20 passages. Studies of the modulation of the BK channel gating by changes in $[Ca^{2+}]_i$, presence of β-subunit, NS1608 and paxilline were carried out using I/O patches. The cells expressed 200-800 BK channels per patch giving rise to macro currents. The BK channel α-subunit was sensitive to changes in $[Ca^{2+}]_i$ in the physiological range 300-1000 nM with 50% channel activity at 18±6 mV (n=6) at 1000 nM $[Ca^{2+}]_i$, as determined by tail current analysis. Presence of the β-subunit increased the voltage sensitivity as seen by a leftward shift of the activation curve by -30 mV (n=9) at 1000 nM $[Ca^{2+}]_i$. The β-subunit had no influence on the time constant for activation which was 1.38ms (α) and 1.33ms (α+β). The time constant for deactivation, however, was increased from 2.53ms (α) to 13.78ms (α+β). 10μM NS1608 shifted the activation curves for the α+β-subunit towards negative membrane potentials by -47.7mV. This is similar to the NS1608-induced shift observed for the α-subunit alone, suggesting that the effects of the β-subunit and NS1608 are mediated by separate mechanisms. The effects of the BK channel blocker paxilline was independent of the presence of the β-subunit.

473.5

DEVELOPMENT OF A COMPETITIVE RT-PCR METHOD FOR QUANTIFICATION OF Slo AND PNMT mRNA IN ADRENAL TISSUE. J. Xie, P. Lappas, W. Yao, E. Carpenter-Hyland, and D.P. McCobb*. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Stress-related stimuli to the adrenal medulla dramatically alter mRNA levels of catecholamine synthetic enzymes such as phenylethanolamine N-methyl transferase (PNMT) in adrenal chromaffin cells. To determine whether transcripts for specific ion channels which control catecholamine secretion in these cells are similarly regulated by stress factors, we have developed competitive RT-PCR methodology for quantification of Slo (BK-type calcium-activated potassium channel) as well as PNMT expression. Specific competitor templates to be added in known quantity to samples of adrenal mRNA containing unknown quantities of native templates were constructed for Slo and PNMT using a simple PCR-based strategy. 20bps were inserted into the wildtype sequence before transcribing in vitro to make competitor RNA. Estimates of native template are made by measuring relative intensities of gel bands of native and competitor RT-PCR products from a series of reactions containing different amounts of competitor. The point at which products would be equal is determined by fitting a line to the log function of product ratios for the series. Corrections for differences in amplification efficiencies and product length are made first. Experiments in which rSlo transcript amounts were measured gave surprisingly consistent results, supporting the quantitative accuracy of the method. RSlo mRNA in six rat adrenal glands was measured twice, and the mean ratio of first to second trials was 1.07 ± 0.15 (SD). The ratio for left and right glands from the 3 animals was 0.94 ± 0.19. Our best estimates of Slo copy number in 25ng of total RNA, obtained by averaging data from left and right glands, were 16,000, 16,100, and 20,200 for the 3 animals. This method will allow us to measure changes in expression accompanying alterations in neural and hormonal inputs to the gland. Supported by Cornell University.

473.6

DISCRETE STEPS IN THE BIOGENESIS OF THE SHAKER K^+ CHANNEL REVEALED BY A MUTATION IN THE S3 TRANSMEMBRANE DOMAIN. C.T. Schulteis*, N. Nagaya, and D.M. Papazian. IDP Neuroscience and Department of Physiology, UCLA School of Medicine, Los Angeles, CA 90095-1751.

The biogenesis of voltage-dependent K^+ channels includes folding and assembly steps that establish tertiary and quaternary interactions. In Shaker K^+ channels, two potential quaternary interactions have been identified: (1) a homotypic N-terminal interaction critical for channel assembly and (2) an interaction between adjacent N- and C-termini detected in native channels by the oxidation of disulfide bonds between cysteine residues C96 and C505 (Schulteis et al., 1995, *Biophys. J.* **68**: A266). The mutation D316K in transmembrane segment S3 of Shaker disrupts an intrasubunit interaction with K374 in the S4 segment, resulting in protein that fails to mature (Tiwari-Woodruff et al., 1996, *Faseb J.* **10**: A145). To test whether the homotypic N-terminal interaction can be established in the presence of this folding mutation, we determined whether D316K subunits have a dominant negative effect on the expression of Shaker-IR subunits. D316K subunits exerted a strong dominant negative effect. This effect was mediated by amino acids 97-196 in the N-terminal as deletion of this domain eliminated the dominant negative effect. We also tested the proximity of the N- and C-termini in D316K by oxidation of protein in intact cells. C96 and C505 failed to form disulfide bonds between D316K subunits. Rescue of the folding defect in the double mutant D316K+K374E restored the C96/C505 interaction. These data indicate that the folding defect introduced by D316K prevents the C96/C505 interaction, but does not preclude the homotypic N-terminal interaction mediated by amino acids 97-196. Our results suggest that biogenesis of Shaker channels involves an initial step mediated by N-terminal amino acid residues followed by the folding of transmembrane segments and the establishment of the N- and C-terminal proximity. Supported by the NIH (GM43459) and the Keck Foundation (DMP), the Howard Hughes Medical Institute (CTS), and the American Heart Association, Greater Los Angeles Affiliate (NN).

473.7

REGULATION OF INACTIVATION IN *SHAKER* POTASSIUM CHANNELS BY OXIDATION. T. Hoshi* and S. H. Heinemann. Physiology and Biophysics, Univ. of Iowa, Iowa City, USA and Max-Planck Society, Res. Unit "Mol. & Cell. Biophysics", Jena, Germany.

Inactivation time course of one variant of the *Shaker* potassium channel, *Sh*C/B, exhibits considerable variability when expressed in *Xenopus* oocytes. At the single channel level, this variability is observed as the modal gating involving the fast inactivation and slow inactivation modes (Aldrich et al., Cold Spring Harbor Symp. *55*, 19-27, 1990). We investigated the mechanism responsible for the variability in the inactivation time course of the *Sh*C/B channels in *Xenopus* oocytes.

The whole-cell *Sh*C/B currents recorded with a two-electrode voltage-clamp amplifier show a complex inactivation time course. Frequently, the inactivation time course could not be readily described by a sum of exponential components. We hypothesized that oxidation may be involved in regulation of the inactivation process and examined the effects of chloramine-T (Ch-T) using the inside-out configuration of the patch clamp. Ch-T (200 μM) dramatically slowed the inactivation kinetics of the *Sh*C/B. We also examined the effects of Ch-T on the *Sh*B channel. The *Sh*B and *Sh*C/B channels differ only in the amino-terminus segment and share all the cysteine residues. However, Ch-T did not alter the *Sh*B inactivation kinetics.

The differential effects of Ch-T on the two *Shaker* channels which share the cysteine residues suggest that other amino acid residues may be oxidized or that the cysteine residues are differentially protected from oxidation. (Supported by HFSP).

473.8

THE SCALING OF PARAMETER MANIFOLDS IN COMPARTMENTAL MODELS OF HIPPOCAMPAL NEURONS. R. M. Eichler West* and G. L. Wilcox. Graduate Program in Neuroscience, Department of Pharmacology, and Minnesota Supercomputer Institute, University of Minnesota, Minneapolis, MN 55455.

We previously demonstrated the use of *Genetic Algorithms* (GAs) for selecting conductance parameters in compartmental models of hippocampal neurons (Eichler West and Wilcox, SfN abstract 21:783.7, 1995). Successful parameter choices demonstrated a high correspondence between simulated and experimentally-obtained behaviors for the fitness criteria chosen. Our previous GA application selected parameter manifolds for the 114 total conductance values (representing six channel types in each of 19 compartments) in the Traub model (J Neurophysiol. 1991). In this present study, we extend this GA approach to select the conductance parameters in a morphometrically realistic compartmental model (> 1500 compartments).

Four results are presented. 1) The channel distributions selected for the full morphometric model are compared to those selected for the 19 compartment equivalent cylinder model. This comparison addresses the suitability of reduced complexity models for network simulations. 2) The GA also searched the morphometric model for calcium buffering terms defined with respect to volume and these results are reported. There was previously no one-to-one correspondence between the calcium terms and the physical dimensions of the model because Traub selected his parameters arbitrarily. 3) A function parameterization was developed for the GA search to reduce the complexity and dimensionality of the representation. This reduced the number of model evaluations needed to obtain a high fitness solution. 4) The contribution of local voltage-gated and/or calcium-dependent channel conductances to the effective synaptic current was studied through a series of simulations along high fitness channel distributions. The results of this analysis address functional consequences for activity-dependent conductance modulations.

(Supported by a MN Supercomputer Institute/Cray Research grant and by access to Cray and SGI supercomputers at MSI and Laboratory for Comp Sci & Engineering)

STRESS III

474.1

GENETIC MAPPING OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH STRESS REACTIVITY IN THE WISTAR-KYOTO HYPERACTIVE RAT, P. Mormède*, H. Courvoisier and M.P. Moisan. Génétique du Stress, INSERM - INRA - Université de Bordeaux II France

In order to better understand the molecular mechanisms of interindividual variability of stress responses and associated pathology we have undertaken a genetic linkage analysis on a F2 intercross between 2 rat strains (Wistar-Kyoto Hyperactive and Wistar-Kyoto) that differ markedly in their stress reactivity. The phenotyping consisted of 3 behavioral tests (activity cages, open-field and elevated-plus maze) and several neuroendocrine measures (corticosterone, prolactin, renin activity) in blood plasma collected after novel environment stress. A multifactorial analysis was performed in order to synthesize these data and to classify each F2 rat according to its degree of reactivity along each independent factor. Each of these 196 F2 rats was also genotyped using 67 rat microsatellite genetic markers that were polymorphic for the parental strains. A major QTL related to the locomotor activity / reactivity was detected on chromosome 8, with a LOD score of 9.5, explaining 29% of the difference between the strains.

Funded by INSERM, INRA, University of Bordeaux II, GREG (Ministry of Research) and Région Aquitaine.

474.2

STRESS ELICITED ACTIVATION OF TYROSINE HYDROXYLASE AND DOPAMINE-β-HYDROXYLASE GENE EXPRESSION IN C-FOS KNOCKOUT MICE: TISSUE AND SEX SPECIFIC DIFFERENCES. E.Sabban[1], L.Serova[1]*, E.Saez[2], B.Spiegelman[2]. [1]Dept. of Biochem. and Mol. Biol., New York Med. Coll., Valhalla, NY 10595. [2] Dana-Farber Cancer Inst., Boston, MA, 02115.

Increased c-fos expression has been reported in many tissues to numerous types of stress. Increased binding of c-fos and c-jun to the AP-1 site of the TH promotor may be one of the regulatory mechanisms involved in mediating the response to stress. To test this hypothesis the effect of repeated immobilization stress (IMO) on tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) gene expression was examined in c-fos knockout mice. Basal levels of TH mRNA in adrenals was similar in wild-type (+/+), heterozygous (+/-) and c-fos null (-/-) mice of both sexes. There was a large induction (6-10) fold in adrenal TH mRNA after 3 daily IMO in all genotypes, suggesting that either c-fos is not essential or another gene product can replace its effect. In contrast, basal levels of DBH mRNA in adrenals appeared to be reduced in the c-fos deficient mice but were increased even more in -/- than in +/+ mice after IMO. In brain stem, DBH induction by stress was reduced in heterozygotes and fos deficient male mice or prevented in female mice. These results indicate that the c-fos gene has tissue specific influences on the regulation of DBH gene expression by IMO. (Supported by NIH grants NS32166 and DK31405).

474.3

CIRCULATING ANGIOTENSIN II IS NOT INVOLVED IN REGULATION OF ADRENAL TYROSINE HYDROXYLASE GENE EXPRESSION INDUCED BY IMMOBILIZATION STRESS. R.Kvetnansky*, M.Rusnak, B.B.Nankova[1], S.Zorad, P.Blazicek, J.Jelokova, O.Krizanova and E.L.Sabban[1]. Inst. Exp. Endocrin., Bratislava, Slovakia; [1]Dept. Biochem. & Mol.Biol., New York Med. Coll., Valhalla, N.Y. 10595.

Immobilization (IMO) stress increases tyrosine hydroxylase (TH) gene expression in rat adrenal medulla (AM). The aim of this study was to examine the mechanism(s) of this change. Our previous results have shown that acetylcholine (ACH) and splanchnic innervation do not play a significant role in the elevation of TH mRNA levels induced by IMO. The rise in TH mRNA occurred even in denervated AM of hypophysectomized rats. These data suggest that messengers other than ACH or ACTH are involved in regulation of TH gene transcription. Angiotensin II (AII) was shown to induce TH gene expression in cell cultures. We evaluated a possible involvement of AII in IMO-induced TH gene expression. Repeated administration of AI (8 ng/kg, i.v.) did not affect levels of TH mRNA in AM. Saralasine, an AII receptors antagonist, did not affect control or IMO-elevated TH mRNA levels. Nephrectomy (N) caused more than a 95% decrease in levels of plasma renin which plays a major role in AII formation. After N no decrease but a significant rise in TH mRNA levels in unstressed rats was found and IMO-induced increase was not reduced. Thus, during IMO stress the adrenal medullary TH gene expression is activated by a nonneuronal and nonpituitary-mediated pathway without involvement of renin-angiotensin system. Supported by NIH Grant NS 32166; Slovak Grant Agency for Science (95/5305/043; 95/5305/272), LV-9301 Stress, and US-Slovak Project 93 024.

474.4

BEHAVIORAL EFFECTS OF PREPRO-TRH$_{178-199}$ IN THE RAT FOLLOWING ICV ADMINISTRATION. R.F. McGivern[1]*, E. Redei[2] San Diego State Univ.[1], San Diego, CA 929120, Univ Pensylvania.[2], Phila., PA 19104

We have recently demonstrated an inhibitory influence of prepro-TRH$_{178-199}$ on ACTH secretion in vivo and in vitro, suggesting that it may function physiologically as a corticotropin release inhibiting factor (CRIF). Processing of the TRH prohormone yields several non-TRH hormones including prepro-TRH$_{178-199}$, a 22 amino acid peptide that is detectable by RIA in several brain regions activated by stress such as amygdala, septum, hypothalamus and periaqueductal gray. Behavioral studies of prepro-TRH$_{178-199}$ were conducted in adult male Sprague-Dawley rats following intracerebroventricular (icv) injection. Animals were tested in a 15" diameter open field for 30 minutes or in a light/dark box for 15 minutes. Each animal was removed from the home cage and injected with CRIF (0.6 ug/kg or 6.0 ug/kg in 3ul volume) or the vehicle 5 minutes prior to testing (N=6-17 animals/dose). Videotape analysis of the behaviors revealed that the 6.0 ug/kg dose of prepro-TRH$_{178-199}$ significantly increased several behaviors in the open field compared to vehicle treatment. These included grooming ($p<0.0001$), locomotor activity ($p<0.01$), rearing ($p<0.05$), and active sniffing ($p<0.001$). The 0.6 ug/kg dose of prepro-TRH$_{178-199}$ significantly reduced rearing behavior throughout the testing period ($p<0.05$) and activity during the initial 5 minutes ($p<0.05$). In the light/dark box, the 6.0 ug/kg dose of the peptide significantly increased the number of entries into the light compartment ($p<0.01$) as well as the total time spent in that compartment ($p<0.01$). Overall, these results indicate that prepro-TRH$_{178-199}$ is behaviorally active and suggest that its effects include some fear-reducing and arousal properties. (Supported by NIAAA 06478 and The Berman Foundation).

474.5

THE BRAIN POLYAMINE-STRESS-RESPONSE; PROGRESSIVE DEPLETION OF POLYAMINES. G.M. Gilad[1], J.M. Rabey[1] and V.H. Gilad. Lab. of Neuroscience, Dept. of Neurology, Tel Aviv-Sourasky Medical Center, and [1]Dept. of Neurology, Assaf Harofeh Medical Center, Tel-Aviv 64239, Israel.

A transient increase in polyamine (PA) metabolism is a common response to acute stressful stimuli. In the brain, repetitive stressor applications result in a recurrent increase in the activity of ornithine decarboxylase (ODC), the enzyme catalyzing the first limiting step in PA synthesis. We now sought to examine the effects of chronic intermittent stress (restraint, 3 h per day for 14 days) on PA concentrations (measured by reversed-phase HPLC method) in the hippocampus and on open field behavior of adult male C57BL/6 mice, and to study their modulation by treatment (intraperitoneal injections twice daily) with the ODC inhibitor α-difluoromethylornithine (DFMO, 200 mg/kg). The findings demonstrate that inescapable chronic intermittent stressors result in a recurring transient increase in putrescine, but lead to a progressive depletion of spermidine and spermine. DFMO treatment reduced the stress-induced putrescine increases, but did not prevent the reductions in PAs. Behaviorally, DFMO treatment led to a reduction in stress-induced open field activity. The study further implicates the PA-stress-response as an adaptive mechanism in the brain reaction to stressful stimuli. *Supported in part by the German-Israeli Foundation for Scientific Research and Development.*

474.6

PIG NEOCORTEX MORPHOLOGY, BEHAVIOR AND IMMUNITY

J.M. McGlone[1]*, J.L. Morrow-Tesch[2], M. K. Jarvinen[3], S.D. Fullwood, T. L. Powley[3], [1]Texas Tech U., Lubbock, TX 79409-2141; [2]USDA-ARS; [3]Purdue U., W. Lafayette, IN 47907.

The domestic pig (*Sus scrofa*) is used for agricultural production and as a biomedical model. Aims were to determine age effects and diverse environments on development of the neocortex, behavior and immunity. Littermates (6 sets, half-sib, female) were randomly assigned to one of 3 treatments: euthanized at birth, or raised crossfostered and in an indoor, simple environment (fan ventilation & concrete floors) an outdoor, complex environment (straw on earth). After 8 weeks, behaviors were observed, blood was collected for immune measures, and pigs euthanized for brain tissue samples. The primary auditory, somatosensory and visual neocortex were sectioned (Golgi-Cox staining). Non-truncated, layer IV neurons (n = 493) were digitized (Eutectics Neuron Tracing System). Soma area increased 15% ($P < .01$) in all 3 neocortex regions from birth to 8 weeks. Auditory cells decreased in length, membrane surface area and had fewer segments (all $P < .001$) at 8 weeks compared with birth. Visual cortex cells increased in length and membrane surface area ($P < .05$) at 8 weeks compared with birth. Pigs reared in more complex outdoor environments had more auditory dendritic segments than pigs reared indoors (4.86 vs. 4.24 ±0.22, $P < .01$). Outdoor pigs were more active and showed greater rooting ($P < .05$) than indoor pigs. Outdoor pigs had more white blood cells ($P < .05$) than indoor pigs, but lymphocyte proliferation, neutrophil chemotaxis and NK activity were similar for each. Diverse environments did not lead to neuroanatomical signs of deprivation.

USDA-ARS, NIH DK27627 and NIMH01023.

474.7

CHRONIC RESTRAINT STRESS CAUSES ULTRASTRUCTURAL CHANGES IN RAT MOSSY FIBER TERMINALS A. M. Magariños*, J.M. Garcia Verdugo° and B.S. McEwen. Laboratory of Neuro-endocrinology, The Rockefeller University, New York, NY 10021; °University of Valencia, 46100, Burdassot, Spain.

Chronic repeated stress induces hippocampal atrophy in the apical dendrites of CA3c pyramidal neurons based on light microscopic Golgi impregnation analysis. This morphological change is correlated with deficits in spatial memory tasks and requires excitatory amino acid mediation since daily administration -before each restraint session- of an NMDA receptor blocker or the antiepileptic, phenytoin, prevents the stress-induced hippocampal atrophy. Since the major excitatory input to CA3 pyramidal neurons is the mossy fiber projection from the granule neurons in the dentate gyrus, we studied the ultrastructure of mossy fiber terminals in the stratum lucidum of restraint-stressed (6h/d/21d) male rats compared to controls. Fixed sections were dehydrated and flat embedded in Durcupan. Ultrathin sections were examined and photographed for ulterior morphometry. Undisturbed control rats showed mossy fibers terminals packed with synaptic vesicles evenly distributed. After 21d of daily restraint stress, animals showed terminals containing compact vesicle clusters that were restricted to 30% of the mossy fiber boutons area. No differences were found in the mean bouton area or mean invaginated dendritic spine area. However, the mean mitochondria area was larger in the mossy fiber terminals of restraint-stressed rats compared to controls. We suggest that repeated stress causes a presynaptic ultrastructural rearrangement that is associated with enhanced glutamate release. This is consistent with data showing by in vivo microdialysis that stress enhances glutamate release. (Supported by MH 41256, the Health Foundation and Servier, France).

474.8

EXPOSURE TO PREDATOR ODOR SUPPRESSES CELL PROLIFERATION IN THE DENTATE GYRUS OF ADULT RATS VIA A CHOLINERGIC MECHANISM. L.A.M. Galea*, P. Tanapat, and E. Gould. Lab. of Neuroendocrinology, The Rockefeller University, New York, NY 10021

The production of granule neurons in the dentate gyrus of the adult rat has been shown to be suppressed by adrenal steroids and excitatory input, factors that are both elevated in response to stress (Gould and Cameron, in press). These experiments were designed to examine the influence of a naturally aversive experience, exposure to predator odor, on the proliferation of cells in the dentate gyrus of adult rats. Using ^{3}H-thymidine autoradiography, the production of cells in the dentate gyrus was examined following exposure to trimethyl thiazoline, a component of fox feces. Exposure to fox odor resulted in a rapid decrease in the number of ^{3}H-thymidine labeled cells in the dentate gyrus. This suppression of cell proliferation was not the result of a stress-induced rise in adrenal steroids because the effect persisted in rats exposed to fox odor following adrenalectomy with low level corticosterone replacement. This effect was, however, prevented by pretreatment with the cholinergic muscarinic receptor antagonist scopolamine. These results indicate that exposure to fox odor inhibits cell proliferation in the adult dentate gyrus through a cholinergic, but not adrenal steroid, mechanism. These findings raise the possibility that suppression of cell proliferation plays a role in the integration of information regarding a potentially threatening experience.

Supported by a NARSAD Young Investigator Award and MH52423 to EG and an NSERC postdoctoral fellowship to LAMG.

474.9

DIFFERENTIAL EXPRESSION OF c-*fos* mRNA IN THE MALE SYRIAN HAMSTER BRAIN FOLLOWING SOCIAL DEFEAT OR RESTRAINT. S. Kollack-Walker*, H. Akil and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720.

Social defeat in the male Syrian hamster is characterized by an increase in the levels of plasma cortisol and corticosterone and by the activation of specific subpopulations of neurons within the central nervous system [Kollack-Walker et al., *Soc. Neurosci. Abstr.*, 21: 501]. To determine if the pattern of neuronal activation observed following defeat is present in other stressful situations, c-*fos* mRNA expression was analyzed in males who were stressed by social defeat or restraint. Beginning one week prior to testing, male hamsters were habituated to handling and to the novelty of exposure to another male's cage. On the day of the experiment, animals were either handled as controls or stressed by placing the animal in a restraint tube or allowing the animal to be defeated by a dominant male. Following 30 min of testing, all animals were killed by decapitation, their brains removed for c-*fos in situ* hybridization, and trunk blood collected for analysis of plasma cortisol and corticosterone levels. Results demonstrated increased c-*fos* mRNA expression within the prefrontal cortex, rostral septum, paraventricular nucleus of the hypothalamus and locus coeruleus of males exposed to either stressor, although the intensity of labeling appeared stronger in defeated males than in restraint males. In addition, c-*fos* mRNA increased selectively within the caudal septum, anterior, ventromedial and ventral premammillary nuclei of the hypothalamus, medial amygdala and dorsal periaqueductal gray in defeated males, and within the lateral hypothalamus and zona incerta in restraint males. These results suggest the presence of a neural circuit that responds to stressors in general, as well as circuits that reflect the unique physical and psychological qualities associated with social defeat and restraint. (Supported by NIMH MH42251, SJW)

474.10

PROLONGED EXPOSURE TO STRESS REDUCES THE DOPAMINERGIC RESPONSE IN THE VENTRAL MEDIAL PREFRONTAL CORTEX TO A SUBSEQUENT STRESS EXPOSURE.

Brian A. Horger*, Bret A. Morrow, John D. Elsworth, and Robert H. Roth, Departments of Psychiatry and Pharmacology, Yale University, School of Medicine, 333 Cedar Street, New Haven, CT 06520.

Stress-associated increases in dopaminergic (DAergic) activity in the ventral aspects of the medial prefrontal cortex (mPFC) have been postulated to have a role in the initiation of a coping response to the stressor. Prolonged exposure to restraint stress results in an initial increase in DA metabolism in the mPFC, peaking 20 min after stress onset, followed by a subsequent decline in DA metabolism, returning to baseline levels after 90 min of stress exposure (Roth et al., 1988, Ann NY Acad Sci, 537,138). Preexposure to a variety of short term (20-30 min) stressors has been shown to augment the DAergic response in the mPFC to a subsequent stressor. The present study examined the effects of prolonged exposure to uncontrollable stress on the DAergic response to a subsequent stress exposure in subregions of the mPFC. Male Sprague-Dawley rats were restrained for 120 min. Controls were unhandled. Twenty-four hours later, all subjects were restrained for 20 min before decapitation. HPLC analysis of tissue concentrations of DOPAC and DA revealed decreased stress-induced DA utilization (DOPAC/DA) in the ventral, but not dorsal, aspects of the mPFC in subjects that had received prolonged stress preexposure relative to controls. Prolonged exposure to uncontrollable stress has been shown to induce a learned helplessness phenomenon where, during subsequent exposures to stress, subjects are less likely to engage in escape avoidance behaviors. Given the postulated role of the ventral mPFC in the initiation of coping behaviors, the present data may provide a relevant neurochemical correlate of learned helplessness.

Supported in part by US PHS (MH 14092).

474.11

PREDATOR EXPOSURE STRESS INDUCES CHANGES IN EXPRESSION OF ZIF/268 MRNA IN THE RAT BRAIN. J.N. Nikulina*, R.P. Hammer, Jr., E. Cooke, D.C. Blanchard, and R.J. Blanchard. Dept. of Psychology, University of Hawaii, Honolulu, HI 96822 and New England Medical Center, Boston, MA 02111.

In a painless model of psychological stress male rats were exposed to an innate threat stimulus, a predator. Rats were moved in their home cages to a test room and placed under an empty cat chamber with a mesh bottom. Five minutes later, a cat was introduced into the chamber for a one hour period. Controls were exposed to a toy cat. Rats were exposed to a predator for one or five daily sessions. Extremely high (80-90%) levels of crouching and freezing were observed during exposure to the cat, but not to the control stimulus. No evidence of behavioral habituation was found over five sessions. The rats were sacrificed two hours after the last predatory exposure and the brains prepared for *in situ* hybridization of zif/268 mRNA. Zif/268 is an immediate early gene that serves as a transcription regulatory factor that is rapidly induced in brain neurons by synaptic transmission. We used a [^{35}S]-labeled oligonucleotide probe for *in situ* hybridization of zif/268 mRNA.

Zif/268 mRNA expression two hours after the single cat exposure was most prominent in basolateral amygdala. As zif/268 mRNA is usually markedly reduced at 120 min post induction, such prolonged expression may be indicative of secondary neuronal activation. After five days of cat exposure zif/268 mRNA expression in basolateral amygdala was not reliably enhanced, suggesting habituation or reduced duration of the response seen after a single exposure. Behavioral habituation did not occur over the same regimen, suggesting either that there is not a one-to-one relationship between zif/268 mRNA expression in basolateral amygdala and behavioral response to the cat, or, that the zif/268 response became more restricted to the actual period of cat exposure over repeated sessions.

Supported by: NSF IBN-11349 and NIH RRO 8125.

474.12

NEUROBIOLOGICAL CORRELATES OF POST-TRAUMATIC STRESS DISORDER. JD Lewine*, J Canive, WW Orrison Jr., JT Davis, JC Edgar, SL Provencal, B Roberts, R Escalona, and D Graeber. The New Mexico Institute of Neuroimaging, The New Mexico Regional Federal Medical Center, 2100 Ridgecrest Dr, SE, Albuquerque, NM, 87111

Post-traumatic stress disorder (PTSD) is a condition precipitated by an unusually threatening event such as combat, torture, or rape. PTSD is unique among psychiatric disorders because the percipitating event can be clearly identified. The core features of PTSD include: (1) re-experiencing the traumatic event, (2) persistent avoidance of trauma-related stimuli or a general numbing of responsiveness, and (3) chronic and persistent symptoms of increased physiological arousal. PTSD is typically conceptualized as a psychogenic condition, but recent data suggest identifiable neurobiological correlates, and perhaps substrates of the condition. In the present study, brain structure and function were evaluated in 42 combat veterans with a diagnosis of PTSD and no history of head trauma or other neurological conditions. Twenty control subjects without PTSD were also evaluated. Eleven PTSD patients showed abnormal cortical atrophy or white matter lesions on magnetic resonance (MRI) examination, whereas none of the control subjects had abnormalities. Electroencephalography and magnetoencephalography were used to examine the relationship between sound intensity and the magnitude of the 200 millisecond component of the auditory evoked response. For control subjects, 19 of 20 showed augmentation of the brain response as a function of sound intensity. The other control subject showed a reducing pattern with response amplitude to the loudest sound being less than that found for intermediate intensity sounds. Only 14 of the 42 PTSD patients showed the normal augmentation pattern. Eight PTSD patients had poor responses at all sound intensities, and twenty subjects were reducers. These data indicate that many PTSD patients have specific neurobiological abnormalities.

Supported in part by grants from NARSAD and Picker International.

VISUAL CORTEX: EXTRASTRIATE—ATTENTION

475.1

TASK-RELATED MODULATION OF NEURONAL RESPONSES IN VISUAL CORTEX OF THE MACAQUE. A.E. Ipata, G. di Pellegrino, G. Gervasoni and L. Chelazzi *. Department of Neurological and Visual Sciences, Section of Physiology, University of Verona and Department of Psychology, University of Bologna, Italy.

Dominant views maintain that visual attention either selects input from a restricted region of space (space-based theories) or individual objects in the scene (object-based theories). The present single-unit recording study tested the hypothesis that visual attention can also lead to selective processing of individual features presented within a single spot of the visual field and belonging to the same object. A single colored, oriented bar (whose pair of features resulted from all possible combinations of 4 colors and 4 orientations) was presented inside the receptive field (RF) of neurons at the V1/V2 border and in area V4 of one macaque, while the animal had to discriminate either feature of the stimulus in different trials. 18 out of 45 cells at the V1/V2 border and 37 out of 63 cells in area V4, selective for color or orientation, or for both, responded differently to the same stimulus according to which feature the animal was required to discriminate. This effect was often specific to one of the colors and/or orientations that drove the neurons. Also, 17 out of 45 cells at the V1/V2 border and 20 out of 63 cells in area V4 varied their baseline firing rate depending on the task. In some neurons we also measured visual responses in a control condition where the animal had to detect a luminance change of the fixation point. 29 out of 38 cells at the V1/V2 border and 28 out of 31 cells in area V4 showed variation of the response to the RF stimuli in the control task relative to either or both peripheral discrimination tasks. These results support either a hierarchical model of selective attention whereby attention can in turn be directed to a certain region of space, then to an individual object within this region and finally to one of its constituent features, or a dynamic model whereby selective processing can be devoted to any kind of representation (spatial regions, integrated objects or individual features) according to current behavioral goals. *Supported by MURST and CNR.*

475.2

ATTENTION ENHANCES NEURONAL RESPONSES WITHOUT ALTERING ORIENTATION SELECTIVITY IN MACAQUE AREA V4. C.J. McAdams* and J.H.R. Maunsell Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

We recorded from individual neurons in area V4 of a macaque monkey while he performed a delayed-match-to-sample task designed to reveal the effects of attention on orientation selectivity and response variability. Two stimuli were presented on each trial, one inside the receptive field and one outside. In the "attend" mode, the animal was required to signal changes in the orientation of the receptive field stimulus. In the "no-attend" mode the animal signalled changes in the color of the other stimulus. By recording responses elicited by sample stimuli of different orientations presented in the receptive field, we obtained two orientation tuning functions for each neuron under conditions of identical visual stimulation: one from the attend mode and the other from the no-attend mode. Most neurons (135/185) had orientation tuning curves for both conditions that were well fit by Gaussian functions. For 73% of these cells, the amplitude of the attend tuning function was greater than that of the no-attend tuning function (median +20.8%, n = 135). In contrast, attention did not sharpen the tuning (median 0.6% narrower). Attention did not significantly alter the function relating the mean rate of firing to its variance (t-test, $p > 0.25$). Because noise does not increase proportionally with the firing rate of cortical neurons, attention, merely by amplifying the responses to oriented stimuli, improved the theoretical discriminability of those stimuli (median change in d' -2.7°).

Thus, attention enhances responses without altering the underlying orientation selectivity or the inherent variability of neurons in area V4.

Supported by NIH EY RO1-05911, T32-07001

475.3

ATTENTION INCREASES CONTRAST SENSITIVITY OF CELLS IN MACAQUE AREA V4. J.Reynolds*, T.Pasternak, and R.Desimone. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892 and Center for Visual Science, University of Rochester.

When two or more stimuli compete within a cell's receptive field, responses are determined by the attended stimulus. Such effects may be the result of an increase in sensitivity to the attended stimulus, which biases the competition between stimuli in its favor. To test for an increase in sensitivity, we placed a single patch of grating within the receptive fields (RF) of V4 neurons and measured responses to sequences of gratings over a range of contrasts. The monkey's task was to indicate when a target grating, which differed from non-target gratings in shape and orientation, appeared in the sequence. One sequence of gratings appeared within the RF, and a second, simultaneous, sequence of gratings appeared at a distant location outside the RF. The monkey's attention was manipulated by cueing it to perform the task at one location or the other.

With attention directed away from the RF, responses of cells increased with stimulus contrast, reaching saturation at higher contrasts (>20-40%). When the monkey attended to the RF, responses to low contrast gratings were typically stronger (17% increase). There was an increase in cells' contrast sensitivity with attention, which was on the order of 30% and was observed in over 70% of recorded neurons. The effects of attention diminished as contrast was increased, with, on average, no effect at the highest contrast. At all contrast levels, the spontaneous firing rate of the cells was higher when attention was directed to the RF. We interpret the boost in sensitivity and the increased baseline activity as evidence of a bias given to cells processing attended stimuli.

Supported by NIMH-IRP, NEI EY06175, and The McDonnell-Pew Foundation.

475.4

ATTENTIONAL MODULATION MAY UNDERLY SHIFT-INVARIANT VISUAL RESPONSES. E. Salinas*, and L.F. Abbott. Volen Center, Brandeis University, Waltham, MA 02254.

Recent neurophysiological results (Connor et al., J. Neurophysiol. 75:1306. 1996) indicate that some neurons in area V4 possess attention fields that are spatially localized but do not necessarily overlap with their classical receptive fields. Because attention modulates the amplitude of the visual responses, the firing rates of these neurons can be modeled as a product of two functions: one that depends on the location and type of visual stimulus, and another that depends on the distance between the current location of the attentional focus and the preferred attentional location for the cell, which is at the center of its attention field. Using theoretical calculations and computer simulations, we show that an array of neurons including different combinations of receptive field and attention field centers can serve as a basis set from which selective responses that are invariant with respect to shifts in the stimulus location can be constructed.

We model an array of neurons with filters similar to those of simple cells, but whose responses are also modulated in a multiplicative way by attention. These cells respond to presented images, and their activity drives an output neuron through synaptic connections. When the synaptic connections satisfy a simple mathematical condition, the output unit responds maximally to a preferred image appearing at the same position as the focus of attention. If the attentional focus is shifted, the location at which the preferred image elicits a maximum response shifts as well, to the current locus of attention. This mechanism does not interfere with the selectivity of the output neuron; its preferred image can be arbitrarily complex.

Thus, cells modulated by attention can drive downstream neurons with translation-invariant responses that can be highly selective for complex visual patterns, e.g., faces (Hasselmo et al., Exp. Brain Res. 75:417, 1989). Supported by NSF-DMS9503261 (L. F. A.) and the Conacyt-Fulbright-IIE program (E.S.).

475.5

MODULATION OF REAFFERENT VISUAL RESPONSES IN MONKEY LATERAL INTRA-PARIETAL AREA (LIP) J. Gottlieb*and M. E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

In natural environments multiple stable visual stimuli are brought in and out of view by saccades. We designed a "stable array task" to mimic such an environment, and used it to study visual LIP neurons that had delay-period responses on standard delayed-saccade tasks (Kusunoki and Goldberg, Soc. Neurosci. Abstr. '95).

In the stable array task a circular 8-element array appeared once and remained on the screen for long blocks of trials (> 10 min). A peripheral fixation target appeared on each trial, from which the monkey made visually-guided saccades to the center of the screen, bringing at least one array element (a "RF stimulus") into the neurons' receptive field (RF). Neurons had no reafferent responses in this condition, regardless of initial fixation position. To examine whether this finding was due to the permanence of the array elements we removed one RF stimulus from the initial array, and turned it on at the onset, and off after the end of each trial. Stimulus onset was 0.5-3 s before the saccade, always outside the RF. Neurons had strong reafferent responses to these newly-appeared, though familiar, stimuli. Responses occurred whether or not the monkey made unrewarded saccades to the stimuli.

We next tested whether stable RF stimuli preselected as saccade targets elicited reafferent responses. We used the 8-element stable array and briefly flashed a cue on each trial during peripheral fixation (outside of the RF). After the initial centripetal saccade the monkey made a second saccade to the array element matching the cue. LIP neurons responded only when the second saccade was directed to a RF stimulus. Responses usually preceded the first saccade (always out of the RF) and lasted through the second (into the RF). We interpret them as predictive visual responses occurring only when a target for a subsequent saccade was brought into the RF. Reafferent visual responses in LIP are thus strongly gated by the behavioral significance and salience of stimuli. Supported by the National Eye Institute.

475.6

CODING THE INTENTION FOR AN EYE OR ARM MOVEMENT IN POSTERIOR PARIETAL CORTEX OF MONKEY.

L.H. Snyder*, A. Batista, R.A. Andersen, Division of Biology, Caltech, Pasadena, CA.

Visual targets elicit responses in the lateral intraparietal area (LIP) and 7a of posterior parietal cortex. This activity may persist after target removal, especially if the animal plans an eye movement to the location of the extinguished target. This may reflect **attention** to that point in space, or an **intention** to move toward that location. Because we often look at or reach towards what interests us, neural correlates of spatial attention and movement intention can be difficult to distinguish. We trained animals to make either eye or arm movements to the same spatial location, reasoning that intention but not attention would depend on movement type.

Peripheral red or green targets were flashed (150 ms) while animals fixated and pointed at a central target. The peripheral cue instructed the type (red=saccade, green=reach) and direction of the upcoming movement. The movement itself was **delayed** until after fixation light offset, which occurred 1-1.6 s later. Neuronal activity depended on intended movement in 62% of 314 cells active during and immediately following cue presentation (100-300 ms after cue onset). In these cells, significant responses were obtained only prior to one of the two types of movement. Delay activity depended on movement intention to an even greater extent (83% of 276 cells active 150-600 ms after cue offset). In LIP, most cells preferred intended saccades, while in a region medial and posterior to LIP, most cells preferred intended reaches. These results show that, in a delayed movement task, the activity of many parietal neurons reflects movement intention.

Support: The Della Martin Foundation, Sloan Foundation, National Institutes of Health, and Office of Naval Research

475.7

NEURONAL RESPONSES IN AREA 7a TO STIMULI THAT ATTRACT ATTENTION. C. Constantinidis* and M.A. Steinmetz Krieger Mind/Brain Institute and Department of Neuroscience, Johns Hopkins Univ., Baltimore, MD 21218.

Neuronal responses were recorded from posterior parietal area 7a of monkeys performing a spatial version of a delayed match-to-sample task. The monkeys were required to maintain fixation while a series of stimuli were presented and to release a behavioral bar when a match stimulus appeared at the same location as the initial stimulus of the series (cue). The cue was either a single square stimulus or a salient stimulus in a 3x3 grid of squares (distractors) centered 25° apart. Salient stimuli differed in color from the identical distractors. The monkeys were trained to remember the location of the cue and to release the bar when a subsequent stimulus appeared at the cued location.

The mean firing rate in 74% (153/207) of the neurons recorded was significantly different depending on whether an element of a cue array presented inside the receptive field was salient. A transient response to a distractor was observed in neurons with short response latencies but was completely absent from neurons with longer latencies. The population response to a salient stimulus, as judged by the average PST histogram, became significantly larger than the response to a distractor 120 ms after the onset of the cue array (t-test, $p<0.05$). Presentation of arrays consisting of 9 identical elements and lacking a salient stimulus to attract attention evoked weak or no responses.

Our previous studies have shown that responses to a match stimulus are suppressed relative to a nonmatch stimulus presented at the same location. We have now observed that the magnitude of this suppression was similar following single and array cues. Responses to a nonmatch stimulus were not suppressed when it followed a cue array even though the nonmatch stimulus appeared at the same location as a distractor element in the array. Our results suggest that area 7a neurons encode the locations of salient stimuli in complex visual scenes, possibly for redirecting visual attention. *Supported by NIH EY09129.*

475.8

PET AND ERP STUDIES OF COMPLEX FORM ANALYSIS AND LUMINANCE DETECTION DURING SPATIAL SELECTIVE ATTENTION G.R. Mangun*[1,2] J.B. Hopfinger[1,2] C. Kussmaul[1,3] E. Fletcher[1,3] H.J. Heinze[4]. Center for Neuroscience[1] & Depts. of Psychology[2] & Computer Sci.[3], University of California, Davis CA 95616; Department of Clinical Neurophysiology, Otto-v-Guericke University, D-39120 Magdeburg, FRG[4].

Previously, we combined positron emmision tomography (PET) and event-related potentials (ERPs) to demonstrate that selective attention to a location in the visual field produced activations in human extrastriate cortex (fusiform gyrus) contralateral to the attended hemifield at a latency of 80 msec (Heinze et al., 1994). Here, using the 15O-water PET method and 3-D acquisition, we replicate and extend these findings by showing that attentional activations at the border of the fusiform and lingual gyri are primarily dependent on spatial selective attention. There was, however, a slight non-significant tendency for the fusiform activity and the P1 component of the ERP to be greater in magnitude during form discrimination (symbol matching) as compared to luminance detection (illuminated pixel present or absent) -- we believe that the more demanding perceptual requirements in the form discrimination task induced the subjects to allocate more attention. In addition, in the present data, there was also activation in the middle occipital gyrus contralateral to the attended location. Ninety-channel ERP recordings showed that the contralateral P1 attention effect showed similar (minor) amplitude changes as did the PET activity, as a function of task (form versus luminance). The present findings significantly strengthen our hypothesis of close associations between early ERP attention effects and neural activity in the human extrastriate cortex. Specifically, they identify an early, spatially-defined selection process that acts to modulate signals from attended regions of the visual scene. [This work was supported by NSF, NIMH, NINDS, the James S. McDonnell Foundation, and a UC Faculty Research Grant].

475.9

DISTINGUISHING CORTICAL AREAS INVOLVED IN VISUAL PROCESSING VERSUS TASK CONTROL USING FMRI E.A. DeYoe*, P. Schmit, J. Kummer & J. Neitz, Dept. Cell. Biology, Medical Col. Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226 (deyoe@post.its.mcw.edu)

Performance of a visual discrimination task involves not only the analysis of the stimulus and selection of a response but also control of attention and other factors that direct the execution of the task. We used functional MRI (FMRI) to distinguish cortical areas having correspondingly different functions. During echo-planar FMRI, each of 4 subjects observed a central fixation point while detecting 3 groups of targets superimposed on a surrounding checkered annulus. During each individual trial, the targets appeared for only 0.5 s. thereby forcing the subject to maintain fixation to perform correctly. Subjects indicated if all 3 groups contained the same or a different number of targets. Within each scan series, the discrimination trials were presented at four different rates: 0, 0.21, 0.75, and 1.21 presentations per second. In occipital visual areas, the FMRI response amplitude increased as the trial presentation rate increased. However, the slope of the response function varied from voxel to voxel. In contrast to occipital cortex, responses in superior parietal and dorsolateral frontal cortex remained relatively constant in amplitude as the trial presentation rate increased. These areas were also poorly activated during passive viewing of the display. Together our results suggest that at least some areas of superior parietal and frontal cortex are largely unaffected by the visual processing "load" (trials per block). Since they are nevertheless strongly activated during task performance, their function may be related to setting up and controlling task execution rather than performing visual computations *per se*. This would be consistent with proposals that superior parietal and dorsal frontal cortex are involved in the control of spatial attention. This approach may provide a novel criterion for identifying areas performing so called "executive" functions. Supported by NIH EY10244.

475.10

FMRI OF HUMAN PARIETAL AND OCCIPITAL AREAS FOR PROCESSING VISUAL MOTION AND THEIR GRADED MODULATION BY SPATIAL AND FEATURAL ATTENTION M. S. Beauchamp* and E. A. DeYoe Graduate Program in Neuroscience, University of California, San Diego(mbeauchamp@ucsd.edu) and Dept. Cell Biology, Medical College of Wisconsin, Milwaukee, WI 53226

Subjects responded to visual stimuli under different attentional conditions while whole brain FMRI was used to record the response of parietal and occipital motion-processing areas. In the baseline condition, subjects' spatial and featural attention was directed away from a motion-containing stimulus. Shifting spatial attention to the motion stimulus without featural attention produced a larger response, while a combination of spatial attention and attention to motion resulted in the largest response.

In the first experiment, the visual task consisted of luminance discriminations made at the center of the display, focusing the subjects' spatial attention on the fixation point and their featural attention on luminance. The visual stimulus consisted of a fixation point with luminance increments/decrements and an annulus consisting of coherently moving points on a background of incoherently moving points, previously demonstrated to activate motion areas. Visual areas located in lateral occipital cortex, the location of human area MT and associated visual areas (hMT+) responded with an average signal change of 2.52 MR intensity units, with an average volume of 394 mm^3 activated cortex. Parietal visual areas totaling 2220 mm^3 responded with a signal change of 2.46 MR units. In the second experiment, subjects fixated at the center of the display but attended to the color of points in an annulus defined by motion and color. hMT+ response increased 35% over experiment 1 to 3.41 MR units, with volume increasing 64% to 647 mm^3. The response of parietal areas increased 122% to 5.46 units with volume increase of 23% to 2730 mm^3. In the third experiment subjects performed speed discriminations, focusing both their spatial and featural attention on the motion stimulus. hMT+ response increased 105% over baseline to 5.16 units, with volume increasing 393% to 1940 mm^3 while parietal intensity increased 202% to 7.44 units with a volume increase of 109% to 4640 mm^3. Supported by an HHMI Fellowship to MSB and NIH RO1-EY10244 to EAD.

475.11

AN IMPAIRMENT OF THE TEMPORAL DYNAMICS OF ATTENTION IN VISUOSPATIAL NEGLECT. M. HUSAIN*, K. SHAPIRO, J. MARTIN and C. KENNARD, Dept. of Clinical Neuroscience, Charing Cross Hospital, University of London, U.K. and School of Psychology, University of Wales, Bangor, U.K.

Identification of a briefly-presented visual stimulus is associated with an impairment in the ability of normal subjects to detect a second stimulus if it appears within 500 ms of the first. This phenomenon has been termed the attentional blink or dwell time. We examined the temporal dynamics of visual attention in a group of seven patients (mean age = 66) with right hemisphere lesions and left-sided visual neglect. In these experiments all stimuli were presented at the same spatial location.

Subjects were asked to view a rapid serial visual presentation (RSVP) in which a stream of letters was displayed successively at fixation. Each letter was presented for 131 ms with an interstimulus interval of 49 ms, yielding a presentation rate of 5.5 items/sec. In each RSVP stream all the letters were black except one which was white (target 1 or T1). T1 occurred 8-16 items after the onset of the stream. On half the trials, a black X (target 2 or T2) was presented at some point, varying randomly from trial to trial, in the ten-item letter stream that followed T1. On the other half of trials, there was no T2.

All subjects were tested under two conditions in which they were given different instructions. In the single-target task, they were asked to say whether thay had detected a T2. In the dual-target task, they were asked to identify the T1 *and* also say whether they had detected a T2. On the single-target task, neglect patients detected T2 on 80-90% of occasions it was presented, irrespective of where it was in the RSVP stream. On the dual target-task, the probability of identifying T1 was 0.71. However, when patients correctly identified T1, their ability to detect a subsequent T2 was significantly impaired (42-67% detection) for >1600 ms afterwards. Age-matched control subjects showed a normal attentional blink which was significantly less protracted and less profound than patients with neglect.

This investigation demonstrates there is an impairment of temporal as well as spatial components of attention in visual neglect. The implications for models of visual attention and neglect will be discussed.

(Supported by a grant from the Wellcome Trust).

475.12

MACAQUE SUPPLEMENTARY EYE FIELD: ENHANCED ACTIVITY DURING EYE MOVEMENTS SELECTED ON THE BASIS OF AN ARBITRARY ASSOCIATION VS. A NATURAL SPATIAL RULE. C.R. Olson* and S.N. Gettner. Dental School, University of Maryland, Baltimore, MD 21201.

Frontal cortex may be selectively involved in generating movements under conditions when the pairing between stimuli and responses is learned rather than hardwired. To test this idea, we recorded from 315 neurons in the supplementary eye field (SEF) of monkeys performing an oculomotor task in which, on interleaved trials, targets were selected either on the basis of a natural spatial rule (go to the target on which the cue flashed) or on the basis of an arbitrary learned association (go to the target associated with the pattern of the foveally presented cue). SEF neurons fired robustly during the delay between the cue and the signal to respond and were selective for the direction of the impending eye movement. Around half of task-related neurons fired differentially as a function of the rule guiding response selection, with a majority favoring the 'pattern' condition. Even in a variant of the task where 'pattern' and 'spatial' cues were presented at identical eccentric retinal locations, a majority of differentially active neurons favored the 'pattern' condition. We conclude (1) that partially non-overlapping populations of SEF neurons mediate the selection of eye movements according to 'spatial' and 'pattern' rules and (2) that activity in the SEF is greater overall when targets are selected on the basis of arbitrary learned associations than when they are selected on the basis of a natural spatial rule.

NIH (RO1 NS27287); NIH (NRSA 1 F32 NS09452)

475.13

NEURONAL ACTIVITY IN MACAQUE SUPPLEMENTARY EYE FIELD IS ENHANCED UNDER CONDITIONS OF STIMULUS-RESPONSE INCOMPATIBILITY. S.N. Gettner* and C.R. Olson. Dental School, University of Maryland, Baltimore, MD 21201.

Human imaging and lesion studies have suggested that frontal cortex is selectively involved in stimulus-response tasks in which stimulus and response are incompatible, as when a cue is presented at a different location from the response target. To characterize neural activity under such conditions, we recorded from the supplementary eye field (SEF) while monkeys performed a task in which the color of a cue (red, green, blue or magenta) indicated which of four targets (right, up, left or down) should be selected for an eye movement. A cue of any color could be presented in superimposition on any of the targets. Conditions could either be 'congruent' - cue location identical to target location - or 'incongruent' - cue location different from target location. We found that neurons were direction selective, and that during the delay period there was a smooth transition from activity reflecting the location of the cue to activity reflecting the direction of the response. In around half of the sampled neuronal population, the mean firing rate during the delay period depended significantly on the type of trial (congruent vs. incongruent). A large majority of neurons exhibiting dependence on trial-type fired more strongly during incongruent trials than during congruent trials. We conclude that the SEF is particularly involved in generating eye movements under conditions of stimulus-response incompatibility.

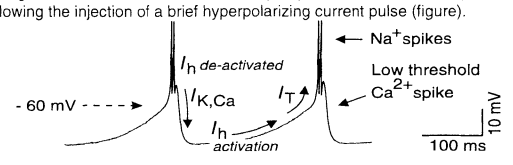
NIH (RO1 NS27287); NIH (NRSA 1 F32 NS09452)

CEREBELLUM: BASAL GANGLIA

476.1

MODULATION OF OSCILLATION IN INFERIOR OLIVARY NEURONS BY A HYPERPOLARIZATION-ACTIVATED CATION CURRENT (I_h). T. Bal* and D.A. McCormick Section of Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510.

Intracellular recordings of guinea pig or ferret inferior olivary neurons (IO) in vitro revealed that these neurons could generate sustained (up to 20s) endogenous 4-8 Hz oscillations at hyperpolarized membrane potentials following the injection of a brief hyperpolarizing current pulse (figure).



Cesium, a specific blocker of I_h when applied extracellularly, had several effects that were indicative of a role for I_h in the generation of oscillation in IO neurons. We propose that I_h contributes to the determination of the resting membrane potential such that reduction of I_h results in hyperpolarization and an increased propensity of oscillation through removal of inactivation of the low threshold Ca^{2+} current (I_T). In this manner extracellular application of Cs^+ resulted in marked "ensemble oscillations" in the inferior olive. In single cell oscillations, I_h contributes to the generation of the afterhyperpolarization through its *de-activation* during the Ca^{2+} spike and its *activation* during the afterhyperpolarization (generated by an apamin-sensitive $I_{K,Ca}$). Thus, I_h contributes to the "pacemaker potential" that times the occurrence of Ca^{2+} spikes in IO cells. Supported by N.I.H..

476.2

WHAT IS THE ROLE OF THE INFERIOR OLIVE IN OCULOMOTOR FUNCTION? E. Marsh* and R. Baker. Department of Physiology and Neuroscience, New York University Medical Center, New York, NY 10016

Climbing fiber activity was analyzed in the goldfish vestibulocerebellum in order to establish a role for the inferior olive (IO) during optokinetic (OKR) and vestibular (VOR) performance and adaptive plasticity. Two prevailing viewpoints, largely based on the presence of error signals and rhythmicity, argue that the IO acts as either a "teacher" or "clock" by directly adapting or commanding Purkinje cell output; however our data do not support either hypothesis. Recording of complex spike activity revealed spontaneous IO discharge to be random and modulation during OKR and VOR to be directionally selective, associated with retinal slip, and independent of Purkinje cell type as determined by simple spike eye movement sensitivity. These IO characteristics remained unchanged throughout either OKR or VOR adaptation. IO sensitivity therefore appears to be independent of any gain state and/or operation in the oculomotor system. After selective IO lesions, as later confirmed with biocytin labeling, OKR and VOR performance and adaptive plasticity were still intact; however, eye position records exhibited a distinct tremor independent of any oculomotor task in either the light or dark. Power spectrum analysis revealed a frequency range from 2-6Hz with eye velocity peaks clustered around 2-4°/s. Lesions that included the mossy fiber projection from Area II to the cerebellum also removed velocity storage and most of the adaptive plasticity. These findings demonstrate that mossy fiber pathways alone, through the cerebellum, are sufficient to produce robust oculomotor plasticity thereby discounting a teaching role for the IO. The lack of complex spike rhythmicity also does not support the IO acting as a "clock" that directly gates cerebellar Purkinje cell output. On the other hand, the eye movement tremor observed after IO lesion may be in agreement with some role in timing. Collectively, these results suggest an alternative hypothesis arguing that IO properties are best designed to regulate intrinsic cerebellar cortical operations by influencing both the mossy-parallel fiber and the inhibitory interneuronal loops that affect the level of activity or 'set point' of Purkinje cell output. Therefore, by acting not only directly on Purkinje cells, but also on the intracerebellar gain loops, the IO controls the dynamics of various mossy fiber loops and hence only indirectly commands oculomotor system function. (NIH NS13742)

476.3

THE ROLE OF THE INTERPOSITUS NUCLEUS IN SACCADES IS DIFFERENT FROM THE ROLE OF THE FASTIGIAL NUCLEUS. F.R. Robinson*[1], A. F. Fuchs[1], A. Straube[2], and S. Watanabe[3]. [1]Dept. Physiol. and Biophys., Univ. of Washington, Seattle, WA 98195, [2]Abteilung Neurologie, Klinikum Grosshadern, Marchioninistrasse 15, 81377 Munich, Germany, and [3]St. Marianna Univ. Sch. of Medicine, Kawasaki, Japan

The ventrolateral posterior interpositus nucleus (VPIN) of the monkey cerebellum contains a "distinct group" of neurons that discharge bursts of action potentials for saccades (Van Kan et al., '93). VPIN is anatomically separate from the saccade-related region in the caudal fastigial nucleus (CFN). Is its role in saccades also distinct?

Recordings show that, like CFN neurons, most VPIN cells (n=14) burst for nearly every saccade. However, unlike CFN bursts which often lead contralateral and lag ipsilateral saccades, VPIN bursts vary their latencies to lead upward (n=4) or downward (n=3) saccades depending on the neuron. Also unlike CFN neurons, some VPIN neurons show a reduction in firing rate late in saccades with a downward component. Finally, some VPIN neurons (n=5) show a burst late in the saccade that is non-directional and outlasts the end of the saccade.

Inactivating VPIN neurons unilaterally with muscimol makes upward saccades slightly too large but does not bend them toward the side of the injection like CFN inactivation does. Bilateral VPIN inactivation makes upward saccades more hypermetric (gain~1.5), downward saccades slightly hypometric (gain ~0.85) and makes horizontal saccades angle upward slightly.

Because saccade-related activity in the VPIN is different from that in the CFN and the consequences of VPIN inactivation are also different we conclude that VPIN function is different from that of the CFN.

Support: EY10578, EY00745 and the Heisenberg Stiftung

476.4

METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION IN CEREBELLAR PURKINJE CELLS AS SUBSTRATE FOR ADAPTIVE TIMING IN EYE BLINK CONDITIONING. D. Bullock*, J.C. Fiala and S. Grossberg. CNS Dept., Boston University, Boston, MA 02215.

To understand how the cerebellum adaptively times the classically conditioned nictitating membrane response (NMR), a mathematical model of the metabotropic glutamate receptor (mGluR) second messenger system in cerebellar Purkinje cells was constructed and simulated. In the model, a spectrum of slow responses, generated postsynaptically by mGluR-mediated phosphoinositide hydrolysis and calcium release from intracellular stores, bridges the interstimulus interval (ISI) between the onset of parallel fiber activity associated with the conditioned stimulus (CS) and climbing fiber activity associated with the unconditioned stimulus (US) onset. Temporal correlation of metabotropic responses and climbing fiber signals produces persistent phosphorylation of both AMPA receptors and Ca^{2+}-dependent K^+ channels. The former mediates long-term depression (LTD) of AMPA receptors; the latter enables CS-induced metabotropic activation to cause a timed reduction in membrane potentials and in the Purkinje population firing rate during the CS-US interval. The Purkinje cell firing decreases disinihibit cerebellar nuclear cells, whose excitatory response constitutes a learned movement command. Purkinje cell learning times this response, while nuclear cell learning can calibrate it. The model reproduces key features of the rabbit's conditioned eye blink response, the NMR: Purkinje cell population response is properly timed; delay conditioning occurs for ISIs of up to four seconds whereas trace conditioning occurs only at shorter ISIs; mixed training at two different ISIs produces a double-peaked response; and ISIs of 200-400ms produce maximal responding.

Supported by ONR N00014-92-J-1309 and N00014-95-10409.

476.5

A COMPUTER SIMULATION OF CALCIUM-INDUCED CALCIUM RELEASE IN A CEREBELLAR PURKINJE CELL

E. De Schutter* Born Bunge Foundation, Univ. of Antwerp, Belgium

Cerebellar Purkinje cells are known to contain extensive calcium stores covered with ryanodine receptors. The physiological role of these stores remains, however, unclear. Application of caffeine, causing activation of the ryanodine receptor, has been shown to result in distinct but rather unspecific changes in the firing pattern of cultured Purkinje cells (Brorson et al. *J. Neurosci.* **11**: 4024, 1991).

We incorporated calcium stores in a standard compartmental model of the Purkinje cell (De Schutter and Bower *J. Neurophysiol.* **71**: 375, 1994) which has been expanded to include detailed calcium dynamics. Calcium uptake and calcium-induced release were simulated with the steady-state equations of Goldbeter et al. (*PNAS* **87**: 1461, 1990) and 100 mM of the buffer protein calsequestrin was included in the stores. This model could replicate the results of Brorson et al. (1991). At low maximum rates CICR caused a transient interruption of bursting followed by slower period bursting. At higher rates simple spike firing was interrupted. These changes showed complex temporal dynamics and were due to the activation of dendritic K^+ channels.

This model of CICR is, however, not very robust because it depends on depletion of the stores to end release, a condition which can only be achieved if the release of bound calcium from calsequestrin is assumed to be very slow. Therefore we will introduce a gating model with inactivation of the ryanodine receptor channel into the Purkinje cell model.

Supported by NIMH MH52903 and NFWO (Belgium).

476.6

CEREBELLAR GRANULE CELL ACTIVITY CORRELATES WITH THE ACTIVITY IN PRIMARY SOMATOSENSORY CORTEX IN THE AWAKE, FREELY-MOVING RAT. M.J. Hartmann* and J.M. Bower, Division of Biology, Caltech, Pasadena, CA, 91125.

Our previous work in awake rats has shown that the cerebellar granule cell layer (GCL) of crus IIa responds to light tactile stimulation of perioral structures. We have recorded simultaneously from primary somatosensory cortex (S1, left hemisphere) and from the GCL of crus IIa (right hemisphere) in awake, freely-moving rats. During initial electrode implantation, we determined the perioral receptive field at each recording site, and later confirmed these fields in the awake rat. Behaviors were videotaped and synchronized with the neural signals.

We compared spatio-temporal patterns of activity in S1 and crus IIa during eating, grooming, drinking, and exploratory behaviors. We will present a video of synchronized neural and behavioral data, showing that cerebellar activity at each electrode site was largest when the rat's behavior resulted in tactile stimulation of the appropriate receptive field. In contrast, activity remained close to background levels during any movements that did not directly stimulate the receptive field (e.g. chewing). When receptive fields in S1 and the cerebellar GCL overlapped, multiunit responses in the GCL were temporally correlated with the multiunit responses in S1.

We carefully examined the GCL activity associated with rat drinking sequences. Each lick elicited highly correlated bursts of activity between S1 and cerebellum at the times when the tongue protruded and retracted past the respective perioral receptive fields. Our results demonstrate that GCL and S1 responses are temporally correlated in the awake animal, and that responses in the GCL of crus IIa are most directly related to tactile stimulation of perioral surfaces, not to motor activity in itself.

Supported in part by NIH grant GM07737-14 and HFSP.

476.7

THE ROLE OF GLUTAMATE IN D1 AGONIST INDUCED INCREASES OF SUBTHALAMIC NUCLEUS NEURONAL FIRING RATE: NORMAL VS. 6-OHDA LESIONED RATS. K.A. Allers[1]*, D.S. Kreiss[2], M.J. Twery[2], J.L. Juncos[1], and J.R. Walters[2]. [1]Dept. of Neurology, Emory University School of Medicine, Atlanta, GA 30322, and [2]Experimental Therapeutics Branch, NINDS/NIH, Bethesda, MD 20892.

Current models of the basal ganglia predict dopamine (DA) agonists decrease subthalamic nucleus (STN) firing via action in the striatum to disinhibit GP neurons allowing greater GABAergic inhibition of the STN. However, we have found that the D1 agonist SKF 38393 and the D1/D2 agonist apomorphine increase firing rate in the STN in normal rats. It has been suggested that local STN presynaptic D1 receptors on cortical glutamatergic afferents may contribute to these excitatory DA agonist effects. A role for glutamate was investigated using in vivo extracellular single unit recording in locally anesthetized rats. Naive and 6-OHDA lesioned animals were pretreated with the NMDA antagonist MK-801 (0.1mg/kg, i.v.) or the AMPA antagonist NBQX (3.7mg/kg, i.v.) followed by SKF 38393 (20mg/kg in naive, 10mg/kg in lesioned, i.v.). SKF 38393 administered alone increased STN firing by 224 ± 36% of basal rates (n=10) in naive rats and 245 ± 63% (n=8) in lesioned rats. Neither MK-801 or NBQX alone had a significant effect on the firing rate of STN neurons. The SKF 38393 induced increase was not attenuated by MK-801 pretreatment (264 ± 35%, n=6) in naive rats, but the increase was completely blocked by MK-801 in lesioned rats (90 ± 11%, n=10). In contrast, NBQX pretreatment significantly attenuated the SKF 38393 induced increase in firing rate in naive rats (149 ± 16%, n=11) but not in lesioned animals (151 ± 59%, n=6). These results suggest that 1) glutamate plays a role in mediating D1 agonist induced increases in STN firing rates; and 2) glutamatergic mechanisms/pathways mediating this effect are dramatically altered by lesion of DA neurons with NMDA receptors playing a more significant role in the lesioned rats. These results have implications for glutamate antagonist pharmacotherapy of Parkinson's disease.

(Funding: NRSA #MH11217-02, NINDS, Fortier and Comstock pvt. cont.)

476.8

ALTERATIONS IN GAD-65 mRNA LEVELS IN THE BASAL GANGLIA AFTER CHRONIC DOPAMINE AGONISTS IN 6-OHDA-LESIONED RATS. K.M.Merchant, L.M.Needham*, N.F.Nichols, D.L.Feldpausch, CNS Diseases Res., Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001.

The present study investigated alterations in the GABA synthetic enzyme, GAD-65, to examine the possibility that chronic intermittent treatment with D1-like and D2-like agonists produce differential neuroadaptive responses in the caudatoputamen (CP), globus pallidus (GP), and substantia nigra pars reticulata (SN-r). Compared to the sham-treated side, GAD-65 mRNA was increased significantly in the ipsilateral CP and SN-r, but was decreased in the ipsilateral GP. These data indicate that increases in striatopallidal GABA activity may contribute to the enhanced GABA output of the nigrothalamic neurons. Chronic treatment with the D1-like agonist (+)SKF 38393, D2-like agonist (-)quinpirole, D2-selective agonist U-95666A, as well as the indirect dopamine agonist L-dopa (plus benserazide) further increased GAD-65 mRNA levels in the lesioned CP compared to the unlesioned side. However, in the GP and the SN-r, the D2 agonists differed significantly from the other agents. Thus, both quinpirole and U-95666A reversed the asymmetry in GAD-65 mRNA levels in the GP and SN-r, SKF 38393 did not reverse the lesion-induced imbalance in either the GP or SN-r, and L-dopa reversed the asymmetry only in the SN-r. Interestingly, behavioral studies also distinguished the effects of the D2 agonists from the others. Thus, quinpirole and U-95666A produced sensitization in the turning rate, SKF 38393 induced tolerance, and L-dopa did not produce time-dependent change in turning. These data indicate that the rate of turning produced by chronic dopamine agonists may be related to their ability to alter the activity of selective GABA projections.

476.9

ULTRASTRUCTURAL LOCALIZATION OF GLUR1 GLUTAMATE RECEPTOR SUBUNIT IN STRIATAL PROJECTION NEURONS. C.L. Veenman, L. Medina, Q. Chen, and A. Reiner.* Anat. & Neurobiol., UT-Memphis, TN 38163; Behav. Biol., Fed. Inst. Technol., Zurich, CH 8603*.

LM immunohistochemical studies have revealed that GluR1 glutamate receptors subunits are sparse on the perikarya of striatal projection neurons in rats and pigeons, but abundant in neuropil. To determine if neuropil labeling for GluR1 reflects dendritic and/or spinous labeling of striatal projection neurons, we carried out LM and EM immunohistochemical studies. In LM studies in pigeon, we found that colchicine treatment led to abundant perikaryal labeling for GluR1 in striatal neurons with a medium spiny morphology. In EM immunohistochemical studies of GluR1 localization in pigeon and rat striatum, we found that GluR1 was abundant and common in the spines of striatal neurons, and also present in scattered dendrites. We quantified the relative abundance of GluR1 labeled structures in pigeon striatum. For comparison, we also quantified GluR2/3 subunit abundance, which appears to be found in the perikarya and dendrites of nearly all striatal projection neurons. At the EM level, we found that spines were labeled as frequently for GluR1 as for GluR2/3, but dendrites were only labeled 33% as commonly for GluR1. Thus, our study indicates that GluR1 and GluR2/3 glutamate receptor subunits commonly localize to the spines of striatal medium spiny neurons, but in contrast to GluR2/3, the GluR1 subunit seems to be much less common on dendrites and perikarya of these neurons. Supported by NS19620, NS28721 (A.R.)

476.10

EVIDENCE FOR SPECIFIC RETINOIC ACID-MEDIATED GENE REGULATION IN THE DEVELOPING AND ADULT BASAL GANGLIA: PRESENCE OF LIGAND, BINDING PROTEINS AND TRANSCRIPTION FACTORS. R.H. Zetterström*, L. Solomin, T. Perlmann, U. Eriksson, L. Olson. Dept. of Neuroscience, Karolinska Institute; and Ludwig Institute for Cancer Research, Stockholm Branch, S-171 77 Stockholm, Sweden.

Retinoic acid (RA) is the main active form of vitamin A. We have used a RA bioassay, in situ hybridization and immunohistochemistry to delineate the possible role of RA in the basal ganglia. RA levels are higher in striatum than several other areas of the developing and adult brain. The cellular retinol-binding protein CRBPI is expressed in patches in the developing striatum and at low levels in the adult; CRBPII is not expressed. The cellular RA-binding protein CRABP1 is strongly expressed in many medium spiny neurons of both the developing and adult striatum, while CRABPII expression is confined to large, presumably cholinergic, neurons in striatum. The RA receptor transcription factor RARα is weakly expressed during development but not in the adult, while RARβ is very strongly expressed in the developing striatum, with lower levels found in small neurons of the adult. RARγ is negative in the CNS. The RXR transcription factor RXRα is not clearly seen, while RXRβ has a general weak CNS expression. RXRγ is strongly expressed specifically in striatum and with a mediolateral gradient during development, while patchy in the adult. The transcription factor NGFI-B, member of a subgroup of the steroid hormone receptor superfamily which also includes NOR-I and NURR-1, can compete with RAR as a heterodimerization partner for RXR, allowing RXR to be responsive to 9-cis-RA. NGFI-B, is expressed in patches in neonatal striatum and in scattered cells in the adult. NOR-1 does not heterodimerize with RXR, but is strongly and distinctly expressed in the classical patch and marginal zone pattern during striatal development and in scattered cells of the adult. NURR-1 can also heterodimerize with RXR to render it 9-cis-RA-responsive, is not found in striatum, but is strongly expressed in the developing substantia nigra from E11.5 into adulthood. Our results demonstrate specific expression of key components of the retinoid system including binding proteins and transcription factors as well as RA itself in the basal ganglia, suggesting important gene-regulatory functions.

Supported by the Swedish MRC and USPHS grants.

476.11

AGE-RELATED DECLINE IN DOPAMINE TRANSPORTERS WITH [I-123]ß-CIT SPECT: ANALYSIS OF GENDER, STRIATAL SUBREGION, AND NEUROPSYCHOLOGICAL CORRELATES. C. H. van Dyck*, J. P. Seibyl, M. Laruelle, K. Sheff, K. L. Marek, S. S. Zoghbi, Y. Zea-Ponce, R. M. Baldwin, D. S. Charney, R. B. Innis, Yale University School of Medicine, New Haven, CT, 06520.

We have previously reported age-related decline in dopamine transporter (DAT) binding in human striatum with [^{123}I]2ß-carbomethoxy-3ß-(4-iodophenyl)tropane ([^{123}I]ß-CIT) single photon emission computed tomography (SPECT) (J Nucl Med 1995;36:1175). We extended this analysis in a larger sample to examine effects of gender and striatal subregion and to investigate neuropsychological correlates in the oldest subjects. [^{123}I]ß-CIT binding in striatum was studied in 72 healthy human subjects (40 M, 32 F) who ranged in age from 18 to 85 y. Following injection with [^{123}I]ß-CIT (mean ± SD = 6.2 ± 1.2 mCi), subjects were scanned with the brain dedicated Picker 3000 or 3000XP SPECT cameras. A reconstructed transaxial slice at the level of the striatum was used to determine tracer uptake in striatal and occipital regions of interest. We estimated the specific-to-nondisplaceable equilibrium partition coefficient, V_3", as (striatal - occipital)/occipital uptake approximately 18 to 24 h post injection. V_3" showed a significant inverse correlation with age (r = -0.78, $p < 0.0001$), declining by 61% over the age range studied or approximately 9% per decade. There were no differences in the rates of decline for males (64%) vs. females (59%) or caudate (65%) vs. putamen (59%). The laterality index was unaffected by age. In the oldest age group (69 to 85 y) V_3" was significantly correlated with simple reaction time (r = -0.46, $p < 0.01$) but not with finger tapping speed. These results confirm previous reports of DAT loss with aging and suggest that humans fail to show the gender differences predicted by animal studies. They further demonstrate that aging, unlike Parkinson's disease is associated with a relatively symmetric loss of DATs in caudate and putamen and in both hemispheres. Finally, they provide the first evidence for a relationship between nigrostriatal dopaminergic function and neuropsychological performance in normal aging. (Supported by AFAR.)

476.12

NITRIC OXIDE INCREASES GAP JUNCTION PERMEABILITY BETWEEN STRIATAL NEURONS. P. O'Donnell* and A.A. Grace. *Depts. Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

Several studies have established a role for nitric oxide (NO) in a diversity of brain functions. Among possible targets of NO are gap junctions, which have a distribution similar to that of NO synthase (NOS). The role of NO in regulating striatal neuronal gap junction permeability was assessed in a brain slice preparation by taking advantage of the ability of the fluorescent dye Lucifer yellow to cross gap junctions, resulting in the labeling of additional cells beyond the one injected (Figure). In control conditions, intracellular injection of Lucifer did not reveal any case of coupling in the dorsal striatum or accumbens core (0/11). Injections in the presence of the NO donor sodium nitroprusside (100 μM) resulted in a significantly higher incidence of dye coupling (6/10, 60%, p=0.004; Fisher exact test). Similarly, stimulation of cortical afferents by means of trains of constant current pulses delivered to corticostriatal fibers resulted in dye coupling in 56% of the cases tested (9/16; p=0.002). The role of NO in the cortical afferent-dependent increase was confirmed by repeating the cortical afferent stimulation experiments in the presence of the NOS inhibitor L-NAME (10-20 μM or 1 mM), which prevented the increase in coupling (0%, 0/13; p=0.001 compared to cortical afferent stimulation in physiological saline). Furthermore, the inactive enantiomer D-NAME failed to block the cortical afferent stimulation-mediated increase in coupling (43%; 6/14). These results suggest that activation of NO, either by exogenous administration or endogenously by activating cortical afferents, results in an increase in gap junction permeability between striatal medium spiny neurons. This action may result in control of afferent-regulated synchronization of striatal neuronal activity via NO-mediated activation of gap junctional conductance. Supported by MH45156, 01055 (AAG), Tourette's Syndrome Association, and a NARSAD Young Investigator Award (P.O'D.).

VISUAL SYSTEM: DEVELOPMENT II

477.1

PRENATAL DEVELOPMENT OF LAYER-SPECIFIC LOCAL CIRCUITS IN MACAQUE PRIMARY VISUAL CORTEX. E.M. Callaway*, A. Sawatari and A.K. Wiser, Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037.

We have studied the specificity of development of layer-specific local circuits in macaque primary visual cortex (V1). Several-hundred neurons were intracellularly labeled in living brain slices prepared from the primary visual cortex of 7 macaque monkeys aged 95 days post-conception (E95) to 2 days postnatal (P2). Axonal and dendritic arbors of labeled neurons were reconstructed to assess their relationships to the cortical layers. We were particularly interested in the development of specificity for "sublayers" dominated by input from magno- (M) or parvocellular (P) streams.

Previous studies have implicated activity independent mechanisms in the development of axonal arbors specific for the 4 main cortical layers - 2/3,4, 5 and 6. In macaque V1, layer 2/3 is subdivided into layers 2/3A, 3B, 4A, and 4B; while layer 4 is subdivided into 4Cα and 4Cβ. We refer to these laminar divisions as "sublayers". Individual neurons in mature animals have axons that are highly specific for these sublayers. Most notably, layer 4Cβ spiny stellate neurons have dense axonal arbors in layers 4A and 3B, but their axons pass unbranched through layer 4B. And layer 6 pyramidal neurons have axons that are specific for the α or β divisions of layer 4C.

We find that spiny neurons in layers 2-4B or layer 5 specifically target superficial and deep layers without forming "incorrect" branches in layer 4C. Similarly, layer 6 pyramidal neurons that target layer 4C do not form "incorrect" branches in layer 5. These results indicate that specific projections to the 4 main cortical layers develop with a high degree of selectivity, just as in other species. However, the development of sublayer-specific projections involves the rearrangement of initially imprecise projections. Most notably, axons of some layer 4Cβ spiny stellates branch in layer 4B at E122-E141. And at similar ages, pyramidal neurons in the upper half of layer 6 have axonal branches in 4Cα as well as 4Cβ, whereas these projections are specific for 4Cβ in more mature animals. There is similar exuberance in axonal arbors of other cell types and in the laminar branching patterns of dendrites. These observations suggest that sublayer-specific projections may utilize activity-dependent mechanisms to identify "correct" targets. Such cues could be provided by different patterns of spontaneous activity of M vs P geniculate afferents. These observations further suggest that the "extra" layers in primate V1 may arise from epigenetic influences.

This research is funded by NIH Grant #EY10742.

477.2

ANISOTROPIC GROWTH OF MACAQUE STRIATE CORTEX DURING THE FIRST MONTHS OF LIFE. G. Blasdel* D. Campbell, Department of Neurobiology, Harvard Medical School, Boston, MA 02115 and L. Kiorpes. Center of Neural Science, New York University, New York, NY 10003

The results of previous optical imaging experiments (Blasdel et al., 1995) suggested that striate cortex grows *anisotropically*, perpendicular to the ocular dominance columns of old world primates during the first weeks of life. Since these results were obtained from different animals, however, one could not rule out the possibility they resulted in part from individual variation. We have now overcome this uncertainty by recording from the same cortical regions sequentially during the first 2.5 months of life. In one animal, recorded at 15 and 75 days of age, the asymmetry of cortical growth was evident from vascular patterns, which expanded 12% and 3% parallel and perpendicular to the border between V1 and V2. Direct measurements of cortical magnification (Campbell and Blasdel, 1995) revealed corresponding changes of 11.2% and 3%, which caused magnification *anisotropy* to increase from 46% to 55% during the same period of time. One unexpected observation concerned the minimum spacing of visual stimuli that were effective at producing cortical maps of visual location. While this could be as small as 0.6° in 75 day old animals, it needed to be at least 1.5° earlier on, at 15 days of age, which suggests that the representation of adjacent spatial locations in separate cortical regions may undergo refinement during the first months of life. Supported by EY06586 and EY10862.

477.3

OPTICAL RECORDING OF FUNCTIONAL CONNECTIVITIES IN ORGANOTYPIC CULTURES OF RAT VISUAL CORTEX AND THALAMUS CONTAINING COMPETING AFFERENTS. Christian M. Müller*, Max-Planck-Institute for Developmental Biology, 72076 Tübingen, Germany

One feature of the visual system in mammals with frontal vision is a segregation of information processing of either eye at multiple levels along the visual pathway. This segregation occurs in an activity-dependent manner during development, largely due to spontaneous activity. In the present study we prepared organotypic cultures from embryonic thalamus and postnatal rat cortex grown at an air-medium interface. Either two thalamic explants or two cortical explants ('source cultures') were co-cultured with one visual cortical slice ('target culture'). Responses in the target cortex to electrical stimulation in the source-structures were visualized after 3 to 6 weeks in vitro using the voltage sensitive dye Di-4-ANNEPS and a 464 photodiode array.

In cortico-cortical cultures responses were most prominent in layers 2/3 of the target cortex. Response amplitudes decayed from close to the apposition site with the stimulated cortex but often propagated over the entire extent of the target cortex. There was no clear evidence for a segregation of functional domains preferentially activated by either of the two competing afferents. In thalamo-cortical cocultures the initial response was localized in layers 6 and 4 followed by a prominent, polysynaptic response in layers 2/3. These responses often revealed a patchy pattern along the extent of the target cortex. In cases where functional interactions between the source-thalami were weak, the two afferents could activate discrete, alternating patches in layers 2/3 of the target cortex. In contrast, when strong functional connectivities were found between the source-thalami, activity-patches were either absent or overlapping. The data suggest that spontaneous activity is not sufficient to drive a significant segregation of domains activated by competing afferents in cortico-cortical cocultures, but may do so in thalamo-cortical cocultures when the source-explants reveal only weak interconnectivities. Supported by the DFG (Mü-908-3-1) and the MPG

477.4

IMMUNOGLOBULINS IN THE DEVELOPING CEREBRAL CORTEX

J.A. Dunn*, E. M. Lukacsi and J.R. Naegele Department of Biology, Wesleyan University, Middletown, CT 06457-0170

Homotypic antisera against IgG have previously been reported to label the subplate zone in developing kitten, rat and mouse visual cortex (Dunn et al., 1995, *Cerebral Cortex* 5:494; Fairen et al., 1992, *NeuroReport* 10:881). The labeling is most intense perinatally then is lost with maturation. Biochemical analyses of perinatal rat cortex have attributed this staining to two polypeptides of MW 53 and 27 kDa (Upender and Naegele 1994, *Soc. Neur. Abstr.* 20:689). Until now, the identities of these cortical antigens were unknown. The 53 kDa polypeptide has now been purified from PND 2 rat cortical cytosol using a combination of differential centrifugation, DEAE affinity and immunoaffinity chromatography. Internal amino acid sequence analyses from multiple polypeptides generated by tryptic digests of the 53 kDa antigen identify it as IgG heavy chain. The largest sequenced polypeptide has 100% homology to residues 226-233 of the rat Ig Gamma 2a chain C region. The source of this IgG in the developing cortex is unresolved.

The pattern of IgG staining in mouse cortex is similar, but not identical, to IgG staining in rat and kitten cortex. IgG immunoreactive peptides have been identified in mouse cortical cytosol. *SCID* (Severe Combined Immune Deficiency) is a mouse mutation resulting in deficient IgG assembly. Although *SCID* serum contains IgG heavy chains, immunocytochemical and biochemical analyses show that *SCID* cortex does not. These data suggest neuronal uptake of IgG via a mechanism requiring intact molecules. Current studies are investigating the source of cortical IgG and comparing laminar and volumetric differences between normal and *SCID* mice. Supported by EY09749.

477.5

ENGULFMENT OF IgG CONTAINING NEURONS BY MACROPHAGES IN THE DEVELOPING BRAIN. M.B. Upender,* P.E. Leckman, and J.R. Naegele. Wesleyan University, Biology Dept., Middletown, CT 06459.

Microglia, or brain macrophages have long been recognized as an important cell type during brain development and during responses of the nervous system to injury. Macrophages are targeted to sites of naturally occurring cell death, retracting axons and degenerating glial scaffolds throughout normal brain development. The molecular signals that enable microglia to distinguish dying from living neurons and axons have not been identified, but it is likely that both cell surface and long-range signaling molecules will be involved. One candidate molecule is immunoglobulin or an IgG-like molecule, since IgG receptors facilitate removal of senescent cells by macrophages elsewhere in the body. Ig-immunoreactivity has been found within neuronal subpopulations in the ventricular, intermediate and subplate zones of the cerebral cortex, the ganglion cell layer of the retina and the deep nuclei of the cerebellum during development. This immunoreactivity declines during the second postnatal week and is undetectable in the adult (Upender and Naegele, Neurosci. Abst. 20, 689; Upender et. al., submitted). Each of the regions undergoes developmental cell death concurrent with the period when intraneuronal Ig-immunoreactivity is present. Recent evidence shows that this Ig-like immunoreactivity is due to uptake or synthesis of IgG in developing brain (Dunn et al., this years abstracts). The present study investigated the role of intraneuronal IgG in mediating microglial phagocytosis in several areas of the developing CNS in rodents. At early postnatal times, activated microglia were identified with the mAb ED-1 or GS-I isolectin B_4 and found closely apposed to Ig-immunoreactive neurons. At later times, Ig immunoreactivity was lost and resting microglia were distributed more uniformly throughout the brain. These observations suggest that macrophages engulf Ig-containing neurons leading to their disappreance. Intraneuronal IgG may serve to recruit and/or activate macrophages in regions of naturally-occurring neuronal death. Supported by NIH EY09749.

477.6

DEVELOPMENTAL PATTERNS OF ON/OFF RETINAL GANGLION CELL ACTIVITY LEAD TO SEGREGATION OF THEIR AFFERENTS UNDER A HEBBIAN SYNAPTIC RULE. C.W. Lee* and R.O.L Wong. Dept. of Anatomy and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

During the development of the ferret visual system, activity dependent synapse elimination leads to laminar segregation of ON and OFF-type retinal ganglion cell (RGC) afferents in the dorsal lateral geniculate nucleus (dLGN). Using calcium imaging and intracellular dye-filling techniques, we previously demonstrated that the ensemble firing patterns of morphologically identified ON and OFF RGCs undergo developmental changes during the segregation period. OFF RGCs exhibit higher burst frequencies than ON RGCs and the correlation between the bursts of ON and OFF RGCs is reduced during the segregation period. In an analysis of these patterns under a variety of linear Hebbian synaptic rules we show that the changes in the bursting patterns lead to ON/OFF segregation in the dLGN. We verify this analysis with computer simulations of synaptic development of RGC inputs to the dLGN using the experimentally recorded bursting patterns, and examine which parameters [e.g., the degree of competition among synapses] lead to segregation. Significantly, we show that the recorded bursting patterns do not support segregation under all Hebbian plasticity rules. For example, we demonstrate that the BCM model of synaptic plasticity does not lead to ON/OFF segregation in the dLGN (though it is important to note that the BCM was designed to model cortical, not LGN, plasticity). These results suggest that the bursting patterns of ON and OFF RGCs during the segregation period can provide the appropriate cues for refinement of their connections in the dLGN. Combining multi-neuronal recordings and computational modeling can thus reveal important insights into the processes of synaptic competition during development, which are not evident with each method alone. (Supported by NIH EY10699 and NIH NS30888)

477.7

THE DEVELOPMENT OF VISION IN THE ZEBRAFISH. S. S. Easter, Jr.* and G. N. Nicola, Dept. Biology, U. Michigan, Ann Arbor, MI 48109

We determined behaviorally when zebrafish (*Danio rerio*) begin to see, and correlated these observations with the development of the retinofugal projection, the retinal image , and the extraocular muscles.

Behaviors: Individual embryos and larvae, 48-96 h post fertilization (hpf), were videotaped, 10-20 naïve animals at each age. Visual startle response (VSR: body twitch) was evoked by a sudden decrease in light intensity, and indicated that the fish sensed light. The optokinetic response (OKR: tracking eye movements) was evoked by rotation of a striped drum, and indicated that the fish extracted and integrated information from the retinal image of the stripes. VSR developed over 68 - 79 hpf, many hours after the the onset of a touch-evoked startle response. Eyeless fish did not make a VSR, ruling out a role for a non-retinal light sense. OKR developed over 73 - 80 hpf, and was always in the direction of drum rotation, which suggests that the coding of the direction of image movement was hard-wired (*i. e.* not determined experientially). **Retinal Image**: The formation of the retinal image was studied in live melanin-deficient fish (reared in dilute phenylthiocarbamide), in which the absence of a pigmented retinal epithelium allowed direct visual access to the image formed by the lens. The image appeared early on the third day, but behind the plane of the photoreceptor outer segments; the eye was initially far-sighted. Gradually the focal length shortened and the best image was at the level of the photoreceptor outer segments by 96 hpf. **Extraocular Muscles**: They were examined immunocytochemically with light- and electron microscopy. They were absent at 66 hpf, evident but immature at 72 hpf, larger and more mature by 96 hpf.

Earlier work showed that retinal photosensitivity and most of the retinofugal projection were present prior to the onset of VSR. The later onset of the OKR is attributable to the delayed maturation of the extraocular muscles and the retinal image. (NIH grant EY-00168.)

477.8

GENETIC BACKGROUND AFFECTS THE OCULAR RETARDATION PHENOTYPE. M.H. Hankin*^, C. Bone-Larson^, S. Basu†, J.D. Radel‡, D. Green† and M. Burmeister†. ^Medical College of Ohio, Toledo, OH 43614, †Univ. Michigan, Ann Arbor, MI 48109, and ‡Univ. Kansas, Kansas City, KS 66160.

Ocular retardation (or^J), a recessive murine mutation caused by a null allele of the *Chx10* homeobox gene (Burmeister et al., 1996), is characterized by severe microphthalmia, a thin poorly laminated hypocellular retina lacking differentiated bipolar cells, optic nerve aplasia and blindness. In addition to or^J/+ mice with normal eyes, the F1 progeny of 129/Sv-or^J/or^J x *Mus musculus castaneous* (CASA/Rk) backcrossed to 129/Sv-or^J/or^J had eyes ranging in size from small to nearly normal, in addition. After selecting for large eye size for 4-6 generations, some of these or^J/or^J mice exhibited functional responses (ERG and pupillary constriction). This suggested that CASA/Rk genes were "modifying" the or^J phenotype. We show that a significant portion of the normal phenotype is recovered in these modified or^J mice, including: increased eye size, retinal thickness and lamination compared to or^J mice, and the presence of an optic nerve with centrally connecting ganglion cell axons. Immunohistochemical staining indicates the presence of poorly differentiated bipolar cells. Although the modified or^J retina is larger than seen in the original mutant strain (i.e., 129/Sv-or^J/or^J), the rate of retinal progenitor cell proliferation is not increased above the depressed levels seen in 129/Sv-or^J/or^J mice. These observations indicate that the CHX10 transcription factor acts on genes which regulate cell proliferation in the early developing retina. We hypothesize, therefore, that the persistence of retinal ganglion cells which are able to form connections with the brain provides trophic support which permits bipolar cell survival in the modified or^J retina. The incomplete differentiation of these bipolar cells, however, may indicate that the expression of CHX10 is essential for attaining and/or maintaining the mature bipolar cell phenotype. *Supported by NIH and the Ohio Lions Eye Research Foundation.*

477.9

DENDRITIC DEVELOPMENT OF PUTATIVE BLUE GANGLION CELLS IN THE *RHESUS MACAQUE*. M.D. Snyder and M.A. Kirby*. Departments of Pediatrics and Natural Science, Loma Linda University, Loma Linda, CA 92350.

We previously reported that the arbors of parasol cells that ramify in the middle layers of the inner plexiform layer (IPL) are transiently bistratified, becoming unistratified during late fetal periods. In this study we examined cells that ramify in the extreme inner and outer layers of the IPL (i.e., receiving S or blue wavelengths) and compared their dendritic growth to cells ramifying in the middle IPL layers.

Timed dated fetal monkeys were delivered by C-section, euthanized, and their retinas rapidly removed and placed into oxygenated media (Eagles, 37° C). Crystals of HRP were inserted into the retinas at various locations and allowed to incubate for 3 hrs. The retinas were then fixed with 2% gluteraldehyde in phosphate buffer (pH 7.4) and processed for HRP histochemistry using diaminobenzidine. Dendritic arbors were measured in wholemounts with quantification of the depth of each portion of the arbor in the IPL.

During mid-gestation ganglion cells with all or part of their arbor in the extreme inner or outer IPL (putative blue ganglion cells) formed four classes. The majority (58%) of putative blue cells were unistratified with their arbors ramifying either in the inner- or outer-most IPL. The second class (26%) corresponded to the adult small-bistratifed cells. These cells had bistratified arbors with one portion in the innermost IPL and the other ramifying in the middle IPL (cells of this class were also observed with portions of the arbor in the outer extreme IPL and the rest in the middle). The third class (13%) were bistratifed with one part of the arbor in the inner most layer of the IPL and the other in the outer most layer of the IPL. Lastly, 3% of putative blue cells were observed to have tri-stratified arbors. These cells had the majority of their dendritic arbor in the middle IPL layers with small portions ramifying the extreme inner and/or outer IPL. In conclusion, putative blue ganglion cells undergo similar development and precision of dendritic growth as other ganglion cells, with the exception of the rarely encountered tri-stratified cells.

477.10

BIOMECHANICAL SIMULATION OF FOVEAL DEVELOPMENT. A.D. SPRINGER*. Department of Cell Biology and Anatomy, New York Medical College, Valhalla, NY 10595.

The mechanisms that lead to the development of the human fovea are unknown. Retinal histogenesis is complete by the beginning of the third trimester. However, a fovea is not apparent at that time. A fovea appears gradually, and it is fully developed by five years of age. Over this time the eye enlarges considerably. Eye growth, in the absence of cell addition to the retina, probably causes the retina to be stretched very slowly over time. The role of considerable eye growth, and its effect on retinal stretching, are difficult to examine experimentally. However, the effect of retinal stretching on foveal development can be simulated mathematically using finite element analysis.

A model having a notch was used to simulate a foveal depression in the ganglion cell (GCL) and inner nuclear layers (INL). As the material was stretched in the X-plane, the notch lifted toward the vitreal surface. The effect of stretching the notched material on a simulated underlying cone photoreceptor cell layer (CCL) was then examined. Elastic trusses, that simulated cone cell axons, were used to link the overlying GCL+INL to the underlying CCL. Stretching both the overlying notched material and the underlying CCL caused the CCL to flow toward the center of the overlying notch in the GCL+INL.

Besides the movement of retinal ganglion cells and inner nuclear layer cells away from the foveal center and the movement of cone photoreceptor cells toward the foveal center, several other aspects of foveal development were simulated in the model. Without adding additional variables, the model was able also to simulate the elongation of foveolar cone photoreceptor cells and the axons of Henle. The simulations suggest that two variables play a significant role in development of the fovea. Eye enlargement generates a small, but sufficient, force that stretches the retina slowly over time. A perturbation in the homogeneous geometry of the GCL+INL, namely the foveal notch, determines the movement of the cone photoreceptor cells toward the foveola, and cone photoreceptor cell morphology.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY V

478.1

GLUTAMATE RECEPTOR ANTIBODIES DESTROY CORTICAL NEURONS BY A COMPLEMENT-DEPENDENT MECHANISM. M. Patel*, X.-P. He, S. Janumpalli, K. Whitney and J. O. McNamara. Depts. of Medicine (Neurology), Neurobiology, and Pharmacology, Duke University Medical Center, Durham, NC 27710.

Rasmussen's encephalitis (RE) is a rare disease of childhood characterized by progressive destruction of one cerebral hemisphere, severe epileptic seizures, hemiparesis, and ultimately dementia. We recently demonstrated that RE is an auto-immune disease and one of the autoantigens is glutamate receptor GluR3 (Rogers et al., *Science* 265:648-651, 1994). We postulated that the interaction of serum GluR antibodies (Abs) with GluRs in the CNS triggered seizures and death of neurons. To begin to test this idea, we engineered a glutathione-S-transferase GluR3 fusion protein (GSTGR3) that contains amino acid residues 246-455 of the N-terminal extracellular domain of rat GluR3. A subset of rabbits immunized with GSTGR3 but not GST developed epileptic seizures and a neurologic syndrome similar to that observed previously. We sought to determine whether GluR Abs destroyed neurons and if so, by what mechanism. Cell death was quantitated by measuring release of LDH from cortical neurons isolated from E18 rats and maintained in primary culture. Glutamate and other low MW substances were removed from plasma by filtration over a molecular sieve. Filtered plasma from GSTGR3-immunized rabbits (n=5) killed cortical neurons in a concentration-dependent manner whereas plasma from GST-immunized rabbits (n=4) did not. The mechanism of cell death was not excitotoxic because neither a competitive (CNQX) nor a noncompetitive (GYKI) antagonist of AMPA receptors blocked the cell death. Isolation of IgG from plasma by an affinity column disclosed that IgG was necessary but not sufficient for the cell death. Addition of a source of complement to the IgG destroyed neurons. Passage of plasma over a GSTGR3 affinity column selectively removed GluR Abs and eliminated the cell death-inducing effects of the GSTGR3 plasma, whereas passage over a GST affinity column failed to remove GluR Abs or eliminate the cell death. We conclude that circulating GluR Abs can destroy cortical neurons by a complement-dependent mechanism. We suggest that one way by which circulating GluR Abs in patients with RE may contribute to neural injury and seizures is by destroying cortical neurons through a complement-dependent mechanism.

Supported by NS 17771 from NINDS.

478.2

FORMATION AND COMPOSITION OF IBOTENIC ACID INDUCED CALCIFICATIONS IN RAT SUBSTANTIA NIGRA. C. Nitsch*, G. Herrmann and A. L. Scotti. Inst. of Anatomy of the University Basel, 4056 Basel, Switzerland.

The neurotoxic glutamate agonist ibotenic acid (IBO) is frequently used to place locally restricted axon-sparing brain lesions. In certain vulnerable areas, as the substantia nigra (SN) e. g., neuronal degeneration is accompanied by the local formation of calcium deposits. Formation of the calcium deposits and their possible association with cartilage-, bone- and brain-specific glycoproteins and components of the extracellular matrix was investigated in the present series of experiments. Male rats received an intranigral injection of 7 µg IBO in 0.7 µl buffered H_2O. One week after IBO application, calcium deposits were detectable in the vicinity of the lesion in the Alizarin stain. Ultrastructural analysis revealed that the deposits probably originate from the calcium accumulated in the degenerating dendrites and perikarya of the GABAergic SN pars reticulata neurons which contain the calcium-binding protein parvalbumin. Astrocytes separating the degenerating neurons from their intact presynaptic boutons express the bone-specific glycoprotein osteocalcin. Laminin and collagen III-positive blood vessels proliferate into the lesion focus. Between degenerating calcium-filled neurons and astrocytes as well as microglia a fibrillar matrix is formed which is positive for heparan sulfate normally absent from brain tissue. During the following weeks this matrix enlarges stepwise and is infiltrated with anorganic calcium salts, thus giving a picture resembling the annual growth rings of trees. The evolving bodies incorporated smaller calcium deposits resulting in large concretions. It is suggested that the different outfit of neurons with calcium-binding proteins together with the response repertoire of glial cells towards modulation of the compounds of the extracellular matrix following excitotoxic cell death, provide the permissive substrate for the calcification process. Supported by funds of the University Basel.

478.3

MECHANISMS OF NEURONAL DEATH DURING EXCITOTOXICITY EFFECTS OF TEMPERATURE. L. Kiedrowski*. The Psychiatric Institute. University of Illinois, Chicago, IL 60612.

The hypothesis tested was that excessive Ca^{2+}-buffering by mitochondria collapses mitochondrial membrane potential ($\Delta\psi$) and plays an important role in excitotoxicity. To this end, effects of glutamate (GLU) on $[Ca^{2+}]_i$ and $\Delta\psi$ were studied simultaneously in primary cultures of cerebellar granule cells loaded with fura-2 and tetramethylrhodamine (TMR), respectively. Neurons were exposed for 15 min to 50 µM GLU and 10 µM glycine applied in a Mg^{2+}-free Locke's buffer at 22 °C and 37 °C. Following GLU removal, conditioned culture medium was reapplied, and dishes were placed in an incubator. After 24 h, the same microscopic field in which $[Ca^{2+}]_i$ and $\Delta\psi$ were earlier monitored was recovered, and the integrity of the plasma membrane was tested using propidium iodide (PI). Nuclei of the same neurons were examined for the presence of chromatin condensation. An increase in temperature from 22 °C to 37 °C during the GLU challenge strongly improved Ca^{2+} buffering and neuronal survival: as many as 65% of neurons failed to buffer Ca^{2+} at 22 °C, while only 15% failed to do so at 37 °C; 60% of neurons died at 22 °C, but only 25% at 37 °C. The failure to buffer Ca^{2+} was well correlated with a collapse of $\Delta\psi$, since neurons that failed to decrease $[Ca^{2+}]_i$, also failed to reaccumulate TMR in mitochondria; at 22 °C, 85% of these neurons stained with PI after 24 h. Unexpectedly, at 37 °C, 57% of neurons that died, upon GLU removal, maintained $\Delta\psi$ and showed a prompt decrease in $[Ca^{2+}]_i$. In these neurons, an evident chromatin condensation was observed after 24 h, but the plasma membrane was still impermeable to PI. It is concluded that there may be at least two mechanisms operative in GLU-excitotoxicity: 1) one that involves a collapse of $\Delta\psi$ and destabilization of Ca^{2+} homeostasis, and 2) one in which neither Ca^{2+} homeostasis nor $\Delta\psi$ are immediately destabilized.

478.4

DISRUPTION OF NEURONAL CA^{2+} HOMEOSTASIS BY NITRIC OXIDE INVOLVES ATP DEPLETION. J.R. Brorson*, R.A. Sulit, and H. Zhang. Depts. of Neurology and Neurosurgery, The University of Chicago, Chicago, IL 60637.

Nitric oxide (NO) has in recent years been recognized as an important mediator of excitotoxicity. We previously reported that NO exposure disrupts Ca^{2+} homeostasis in cultured hippocampal neurons. Using patch clamp and $[Ca^{2+}]_i$ microfluorimetric methods, we have studied the mechanism of this effect.

The NO releasing agent *S*-nitrosocysteine (SNOC, 300µM) over 20 minute exposures gradually produced a delayed rise in $[Ca^{2+}]_i$ accompanied by a slowing of recovery from 50mM K^+ depolarization-induced $[Ca^{2+}]_i$ transients. These effects of SNOC were not mimicked by inactivated SNOC, nor by the components of SNOC, L-cysteine and $NaNO_2$, but they were blocked by the NO scavenger oxyhemoglobin, confirming NO as the responsible agent. They were not mimicked by 8-bromo-cyclicGMP, and they were blocked by superoxide dismutase, suggesting that they involved peroxynitrite formation. The poly-(ADPribose) synthetase (PARS) antagonist benzamide did not prevent the effects of SNOC on slowing of $[Ca^{2+}]_i$ recovery; however, the effects of SNOC could be mimicked by the mitochondrial ATP synthase inhibitor oligomycin. Similar effects on $[Ca^{2+}]_i$ homeostasis were seen in combined perforated patch-$[Ca^{2+}]_i$ fluorimetry experiments, in which the $[Ca^{2+}]_i$ transients were induced by direct depolarization, but not in combined whole cell patch clamp- $[Ca^{2+}]_i$ fluorimetry experiments in which an ATP-regenerating system was supplied via the patch pipette.

We conclude that prolonged exposures to NO disrupt $[Ca^{2+}]_i$ homeostasis in hippocampal neurons by impairing Ca^{2+} extrusion from the cytoplasm, apparently as a result of inhibition of mitochondrial metabolism leading to depletion of ATP. The disruption of $[Ca^{2+}]_i$ homeostasis may potentially contribute to the delayed neurotoxicity of NO. *Supported by NIH grant NS01630, the Schweppe Foundation, and the Brain Research Foundation.*

478.5

CULTURED MURINE OLIGODENDROCYTES ARE HIGHLY VULNERABLE TO AMPA/KAINATE RECEPTOR - INDUCED DEATH. J.W. McDonald*, S.P. Althomsons, D.W. Choi and M.P. Goldberg. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

CNS demyelination and oligodendrocyte death may be important function-limiting sequelae in many neurological disorders including stroke, spinal cord and brain trauma, multiple sclerosis, and white matter encephalopathies. We established an in vitro co-culture system of well differentiated oligodendrocytes on an astroglial monolayer, a system that supports oligodendrocyte survival and differentiation. DIV 14-28 oligodendrocytes were selectively Gal-C immunoreactive, expressed high levels of AMPA receptor protein (GluR B/C) subunit by immunohistochemistry, and were highly susceptible to exposures of AMPA for 24 hrs. Little astrocyte death was present under these conditions. The EC_{50} for AMPA-induced oligodendrocyte death was approximately 10 μM, comparable to that of cortical neurons. This death was associated with early cytoplasmic swelling and was sensitive to co-application of the AMPA receptor antagonist, NBQX, observations consistent with excitotoxic form of death. The NMDA antagonist MK-801 or the growth factors CNTF or IGF-1 did not reduce death. Inhibition of AMPA receptor desensitization by cyclothiazide enhanced AMPA-induced death. Although exposure to glutamate was not very toxic, blockade of glutamate uptake with t-PDC, markedly augmented death. As we have previously demonstrated for neurons, AMPA-induced death of oligodendrocytes was markedly enhanced by reduction of extracellular pH to 6.6. These data suggest that high vulnerability to death induced by low concentrations of excitotoxins is not unique to neurons, and raise the specific possibility that excitotoxic oligodendrocyte death may play an important role in the pathogenesis of certain neurological disorders.

Supported by NIH NINDS grants NS 32140 (MPG) and 36636 (MPG and DWC).

478.6

ZINC TOGETHER WITH LOW pH POTENTIATE KAINATE-INDUCED DEATH OF ASTROCYTES. S.L Sensi*, L.M.T. Canzoniero, J.Y. Koh, and D.W. Choi. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Although astrocytes express AMPA and kainate receptors (Holzwarth et al., J.Neurosci.,14:1879, 1994), they are highly resistant to death induced by excitotoxins when tested by themselves at normal pH. However, during ischemia in vivo, extracellular pH decreases. Further, it is likely that zinc, normally present in the presynaptic vesicles of central glutamatergic neurons, is released. We examined the effect of adding zinc and lowering pH, on the vulnerability of cortical astrocytes to kainate exposure.

Murine cortical astrocytes (DIV 15-21) exposed to 1 mM kainate for 1 hr at normal (7.4) or acidic (6.2) pH did not exhibit degeneration assayed 24 hr later. While astrocytes are vulnerable to injury induced by high concentrations of zinc (300 μM)(Yokoyama et al., Neurosci .Lett. 71:351, 1986), 1 hr exposure to 50 μM zinc at either pH 7.4 or 6.2 was also non-toxic.
However, 1 hr exposure to 1 mM kainate in the presence of 50 μM zinc and lowered pH to 6.2, produced extensive glial injury.

Further studies will be necessary to understand the mechanisms underlying this toxic potentiation, but present data raise the possibility that AMPA/kainate receptor activation on astrocytes can be lethal under ischemic conditions, or other conditions where extracellular zinc and protons are increased.

Supported by NIH NINDS grant NS 32636 (DWC).

478.7

Ca^{2+} changes driven by electrical activity are depressed, not increased, in post-ischemic hippocampal CA1 neurons destined for death. J. A. Connor*, J.J. Petrozzino, S. Razani, R. Cormier & R.C.S. Lin ^. The Lovelace Institutes, Albuquerque NM 87108 ^Med. College of. PA. & Hahnemann University, Philadelphia, PA 19102.

CA1 neurons subjected to a 5-10 min global ischemia initially survive the insult but, for undetermined reasons, up to 90% die over the next few days. It is widely agreed that large Ca^{2+} increases, during the ischemic period, initiate death dealing processes, and it has been speculated that delayed increases in Ca^{2+} loading during electrical activity or the loss of Ca^{2+} regulation are proximal causes of death. We have measured Ca^{2+} homeostasis of CA1 neurons in brain slices from adult gerbils following 5 min, *in vitro*, ischemia. Brain slices were prepared 1, 2, &3 days after the insult and examined by microelectrode recording and fluorescence imaging of fura-2. At 1 day after the insult (P-i 1), neurons were indistinguishable from controls with respect to maximum Ca^{2+} changes evoked by current injection, time course of Ca^{2+} recovery, and resting Ca^{2+} levels. Over the next two days however, the maximum changes during firing were suppressed, by ≈ 65% on average at P-i 3. Resting Ca^{2+} trended to lower levels. Recovery time course following spike induced loading was unchanged, indicating that buffering and uptake mechanisms were functional. Dendrite beading, an anatomical correlate of CA1 neuron degeneration was confirmed for a sub population of the experimental neurons by co-injection of biocytin with fura-2 and later examination. The results point to Ca^{2+} 'starvation' not delayed overload as a factor in the CA1 death program.

Support from Alzheimer Foundation to RCSL

478.8

CREATINE REDUCES GLUTAMATE-MEDIATED APOPTOTIC CELL DEATH IN A NEURONAL CELL LINE. Zun Lee* and Mario D. Saltarelli. Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322.

Excitotoxicity, defective cellular energy metabolism, and oxidative stress have been implicated as interrelated pathological mechanisms underlying various neurological disorders. Neuroblastoma hybrid cells (N18-RE-105) undergo apoptotic cell death via a slow oxidative mechanism involving glutathione depletion when they are exposed continuously to glutamate *in vitro*. To investigate the role of the phosphocreatine/ creatine kinase (PK/CK) system in neuronal death, we determined the effect of creatine supplementation on glutamate-mediated N18 cell death *in vitro*. As previously reported (*Br. Res.* 444:325), glutamate-mediated cell death (as determined by fractional LDH release) increased in a time- and concentration-dependent manner. However, when either creatine (5 mM) or the creatine analog β-guanidinopropionate (β-GPA; 1mM) was added to the incubation medium, cell death significantly decreased (40% ± 3% reduction; X ± S.E.M.; N = 12; $p < 0.001$). Similar protection by both creatine and β-GPA was also observed when cell death was determined by direct cell counting. L-Buthionine-sulfoximine (100 μM), an irreversible inhibitor of γ-glutamylcysteine synthetase, completely reversed the protective effects of creatine and β-GPA, suggesting potentiation of intracellular glutathione as the underlying protective mechanism. We hypothesize that creatine administration inhibits neuronal apoptosis through the enhancement of intracellular energy and antioxidant reserves. Supported by NINDS CIDA NS01651 (MDS).

478.9

ENHANCED RECOVERY FROM OXYGEN/GLUCOSE DEPRIVATION OF HIPPOCAMPAL CA1 EPSPS IN CALBINDIN KNOCKOUT MICE. G.J. Klapstein[1]*, P. Gray[1], J. Mody[1], M.S. Airaksinen[2], M. Meyer[2] and H. Thoenen[2] Dept. of Neurology, UCLA School of Medicine, Los Angeles, CA[1], and Dept. of Neurochemistry, Max-Planck Institute for Psychiatry, Martinsried, F.R. Germany[2]

Calcium binding proteins are thought to act as intracellular calcium buffers, thereby shielding against Ca^{2+} mediated pathology. It has been speculated that the selective vulnerability of certain neurons to Ca^{2+} dependent injury may stem from a lack of calbindin (CB) content. We tested this hypothesis by examining *in vitro* the effects of transient oxygen/glucose deprivation (OGD) on synaptic responses of CA1 pyramidal cells, one of the most vulnerable neurons to ischemia in the mammalian brain. We compared the effect of OGD in 5 CB nullmutant mice (CB–/–) with their heterozygote littermates (CB+/–, n=5) and wild-type controls (CB+/+, n=5).

Coronal brain slices (350μm thick) were incubated, submerged at 35°C in a recording chamber and perfused constantly with ACSF in an atmosphere of warm, moist carbogen (95% O_2, 5% CO_2). Extracellular field EPSPs were recorded from stratum radiatum of area CA1. All experiments and analyses of EPSP slopes were conducted blindly with respect to the genotype of the animals. Following a control period of at least 10 minutes, OGD conditions were imposed for either 6 or 8 minutes by substituting sucrose for glucose (equimolar) in the perfusion solution, and 95% N_2, 5% CO_2 for carbogen, resulting in a rapid and dramatic reduction (91-100%) in EPSP slope for all genotypes. All genotypes recovered well following 6 minute OGD (n = 8-15), with little difference in either recovery rate (time constant of recovery; τ, min: CB–/– 4.45; CB+/– 6.08; CB+/+ 9.26) or degree of recovery (normalized % recovery ± sem, measured at 30 min post-OGD: CB–/– 85 ± 13; CB+/– 65 ± 12; CB+/+ 93 ± 11) following return to control conditions. Following 8 minute OGD (n = 11-16), however, EPSPs in CB–/– and CB+/– recovered faster (τ=6.17 min and 5.71 min) than wild type (τ=29.6 min) but CB–/– recovered to a significantly greater degree (72 ± 10%) than either of the other two genotypes (CB+/– 26 ± 9%; CB+/+ 21±19%).

Our data are inconsistent with a purely neuroprotective role of calbindin.

Supported by grant NS-27528. GJK holds an AHFMR postdoctoral fellowship.

478.10

LACTATING RATS ARE PROTECTED AGAINST THE EXCITOTOXICITY OF KAINATE D.A. Cruz, K. A. Berghorn, and G.E. Hoffman*. Department of Neurobiology, Univ. Pittsburgh, Pittsburgh, PA, 15261.

Use of kainic acid (KA) serves as a model for temporal lobe epilepsy. KA produces seizures in the limbic system, and when severe, the seizures lead to exitotoxic cell death. Areas most vulnerable to damage are the CA1 and CA3 regions of the hippocampus. KA acting on non-NMDA receptors promotes the initial depolarization; actions involving NMDA receptors later lead to cell death. We determined earlier that lactating rats were resistent to behavioral and transcriptional effects of an NMDA agonist, raising the question of whether seizures and the excitotoxicity of KA could also be attenuated in the lactating animal. Lactating rats (day 9 post-partum) and cycling rats selected on diestrus I (DI) were given a single i.p. injection of 9 or 12 mg/kg KA. Three days after KA injection, the rats were anesthetized, perfused and examined for neuronal morphology. Seizure activity was evident in all rats. All lactating rats (n=6) survived treatment. The lactating animals that received 9 mg/kg resumed nursing within 24 of treatment. None of the DI rats (n=3) survived 12 mg/kg KA; 1 of 3 DI rats receiving 9 mg/kg of KA died within 12 hrs after injection. Seizures persisted intermittently for 48 h in surviving DI rats; in lactating rats seizures subsided within 24 h. Examination of the hippocampus using neutral red and acid fuchsin revealed damage to CA1 and CA3 in the surviving DI rats that exceeded that in the lactating rat, irrespective of the KA dose. Lactating rats receiving 9 gm/kg KA had little evidence of excitotoxic cell injury or cell death. These experiments show that lactation provides protection against KA induced neuronal excitotoxicity. Supported by NS28730 and APA Minority Fellowship in Neuroscience.

478.11

HIGH-POTASSIUM-INDUCED NEURONAL DAMAGE *IN VIVO*: EFFECTS OF GLUMATATE-RECEPTOR AND SODIUM-CHANNEL BLOCKADE. D. G. Fujikawa*, T.B. Sohn and A.F. Alcaraz. Exp. Neurol. Lab., VA Med. Ctr., Sepulveda, CA and Dept. of Neurology and Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA.

Microdialysis probe delivery of an isotonic high-K^+ solution to the rat amygdala for 60 min produces neuronal necrosis, edema and increases in extracellular glutamate (GLU_o) (3-4-fold) and aspartate (ASP_o) (2-fold) (Fujikawa *et al., Neuroscience*, 1996). The competitive NMDA-receptor antagonist CGP 40116 dialysed unilaterally in rats with high-K^+ perfusion of both amygdalae is neuroprotective but does not affect the severe edema. In this study we determined the effect of CGP 40116 on extracellular amino acid concentrations during high-K^+ perfusion and the effect of reduced NaCl perfusion and Na^+-channel blockade, with and without combined NMDA- and AMPA-receptor blockade. Bilateral CMA/11 guide cannulae were placed in the basolateral amygdaloid nuclei of adult Wistar rats. The next day CMA/11 microdialysis probes were inserted and perfused with KRB solution for 2 h in freely-moving rats. The solution was then switched bilaterally for 60 min to 100 mM KCl in modified KRB (of which 12% was extracted by tissue). In the CGP 40116 experiments, one side contained either 36 μM (n=6) or 360 μM (n=7) CGP 40116 during baseline and high-K^+ perfusion; 42% of the 36 μM and 48% of the 360 μM CGP 40116 were extracted by tissue. In the reduced NaCl + TTX experiments, one side contained 15 mM NaCl + 3.3 mM TTX (42% was taken up by tissue) during high-K^+ perfusion, with (n=6) or without (n=7) bilateral 360 μM CGP 40116 + 500 μM NBQX during baseline and high-K^+ perfusion. 36 μM CGP 40116 alone had no effect on high-K^+-induced increases in GLU_o and ASP_o, whereas the neuroprotective 360 μM concentration prevented the high-K^+-induced elevation of GLU_o (290 ± 41% on the control side vs. 149 ± 15% on the CGP 40116 side; $p < 0.001$). Reduced NaCl + TTX alone decreased tissue edema slightly but did not protect neurons, and it did not add to the protective effect of CGP 40116 + NBQX. The reduction in GLU_o by CGP 40116 may play a role in its neuroprotective effect. Na^+/Cl^- influx may not contribute to high-K^+-induced neuronal death *in vivo*. (VA-supported.)

CELL DIFFERENTIATION AND MIGRATION VIII

479.1

INVOLVEMENT OF THE DROSOPHILA LIM HOMEOBOX GENE *islet* IN NEURONAL DIFFERENTIATION. S. Thor and J.B. Thomas*, Molecular Neurobiology Laboratory, The Salk Institute, 10010 N. Torrey Pines Rd. La Jolla, CA 92037.

The LIM homeobox genes encode a distinct family of transcriptional regulators. One distinguishing feature of these genes is that they appear to be expressed in discrete subsets of differentiating neurons. To elucidate the possible role of these genes in controlling neuronal differentiation we have cloned the Drosophila homolog of the vertebrate *islet* genes. From a series of promoter fusions to an axon-targeting tau-myc reporter, we have found that *islet* is expressed in a subset of differentiating motor- and interneurons. In *islet* mutants a subset of the *islet* expressing neurons show pathfinding defects. In addition, some of the *islet* expressing interneurons are dopaminergic and can be visualized by the expression of tyrosine hydroxylase (TH). The expression of islet precedes the onset of TH expression and in *islet* mutant embryos we observe an apparent loss of TH expression, suggesting that *islet* may be involved in the direct transcriptional regulation of TH.

Supported by grants from the NIH, a March of Dimes Basil O'Connor Scholar Research Award and a Pew Scholars Award from the Pew Memorial Trusts to J.B.T. and by a Human Frontiers Science Program Fellowship to S.T.

479.2

MAMMALIAN NEURAL CREST CELLS EXPRESS FUNCTIONAL GAP JUNCTIONS - EVIDENCE THAT GAP JUNCTIONS MODULATE THE SURVIVAL OF NEURAL CREST CELLS, P.G. Bannerman,* T.M. Oliver, Z. Xu, and D.E. Pleasure . Neurology Research, Children's Hospital of Philadelphia, Philadelphia, PA 19104.

Connexons are the functional units which comprise supramolecular complexes termed gap junctions. Gap junctions are widely held to perform pivotal role(s) in mediating intercellular communication during the development of eukaryotic organisms. We show for the first time that mammalian neural crest cells (NCC), the progenitor cells of the peripheral nervous system (PNS), assemble demonstrable gap junctions as assayed by dye (lucifer yellow) transfer experiments. Immunohistochemical techniques demonstrated that NCC express the connexon protein, connexin 43 (Cx43) both in vivo and in vitro.

We hypothesized that Cx 43^+ connexons participate in the regulation of intracellular communication during the early development of the mammalian PNS. We tested this hypothesis by perturbing the function of NCC gap junctions in a neural tube explant culture paradigm A) using the uncoupling agent octanol and B) inhibiting the synthesis of Cx 43 connexons by antisense methodology. Our data shows that octanol(1mM) but not hexanol(1mM) (which does not uncouple gap junctions), markedly decreases the survival of a subset of NCC. Studies with phosphothiolated antisense/sense oligomers (22mer,150uM) which overlap the initiation and termination sites of the rat sequence for Cx43 (Genebank Accession #M19317), provide strong evidence that perturbation of Cx43 synthesis also decreases the survival of a subpopulation of NCC. These results clearly demonstrate a role for gap junctions in the development of NCC.

This work was supported by NIH grants NS25044, NS08075 and CA 16420, the National Multiple Sclerosis Society (RG2556A-A-1) and a Florence Murray Fellowship.

479.3

STRENGTH OF INTERCELLULAR COMMUNICATION HAS A PRONOUNCED INFLUENCE ON NEURONAL DIFFERENTIATION. R. Rozental, M. Urban, A. Andrade-Rozental, G.I. Fishman, E.M. Eves, M.R. Rosner* and D.C. Spray. Depts. Neurosci. & Med., A. Einstein Coll. Med., Bronx, NY 10461; Inst. Biophysics "Carlos Chagas Filho", Fed. Univ. Rio de Janeiro, Brazil; Ben May Inst., Chicago, IL 60637.

We have shown that electrotonic coupling (g_j) *via* Cx43 is quite strong among immature neuroblasts and that Cx43 mRNA progressively decreases during neuronal differentiation. To examine the possible coordinate regulation between gap junction down-regulation and acquisition of neuronal phenotype, we have used an SV40 immortalized rat hippocampal neuronal cell line that is highly coupled by a single connexin-type (Cx43) and that can be induced to differentiate with retinoic acid (0.3 μM) as evidenced by immunocytochemical and functional criteria; these were compared with cells further transfected with Cx43 by electroporation, to cells treated with heptanol (2-3 mM) or 18-α-glycyrrhetinic acid (10-40 μM), inhibitors of gap junctional coupling, and to cells treated with hexanol (2-3 mM) or glycyrrhizic acid (10-100 μM), respective controls. We show that constitutive over expression of Cx43 in transfectants antagonizes neuronal differentiation; in contrast, uncoupling treatments lead to more rapid neuronal maturation. Thus, decreased intercellular coupling appears to be permissive for early steps in neuronal differentiation.

479.4

SEROTONIN MODULATES *IN VITRO* NEURONAL MIGRATION IN RODENT CEREBRAL CORTEX. J.-P. Hornung* and J. Durig. Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

We tested the influence of serotonin on the migration of layers II/III neurons, which leave the cell cycle late (E16/17 in mouse, E19 in rat) and reach their final laminar distribution 3 to 5 days later. Both in rats and mice, neurons destined to the upper layers were labelled by systemic BrdU injection to the dam on the day of their final division. One to three days later, embryos were harvested and slices of the cerebral cortex were maintained in organotypic cultures for 7 days. Culture medium contained various concentrations of serotonin (0,1,5, or 10 μM) or 5 μM of agonist or antagonist of the 5-HT1a (8-OHDPAT/NAN-190), or of the 5-HT2 (alpha-methyl-5-HT/ritanserin) receptors. After one week in culture, slices were fixed and immunostained for BrdU. Brains of embryos of the same litters were fixed, cut, and stained in parallel to verify that labeled neuroblasts had not migrated prior to the culture period. Compared to the situation *in vivo*, many neuroblasts did not complete their migration to their expected final laminar position in layer II (with the present conditions of labeling). However, the proportion of labeled neuroblasts which succeeded in reaching layer II was directly proportional to the concentration of serotonin in the medium. This proportion was also high after 5-HT1a agonist treatment but was low in the presence of the 5-HT1a antagonist (combined with 5 μM 5-HT) or the 5-HT2 agonist. Neuronal migration in cultures treated with medium supplemented with dopamine or noradrenaline (10 μM) was comparable to that of controls (with 0 μM 5-HT). These data suggest that serotonin has a selective promoting function (5-HT1a-mediated) on neuronal migration in the cerebral wall which could ultimately affect laminar cortical organization in cases of monoamine metabolism disturbances. Suppported by SNF grant 31-40852.94

479.5

DOPAMINE ALTERS THE EXPRESSION OF PARVALBUMIN DURING CORTICAL DEVELOPMENT *IN VITRO*. L.L.Porter*, J.-P.Hornung. Inst. Anat., Univ.of Lausanne, 1005 Lausanne, Switzerland.

We examined the effects of dopamine (Da) on the morphological and temporal patterns of parvalbumin (Pv) expression during neocortical development. Organotypic slice cultures of the cerebral cortex were prepared from neonatal rats (PN 2/3) and maintained for 6 to 35 days-*in-vitro* (DiV) in serum-enriched medium. Slices were fixed and immunostained for Pv immunoreactive (IR) neurons. Under control conditions, Pv IR cell bodies are first noted in cingulate and perirhinal cortex at 6 DiV and are restricted to these regions. In Da (10 μM) enriched medium, Pv IR somata are detected not only in cingulate and perirhinal cortices, but also throughout frontal and parietal cortices at 6 DiV. The labeled somata are distributed throughout the deep cortical laminae.This pattern of Pv IR in Da enriched cultures at 6 DiV is similar to that in control cultures at 14 DiV. Da enriched cultures at DiV 14 exhibit an increased density of labeled somata in the deep laminae, as well as, labeled neurons throughout all cortical laminae. Also, dendritic labeling is more extensive and a dense network of axons is evident in the deep laminae. This pattern emerges in control cultures by DiV 21. The differential development of Pv expression is apparent at all time intervals examined. Lower doses of Da (1 μM) appear to be less effective in altering Pv expression at earlier times. To determine if these effects are mediated by specific dopamine receptor subtypes, the cultures were maintained in SKF 38393 or quinpirole (D1, D2 agonists), or in Da with SCH 23390 or eticlopride (D1, D2 antagonists). These treatments indicate that the Da induced alteration of Pv expression is not mediated by D1 receptors. However, the D2 antagonist appears to block the effects of Da and the D2 agonist mimics Da. Thus, a D2 receptor-mediated effect may be the mechanism by which Da alters the pattern of Pv expression during cortical development. The relationship between the onset of Pv expression and functional activity in the cortex raises the notion that activation of the D2 receptor system may stimulate cortical activity, which in turn leads to enhanced Pv expression. Supported by the CIBA-GEIGY and by the SANDOZ Foundations.

479.6

INTRA-UTERINE TRANSPLANTATION OF NEURAL PRECURSORS ALLOWS THE STUDY OF NEUROLOGICAL AND TARGETED MOUSE MUTANTS IN A WILD-TYPE BACKGROUND. U Maskos*, O Brüstle, and RDG McKay, LMB/NINDS/NIH, Bethesda MD 20892-4092.

Neural precursors derived from E14 wild-type and transgenic mouse embryos were transplanted into the ventricular system of E16 to E18 rat embryos using intra-uterine transplantation [O Brüstle, U Maskos & RDG McKay, Neuron 15, 1275-1285, 1995], thus distributing them over the entire neuroepithelium. The transplanted cells were followed using genetic marking methods, i.e. the expression of a lacZ reporter gene, in situ hybridisation to mouse DNA, and mouse-specific neural antigens.The precursors were able to leave the ventricle and migrate into the parenchyma where they differentiated into neurons in a region-specific manner.

This method was used to analyse NMDA receptor R1 mutant mice. The NMDA receptor is one of the most intensely studied proteins in the brain because of its involvement in neurotransmission, synaptic plasticity and neurodegeneration. Homozygous mutant animals die on the day of birth, which precludes their analysis at the most interesting later stages of development and maturity.

Embryos from matings of heterozygous animals were dissected and genotyped by PCR. Single cell suspensions from homozygous null embryos were injected into E18 rat embryos. Analysis of young and adult transplanted animals showed that the mutant cells survive, migrate and differentiate in the wild-type background.

This new method now allows to study the phenotype of fully differentiated cells that would otherwise not be accessible because of embryonic or neonatal death of the animal, and, as a transplantation technique, allows the heterochronic and heterotopic placement of cells into a large number of brain areas.

Supported by the Deutsche Forschungsgemeinschaft and NINDS.

479.7

DEVELOPMENTAL CHANGES OF PROTEIN KINASE C ISOFORMS IN CEREBELLAR GRANULE CELLS: IMPLICATION FOR NEURONAL DIFFERENTIATION AND SURVIVAL. W.W. Lin*, C.W. Wang and D.-M. Chuang. Department of Pharmacology, National Taiwan University, Taipei, Taiwan and Section on Molecular Neurobiology, NIMH, NIH, Betheda, Maryland 20892-1272.

The primary cultures of cerebellar granule cells (CGC) grown in high K^+ (25 mM) medium progressively differentiate *in vitro* by the appearance of neuronal aggregation and neurite outgrowth. These differentiation features occur after 3- to 4-day *in vitro* (DIV) and reach a mature stage after 8 DIV. Longer cultivation of CGC (≥13 DIV) triggers the processes of spontaneous cell death. To address the role of protein kinase C (PKC) in the development of CGC, we measured the kinase activity as well as the protein level of kinase isoforms. As the CGC culture proceeded, we found that the PKC activity time-dependently increased by 3.2 fold, reaching a steady state at 8 DIV. Western blot analysis using PKC isoform-specific antibodies revealed an increase in PKCα,γ,μ,λ and ι from 2 to 8 DIV. A slight change was observed for PKCε, while the isoforms of δ,θ and ζ were not detected. Compared to the 8 DIV cultures, the immunoactivity of PKCι, ε and λ was decreased and that of PKCα or γ was unchanged at 13 DIV, while that of PKCμ was still increased. In cultures grown in 25 mM KCl containing MK801, a NMDA receptor antagonist, CGC survived more than 20 DIV and all six PKC isoforms except PKCα, were increased more robustly and persistently up to 13 DIV. On the contrary, when CGC grown in 3 mM KCl medium, the immunoactivity of each PKC isoform at 2-4DIV was similar to that observed in K25 cells, although no remarkable differentiation features were observed. Coordinated with the appearance of cell death at 8 DIV in low K^+ cultures, PKCα, μ, λ and ι, but not γ, ε, were markedly decreased. Taken together, induction and accumulation of PKC isoforms may play a more important role in the maintenance of neuronal survival than initiation of neurite outgrowth.

479.8

DIFFERENTIATION OF OLIGODENDROCYES FROM MULTIPOTENT SUBVENTRICULAR ZONE PROGENITOR CELLS IN VITRO IS REGULATED BY CNTF/IL6 gp130 CYTOKINES AND INTRINSIC CELLULAR SIGNALS. R. Marmur*, M. F. Mehler, P. Mabie and J. A. Kessler, Dept. of Neurology and Neuroscience, Albert Einstein Col. Of Medicine, Bronx NY, 10461.

Little is known about epigenetic signals that regulate the differentiation of early oligodendrocyte progenitors at the pre-OLP stage (A2B5-/O4-). Embryonic day 17 multipotent subventricular zone (SVZ) progenitor cells were processed for A2B5 immunoselection and the A2B5- SVZ progenitors were utilized for our experimental paradigms. Compared to moderate density cultures, high density preparations gave rise to a higher proportion of post-mitotic OLs (galactocerebroside, GC+; 5±3% vs. 15±6% of the total cells) and accelerated the generation of GC+ OLs (10 vs. 6 DIV). Application of CNTF but not IL6 enhanced the relative proportion of OLs (30±7% of total cells) and further shortened the temporal interval for oligodendrocyte differentiation (4 DIV). In the absence of added factors, no proliferating OLPs (O4+/BrdU+) were detected at 4 DIV. Application of bFGF (1ng/ml) resulted in the generation of proliferating OLPs (9-11% of the total O4+ cells), while combined application of bFGF/IL6 enhanced these proportions (16-19% of total O4+ cells), whereas bFGF/CNTF co-treatment significantly decreased these proportions (1-2% of the total O4+ cells). Subsequent factor withdrawal (4 DIV) and propagation in defined SFM for 5 additional days demonstrated that bFGF/IL6 pre-treatment further enhanced, while bFGF/CNTF pre-treatment restricted the number of GC+ OLs compared to bFGF alone. We conclude that intrinsic factors within the SVZ culture system as well as specific cytokine subgroups regulate the mitogenic capacity of nascent OLPs as well as the temporal development of OLs. (Funded by MDA and Hirschl Trust (MFM) and NIH (JAK) grants).

479.9

A MODEL FOR THE DIFFERENTIATION AND MAINTENANCE OF THE EMBRYONIC RADIAL GLIAL SCAFFOLD IN MAMMALIAN FOREBRAIN K.E. Hunter* and M.E. Hatten, Laboratory of Developmental Neurobiology, The Rockefeller University, 1230 York Avenue, New York, New York 10021.

The radial glial scaffold is a transient structure in the mammalian forebrain, first appearing at the onset of cortical plate formation, being maintained throughout embryogenesis, and disappearing perinatally, at which time the radial glia transform into mature astrocytes. The scaffold plays a key role in corticogenesis, since the glial fibers provide the primary substrate and template for neuronal migration. We have previously reported the existence of a soluble protein whose effects and *in vivo* expression suggest that it may function as a radial glial maintenance factor (*Proc. Natl. Acad. Sci. USA*, **92**:2061-2065). The molecular identity of the protein is being elucidated but the intriguing question of how it might function *in vivo* remains. In addressing this question we have utilised the *reeler* mouse, in which there is a disturbance of glial guided neuronal migration during corticogenesis. Using immunohistochemical analysis we have found that the radial glial scaffold in *reeler* cortex is disorganised and poorly differentiated, that it disappears prematurely, and that astrocytes appear in *reeler* cortex earlier than normal. Together with our previous observation that *reeler* astrocytes cannot respond to our differentiation factor *in vitro* although the activity is present in *reeler* cortex (Soc. Neurosci. Abs. 315.6), these findings suggest that radial glia themselves may be a direct site of *reeler* gene action. Transplanting astrocytes into embryonic forebrain has revealed differences in the capacity of normal astrocytes to incorporate into either a normal or *reeler* cortical environment, and has suggested there may be a defect in the way in which the differentiation factor is presented in *reeler* cortex. These findings begin to illuminate the question of how the radial glial cell scaffold is initially established and maintained, and subsequently transforms, in the mammalian forebrain. (Supported by NIH Grant NS 15429 awarded to MEH.)

479.10

A REGIONALLY DISTRIBUTED RADIAL GLIAL ANTIGEN: A CANDIDATE FOR SIGNALING AN END TO NEURONAL MIGRATION. E. S. Anton*, W. D. Matthew^, and P. Rakic°. ° Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06514 . ^Dept. of Neurobiology, Duke University School of Medicine, Durham, NC 27710.

During the development of cerebral cortex, neurons generated in the ventricular zone sequentially migrate to the interface between cortical plate and marginal zone (layer 1), where they stop, detach from the radial glial fibers, and become part of the laminar architecture of the cerebral cortex. To elucidate mechanisms that signal neurons to end their migration, a panel of antibodies previously known to recognize embryonic CNS antigens was screened in developing rat cerebral wall. One of the antibodies, PC2A1, recognizes an antigen (a doublet of ~ 330 kDa) that is primarily localized to the developing cortical plate. During corticogenesis, the superficial strata of the cortical plate appear to label more intensely than the lower strata of the cortical plate. Interestingly, the marginal zone is devoid of labeling. In vivo and in vitro, double labeling studies using various glial markers and PC2A1 mAbs, indicate that PC2A1 recognizes a radial glial cell surface antigen. The prominent spatial and temporal distribution of PC2A1 antigen in the region of radial glia spanning the developing cortical plate suggests that it may function as a positional indicator for cessation of neuronal migration. Preliminary results from in vivo studies using BrdU- labeled migrating neurons support this hypothesis, since exposure to PC2A1 mAbs disturbs the settling of neurons at the interface between the cortical plate and marginal zone. [Supported by NS22807 (PR) and a Public Health Service Fellowship (EA)].

479.11

THE FORSE-1/LeX CARBOHYDRATE EPITOPE IS INVOLVED IN MIGRATION OF DEVELOPING CORTICAL NEURONS. K.L. Allendoerfer,* B.S. Kim, and P.H. Patterson. Division of Biology, Caltech, Pasadena, CA 91125.

During cortical development, newly-generated neurons migrate away from the ventricular zone through diverse extracellular environments to their final positions in the cortical plate, which will become the adult cortex. Cell-surface carbohydrate moieties, which mediate adhesion and recognition events in the immune and hematopoietic systems, could provide a high degree of complexity to the environment through which the young neurons move. We have recently shown that the FORSE-1 monoclonal antibody (mAb), which labels the proliferative neuroepithelium of the telencephalon, specifically binds to the LewisX (LeX) carbohydrate epitope. We find the FORSE-1/LeX carbohydrate epitope on two types of molecules in embryonic rat brain: neutral glycolipds in the early embryo, and the chondroitin sulfate proteoglycan, phosphacan, at late embryonic and early postnatal stages. In order to study the function of the FORSE-1/LeX carbohydrate in the brain, we injected FORSE-1 mAb, control mAbs, and oligosaccharide agonists and antagonists into the ventricles of the E15.5 embryonic rat brain. FORSE-1 mAb-producing hybridoma cells survive well after injection, and they secrete mAbs that penetrate throughout the developing cortex. Effects of FORSE-1 mAb treatment on neuroblast migration are assayed in situ by labeling a cohort of neuroblasts through BrdU injection into the mother and measuring the distance this cohort of cells has migrated in treated and control embryos by E21.5 (after 6 days of mAb exposure). Our initial experiments indicate that in the presence of FORSE-1, neurons generated on E16.5 have migrated a much shorter distance from the ventricular zone by E21.5 than those in control brains (uninjected, mock-injected, and those injected with a control mAb, HNK-1). These results suggest that the FORSE-1/LeX carbohydrate epitope is important for neuroblast migration in the developing cerebral cortex. Supported by the Helen Hay Whitney Foundation (KLA), the NIH (PHP), and Caltech SURF (BSK).

479.12

NEURONAL CHANGES IN GRAY MATTER OVERLYING WHITE MATTER LESIONS IN DEVELOPING HUMAN NEOCORTEX. M. Marín-Padilla*. Dept. Pathology. Dartmouth Medical. School, Hanover, NH 03755.

Perinatal brain damage in prematurely born infants who suffered perinatal asphyxia requiring prolonged hospitalization has been associated with the late occurrence of neurological sequelae (cerebral palsy, epilepsy, mental retardation). Rapid Golgi study of the neocortex of 22 infants who survived clinically documented white matter damage (necrosis) -for hours, days, months, and years- has demonstrated secondary neuronal alterations in the overlying and still developing gray matter (acquired neocortical dysplasia). The main consequence of white matter damage (infarction) is the local destruction of the axis-cylinders (axons) of corticipetal, corticofugal, and association fibers affected by the lesion. Consequently, the overlying and still developing gray matter is deprived of some afferent fibers needed for its 'normal' maturation and the severed axons of its projective neurons will failed to reach their targets. The following observations have been made in this secondarily affected gray matter. Its developing neurons are able to survive this type of injury (deprivation) and to continue their structural and functional 'albeit abnormal' maturation. Affected gray matter neurons undergo progressive differentiation (transformation) of their dendritic and axonic arborizations; some of them become large hypertrophic meganeurons. The axons and collaterals of affected projective neurons are exclusively distributed intracortically. These neurons are subsequently transformed into local-circuit interneurons. Accordingly, the intracortical neuropil of the affected gray matter region increases significantly. The direct vascularization of the gray matter by leptomeningeal perforating vessels remains unaffected. The evolution of these acquired cytoarchitectural alterations in the overlying gray matter will be illustrate by exploring simultaneously acute, subacute (healing), and chronic (repaired) underlying white matter lesions. The study proposes that acquired gray matter alterations could cause local cortical dysfunction which could play a role in the pathogenesis of neurological disorders. Supported NINDS Grant # NS-22897.

DEGENERATIVE DISEASE: ALZHEIMER'S-BETA-AMYLOID—PATHOGENESIS

480.1

ENHANCED PATHOLOGIC PROPERTIES OF DUTCH-TYPE MUTANT AMYLOID ß-PROTEIN. W.E. Van Nostrand* and J. Davis. Dept. of Medicine, Health Sciences Center, State University of New York, Stony Brook, NY 11794-8153.

Cerebrovascular amyloid ß-protein (Aß) deposition is a pathological feature of several related disorders including Alzheimer's disease and hereditary cerebral hemorrhage with amyloidosis Dutch-type (HCHWA-D). This latter disease is caused by a point mutation in the gene that encodes the amyloid ß-protein precursor (AßPP) and results in a Glu -> Gln substitution at position 22 of Aß. In comparison to Alzheimer's disease, the cerebrovascular Aß deposition in HCHWA-D is generally more severe, often resulting in intracerebral hemorrhage when patients reach fifty years of age. We recently reported that $A\beta_{1-42}$, but not the shorter $A\beta_{1-40}$, induces pathologic responses in cultured human cerebrovascular smooth muscle (HCSM) cells including cellular degeneration that is accompanied by a marked increase in the levels of cellular AßPP and soluble Aß peptide. In the present study, we show that the HCHWA-D mutation converts the normally nonpathologic $A\beta_{1-40}$ into a highly pathologic form of the peptide for cultured HCSM cells resulting in enhanced cell death and higher levels of cellular AßPP compared to wild type $A\beta_{1-42}$. These findings suggest that these altered functional properties of HCHWA-D mutated Aß may contribute to the early and often severe cerebrovascular pathology that is the hallmark of this disorder.

This work was supported by grants from the National Institutes of Health and the American Heart Association.

480.2

HYDROGEN PEROXIDE INDUCES Aβ ACCUMULATION IN NEURONS
Y. Ohyagi* and S. G. Younkin. Mayo Clinic Jacksonville, Jacksonville, FL 32224

Amyloid β (Aβ) protein is a soluble 4 kD protein, which is normally secreted but characteristically deposited in the Alzheimer's disease (AD) brain. We reported at the last Neuroscience Meeting (Ohyagi et al. '95) that fetal guinea-pig primary mixed brain cell cultures vigorously secrete Aβ. We now present studies of Aβ metabolism in those cells using Aβ-end-specific monoclonals.

The mixed primary cultures were prepared from fetal brain and maintained in medium containing 5% calf serum. Sandwich ELISAs revealed that 90% of Aβ in the medium was $A\beta_{1-40}$ while 10% was $A\beta_{1-42(43)}$, and that Aβ was detectable even in the cells, but by contrast, 60% of Aβ was $A\beta_{1-42(43)}$. This finding is of considerable interest because $A\beta_{1-42(43)}$ is the major Aβ that is deposited in AD brain. We then treated these cultures with H_2O_2 at various concentrations. After H_2O_2 treatment, secreted Aβ levels were substantially decreased, but the cellular Aβ content was gradually increased, and the increase in cellular $A\beta_{1-42(43)}$ was larger than that in cellular $A\beta_{1-40}$. These effects of H_2O_2 were significantly prevented by antioxidants such as α-tocopherol or N-tert-butyl-2-sulpho-phenylnitrone (S-PBN). Immunocytochemically, $A\beta_{42(43)}$ was clearly deposited in the damaged cells, and most of those cells seemed to be neurons. Combined analysis with Perls' stain for Fe^{3+} and appropriate immunocytochemical markers revealed that Fe^{3+} appeared in the damaged neurons. These results may indicate that an iron-related radical generation caused by H_2O_2 such as the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + \bullet OH$) occurs in the neurons, and that hydroxyl radical (•OH) formation may contribute to Aß accumulation. In AD brain, Fe^{3+} and $A\beta_{42(43)}$ were found in some neurons as well as senile plaques. This suggests that our *in vitro* finding of intraneuronal Aβ accumulation caused by H_2O_2 may be related to AD pathology.

(Supported by French Foundation for Alzheimer Research)

480.3

AMYLOID β CYTOTOXICITY IS ASSOCIATED WITH DEFICIENT ACTIVATION OF AN OXIDATIVE STRESS REGULATORY PATHWAY IN NEURAL CELLS. A. Kopp* and R.C. Stanton. Dep't of Med., Deaconess Hosp., Boston, MA 02215

Amyloid β (Aβ) stimulates H_2O_2 production. Aβ cytotoxicity is caused, at least in part, by H_2O_2. Aβ circulates, yet the main pathologic changes occur only in neural cells. We studied whether there was a difference in how a neural cell line (B12) regulates oxidative stress versus non-neural cells (Hep G2, a liver cell line). After 24 hrs. of Aβ(25-35) exposure, 55% of the HepG2 cells were viable; only 11.1% of B12 cells were viable. Aβ stimulated H_2O_2 production in both cell lines: (B12 - 3 x control; HepG2 - 2 x control). Activity of the hexose monophosphate (HMP) shunt, source of the main intracellular reductant, NADPH, was measured. Aβ caused a 67% increase in the HMP shunt in HepG2 cells. In B12 cells Aβ caused no increase in HMP shunt activity; yet exogenous H_2O_2 increased HMP shunt activity in B12 cells. Catalase decreased HMP shunt activity in both cell lines. Conclusion: Aβ cytotoxicity of B12 cells is mediated, at least in part, by lack of activation of a critical oxidative stress defense mechanism.

American Cancer Soc.&Departmental FUND

480.4

β-AMYLOID-ASSOCIATED FREE RADICAL OXIDATIVE STRESS: RELEVANCE TO ALZHEIMER'S DISEASE. D. A. Butterfield*, R. Subramaniam, K. Hensley, M. Aksenova, M. Aksenov, M. Harris, K. Allen and J. Carney. Dept. of Chemistry and Pharmacology and Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506.

β-amyloid peptide (Aβ) is the central constituent in senile plaques (SP) in Alzheimer's disease (AD) brain. Our group first demonstrated Aβ-associated, oxygen-dependent free radicals using electron paramagnetic resonance (EPR) spin trapping methods, a result recently confirmed by others. A free radical-based model of Aβ-induced neurotoxicity in AD was developed to account for and unify into one theoretical framework the numerous reports of multiple membrane protein and lipid alterations in brain membranes in AD. Explicit tests of the model, which states that Aβ-derived free radicals react with key components of neuronal and glial cell membranes leading to their dysfunction and subsequent cell death, have been conducted. EPR spin trapping and spin labeling, histofluorescence, measurements of enzyme activity and protein carbonyl levels, etc. were used. We found that Aβ-associated free radicals in brain membranes cause: lipid peroxidation (consistent with the findings of others that Aβ(25-35) inserts into the lipid bilayer domain of brain membranes); protein oxidation; inhibition of multiple transmembrane transport systems; increased reactive oxygen species; large accumulations of intraneuronal Ca^{2+}; and brain cell death. All these Aβ-induced effects are inhibitable by free radical scavengers, consistent with a free radical basis for these findings. Our model predicts, and we found, that in AD brain the hippocampal and inferior parietal regions, rich in SP, exhibit significant protein oxidation, while the cerebellum, poor in SP, does not, relative to age-matched controls in rapid autopsy material. An important role of Aβ methionine in these processes is emerging, and our data to support this role will be presented. Our findings are consistent with Aβ-associated free radical oxidative stress being a fundamental process in AD. Supported by NIH grants AG-10836; AG-05119.

480.5

MITOCHONDRIAL FAILURE INDUCES ABNORMAL CEREBRAL ZINC HOMEOSTASIS: A PRELUDE TO AMYLOID FORMATION? C.S.Atwood¶, X.Huang¶, R.Matthews†, R.D.Moir, M.F.Beal†, R.E.Tanzi, A.I.Bush*, Genetics & Aging Unit, &†Neurology Res., Mass. Gen. Hosp., Boston, MA. ¶first-authors

Aß, the main component of plaque amyloid in Alzheimer's disease (AD), specifically binds zinc, when, at physiological concentrations, it increases the peptide's resistance to proteolysis and rapidly induces amyloid formation (Bush *et al.*, Science, 265, 1464; 1994). Rat Aß is not as sensitive to the effects of zinc. We have confirmed our initial findings using various techniques including turbidometry as well as sedimentation analysis of tritium-labeled peptide, and concluded that aggregation of a 10 µM solution of Aß1-40 by 10 µM Zn(II) at pH 7.4 is a robust test of the patency of the synthetic peptide, but is contingent upon (a) the presence of isotonic NaCl, (b) the freshness of the peptide stock, since aged peptide (eg frozen and thawed) has attenuated interaction with zinc, (c) the conformation of the peptide, since α-helical promoting solvents enhance zinc-induced precipitation and its reversibility.

These *in vitro* data are important since they describe pathophysiological conditions that could be emulated. The brain compartmentalizes high concentrations of zinc in glutamatergic boutons by an energy dependent mechanism, so we studied the effect of 3-nitropropionic acid, a mitochondrial (succinate dehydrogenase) inhibitor, upon cerebral zinc homeostasis in rats. Zinc in frontal cortical tissue samples fell 70% within 9 hours, but copper levels in the frontal cortex were unaffected. This change probably reflects the loss of reuptake of zinc into synaptic boutons following neurotransmission. Similar changes in zinc levels in AD-affected neocortex have been reported. Failure of reuptake of zinc following neurotransmission may cause an elevation in extracellular zinc levels in the vicinity of the synapse (which cannot be appreciated by this assay), perhaps explaining the focal deposition of amyloid. Zinc levels in the striatal grey matter and motor cortex were only marginally reduced. The neuroanatomical specificity of this lesion upon zinc levels is significant because the disturbance of zinc is greatest in frontal cortex >> motor cortex > striatum, correlating well with the pattern of amyloid deposition in AD.

Supported by: NIH (1R29AG12686̈01), AFAR (Paul Beeson Award), Alzheimer's Association (IIRG-94110), International Life Sciences Institute.

480.6

^{65}ZINC BIODISTRIBUTION IN RAT AND BINDING TO HUMAN ALZHEIMER'S DISEASE CORTICAL LESIONS. A.W. Lyckman*[1], K.L. Moya[2], L. Di Giamberardino[3], and B. Tavitian[1,3]. CEA-SHFJ(1), CNRS URA2210 (2) & INSERM U334 (3), 4 place du Général Leclerc, 91401 Orsay, France.

To date there is no in vivo diagnostic technique for Alzheimer's disease (AD). Zn^{2+} is a ubiquitous protein cofactor with important links to CNS function, notably as a neuromodulator in hippocampus and cortex. Among the potential Zn^{2+}-binding sites is soluble amyloid peptide βA4. To begin evaluating Zn^{2+} as a tracer for brain function and pathology by positron emission tomography (PET), we examined the biodistribution of $^{65}Zn^{2+}$ by γ-counting in adult rat, and the specificity of metal cation binding to postmortem cortex from AD patients using Timms staining and storage phosphor autoradiography.

Systemic injection of a trace dose of $^{65}Zn^{2+}$ (20 µCi; 600 µCi/mg; 0.1 µmol) in rat revealed at 15 min a relatively high uptake (%-injected dose/kg) in peripheral organs, e.g., liver (38.6), blood (6.2) and plasma (10.5), which *decreased* 32-86% over a four hour survival time. Uptake was lower at 15 min in frontal cortex (0.6), posterior cortex (0.7), striatum (0.5) and hippocampus (0.7) but *increased* 31-51% over the same period.

Preincubation of AD cortex with Zn^{2+} (but not Cu^{2+}, Ni^{2+}, or Pb^{2+}) at concentrations ≥ 100µM, resulted in specific argentophilic colabeling of thioflavin S deposits in parenchyma and vessels, as well as specific staining of white matter. Highly selective $^{65}Zn^{2+}$-binding to white matter in AD cortex, which was competed by exogenous cold Zn^{2+}, was revealed by storage phosphor autoradiography.

Our data demonstrate selective Zn^{2+} uptake in CNS in vivo and high selectivity for cortical amyloid deposits and cortical white matter in AD brain in situ. ^{63}Zn, a potent β$^+$-emitter with 38 min half-life, may be useful as a brain PET tracer, with particular implications for white matter pathology, hippocampal dysfunction and AD.

We thank Dr. André Delacourte for kindly providing AD brain tissue; M. Clavier, I. Doignon and R. Hässig for technical assistance. Supported by CEA, CNRS & INSERM.

480.7

PYROGLUTAMATE-MODIFIED AMYLOID β-PEPTIDE IN ALZHEIMER'S DISEASE AND DOWN'S SYNDROME. C. Russo, L. M. DeBusk, T. C. Saido#, X. M. Xu, M. Tabaton, P. Gambetti and J. K. Teller*. Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106, U.S.A. and #Tokyo Metropolitan Institute of Medical Science, Tokyo 113, Japan.

We have recently demonstrated that soluble amyloid β-peptides (sAβ), ending at residue 42 precede amyloid plaque formation in Down's syndrome (DS). The same pathogenic mechanism seems to operate in Alzheimer's disease (AD). The soluble peptides can be resolved into at least three forms, one being modified by cyclization of the N-terminal glutamate at the third residue (pyrAβ). We have characterized this form by electrophoresis, immunoblotting with a pyrAβ-specific antibody, HPLC and enzyme analysis in sporadic and familial AD and DS brains. In DS pyrAβ was already present in the youngest brain under study (four days old). It increased, relatively to the full length sAβ, with age becoming the dominant peptide in plaque-rich DS brains. In all AD brains, both sporadic and familial, carrying various mutations in the Aβ precursor protein, the peptide was consistently the most abundant form. This result was confirmed by immunocytochemical analysis. The identity of the peptide and its modification was confirmed by chromatographic analysis followed by ELISA and by abolishing its immunogenicity after an enzymatic removal of the N-terminal residue. The pyroglutamate-modified sAβ could not be detected in the cerebro-spinal fluid, blood serum or cultured cells overexpressing Aβ precursor protein indicating that the peptide is uniquely specific for the brain parenchyma. The facts that the peptide appears in DS long before Aβ deposits are detectable, is dominant in all AD brains, is more hydrophobic than Aβ(1-42), and is stably N-terminally blocked point to its crucial role in both plaque formation and pathogenesis of AD.

Supported by NIA grants AG0812, AGNS08155, AG08992 and the Britton Fund.

480.8

Amyloid β Peptides in Cerebellar Preamyloid and Cortical Neuritic Plaques of Down's Syndrome Patients M.Lalowski[1], A.Golabek[2], C.A.Lemere[3], D.J.Selkoe[3], E.Kolodny[2]*, B.Frangione[1], T.Wisniewski[2]. Dept.Pathol [1]and Neurol [2], NYU Medical Center, NY 10016; Harvard Med.Sch.[3], Boston, MA 02115

Alzheimer's disease (AD) and Down's Syndrome (DS) are both associated with the deposition of amyloid β (Aβ) in senile plaques. An early stage in the development of senile plaques is thought to be "preamyloid" or diffuse plaques. This is based, in part, on studies in DS individuals where diffuse plaques occur at a very early age. After the age of 40 most DS patients develop the neuropathological features of AD in the cerebral cortex; in addition, many DS patients also have extensive preamyloid deposits in the cerebellum. These cerebellar preamyloid lesions do not progress to neuritic plaques. We have studied biochemically and immunohistochemically (using epitope specific anti-Aβ antibodies) the composition of DS cerebellar preamyloid in 5 patients ranging in age from 46 to 65. In two of these DS patients aged 57 and 62 we also identified the composition of neuritic plaque Aβ. The major preamyloid Aβ peptide in the cerebellum was Aβ17-42 (70-95% of total Aβ),which correlated with our immunohistochemical studies. Other minor preamyloid Aβ peptides included Aβ1-42, 8-42, 11-42 and 1-40. Aβ17-42 was also found as a minor component of cortical neuritic plaque amyloid (3-4%); however, the Aβ17-42 from neuritic plaques was partially oxidized by mass spectroscopic analysis, while cerebellar Aβ17-42 was not. In vitro, we show that Aβ17-42 peptides are less fibrillogenic then Aβ1-42 by Thioflavin T assay and electron microscopy, with oxidation increasing the fibrillogenic potential. These studies, our previous biochemical extractions in aged dogs and other work all suggest that the initially deposited Aβ peptide in preamyloid is Aβ17-42. Since Aβ17-42 was the major peptide in DS cerebellar preamyloid, where progression to neuritic plaques does not occur, our data also indicate that the presence of Aβ peptides extending to residue 42 does not itself lead to the development of neuritic plaques and other factors are also likely to be important.

Supported by NIH grants: AG05891, NS30455, AG00542 and AG08721.

480.9

MOLECULAR FACTORS CONTROLLING Aβ FIBRIL NUCLEATION AND ELONGATION.
D.B. Teplow*[Δ†], A. Lomakin[Δ†#], G.B. Benedek[#], and D.M. Walsh[Δ†]. [Δ]Department of Neurology, Brigham and Women's Hospital; [†]Department of Neurology (Neuroscience), Harvard Medical School, Boston, MA 02115; [#]Department of Physics, and Center for Material Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA, 02139.

One strategy for treating Alzheimer's disease is to inhibit amyloid fiber formation. Successful implementation of this strategy requires an understanding of the key molecular factors controlling the kinetics of fibril nucleation and elongation. We have developed a model system in which the kinetics of fibrillogenesis is reproducible and experimentally manipulable. This has allowed us to use quasielastic light scattering spectroscopy to continuously monitor fibril nucleation and growth. Analysis of the temporal change in fibril length in the context of a simple, powerful mathematical model of fibrillogenesis produces quantitative information on the equilibrium, kinetic, and geometric parameters describing the process, including nucleation and elongation rates. We report here our initial findings on the relative roles of Aβ primary structure, solvent conditions, and exogenous molecules (chaperones) in controlling the kinetics of fibrillogenesis. This information contributes to the knowledge base necessary for the rational design of therapeutic agents targeted to critical steps in the process of Aβ fiber formation.

480.10

AGGREGATION OF Aβ IS ENHANCED BY GLYCEROPHOSPHOCHOLINE, A METABOLITE GREATLY INCREASED IN ALZHEIMER'S DISEASE BRAIN. WE Klunk,* CJ Xu, RJ McClure, & JW Pettegrew. *Laboratory of Neurophysics, Dept. of Psychiatry, WPIC, Univ. of Pittsburgh, Pittsburgh, PA 15261*

There is increasing evidence that deposition of the Aβ peptide in Alzheimer's disease (AD) brain plays an important role in the pathophysiology of this dementia. There is evidence that, at least in cell culture, Aβ is toxic to neurons. This toxicity is totally dependent on the state of aggregation of the peptide. Monomers have little or no toxicity while beta-sheet fibrillar aggregates are toxic. Alzheimer's disease could be viewed as an abnormality in the kinetics of aggregation of Aβ. Anything which accelerates aggregation beyond a certain threshold could produce the disease phenotype. A third copy of the APP gene (Down syndrome) or the presence of the "Swedish mutation" may accelerate the kinetics by increasing production of Aβ. The codon 717 mutations may accelerate the kinetics by increasing the percentage of the more rapidly aggregating Aβ(1-42/43). Other endogenous factors such as zinc can accelerate aggregation. In this study, we examined several metabolites whose levels are altered in post-mortem AD brain. Several metabolites, including glycerophosphocholine, glycerophosphoethanolamine, and α-glycerophosphate, accelerated the rate of aggregation of Aβ(1-40) as measured by light diffraction. The acceleration was concentration-dependent and occurred at metabolite concentrations found in AD brain. This finding may explain the progressive deposition of Aβ with normal aging and the exaggerated deposition in AD since PDE levels increase with normal aging and to a much greater extent in AD.

Supported by NIA LEAD Award AG08974 (JWP)

480.11

PRO-INFLAMMATORY RESPONSE MOLECULES ARE DIFFERENTIALLY ASSOCIATED WITH APOPTOSIS AND CEREBRAL INFLAMMATION IN AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE. S.A.L. Bennett*[1,2], J. Lakins[2], T. Fortin[1], M.P.R. Tenniswood[2], J. de la Torre[1], and B. Pappas[1]. [1]Neuroscience, Carleton University, Ottawa, Ont, K1S5B6 [2]W.Alton Jones Cell Science Center, Lake Placid, NY, 12946.

Complement proteins are upregulated in and around senile plaques during Alzheimer's' Disease indicating that pro-inflammatory molecules may contribute to progressive neurodegeneration. This upregulation is also associated with hypoperfusion of cerebral tissue or chronic vascular insufficiency (CVI). To further explore this relationship, we induced CVI in rats by permanent bilateral ligation of the common carotid arteries (2-VO). Behavioural, immunohistochemical, Western, and Northern data demonstrates that 2-VO results in progressive behavioural dysfunction, increased neuronal apoptosis, accumulation of carbohydrate-containing lesions, and upregulation of both the pro-inflammatory response molecule, platelet activating factor receptor (PAFR), and the complement regulatory protein, clusterin. These pathologies are time-dependent. Between 2-27 weeks of 2-VO, increases in PAFR and clusterin expression are localized to brain regions apparently undergoing apoptosis as assessed by morphological examination and TUNEL. By 27-40 weeks of 2-VO, enhanced PAFR and clusterin expression are associated with brain regions exhibiting infiltration of peripheral inflammatory cells, reactive gliosis, and senile plaque formation. These data suggest that pro-inflammatory response and complement regulatory molecules are differentially associated with both apoptosis and inflammation during progressive neurodegeneration. [Supported by grants from HFSPO, Ontario Heart and Stroke, and Alzheimer Society of Canada.]

480.12

THE ALZHEIMER'S Aβ1–42 PEPTIDE INDUCES CA1-SPECIFIC Aβ IMMUNOSTAINING AND SYNAPTIC DECAY IN HIPPOCAMPAL SLICE CULTURES. B.A. Bahr[1], K.B. Hoffman[1], P.W. Vanderklish[1], B.T. Kawasaki[1], A.J. Yang[2], U.S. Hess[3], K. Guthrie*[3], C.M. Gall[3], C.G. Glabe[2] & G. Lynch[1]. [1]Ctr. for Neurobiol. of Learning & Memory, [2]Dept. Molec. Biol. & Biochem, [3]Dept. Anatomy & Neurobiol, Univ of Calif, Irvine, CA 92717.

Buildup of the amyloid β-peptide (Aβ), deterioration of synaptic contacts, and the loss of neurons are well known characteristics of Alzheimer's disease and are especially pronounced in the hippocampal CA1 region (see Honer et al., *Neurobiol. Aging* 13:375, 1992; West et al., *Lancet* 344:769, 1994). Previous studies have indicated that internalized Aβ is sequestered into lysosomes and induces the production of potentially amyloidogenic fragments (Knauer et al., *P.N.A.S. USA* 89:7473, 1992; Yang et al., *J.B.C.* 270:14786, 1995), and that similar amyloidogenic accumulations are linked to the decay of hippocampal synapses (Bahr et al., *Exp. Neurol.* 129:81, 1994). The present study tested whether Aβ1-42 targets specific cells and affects synaptic function in long-term hippocampal slices, a culture system that possesses stable, adult-like synapses for months. Aβ1-42 (30 μM) pre-incubated in serum-free media was applied daily to 68 cultured slices for 8-10 h and then overnight at 17 μM with 20% heat-treated serum. Over one to nine days of treatment, selective labeling with anti-Aβ antibodies increased gradually in dense bodies in the somata and apical dendrites of CA1 pyramidals and in numerous puncta in the stratum lacunosum-moleculare; 12- to 18-day treatments produced less than expected CA1 effects and trace stratum granulosum labeling. Control slices treated 6-18 days with random1-42 or Aβ1-28 peptides exhibited no anti-Aβ staining. The Aβ slices also displayed evidence for carboxy-terminal APP fragments when stained with the 13G8 monoclonal antibody, and contained 4 and 16 kDa bands recognized by anti-Aβ (the latter band being produced over 6-12 days). In addition, autoradiography showed region-specific buildup of [^{14}C]Aβ1-42 increasing over 1-3 days. EPSPs recorded from CA1*a*, which exhibits robust Aβ effects, were 25% smaller in Aβ slices than in the same area of control slices ($p<0.01$, *t*-test; n=8); no difference was seen when recording from CA1*c* where the immunostaining was minimal. Correspondingly, Aβ slices contained 23-45% less synaptophysin over 6-18 days than random1-42 slices ($p<0.001$ at day 18). Thus, Aβ uptake and/or its induction of amyloidogenic species produce pathophysiology with some similarities to that in Alzheimer's disease (supported by NIH AG00538 & NS31230).

480.13

DENDRITIC LOCALIZATION OF PRESENILIN-1 IN RAT BRAIN: COMPARISON WITH PRESENILIN-2 BY IMMUNOCYTOCHEMISTRY. M.N. Gordon*, L.M. Refolo, Y. Harigaya, C. Eckman, M. Prada, S. Younkin and D.G. Morgan. Dept. of Pharmacology & Therapeutics, Univ. of South Florida College of Medicine, Tampa, FL 33612-4799 and The Mayo Clinic, Jacksonville, FL 32224.

Polyclonal antisera were generated against synthetic amino acid sequences of presenilin-1 (PS-1) and presenilin-2 (PS-2) deduced from published gene sequences. Four independent antisera, recognizing 2 distinct epitopes (amino acids 1-14 and 57-70) of PS-1 stained (1) a 52kDa protein on western blots of rat brain homogenate and (2) dendritic profiles of large pyramidal neurons in the cerebral cortex and hippocampal CA fields. Specific staining was abolished by preabsorption of the antisera with the relevant peptide. In addition, antisera purified by affinity for the antigenic peptide retained the dendritic localization of immunostaining and selectively immunoprecipitated a 52 kDa protein from rat brain homogenates. This dendritic staining with PS-1 was strikingly similar to that observed for microtubule-associated protein-2 (MAP2). The large number of PS-1 mutations capable of causing familial AD coupled with the shift toward more amyloidogenic forms of Aβ in carriers of these mutations will focus attention on the dendritic compartment as sites where PS-1 mutations modify APP metabolism. PS-2-like immunoreactivity was localized to neuronal somata in initial studies using antisera directed against amino acids 17-33 of PS-2. Most neurons were labeled, with strongest immunoreactivity in hippocampal pyramidal and granule cells and in entorhinal cortex. No positive staining was observed in the molecular layer of the cerebral cortex or white matter. Thus, PS-2 immunoreactivity appears most intense in those brain regions most affected in AD. Supported by Pfizer Pharmaceuticals and the Alzheimer's Association IIRG93-083 to DGM.

TRANSPLANTATION III

481.1

28 MONTH OLD RATS WITH A 16 MONTH HISTORY OF UNILATERAL NIGROSTRIATAL LESION: DRUG-INDUCED BEHAVIOR AND RESPONSE TO GRAFTED DOPAMINE NEURONS AND SCHWANN CELLS. T.J. Collier* and K. Steece-Collier. *Dept. Neurological Sci., Rush Presbyterian Med. Ctr., Chicago, IL 60612; and Dept. Neuroscience, The Chicago Medical School, North Chicago, IL 60064.

The parameters of old age and protracted history of neural deficits are seldom included in animal models of neurodegenerative disease. In order to address the influence of these factors in the response to cell grafting therapies for Parkinson's disease, we are studying male F344 rats that received large unilateral lesions of the nigrostriatal dopamine (DA) pathway at 12 months of age, and reached the age of 26 months prior to testing the efficacy of cell implants for DA replacement. Over the 14 months following lesion and prior to grafting, rats were tested for rotational behavior in response to amphetamine at 1 month intervals, and rotation to apomorphine at 2 month intervals. Animals exhibited high rates of rotation to amphetamine over the first 7 months after lesion. From 8 months post-lesion onward, rotation to amphetamine declined coincident with the development and progressive increase in behaviors we have tentatively classified as dyskinesias. Following intrastriatal implantation of a single DA neuron-rich cell suspension or a co-graft of DA cells + Schwann cells (SC), rats receiving co-grafts exhibited a reduction in rotation that was more consistent and greater in magnitude than rats receiving a DA graft alone. Neither type of implant affected 'dyskinetic' behavior. Our evidence suggests that the variables of advanced age and prolonged lesion duration significantly affect the response of rats to neural grafting in the DA system. The better success of the DA-SC co-graft supports the utility of SC as sources of DA neurotrophic activity, and suggests that replenishment of the trophic environment of grafts in aged individuals with a long history of neural deficits may be required for optimal therapeutic outcome. Supported by NIA AG10851 (TJC).

481.2

SUCCESSFUL GRAFTS OF CRYOPRESERVED MESENCEPHALIC, DOPAMINERGIC NEURONS IN PRIMATES. J.R. Sladek Jr.[1]*, B.C. Blanchard[1], J.D. Elsworth[2], T.J. Collier[3], J.R. Taylor[2], R.H. Roth[2], and D.E. Redmond Jr.[2]. [1] Dept. of Neuroscience, Chicago Medical School, North Chicago, IL 60064; [2] Depts of Psych. & Pharm, Yale University School of Medicine, New Haven, CT 06510; [3]Dept. of Neurol. Sci., Rush Medical School, Chicago, IL 60612

The ability to store embryonic neural tissue is important for the application of grafted tissue in the treatment of Parkinson's disease. Transplants of fresh and cryopreserved dopamine (DA) neurons were made into six, male, MPTP-treated African green monkeys. Each received E44 tissue from one donor; half was implanted as fresh, solid grafts; the other half was cryopreserved, stored briefly at -80°C, thawed and grafted into the contralateral side. Thus, three fresh grafts were placed into the right caudate nucleus, while three equal-sized, cryopreserved grafts from the same donor were implanted into the left caudate nucleus within 3-4 hours of placement of the fresh grafts. Grafts were allowed to grow for 3 months. Brains were analyzed for tyrosine hydroxylase cell and fiber patterns. Three animals received manually frozen cells and three received cells that were cryopreserved with an automated apparatus.

All six animals had grafts of cryopreserved DA neurons. Some were comparable to fresh grafts, and two grafts in one animal each contained over 1000 DA neurons. Fiber outgrowth was comparable to fresh grafts. Some grafts were found deep in the caudate and were indistinguishable from fresh grafts. A second animal had a graft with over 1000 DA neurons. Our prior studies with fresh tissue indicate that 1000 DA neurons/graft is adequate for functional improvement following MPTP. In one animal, 42% of the total DA neurons in grafts were derived from the cryopreserved tissue. On average, cryopreserved grafts had fewer total DA neurons.

It is possible to cryopreserve primate neural tissue and to obtain substantial DA neuron survival after transplantation. The findings also reveal a variability that suggests that further studies are needed to develop an effective cryopreservation procedure for primate dopamine neuroblasts in order to maintain maximal viability and function after transplantation. Supported by NS24032 and RSA MH00643 (DER).

481.3

EIGHT YEARS EXPERIENCE WITH FETAL NEUROTRANSPLANTATION IN PATIENTS WITH ADVANCED PARKINSONS DISEASE. CR Freed,*[1] RE Breeze,[1] MA Leehey,[1] SA Schneck,[1] CF O'Brien,[1] LL Thompson,[1] LO Ramig,[1] CA McRae,[1] JC Mazziotta,[2] RS Miletich,[3] and D Eidelberg [4]. 1. University of Colorado School of Medicine, Denver, CO 80262; 2. UCLA Medical Center, Los Angeles 90024; 3. National Institutes of Health, Bethesda, MD 20892; 4. North Shore University Hospital, Manhasset, NY 11030.

Since November 1988, we have performed embryonic mesencephalic transplants in 25 patients, 17 males and 8 females, with a mean age of 58 and duration of Parkinson's disease of 14 years. Most patients have received putamenal implants (23/25) with simultaneous bilateral placement of strands of tissue from one to six embryos 7 to 8 weeks post conception which had been in tissue culture prior to transplant. The current surgical approach uses needle passes through the forehead, with a superior and inferior pass into each putamen bilaterally. Activities for Daily Living scores provided the best clinical measure of patient outcome. At one year after transplant, this value fell 30% in the "off" state and 40% in the "on" state. Drug doses were reduced an average of 40% and one patient discontinued all medication by 18 months after surgery. Hoehn and Yahr scores improved about 1.1 unit from 3.5 to 2.4. Pre and postoperative ^{18}fluorodopa PET scans have been obtained in 10 patients with 8/10 showing improved putamenal uptake bilaterally. Complications included one death from brain hemorrhage. There have been no complications from the frontal approach (n=8). After improving progressively for 3 to 4 years after a unilateral implant in caudate and putamen, our first patient has deteriorated during years 6 to 8 as shown by clinical exam, PET scans, and increased L-dopa requirements. Retransplant on the unoperated side is planned. Unilateral pallidotomy is being considered for patients with persistent drug induced dyskinesias. We conclude that embryonic neurotransplants provide substantial and sustained benefit to patients with advanced Parkinson's Disease. Supported by grants from Robert E. Stanton, NIH, and the National Parkinson Foundation.

481.4

REASONS FOR LIMITED CLINICAL BENEFIT FOLLOWING FETAL NIGRAL TRANSPLANTATION IN THE TREATMENT OF PARKINSON'S DISEASE. T.B. Freeman*, J.H. Kordower, R.A. Hauser, B.J. Snow, P.R. Sanberg, C.W. Olanow. University of South Florida, Div. of Neurosurgery, Tampa, Florida.

Over 200 patients have received fetal nigral transplants for the treatment of Parkinson's disease (PD) in 17 centers worldwide. Preliminary evidence of clinical efficacy has been demonstrated. In our patients, significant improvement from baseline to latest evaluation (mean of 18 months) was noted in UPDRS motor (mean ± S.E. = 50.8 ± 7.2 vs 35.7 ± 5.5, p = .028) and UPDRS ADL scores (32.1 ± 2.9 vs 21.2 ± 3.1, p = 0.28) while "off". Also percent time "off" significantly decreased from 34.8 ± 5.1% to 16.5 ± 4.9% (p = .046). This correlates with reliable increased fluorodopa uptake on PET scan (Ki = 0.0069 ± 0.0005 vs 0.0111 ± 0.0007, p = .003) and autopsy-proven graft survival with organotypic host reinnervation.

However, it is not appropriate at this time to offer fetal nigral transplantation as a definitive therapy for PD. Only three centers have demonstrated reproducible preliminary improvement in both clinical outcome and FD-PET. Although supranormal DA neuronal survival has been demonstrated at autopsy, only 53% of the limited target area has been reinnervated. The survival rate of transplanted DA neurons is only 5%, limiting the practicality of widespread use of the technique. Many symptoms of PD have not been improved and many symptoms improved significantly but not completely. This most likely correlates to the limited regions of reinnervation, multisystem degeneration in PD and possible inability of DA grafts to influence "non DA" symptoms. These and other issues remain to be studied further in the laboratory and with well-designed clinical trials. Although preliminary results are encouraging, neural transplantation remains an experimental therapy.
SUPPORTED BY NIH GRANT #1 RO1 NS32842-01

481.5

SIX TO NINE YEAR FOLLOW-UP OF PARKINSON'S DISEASE PATIENTS AFTER UNILATERAL AUTOLOGOUS ADRENAL MEDULLARY AND HUMAN FETAL VENTRAL MESENCEPHALIC BRAIN GRAFTING. I. Madrazo, R.E. Franco-Bourland*, H. Castrejón, C. Cuevas, F. Ostrosky-Solís, E. Magallón, C. Zamorano, I. Grijalva, G. Guízar-Sahagún. IMSS, INNSZ, UNAM México, D.F., México

This year marks the tenth anniversary of the first two successful cases of Parkinson's disease (PD) treated with autologous adrenal medullary (AM) brain transplants (New Engl J Med 316 (1987) 831-834). The purpose of human brain grafting is to deliver sufficient endocrine or neural tissue to neurodegenerate areas of the brain, in order to supply adequate amounts of the missing neurotransmitters, as well as other neuroprotective and neurotrophic factors for functional recovery. In an attempt to restore the dopaminergic deficiency of PD, we have tested the clinical potential of the unilateral striatal implantation to PD patients of dopamine-rich autologous AM and human fetal ventral mesencephalic (VM) grafts. Using open microsurgery for the implantation of tissues to the ventricular wall of the caudate nucleus (CN), the strategies for grafting one gram of autologous AM tissue included placement: to one cavitated site, to multiple sites without precavitation, and cografting with peripheral nerve tissue (intercostal nerve) to one site, after precavitation; in the case of homotransplantations, the total VM tissue from one fetus was embedded to one site in the CN. Using the Unified Parkinsonism Rating Scale, the Hoehn and Yahr, and the Schwab and England evaluation scales, transplanted PD patients from all groups have been followed by us now for over 6-9 years. All transplantation groups compared, those patients cografted with AM/peripheral nerve tissue, and those with fetal VM grafts appear to have benefitted the most from surgery. This work was funded by the Interinstitutional Research Foundation for the Regeneration of the Central Nervous System, México.

481.6

WITHDRAWN

481.7

ASTROCYTES ENGULF IMPLANTED DOPAMINE MICROSPHERES: MECHANISMS FOR FIBER GROWTH? A. McRae*[1], E.A. Ling[2], S. Hjorth[3], A. Dahlstrom[1], D. Mason[4], T. Tice[4], Inst. of Anatomy & Cell Biology[1] and Pharmacology[3], Univ. of Göteborg, Medicinaregatan 3-7, S-41390 Göteborg, Sweden; National Univ. of Singapore[2], Singapore 119260; Southern Res. Institute[4], Birmingham, AL 35255, USA.

Controlled-release dopamine (DA) loaded microspheres (MS) made with the biocompatible biodegradable polyester excipient poly (DL-lactide-co-glycolide) (PLG) induce dopamine (DA) fiber growth in the 6-OHDA denervated rat striatum. Functional recovery appears related to the degree of fiber growth. Unexpectedly electron microscopic (EM) investigations revealed that astrocytes engulf MS implanted in the host striatum. Scanning EM and confocal laser microscopy confirmed that cultured striatal astrocytes take up DA loaded MS and have a preference for small diameter MS. Unilateral 6-OHDA denervated rats displaying 400 or more contralateral rotations to apomorphine were implanted with DA loaded MS having diameters less than 45 µm or greater than 45 µm. Small diameter MS induced significant reductions both in the number of apomorphine rotations and DA fiber growth. In contrast, rats bearing larger diameter MS displayed only slight reductions in apomorphine induced rotational behavior and had rare fiber growth. These results suggest that astrocytes may play a role in the ability of implanted DA MS to promote fiber growth and assure functional recovery. In view of the numerous trophic factors associated with astrocytes these mechanisms could be of therapeutic importance in Parkinson's disease or other neurodegenerative disorders.

Supported by the Swedish MRC (#7486 to S.H., #2207, to A.D.), Riksbankens Jubileumsfond, Handl. Hj. Svenssons Stiftelse, King Gustav V's and Queen Victoria's Research Foundation.

481.8

CORTICAL INTERNEURONS UPREGULATE NEUROTROPHIN EXPRESSION IN RESPONSE TO TARGETED APOPTOTIC DEGENERATION OF NEIGHBORING PYRAMIDAL NEURONS *IN VIVO*. Y. Z. Wang*, V. L. Sheen and J. D. Macklis. Department of Neurology and Program in Neuroscience, Harvard Medical School, and Division of Neuroscience, Mental Retardation Research Center, Children's Hospital, Boston, MA, 02115

Embryonic neocortical neurons and multipotent neural precursors transplanted into regions of adult mouse neocortex undergoing photolytically induced, synchronous apoptotic degeneration of callosal pyramidal neurons can respond to reexpressed developmental signal molecules, selectively migrate into these regions, differentiate into pyramidal neurons, accept afferent synaptic input, and re-form distant projections with specificity. The signal molecules include spatially and temporally specific upregulation of the neurotrophins BDNF, NT4/5, and NT-3, and the TrkB receptor, uniquely during the 3 week period of ongoing apoptosis and directed differentiation within the experimental regions. In the current experiments, we have begun to examine the specific cellular localization of these upregulated messages following induction of targeted apoptosis, using *in situ* hybridization. Pyramidal neurons in C57B/6J mice (n=10) were retrogradely targeted with photoactive nanospheres and noninvasively activated at 4 weeks of age to initiate apoptotic degeneration in lamina II/III of neocortex. We performed *in situ* hybridization with ^{35}S radiolabeled oligoprobes and riboprobes 3 and 6 days after induction. BDNF mRNA expression was specifically increased in local interneurons surrounding degenerating pyramidal neurons, uniquely within the regions of ongoing degeneration in lamina II/III of neocortex. BDNF expression was not increased in the pyramidal neurons themselves, nor in local glia. Control regions from intact (n=6) and experimental mice showed no mRNA upregulation. These findings suggest that the induction of BDNF mRNA (and potentially the other upregulated neurotrophins) is a specific effect of targeted apoptosis that may result from intercellular signaling between degenerating pyramidal neurons and surrounding interneurons. These results, and analysis of the cellular localization of other upregulated molecules, may provide insight regarding intercellular signaling with apoptosis, molecular controls responsible for neuronal migration and differentiation during neocortical development, and toward potentially therapeutic neural precursor transplantation within neocortex. Supported by HD28478, Alzheimer's Association IIRG-93-143, training grant MH18012, MRRC HD18655, and The Rita Allen Foundation.

481.9

VERSATILE EXPRESSION OF LONG-TERM CULTURED EGF RESPONSIVE PROGENITAL CELLS FROM FETAL BRAIN. F. C. Zhou* and Y. H. Chiang, Depts. Anatomy and Neurobiology Program, Indiana Univ. Sch. Med., Indianapolis, IN 46202

Epidermal Growth Factor (EGF) responsive progenital cells from fetal mesencephalon cells can be isolated and cultured in the form of neurospheres (Reynold and Weiss, 1992). We previously isolated the EGF-responsive neurospheres from embryonic 12-16 days old fetal brain, and characterized the differentiation of astroglial cells (Chiang, Silani and Zhou, 1996). In this study, we report the longevity and versatility of neuronal /glial expression of the non-passage neurospheres. Non-passaged neurospheres can be maintained in 10 ng/ml EGF supplemented DMEM / F12 culture medium for more than a year. When plated on poly-D-lysine and laminin substrate in neurobasal medium with 2% fetal calf serum, the neurospheres grow and differentiate into glia and neurons with similar expression (described below) regardless of age (3, 6, and 9 months old). Many progenitor cells still remain undifferentiated (Nectin-positive), capable of dividing (PCNA-positive), and maintain immature features (S100-positive radial fibers). Glial marker (glial fibrillary acidic protein, GFAP) and neuronal marker (microtubule associate protein, MAP2) are more often expressed individually, but can also be found in a small population within the same cells. In addition, the GFAP-staining indicative of mature astrocytes was found in cells bearing neuronal varicosity-like structures. Immunocytochemical staining against 5-HT, 5-HT transporter (5-HTT), and 5-HT1A, indicate that many 5-HT-, 5-HTT-, and 5-HT1A immunoreactive (IR) cells exist in the long-term cultured mesencephalic neurospheres. In addition, a smaller population of 5-HT-, 5-HTT-, and 5-HT1A positive cells were also found in the striatal and cortical neurospheres, but not in our primary culture. In contrast, tyrosine hydroxylase was expressed in small size neurons in a small proportion of the mesencephalic neurospheres. This indicates that the progenitor cells we isolated are versatile in their expression in a number of phenotypes. On the other hand, the low expression of TH was also noticed. The use of differentiation factors to increase TH expression is currently in progress in our laboratory. (PIRC 22-821-01)

481.10

Grafts of Cells Genetically Modified to Produce NT-3 Promote Corticospinal Axon Sprouting After Spinal Cord Injury. R Grill[1,*], K. Murai[1], A Blesch[1], FH Gage[2], M H Tuszynski[1,3]. [1]Dept Neurosci., Univ Calif-San Diego, La Jolla, CA; [2]Salk Institute, La Jolla, CA; [3]VA Med Ctr, San Diego, CA.

Little spontaneous recovery of adult corticospinal motor projections occurs after spinal cord injury in mammals. To assess neurotrophin responsiveness in this projection, primary Fischer 344 rat fibroblasts were genetically modified with MLV retroviral vectors to produce and secrete human NT-3. Transduced cells produced hNT-3 mRNA and biologically active protein in vitro. These cells were embedded into collagen matrices and grafted to spinal cord dorsal hemisection lesion sites in 18 adult Fischer rats. 10 control rats received identical grafts lacking the NT-3 transgene. Animals survived from 1-7 mo, then received WGA-HRP cortical injections to label corticospinal projections.

From 1-7 mo later, NT-3 graft recipients showed significantly elevated corticospinal axon sprouting in the ventral grey matter at the spinal cord lesion site (assessed by densitometry of HRP-TMB reaction product on NIH Image software, corrected for variability in labeling intensity between subjects); NT-3: 3.01±0.79 pixels vs. 1.71±0.23 pixels in controls, p=0.05. Significant differences were also present at the mid-point and distal to the lesion site ($p<0.05$ in both cases). Lesion extent was verified in all subjects.

These findings indicate that lesioned adult corticospinal axons show long-term responsiveness to hNT-3 delivered by gene therapy.

[Supported by Dept. of Veterans Affairs, International Spinal Research Trust, and the Hollfelder Foundation.]

481.11

SCHWANN CELL MICROIMPLANTATION INTO THE ADULT CENTRAL NERVOUS SYSTEM: IMMUNOLOGICAL RESPONSES. C.C. Stichel*, G. Wunderlich, S. Hermanns, C. Rosenbaum, C.O. Hanemann, H.W. Müller Molecular Neurobiology Lab., Dept. of Neurology, Univ. of Düsseldorf, D-40225 Düsseldorf, Germany.

Over the past decade experimental studies have suggested that regeneration of lesioned CNS axonal pathways through Schwann cell (SC) implantation might be developed into a useful therapeutic approach in human neurodegenerative disorders. Although it is known that inflammatory reactions may be a significant impediment to the survival, integration and effect of implanted SC a detailed analysis of SC induced immune response in the host tissue is still missing. Moreover, with respect to the clinical perspective, it seems also important to address the nature of immune responses to a delayed or xenogeneic SC implantation.

The present study was designed to examine the immune response to different SC transplantation paradigms. As a lesion and implantation model we used the stereotactically transected postcommissural fornix of the adult Wistar rat (Wr). One group of animals received immediately after transection either an injection of (1)allogeneic(Wr)-neonatal; (2)allogeneic(Wr)-adult or (3)xenogeneic(human)-adult SC. A second group received an (4) allogeneic(Wr)-neonatal SC implant 2 days to 4 weeks after transection and (5)control animals were lesioned only. Animals were allowed to survive up to 2 months before immunocytochemical analysis of T-cell infiltration and MHC-expression was performed.

Mechanical transection induced (i) a rapid expression of MHC antigens and (ii) an infiltration of few T-cells, that were all of the helper/inducer subset. While no change in MHC- and T-cell responses were found after immediate or delayed allogeneic SC-implantation, xenogeneic human SC induced a prominent host-defense response. Supported by the DFG.

481.12

IMPROVED SURVIVAL OF NEURONAL PRECURSORS TRANSPLANTED AS MIXED NEURONAL-GLIAL AGGREGATES EA Bonaroti, G Wang, J Hook, M White, S Meriney*, C Achim. Div. of Neuropathology, Univ. of Pittsburgh, Pittsburgh, PA, 15213.

Neuronal precursors and stem cells may be a useful alternative to primary fetal tissue for CNS grafting. Others have shown poor survival when pure populations of precursors are transplanted. We hypothesized that neuro-glial interactions, and neurotrophic factors supplied by microglia would improve the survival of precursors transplanted as mixed reaggregates. We have previously described a subset of cells present in reaggregate mixed cultures derived from second trimester human fetal tissue which have the characteristics of precursor cells. These cells are morphologically undifferentiated, mitotically active, and give rise to differentiated neurons and glia in vitro. A pure population of EGF responsive cells from the subventricular zone of second trimester tissue was generated, labeled with BrdU, and transplanted into the striatum of 12 outbred male SCID mice. An identical volume (10 ul) of mixed aggregate culture was injected into the contralateral hemisphere. Animals were sacrificed at 2, 4, and 6 weeks. Comparison of overall graft volume, and cell counts of BrdU labeled cells using anti-BrdU antibodies and confocal laser microscopy showed an increased survival of precursor cells using mixed aggregate cultures. To investigate the role of microglia in the support of precursor cell survival, mixed aggregate cultures were enriched with microglia and graft survival after transplantation assessed. Graft volume measurements and BrdU positive cell counts are currently underway. Glia appear to perform an important role in supporting survival of transplanted CNS tissue. (Supported by CMRF, Univ. of Pittsburgh)

481.13

A HELPER VIRUS-FREE HSV-1 PLASMID VECTOR SYSTEM; IMPROVED TITERS AND LONG-TERM EXPRESSION. A. Geller*, Y. Wang, L. Yu, E. Gussoni[1], L. Kunkel[1], and C. Fraefel, Div. Endocrinology and [1]Genetics, Children's Hosp., Boston, MA 02115.

Vectors based on Herpes Simplex Virus Type 1 (HSV-1) have potential for gene transfer into the brain to perform gene therapy of neurological disorders and to study neuronal physiology. However, a number of unresolved problems with HSV-1 plasmid vectors, especially cytopathic effects and immune responses, have limited their potential utility. The majority of these problems appear to be caused by the helper virus, so we developed a helper virus-free packaging system for these vectors (reported last year). Following transfection into cultured fibroblast cells, a HSV-1 cosmid set deleted in the DNA cleavage/packaging signals can provide all the required functions for replicating and packaging cotransfected vector DNA. The resulting vector stocks lack detectable helper virus, and therefore gene transfer into the rat brain is more efficient and is associated with markedly reduced cytopathic effects and immune responses compared to vector stocks that are prepared using a helper virus.

We now report data from 3 recent experiments that were designed to further improve and characterize this helper virus-free vector system: (i) By optimizing the transfection protocol we obtained vector titers of $>10^6$ infectious vector particles (IVP)/ml from the packaging procedure, and after concentration the titer was $\sim 10^8$ IVP/ml. This represents ~10-fold improvement. (ii) We examined a number of promoters for both the efficiency of gene transfer and the stability of long-term expression in the brain. The promoters which were studied included the neurofilament heavy gene promoter, the tyrosine hydroxylase (TH) promoter, and the cytomegalovirus immediate-early (CMV IE) promoter. Both the TH promoter and the CMV IE promoter supported surprisingly high efficiencies of long-term gene expression. (iii) We developed a method to colocalize vector DNA and a reporter gene product in cells in the brain. Vector DNA was detected by fluorescent in situ hybridization and the reporter gene product was detected by immunohistochemistry.

Supported by Burroughs Wellcome Fund, NARSAD, Neurovir.

481.14

REGULATABLE TRANSGENE EXPRESSION IN BRAIN USING A HERPES SIMPLEX VIRUS VECTOR CONTAINING A STEROID RESPONSIVE INDUCIBLE PROMOTER. P.L. Poliani, T. Oligino, Y. Wang, S.Y. Tsai, B.W. O'Malley, J.C. Glorioso and D.J. Fink*. Departments of Molecular Genetics and Biochemistry and Neurology, U. Pittsburgh, and VA Medical Center, Pittsburgh, PA 15261 and Baylor College of Medicine, Houston TX 77030.

We have previously demonstrated that recombinant HSV-based vectors can be used to transfer and express transgenes in neurons in brain. In order to regulate the expression of the transgene, we constructed an HSV-based vector containing a drug inducible transactivator. A cassette coding for a tripartite transactivator consisting of the yeast GAL4 DNA binding domain, the HSV VP16 acidic transactivation domain, and a mutated hormone binding domain of the progesterone receptor responsive to the drug RU486 (PNAS 91:8180, 1994) under the transcriptional regulation of the human cytomegalovirus immediate early promoter was inserted into the gC locus of an ICP4-deleted replication-incompetent HSV genomic vector. A *lacZ* reporter gene under the transcriptional control of a minimal TATA box-containing promoter downstream of 5 GAL4 binding sites was placed in the TK locus of the same vector.

Vero cells transfected with the vector showed a 30-fold induction in β-gal expression measured by ONPG asssay following 24 hrs exposure to 10^{-7} M RU486. To assess expression *in vivo* 5 µl containing $2x10^6$ pfu of virus was injected stereotactically into the hippocampus of 200-250 gm Sprague Dawley rats. At the time of injection and 24 hrs later the animals were treated with either RU486 (15 mg/kg IP) or vehicle alone. At 48 hrs the animals were sacrificed and for β-gal expression determined by X-gal staining. Drug-treated but not control animals showed expression of β-gal in neurons of the injected hippocampus.

This is the first demonstration of drug-inducible transgene expression in brain following vector-mediated gene transfer.

Supported by grants from the NIH, Department of Veterans Affairs, and GenVec.

482.1

MEMBERS OF THE JAK-STAT PATHWAY ARE EXPRESSED IN VIVO IN THE DEVELOPING CNS AND BECOME RECRUITED IN ST14A CNS PROGENITOR CELLS BY ACTIVATED SURFACE RECEPTORS

E. Cattaneo*, L. Conti, S. Govoni° and C. De Fraia Inst. of Pharmacol. Sciences, Via Balzaretti 9, 20133 Milano; °Inst. of Pharmacol., Univ.of Pavia, Pavia-Italy

The presence and activation of members of the JAK-STAT families of proteins in response to specific cytokines is being deeply investigated in the hematopoietic system. Although some evidences suggested that cytokines could play an important role in the brain, very limited informations are available on their presence and possible involvement in CNS functions. We found that among the JAKs, Jak2 is present at high levels in the different brain regions analyzed, its expression being more aboundant at early stages of fetal life. Furthermore Stat1,Stat3, Stat5 and Stat6 were also present in vivo in CNS tissues. We were interested in whether members of the JAK-STAT families can become activated in CNS progenitor cells in response to specific stimuli. For this purpose different exogenous cytokine receptors were expressed in the striatal-derived ST14A CNS progenitor cells. Clonal populations of IL3 receptor or GMCSF receptor (of murine or human origin) bearing ST14A cells were isolated. Western Blot analyses and binding assay with iodinated ligands revealed that stable transfectants expressed high affinity receptors. Transfected cells exposed to the appropriate cytokine in the absence of serum, were capable of extensive proliferation. Immunoprecipitation studies showed that 10 min. after addition of a specific ligand (IL3 or GMCSF), phosphotyrosine containing proteins of different m.w. could be detected. When the same immunoprecipitated material was immunoblotted with anti-JAK2 antibody a tyrosine phosphorylated band of 130kDa corresponding to JAK2 could be detected in lysates from stimulated cells. Furthermore also Stat5, one of the effectors of JAK2 in hematopoietic cells, was found phosphorylated in the stimulated cytokine receptor-bearing ST14A CNS progenitors. These results indicate that members of the JAK-STAT families are expressed in vivo in the CNS and may be activated in CNS progenitor cells (partially funded by the Hereditary Disease Foundation,USA).

482.2

PROLACTIN ACTIVATES C-JUN AND STIMULATES PROLIFERATION IN PC12 CELLS. Y. Cheng, R.L. Perlman* and D. Mangoura. Department. of Pediatrics, The University of Chicago, School of Medicine, Chicago, IL 60637.

The anterior pituitary hormone prolactin not only participates in osmoregulation and reproduction but also has important actions in the nervous system. The prolactin receptor belongs to the super-family of cytokine/growth hormone/prolactin receptors. Prolactin caused a significant increase in c-jun phosphorylation in undifferentiated PC12 rat pheochromocytoma cells. The increase in c-jun phosphorylation was evident within 15 min of incubation with prolactin and was accompanied by an increase in c-jun levels, which peaked at about 2 hr and lasted for several hours. The activation of c-jun was specific, in that prolactin did not stimulate the phosphorylation of c-fos in these cells. In addition, prolactin caused little or no increase in Erk2 activity. Therefore, prolactin probably activated c-jun in PC12 cells through the JNK signaling pathway. This pathway is activated after NGF withdrawal in PC12 cells, but the importance of its activation by physiological stimuli is unknown. In the long term, prolactin was mitogenic for PC12 cells as measured by direct cell counts. The growth rate of PC12 cells after two days of prolactin treatment (100 nM) is 1.80:1= prolactin treated cells ($5.45 \times 10^5 \pm 0.63$):control cells ($3.02 \times 10^5 \pm 0.15$) and 1.75:1 after 72 hr stimulation. Prolactin thus appears to regulate the proliferation and growth of PC12 cells. Prolactin may participate in the regulation of adrenal medullary function in pregnancy and lactation. (HD 09402 and BRF).

482.3

INSULIN-LIKE GROWTH FACTOR-I SIGNALING PATHWAYS IN NEURITE OUTGROWTH. B. Kim*, and E.L. Feldman, Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, MI 48109

We have previously shown that insulin-like growth factor-I (IGF-I) can enhance neurite outgrowth and are now investigating its role in lamellipodia formation and neurite initiation. As part of these studies, we are characterizing the signaling pathway that mediates IGF-I's effects on neurite elongation. In our studies, we employ a cell culture model of neuronal growth, the SH-SY5Y human neuroblastoma cells. When SH-SY5Y cells were treated with IGF-I, there was a dose-dependent increase in the tyrosine phosphorylation of the type I IGF receptor (IGF-IR), and 2 MAP kinases: ERK1 and ERK2. While IGF-IR phosphorylation was immediate, maximal phosphorylation of ERK1 and ERK was not reached for 30 min and maintained for up to 4 hr. IGF-IR, ERK1 and ERK2 phosphorylation were inhibited by the type I IGF receptor blocking antibody, α-IR3, while ERK1 and ERK2 phosphorylation were inhibited by a compound which specifically blocks MEK activity (PD98059). In parallel, PD98059 blocked neurite outgrowth. Inhibition of neurite outgrowth by PD98059 was present only if neurons were cultured for a sufficient period of time to allow for extracellular matrix deposition. Collectively these studies show that IGF-I 1) induces a unique pattern of MAP kinase phosphorylation in neurons which results in neurite outgrowth, and 2) may play a role in the organization of extracellular matrix.

Sponsored by R29 NS32843 and grants from the American Diabetes Association and the Juvenile Diabetes Association.

482.4

MOLECULAR CLONING OF CDGF, A NEUREGULIN-LIKE GENE ENCODING NOVEL LIGANDS FOR ERBB-RECEPTORS. H. Chang, W. Gilbert†* and U. J. McMahan. Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305 and †Bio. Labs, Harvard Univ., Cambridge, MA 02138.

Neuregulins (also called ARIA, GGF, Heregulin, and NDF) are a group of polypeptide factors that arise from alternative RNA splicing of a single gene. Through interaction with the erbB-receptors (erbB2, erbB3, and erbB4), neuregulins are thought to play important roles in the regulation of cell growth and differentiation in many tissues including the nervous system. Here we report the cloning of a second neuregulin-like gene, which we named CDGF. Sequence analysis of CDGF cDNA clones shows that they have a motif structure similar to that of neuregulins. Northern blot analysis of adult rat tissues indicates that CDGF transcripts are detected mostly in neural tissues. An alternative splicing site in the EGF-like domain gives rise to two isoforms of CDGF (α and β).

Recombinant CDGFα protein induces the tyrosine phosphorylation of erbB2, erbB3 and erbB4 in cell lines expressing mixtures of these erbBs, indicating that it is a functional ligand for erbB-receptors. A detailed comparison of CDGFs and neuregulins, such as the temporal and spatial regulation of their expression, and their interactions with erbB-receptors, will be crucial for understanding the biological functions of this multi-ligand/multi-receptor signaling pathway. (supported by NIH grant NS14506 to UJM).

482.5

REK, A NOVEL RECEPTOR TYROSINE KINASE IN THE AXL/TYRO3 FAMILY. James J. Fiordalisi, Stephen Crews* and Patricia F. Maness. Dept. Biochemistry, University of North Carolina Sch. of Med., Chapel Hill, NC 27599.

Rek (retina-expressed kinase) is a receptor tyrosine kinase identified by reverse-transcriptase polymerase chain reaction in cultured chick retinal Müller glia. The Rek extracellular region contains two Ig domains and two fibronectin (FN) domains placing it in a family of receptor tyrosine kinases, which includes *axl, tyro3*, c-*mer*, and c-*eyk*. The intracellular domain of Rek contains consensus SH2 binding motifs for PI-3-K (YDLM) and for Grb2 (YVNI) suggesting that Rek triggers several signal transduction pathways. Rek transcripts are restricted primarily to developing brain and retina where Rek may play a role in the differentiation of retinal neurons and glia. Overexpression of *rek* cDNA in mouse NIH3T3 fibroblasts resulted in ligand-independent activation of the 140kD Rek kinase and induction of morphologically transformed foci demonstrating that Rek has oncogenic potential. Rek-transformed mouse NIH3T3 fibroblasts were found to aggregate as a result of cell-cell interactions via the Rek extracellular region, suggesting that Ig and FN domains may mediate cell contact signaling in developing neural cells.

These findings indicate that the normal function of Rek is likely to be in mediating cell-cell recognition events governing the differentiation or proliferation of neural cells. It's ability to induce transformation in NIH3T3 cells upon overexpression suggests that mutations in *rek* causing overexpression or kinase activation may contribute to the development of malignant nervous system tumors.

This work was supported by NIH grant NS52269.

482.6

CERAMIDE: A NEGATIVE REGULATOR OF NEURITE GROWTH IN RAT SYMPATHETIC NEURONS. M. Bussière*, E.J. Posse de Chaves, J.E. Vance, R.B. Campenot and D.E. Vance. University of Alberta, Edmonton, Alberta, Canada, T6G 2S2.

Recent studies suggest that the agonist-induced generation of the lipid second messenger ceramide is an anti-mitogenic signal leading to the inhibition of cellular proliferation, cell differentiation and apoptosis. We are investigating the role of ceramide in neurite growth using compartmented cultures of rat sympathetic neurons. We have previously shown that a local increase in the intracellular level of ceramide within the distal neurites of sympathetic neurons, induced by *threo*-PPMP (D,L-*threo*-1-phenyl-2-hexadecyl-3-morpholino-1-propanol) treatment, results in the inhibition of neurite growth. In agreement with these results, treatment of distal neurites, but not cell bodies/proximal neurites, with 10 mM exogenous cell-permeable C_6-ceramide inhibited neurite growth by 60%. A similar concentration of the closely-related molecule, C_6-dihydroceramide, had no effect on neurite growth. The effect of ceramide on neurite growth was not reversible by the concomitant treatment of neurons with ceramide and a molar excess of either diC$_8$-glycerol (a cell-permeable diacylglycerol) or ceramide-1-phosphate, which have been previously shown to reverse certain effects of ceramide on cells. To determine if a metabolite of ceramide might be responsible for the inhibition of neurite growth observed upon ceramide-treatment, the metabolism of a fluorescent analog of ceramide, C_6-NBD-ceramide, was examined. Greater than 90.2% of the C_6-NBD-ceramide taken up was recovered as ceramide, 2.3% was recovered as ceramide-1-phosphate and 2% as sphingomyelin. Less than 3% of the fluorescence was recovered as free NBD, representative of the product of the hydrolysis of ceramide, sphingosine. These results suggest that ceramide plays a key role as a negative regulator of neurite growth and underscores the finding that neurite growth is regulated by local mechanisms within growing neurites. The mechanism of ceramide-mediated inhibiton of neurite growth and its relationship to the promotion of neurite growth by NGF will be examined. (Supported by the Rick Hansen Foundation, AHFMR, MRC)

482.7

DOES NEURONAL MODULATION OF P21RAS ACTIVITY INDUCE NEUROTROPHIC EFFECTS? R. Heumann*,F. Narz[1], Y. Algür[1], D. Bartsch[1], E. Wagner, H. Berns[1],K. Obst[2] and P. Wahle[2], Ruhr-Univ.Bochum Molekulare,Neurobiochemie[1] or Neurobiologie[2],44780 Bochum, Germany

We have previously shown that p21ras activity mediates nerve growth factor induced survival in peripheral sensory neurons of the chick. Here we have produced transgenic mice expressing permanently activated Val12-Ha-ras (ras-oncogene) under the control of the neuronal promoter for the synapsin-1 gene. During development the transgene protein product was detected perinatally in total brain extracts reaching high levels at P40. In the adult animals high levels of Val12-Ha-ras protein were found in the cortex, hippocampus, septum and lower levels in the olfactory bulb and mesencephalon. The regional and cellular transgene expression pattern correlated with that of the endogenous synapsin protein. Macroscopic inspection of brain sections revealed a 20% enlargement of the cerebral hemispheres displacing the tectum and cerebellum further posterior. Consistently, the cell somata of layer V pyramidal neurons showed an increased diameter. Furthermore, in the cortex there was an increase in neuropeptide Y (NPY) mRNA levels, while the mRNAs coding for glutamate decarboxylase or parvalbumin remained unaltered. In the septum of adult transgenic mice we find elevated choline acetyltransferase activities indicating that neuronal p21ras may regulate this behaviourally relevant enzyme. We tested if transgenic activation of p21ras may lead to a protection of neurons against (excito)toxic damage in dissociated cultures: Embryonic mesencephalic cells were kept in culture for 9 days and identified dopaminergic neurons were partially resistant to the toxic effectes of 6-OHDA, thereby mimicking the effect of neurotrophin-4/5. In cultures of postnatal hippocampal activated p21ras expressing neurons a partial protection against long-term treatment with glutamate (1mM) was established. We conclude that p21ras activation mimics important functions of neurotrophins.

487.8

TRKA EXPRESSION ATTENUATES C6-2B GLIOMA CELL GROWTH: *IN VITRO* AND *IN VIVO* STUDIES. S.J. Rabin, C. Tornatore, B.Baker-Cairns, S. Keir and I. Mocchetti*, Dept. of Cell Biology, Georgetown Univ. Sch. of Med., Washington DC 20007, and LMMN, NINDS, NIH, Bethesda, MD 20892.

A novel approach to the treatment of glioblastoma multiforme (GMB) would be to induce the tumor into a differentiated, post-mitotic phenotype to attenuate tumor progression followed by treatment with conventional therapies. Nerve growth factor (NGF) characteristically induces a differentiated, post-mitotic state in cells which express the high affinity NGF receptor trkA. C6-2B rat glioma cells express the low affinity NGF receptor p75 but not trkA and thus are unresponsive to NGF. Upon stable transfection with trkA rat cDNA, C6-2B cells (C6trk+) exhibit novel NGF-mediated biochemical and morphological responses. Most important, in these cells, NGF accelerates differentiation and decreases the growth rate, suggesting that expression of trkA attenuates C6-2B cell proliferation. We grafted wild type and C6trk+ cells into 20 ACI nude rats. By 7 days post-transplantation, the wild type C6-2B cells had formed a 1x1 mm tumor in the striatum which was MHC class I positive and Thy1.1 negative, with patchy vimentin immunoreactivity. Scattered CD4, CD8 and CD11b cells could be identified within the tumor mass as well. The transplant was positive for the low affinity NGF receptor p75, and negative for trkA, confirming the identity of C6-2B glioma cells. By day 14 the tumor had doubled in size and by day 28 half the striatum was replaced with the gliomatous cells. In contrast, growth of the C6trk+ cells was markedly attenuated. By 7 days and up to 28 days, these cells had formed only a small nidus of approximately 1x1 mm. Not until day 71 post-transplantation had the C6trk+ formed a significant tumor mass which measured 1x3 mm. Transplanted C6trk+ cells were MHC class 1 positive, Thy1.1 negative and, interestingly, these cells remained trkA positive up to 71 days *in vivo*. Our data encourage the speculation that expression of trkA in GMB *in vivo* will attenuate tumor progression. [Supported by NIH-NS01675 and T32HD07459].

482.9

IN VIVO IMAGING OF TrkA WITH SMALL MOLECULE ANALOGS OF NERVE GROWTH FACTOR. L. LeSauteur, R. Lisbona, N-K. Cheung, A. Ribeiro-da-Silva* and H. U. Saragovi. Dept. of Pharm.& Therap., McGill Univ., Mtl, QC, CANADA.

The Nerve Growth Factor (NGF) receptor TrkA, is a 140kDa cell surface protein encoded by the trk proto-oncogene. NGF binding to the extracellular domain of TrkA leads to neuronal survival and differentiation, but can also play a role in neuronal and non-neuronal neoplastic disease including neuroblastoma, small cell lung carcinoma and melanoma.

Scintigraphy using mAbs has proven useful as a non-invasive method to detect neoplasias associated with elevated receptor expression. However, the use of small designed molecules would be more desirable because of increased tissue penetration, reduced immunogenicity and lack of pleitropic effects. The in vivo targeting efficacy (kinetics, tissue penetration, and distribution) of small molecule analogs of NGF (LeSauteur et al., JBC 270:6564,1995) that bind TrkA was evaluated; and compared with an anti-TrkA monoclonal antibody (mAb 5C3)(LeSauteur et al., J. Neurosci. 16:1308, 1996). Nuclear imaging studies were done after injection of 99m-Tc labeled compounds in mice bearing tumors. mAb 5C3 and small mimics of NGF were bioavailable and targeted human TrkA-expressing tumors but not other tumors demonstrating the TrkA specificity of NGF mimics in vivo.

Receptor specific small molecule analogs designed from large polypeptides may be as useful as antibodies, and may be effective agents for the detection, diagnosis and possible treatment of tumors overexpressing TrkA.(Supported by MRC of Canada).

482.10

MECHANISMS OF SIGNAL TRANSDUCTION THROUGH THE P75 NEUROTROPHIN RECEPTOR. Bruce D. Carter*[1], Christian Kaltschmidt[2], Georg Dechant, Elisabeth Casademunt, Moses V. Chao[1], and Yves-Alain Barde. [1]Dept. Cell Biol. and Anatomy, Cornell Univ. Med. College, New York, NY 10021. [2]Inst. for Biochem., Freiburg Univ., 79104 Germany. Dept. Neurobiochem., Max-Planck Inst. for Psychiatry, 82152 Germany.

The neurotrophins bind selectively to distinct tyrosine kinase receptors referred to as the trks, however, they all bind with similar affinity to the p75 receptor. The significance of neurotrophin binding to p75 is not yet fully understood. We show that NGF binding to p75, in the absence of trkA, results in the activation of NFκB in Schwann cells from rat sciatic nerve. NGF activation of NFκB was also seen in L-cells expressing p75. Surprisingly, the effect was selective for NGF, neither BDNF nor NT3 activated NFκB. These results indicate that p75 is able to signal in non-transformed cells and can discriminate among neurotrophins. To understand the mechanisms of p75 signal transduction, the intracellular domain of the receptor was used in the yeast 2-hybrid system (Gyuris et al., Cell 75:791) to look for associating proteins. The p75-intracellular domain-lexA fusion "bait" was tested for interaction with a fetal human brain expression library. Among the clones fished out was a previously unknown zinc finger protein we termed "neurotrophin receptor interacting factor" (NRIF). The interaction in yeast was shown to be specific for p75 when compared with several other "false baits," none of which were able to interact, as determined by growth selection. To further demonstrate the interaction between p75 and NRIF, recombinant NRIF coupled to agarose beads was produced and shown to be capable of precipitating full-length p75 receptor from cell lysates. The mouse homolog of NRIF was cloned and shown to encode for a protein of approximately 35kD. The biological function of NRIF-p75 interaction is currently under investigation. (Funded by the Max Planck Society).

482.11

THE LOCALIZATION OF THE p75LNTR IN THE RETINA OF THE ADULT SPRAGUE-DAWLEY RAT. B. Hu, H. K. Yip* and K. -F. So. Developmental Neurobiology & Neuroregeneration Lab., Anatomy Dept., Univ. of HongKong. HongKong.

The low affinity neurotrophin receptor, p75LNTR or p75 is a 75Kd cell surface glycoprotein that binds all neurotrophins with similar affinity and is thought to help to ensure the specificity of each neurotrophin. Previous studies have found p75 in the retina ganglion cell (RGC) layer as well as on Müller cells. The exact function of p75 in both normal and lesioned retina was less certain and controversial. To investigate the role of neurotrophins and the p75 in the maintenance for RGC, we have examined the localization of p75 in the adult rat retina by immunocytochemistry at both light and electron microscopy levels.

Retinal wholemounts with RGC retrogradely labeled by a fluorescent dye Fluoro-Gold were prepared for 192-IgG indirect double fluorescent immunocytochemistry. Parasagittal paraffin (4μm) and cryostat (10~15μm) retinal sections were also processed for 192-IgG immunocytochemistry by the enhanced DAB (ABC) method. Pre-embedded immuno-electron microscopy study was applied in this study.

Previous studies only distinguished the p75 positive cell type in the retina by simple morphological criteria and by their localization in different retinal layers. It is very difficult to distinguish whether the positive stained membranes belong to RGCs or to the surrounding Müller cell processes at light microscopy level. The failure to detect p75 in retinal wholemount implied that it might not be expressed on RGCs. The similarity between the staining pattern of p75 with that of the distribution of Müller cell processes suggests that p75 is on the Müller cell processes and not on the RGCs as previously reported. Electron microscopic studies has further supported our observations. The p75 immunopositive staining are localized on Müller cell processes which not only form the inner limit membrane (ILM), but also wrap around the RGC bodies. The immunopositive staining on RGCs that have previously reported might belong to the surrounding Müller cell processes. We proposed that neurotrophic effects on RGCs mediated by p75 might be through a glia-neuronal pathway rather than on neuron directly.

[Grant support: CRCG 361.031.3084 and 337.031.0022]

482.12

p75-BINDING IS NECESSARY AND SUFFICIENT FOR THE ANTI-APOPTOSIS EFFECTS OF NGF ON NEUROBLASTOMA CELLS. N. F. Schor*. Depts. of Pediatrics, Neurology, and Pharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Prevention by nerve growth factor (NGF) of apoptotic death in neural cells has been variously ascribed to binding of NGF to its low-affinity (p75) or high-affinity (trk A) receptor or to a cooperative interaction between the two. We have previously demonstrated that SH-SY5Y human neuroblastoma cells undergo apoptosis when treated with antimitotic agents such as the antineoplastic agent, neocarzinostatin (NCS). SH-SY5Y cells express both p75 ($1.3x10^5$/cell) and trk A ($2.6x10^5$/cell), and bind NGF at both of these receptors. We have shown that SH-SY5Y cells are completely protected by NGF from NCS-induced apoptosis. The present studies test the hypothesis that the protective effect of NGF on neuroblastoma cell lines is the result of NGF binding to p75. In contrast to SH-SY5Y cells, NGP cells express p75 ($2.7x10^4$ receptors/cell), but not trk A. NGF (40 nM) completely protects NGP cells from the effects of NCS. In addition, continuously treating SH-SY5Y cells with a mutant NGF that binds to p75 with a 100-fold greater affinity than to trk A (4.2 nM) completely protects SH-SY5Y cells from the effects of NCS. Conversely, two NGF mutants that are 100-fold selective for trk A relative to p75 offered no protection to SH-SY5Y cells from NCS-induced apoptosis. Finally, SH-SY5Y cells were treated with NGF and NCS in the presence or absence of Ab 9651, an antibody that blocks binding of NGF to p75. Ab 9651 abrogates the protective activity of NGF in neuroblastoma cells treated with NCS. (Supported by NIH Fellowship #CA67421)

483.1

INTEGRATIVE PHYSIOLOGY OF VOLTAGE-GATED CONDUCTANCES ON THE DENDRITES OF CORTICAL PYRAMIDAL CELLS. M. M. Millonas and P. S. Ulinski*. James Franck Institute and Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL 60637.

The dendrites of pyramidal cells typically have potassium and fast sodium voltage-gated conductances, but the role of dendritic conductances in the integrative physiology of pyramidal cells is not understood. In an earlier study using a compartmental model (CNS*96), we suggested that the fast prepotentials or "spikelets" present in pyramidal cells in turtle visual cortex result from fast sodium and delayed rectifier conductances located in spots on dendrites, distant from the soma. These "hotspots" generate dendritic action potentials that propagate into the soma where they are seen as small spikes. This study uses simulated AMPA- mediated conductances constrained by data on geniculocortical EPSPs to investigate the interaction between synaptic inputs and hotspots. Dendritic synapses can trigger rapidly firing dendritic action potentials which effectively voltage clamp the dendrite, limiting the amplitude of the resultant EPSP at the soma. EPSPs arriving on two different dendrites with hotspots within a time window on the order of 25 msec produce a larger somatic EPSP than if they arrived within the same time window on one active dendrite. All of these results were reproduced by a 4-variable model composed of two coupled Fitzhugh-Nagumo type oscillators, showing that they are robust features of neurons with dendritic hotspots. These results demonstrate the potential importance of interactions between multiple dendritic hotspots and the soma in the integrative physiology of pyramidal cells. MM is supported by a grant from the Office of Naval Research.

483.2

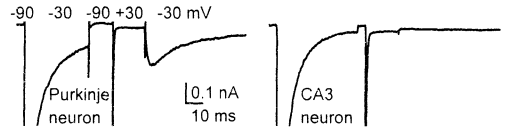
RELATIONSHIP BETWEEN Na^+ INFLUX AND INTRACELLULAR $[Na^+]$ IN ISOLATED NERVE ENDINGS.
D. Turner and E.L. Stuenkel*. Department of Physiology, University of Michigan Medical School, Ann Arbor, MI 48109

A change in $[Na^+]_i$ has been implicated as a modulatory influence in certain forms of short term synaptic plasticity. However, little quantitative information exists concerning the temporal and spatial characteristics of depolarizing impulse-induced changes in intracellular free $[Na^+]$ at nerve endings. We have, therefore, attempted to quantitate the voltage-dependent properties of Na^+ channel activation and inactivation and determine the relationship between time integrated Na^+ influx and changes in spatially averaged $[Na^+]_i$ in single rat neurohypophysial nerve endings using whole cell voltage clamp in conjunction with fluorometric monitoring of $[Na^+]_i$. Basal $[Na^+]_i$ in intact nerve endings was found to be ≈ 15 mM. Brief repetitive depolarizing pulses (-90 mV holding, to -10 mV step) of 2 ms duration and frequency similar to that of action potential bursting in vasopressinergic nerve endings (13-40 Hz), evoked inward Na^+ currents (1.47 ± 0.11 nA) that resulted in $[Na^+]_i$ increases, and were blocked by 0.3 μM TTX. The $\Delta[Na^+]_i$ was shown to be proportional to the applied stimulation-frequency for all periods of stimulation and ranged from 0.35 mM to 2.73 mM. Initially, sodium influx followed a near proportional increase in $[Na^+]_i$ whose rate increased as terminal diameter decreased. However, when sodium influx exceeded ≈ 2.2 mM, regardless of terminal diameter, this relationship became non-linear suggesting efficient buffering and extrusion of Na^+_i. Nerve endings of smaller diameter had greater charge density (pC/μm^2) than those of larger diameter. The data suggest that action potential invasion of small diameter nerve endings can transiently elevate the intracellular $[Na^+]$. (Supported by NIH grant to ELS.)

483.3

SODIUM CURRENTS NEAR THRESHOLD VOLTAGES IN CEREBELLAR PURKINJE NEURONS. Indira M. Raman* and Bruce P. Bean. Vollum Institute, Portland, OR 97201.

The distinctive firing pattern of Purkinje neurons is influenced by persistent sodium current active near threshold voltages (Llinás & Sugimori, J. Physiol. **305**:171,1980). We studied TTX-sensitive Na^+ current in freshly dissociated rat Purkinje neurons. A depolarization from -90 mV to -30 mV induced a transient current of ~-4 nA (50mM $[Na^+]_o$) followed by a steady-state current of ~-40 pA. Unexpectedly, when cells were depolarized to +30 mV and returned to -30 mV, a slow "tail" current developed (rise time 8 ms, decay τ 30 ms). Relative to the peak transient current, the slow tail was 3.5±2% and the steady-state current was 1.1±0.7%. The charge transfer in the first 40 ms of the slow tail was 31±14% of that of the transient current. Hippocampal CA3 neurons had transient and steady-state currents similar to Purkinje neurons but no slow tails. The slow tail current may promote repetitive firing in Purkinje neurons by providing a surge of Na^+ current following an action potential. In single channel patches, the channels underlying the slow tail current had a conductance of ~20 pS (140mM $[Na^+]_o$) and also produced conventional transient currents. NIH (HL35034)



483.4

NA^+-DEPENDENT PLATEAU POTENTIALS AND NA^+-DEPENDENT K^+ CONDUCTANCE IN THE NUCLEUS PRINCIPALIS TRIGEMINI NEURONS. V.M. Sandler, E. Puil, D.W.F. Schwarz*. Rotary Hearing Center, Dept. of Surgery (ENT) and Dept. of Pharmacology & Therapeutics, Univ. of British Columbia, Vancouver, B.C., Canada. V6T 2B5

Little is known about the electrophysiological properties of the nucleus principalis trigemini (PrV) neurons. We have investigated PrV neurons using whole-cell recordings in *in vitro* brainstem slice preparation. Of three electrophysiologically distinct cell types recorded in the PrV we concentrate on some properties of the spontaneously active bursting neurons. Depolarization by current pulse injection occasionally evoked "plateau potentials" in these neurons. Such plateau potentials were observed consistently during perfusion with Ca^{2+} free media, with or without the Ca^{2+}-channel antagonists, Co^{2+} or Cd^{2+} (1 mM), and during external TEA (1 mM) application. TTX application (e.g., 0.6 nM for 6-10 min.) increased the latency to onset and decreased the duration of the plateau potential without affecting the action potentials shape. TTX also enhanced the negative slope of the plateau potential in a dose-dependent manner. Higher concentrations of TTX (e.g., 60 nM for 10 min.) abolished the plateau potential before completely blocking action potentials. Low $[Na^+]$-perfusion simultaneously reduced the amplitudes of plateau potentials and fast spikes. Both subthreshold and suprathreshold depolarization in Ca^{2+} free ACSF with Co^{2+} (1mM) evoked a long-lasting hyperpolarization at the offset of the depolarizing current pulse. This hyperpolarization was blocked by TTX (more than 5 nM). The magnitude of the hyperpolarization depended on the level of neuronal depolarization. We conclude, therefore, that Na^+ entry during a depolarization can increase a K^+ conductance in type 1 neurons.
Supported by MRC Canada. and Lion's MD19 Hearing Foundation.

483.5

THE T698M-HyperPP Na CHANNEL MUTATION DOES NOT ENHANCE NON-INACTIVATING CURRENTS, BUT CHOLERA TOXIN DOES. T. R. Cummins* and F.J. Sigworth, Interdept. Neurosci. Prog. and Dept. of Cell. & Mol. Physiol., Yale Univ. Sch. of Med., New Haven, CT. 06520.

Mutations in the skeletal muscle sodium channel gene are believed to cause hyperkalemic periodic paralysis (HyperPP). The T704M mutation is the most common HyperPP mutation. We have shown that the corresponding mutation in the rat channel (T698M) alters activation and impairs slow inactivation, and proposed that these changes underlie the pathophysiology of HyperPP. However, it has also been proposed that the T698M mutation disrupts fast inactivation. Therefore we examined fast inactivation of WT and T698M channels stably expressed in HEK293 cells.

Large non-inactivating currents were sometimes observed in WT and T698M cells just after beginning whole-cell recording. At -10 mV, the relative size of the non-inactivating component was 2±2% (mn±SD) of the peak current for both WT (n=47) and T698M (n=59) cells. However, the non-inactivating current rapidly ran down and after 10 min was less than 0.5% of peak in both WT and T698M cells. While we were unable to prevent this rundown, we found that treating the cells overnight with cholera toxin (ChTx) dramatically increased the size of the non-inactivating current. After ChTx treatment the non-inactivating component was about 20% of the peak current. Surprisingly the S1321A mutation, which removes a putative phosphorylation site in the III-IV linker, did not prevent the ChTx effect.

These data indicate that the T698M mutation does not directly affect the size of the non-inactivating component. Interestingly, this component can be modulated, possibly by G-proteins, in cells expressing either WT or T698M channels. (Supported by NIH grant NS21501 to FJS).

483.6

SLOW INACTIVATION OF MUTANT SODIUM CHANNELS IN NEUROMUSCULAR DISEASE. L. J. Hayward[1]*, R. H. Brown, Jr.[1], and S. C. Cannon.[1,2] Departments of [1]Neurology and [2]Neurobiology, Mass. General Hospital and Harvard Medical School, Boston, MA 02114.

Missense mutations of the Na channel α subunit cause a spectrum of neuromuscular disorders with myotonia (SCM), paralysis (HyperPP) or both (PMC, most forms of HyperPP). Defects of rapid inactivation have been identified for most mutants and can account for many of the observed abnormalities in muscle excitability. Ruff (Biophys J. 66:542, 1994) postulated that slow inactivation (SI) must also be impaired to produce sustained paralysis. Cummins and Sigworth (Biophys J. 70, 1996) showed a disruption of SI for a HyperPP mutant (rT698M, homolog of hT704M). We tested whether a defect of SI is a necessary condition for paralysis. Na currents were recorded from HEK293 cells transfected with $h\beta_1$ and either wildtype rSkM1 or a mutant α subunit: one of three HyperPP (rT698M, rM1585V, rM1353V), one PMC (rT1306M), and one SCM (rG1299E). SI was defined as the fraction of current that failed to recover within 20 ms at -100 mV (where recovery from fast inactivation is >95% complete). Entry to SI was measured after variable-duration conditioning pulses to -10 mV, extent of SI was measured after a 60 sec conditioning pulse, and recovery was measured over 0.1 ms to 10 sec at -100 mV. Impaired SI was defined as: >2-fold slower entry rate, >2-fold increase in residual current that fails to SI at -10 mV, or >3-fold faster recovery rate. The two most prevalent HyperPP mutations (rT698M and rM1585V) both had clear defects of SI. However, two other mutations associated with paralytic phenotypes (PMC: rT1306M; HyperPP: rM1353V) did not have significantly impaired SI. The SCM mutant (rG1299E), which is not associated with paralysis, also had no defect in SI. We conclude that a disruption of SI probably contributes to the HyperPP phenotype but is not necessary for sustained weakness to occur. Supported by HHMI (LJH), RO1-AR42703 (SCC), and the Klingenstein Foundation (SCC).

483.7

CLUSTERING OF SODIUM CHANNELS ON AXONS IS INDEPENDENT OF MYELINATION IN THE DYSTROPHIC MOUSE 129/ReJ-Lama2^{dy}. T.J. Deerinck[1], V.M. Edelman[1], S.R. Levinson[2] and M.H. Ellisman[1]*. [1]Dept. Neurosciences, UCSD, La Jolla, CA 92093; [2]Univ. Colorado Health Sci. Center, Denver, CO 80262.

The processes involved in myelination and the development of nodes of Ranvier have been extensively studied. Despite this, there is no clear consensus as to whether it is the Schwann cell or the axon which defines the sites destined to become nodes of Ranvier in developing and remyelinating nerve. Dystrophic mice of the strain 129/ReJ-Lama2^{dy} have a genetic defect in Schwann cell proliferation and myelinogenesis. In the dorsal and ventral spinal roots of these mice, many axons are either amyelinated or partially myelinated. In previous studies, observations of freeze-fracture replicas have revealed node-like patches of intramembranous particles on axons without nearby Schwann cells, suggesting the possibility of axonal definition of such sites (M. Ellisman, J. Neurocytol. 8:719-735.1979) In addition, the distribution of intramembranous particles at partially myelinated heminodes of Ranvier as revealed by freeze-fracture analysis are similar to that found at normal nodes of Ranvier. In the present study we have identified discrete clusters of sodium channels on axons completely devoid of myelin with a peptide-derived polyclonal antibody against the voltage dependent sodium channel using immunocytochemistry. These clusters appeared as either circumferential rings around bare axons or as asymmetrical patches. In bundles of these amyelinated axons, adjacent regions of neighboring axons could be seen to contain such clusters. Apart from these regions, no significant staining of the axolemma was observed. In addition, partially myelinated axons or heminodes and fully formed nodes of Ranvier also showed discrete positive immunoreactivity for sodium channels. We have determined that axons which develop in an environment deficient in Schwann cells produce focal concentrations of sodium channels. The appearance of these clusters without associated Schwann cells demonstrates that axons define the initial locations of these aggregations and that these sodium channels are not supplied to the axon by transcytosis from Schwann cells. Supported by: NIH NS14718, NS24635, RR04050 (to MHE) and NS15879 (to SRL).

PATTERNING II

484.1

FIBROBLAST GROWTH FACTORS AND SPATIAL PATTERNING IN THE DEVELOPING NEURAL TUBE. I.Mason*, G.Grasso, R.Mahmood, N.Morton, K.Robertson and H.Sheikh. MRC Brain Development Programme, Dept. Developmental Neurobiology, UMDS Guy's Hospital, London SE1 9RT, UK.

Studies by this group and others have implicated fibroblast growth factors (FGFs) in the regulation of early neural tube development. In particular, FGF-3 may function in the organisation of the developing hindbrain and FGF-8 may be part of the activity from isthmus that is responsible for patterning the posterior midbrain.

We have shown that Fgf-3 is expressed in a dynamic spatial and temporal manner in the developing hindbrain which is conserved between chick and mouse embryos (Development [1995] 121: 1399; Anat Embryol [1996] in press). This pattern of RNA distribution is consistent with an early role relating to the development of rhombomeres 4, 5 and 6 and a later function in the development of rhombomere boundaries. We have identified the high affinity receptors for FGF-3 as the IIIb splice variants of FGFRs 1 and 2 (J.Biol.Chem [1995] 270: 6779; 270: 24197; Oncogene [1996] 12: 1503). Examination of the expression of transcripts for these receptors is consistent with the proposed roles for FGF-3 in the hindbrain. We are investigating the functions of FGF-3 in both mouse and chick embryos and data will be presented concerning its role in hindbrain development.

The isthmus (midbrain/hindbrain junction) is an organiser responsible for paterning the posterior midbrain. Fgf-8 is expressed in the isthmus of both chicken and mouse embryos in a spatial and temporal manner which prompted speculation that it might be involved in the organising properties of that tissue (Current Biology [1995] 5: 797). Consistent with this suggestion, we find that ectopic FGF protein can convert diencephalic tissue to a midbrain fate, a property also exhibited by grafted isthmus tissue. Further studies concerning FGF-8 function will be presented.

Supported by programme awards from the MRC and International Human Frontier Science Program and projects grants from the MRC and Wellcome Trust.

484.2

DIFFERENTIAL EXPRESSION OF BONE MORPHOGENETIC PROTEINS IN THE DEVELOPING VESTIBULAR AND AUDITORY SENSORY ORGANS. Doris K. Wu* and Seung-Ha Oh National Institute on Deafness and Other Communication Disorders, NIH, Rockville, MD 20850.

Our previous study showed that BMP4 (bone morphogenetic protein) is an early marker for all sensory organs of the inner ear (Mol. Biology of Hearing and Deafness Meeting, 95', Abstr#2). In this report, we investigated the gene expression of BMP4 during sensory organ differentiation and compared its expression pattern to that of BMP7, since different BMPs may have a co-operative function during development. Among the different BMP genes characterized in the chick inner ear including BMP4, 5 and 7, BMP7 was the earliest one to be activated, starting in the otic placode. By embryonic day 4 (E4), all presumptive sensory organs were within a subset of BMP7 positive areas in the otocyst, except for a portion of the macula utriculus. At this age, BMP7 seemed to be a marker for most of the presumptive sensory and non-sensory portions of the inner ear. Starting at E5, gene expression patterns of BMP4 and 7 showed differences for the vestibular and auditory sensory organs. In the vestibular sensory organs, BMP7 gene expression segregated from the sensory tissues at the onset of differentiation, while BMP4 expression remained in supporting cells, but not hair cells. However, in the cochlea, BMP7 gene expression became restricted to sensory tissue over time, and eventually concentrated in supporting cells while BMP4 gene expression was localized to hair cells.

484.3

MULTIPLE WNT GENES EXPRESSED AT THE MEDIAL MARGIN OF EMBRYONIC MOUSE CEREBRAL CORTEX. E.A. Grove*, L. Yip,† S. Tole, and C.W. Ragsdale. Dept. Pharm. Phys. Sci. and Pritzker School Med.†, Univ. Chicago, Chicago, IL 60637.

To identify developmental control genes for the growth and patterning of cerebral cortex, we employed the PCR procedure of Gavin et al. (1990) to characterize *Wnt* gene family members expressed in embryonic mouse telencephalon. Five different *Wnt* fragments were isolated from cDNA derived from embryonic day 12.5 (E12.5) CD-1 mouse cerebral hemispheres: *Wnt*-3a, -4, -5a, -7a, and a previously unreported mouse *Wnt* gene. Riboprobes were generated to examine gene expression by *in situ* hybridization in whole embryos and tissue sections.

At E12.5, *Wnt*-3a, -4, -5a, and the novel mouse *Wnt* are expressed along the medial margin of the cerebral cortex, in a band of tissue that extends through the caudal two-thirds of the hemisphere. This band of strong *Wnt* gene expression lies adjacent to the developing choroid plexus (CP), which itself expresses only low or undetectable levels of *Wnt*-3a, -4, -5a, and the novel mouse *Wnt*. Tissue within the band of *Wnt* gene expression appears similar to contiguous pseudostratified neuroepithelium in morphology, but differs in its expression of class III β-tubulin mRNA, a marker of differentiating neurons. At E12.5, a layer of β-tubulin-expressing cells already extends throughout the cerebral hemisphere. This cell layer undergoes a striking transition at the medial margin of the hemisphere, such that at least part of the *Wnt*-positive band contains only scattered cells expressing β-tubulin at markedly reduced levels.

Multiple *Wnt* genes are expressed at the medial margin of the cerebral hemisphere, between the developing CP on one side and fully typical cortical neuroepithelium on the other. We hypothesize that *Wnt* genes may be involved in setting up a boundary in the medial telencephalon between developing cerebral cortex and the non-neural CP, and in regulating the subsequent growth of these tissues.

Funding provided by the University of Chicago and the Brain Research Foundation.

484.4

RETINOID-MEDIATED INDUCTIVE SIGNALING IN THE MOUSE SPINAL CORD FROM EMBRYO TO ADULT. W. W. Rubin* and A.-S. LaMantia. Dept. of Neurobiology, Duke University Med Center, Durham, NC 27710.

We have hypothesized that retinoid-mediated induction is important for early spinal cord development. As early as embryonic day 8.5 (E8.5), proliferative cells throughout the anterior-posterior and dorsal-ventral extent of the cord express a retinoid-sensitive transgene. At E12.5, the expression of the transgene becomes localized to precursors and early post-mitotic cells in cervical and lumbar cord but is excluded from thoracic and sacral cord. We asked whether retinoid-mediated induction contributes to subsequent differentiation and ongoing plasticity in the perinatal and adult spinal cord. At E14.5 through birth, expression is enhanced in the alar plate of the cervical and lumbar cord. In the second postnatal week, however, the transgene is expressed at low levels in a restricted region of the dorsal horn in the thoracic as well as cervical and lumbar regions. As postnatal development progresses, expression of the transgene becomes restricted to a subset of cells in the superficial layers of the dorsal horn. It gradually increases in thoracic cord until adulthood when expression is qualitatively similar to that in the cervical and lumbar regions. Thus, retinoid signals in the spinal cord may play different roles in development and maturity. At early stages, retinoids may play a role in neural differentiation throughout the cord. From midgestation through the first postnatal week, they may be involved in regional differentiation of spinal neurons and circuits, including regionally-specific patterns of afferent innervation. From the second week of life, retinoid-signaling may contribute to plasticity of a subset of dorsal horn neurons in response to changes in the periphery. (Supported by NIH grant HD29178)

484.5

A FAILURE OF RETINOID RESPONSIVENESS ACCOMPANIES THE FAILURE OF OLFACTORY BULB MORPHOGENESIS IN THE GLI-3 MUTANT MOUSE *EXTRA TOES* (*Xt*) A-S. LaMantia,* Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710

Local retinoid signaling in the forebrain and presumptive olfactory placode prefigures the morphogenesis of the olfactory bulb and epithelium. In homozygous *extra toes* mutant mice (*Xt/Xt*: a deletion of the Gli-3 transcription factor) the olfactory bulb is absent, while the epithelium can still be recognized. Accordingly, if retinoid signaling is necessary for bulb and epithelium morphogenesis, one would predict that retinoid-mediated gene expression would be absent in the forebrain while persisting in the olfactory pit/placode of *Xt/Xt* embryos. To assess this issue a retinoid indicator transgene was bred into *Xt/+* mice and the pattern of retinoic acid (RA)-mediated gene expression in *Xt/Xt* mutant/transgenic embryos was evaluated. At embryonic day 10.5 RA-mediated gene expression is absent from the forebrain, but persists in the olfactory pit/placode. When embryos of this age are exposed to exogenous RA via maternal circulation, the forebrain remains unresponsive, even though ectopic or enhanced expression of the indicator transgene can be seen in other regions of *Xt/Xt* embryos including the olfactory pit, as well as throughout the forebrain in littermate +/+ or *Xt/+* controls. Apparently, a failure of retinoid responsiveness in the forebrain—due either to the inability of the forebrain to receive or respond to retinoid signals—accompanies the loss of the olfactory bulb in *Xt/Xt* mice. In contrast, a persistence of retinoid-mediated gene expression accompanies the differentiation of the olfactory epithelium in the absence of its normal target. Supported by NIH-HD 29178.

484.6

CEREBELLAR DEVELOPMENT REGULATED BY RETINOIC ACID P. McCaffery*, M. Yamamoto and U.C. Dräger E.K. Shriver Center, Waltham, MA 02254 and Harvard Medical School, Boston, MA 02115

Retinoic acid (RA) is a potent activator of gene transcription, and it interacts with other ligands of the nuclear receptor family to guide patterning and differentiation within the developing nervous system. The localization of RA's action is determined by: (1) the distribution of RA receptors, (2) the area of expression of RA binding proteins, and (3) the activity of RA synthesizing and degrading enzymes. The developing cerebellum is a region of the brain where all three modes of regulation act to control developmental patterning. RA synthesis in the developing cerebellum is highest in two restricted locations, the associated meninges and the choroid plexus. Changes in the expression of RAR and RXR receptors correlate with discrete events in cerebellar development. With a novel assay for RA binding proteins we find evidence for the expression of a new binding protein in the cerebellar vermis and hemispheres. With a new, sensitive assay for the NADPH-dependent microsomal enzymes that degrade RA, we find spatially and temporally regulated expression of RA-degrading activity. The regionalization of RA synthetic and degradative enzymes will create spatial patterns in RA levels that are likely to have morphogenetic significance for the developing cerebellum. It would be expected that systemic application of RA will distort these patterns. We injected RA into early postnatal mice, during the period of most active cerebellar morphogenesis. Such injections cause a general reduction in cerebellar size, down to 70% of control. Most affected are lobules VIII and XI, where a portion of the granule cells remain undescended in the external granular layer even 6 weeks after birth. These observations support the notion that normal cerebellar development is dependent on temporally and spatially regulated patterns of RA levels within the cerebellar anlage. Grant support: HD05515, EY01938 and EY04801

484.7

STABILIZATION OF A HOX GENE PATTERN IN THE LUMBOSACRAL SPINAL CORD OF THE CHICK EMBRYO. C. Lance-Jones* and K. Sharma. Dept. Neurobiology, Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15261.

In the chick embryo, analyses of motor projections after spinal segment reversal indicate that axial differences exist within the lumbosacral (LS) neural tube prior to axon outgrowth. These axial differences appear to be fixed at stage (st) 15 but not at st 13-14. In the cranial neural tube, a unique Hox code identifies individual axial levels. We have asked if the acquisition of axial identity regarding motoneuron projections is temporally correlated with the acquision of a unique Hox identity in the LS spinal cord. To address this question, two types of surgeries were performed at both st 15 and st 13-14. In one set of embryos, 3-4 neural tube segments at the thoracic (T)-LS boundary were reversed. In another, 2-3 segments of T or LS neural tube were exchanged. The expression pattern of Hoxd-10 was assessed by whole-mount *in situ* hybridization with digoxigenin-labeled probes (cDNA kindly provided by C. Tabin) at st 25-30. In unoperated embryos, Hoxd-10 is expressed in the LS cord with a peak in midLS regions (LS3-5) . No expression is detected in T segments. Following st 15 reversals/exchanges, Hoxd-10 expression is not detected in the original T segments, as in unoperated embryos. The original LS segments express Hoxd-10 at high levels, comparable to that within LS segments in unoperated embryos. Following st 13-14 reversals/exchanges, Hoxd-10 expression is not detected in the original T segments, as in unoperated and st15 surgery embryos. In contrast, the original LS segments express Hoxd-10 at levels that are much lower then those in unoperated or st 15 surgery embryos. This finding suggests that at st 13-14 environmental factors can alter Hoxd-10 expression within LS segments. The fixation of this Hox pattern appears to occur just before st 15, like the stabilization of axial differences relating to motoneuron target identity. Supported by NIH (HD25676 to CLJ).

484.8

EXPRESSION PATTERN OF BCL-2, BCL-X AND BAX GENES IN THE DEVELOPING MOUSE NERVOUS SYSTEM. J.K. Mai*, M. Krajewska, J.C. Reed and S. Krajewski , [1]Dept. Neuroanatomy, H.-Heine University, Duesseldorf, F.R.G.and the Burnham Institute, La Jolla, CA 92037

The pre- and postnatal development of the expression of several members ofthe BCL-2 family was studied on a closely spaced ontogenetic series of paraffin embedded specimens. Immunohistochemical localization of Bcl-2, Bcl-x and Bax was performed using polyclonal antibodies against the respective proteins. A first phase of ubiquitous expression involving all members of the Bcl-2 family lasted from the time of the formation of the germinal layers until about E10.5 when the migration of postmitotic neurons is advancing. After E11.5 regional expression patterns, typically confined to settling and differentiation areas of neurons, became manifest. The timing and distribution of the expression of the three members of the BCL-2 family showed substantial overlap in some areas (like the lower motor neuron pool of the spinal cord, the developing motor nuclei of the brain stem, the cerebellum and thalamus) but also numerous locations where a mismatch or reciprocal expression patterns were observed (striatum, cortex, olfactory bulb). Around birth the expression all three members is gradually declining albeit the topographic relation to particular nuclear structures remains evident. The time related expression pattern thus follows a biphasic course: 1. a general expression until the period of neuronal migration and 2. a focussed expression during the late migration period and beginning differentiation of some (selected) neuronal cell groups, which is compatible with the idea of expression of Bcl-2 during the period of the formation and establishment of interneuronal contacts. The changes in the time related expression patterns and immunohistochemistry with antibodies against HNK-1, CD15 and calbindin on adjacent sections allowed the correlation with defined maturation processes in the CNS, which influence the expression pattern of Bcl2 protein family.

NIH RO1.CA67329-02

484.9

SEPARATION OF STRIATAL PATCH AND MATRIX COMPARTMENTS *IN VITRO*. A. Dori, A. Maric[1], D. Maric[1], J.L. Barker[1], A. Alfahal and W. F. Silverman* Zlotowski Center for neuroscience, Ben-Gurion University of the Negev, Beer Sheva, ISRAEL and [1],Laboratory for Neurophysiology, National Institute of Neurological Diseases and Stroke, NIH.

The mechanisms governing the establishment of patch/matrix compartments in the striatum and its functional significance are not yet understood. The pattern assembles following two separate waves of neurogenesis in the ventricular area: Cells which produce the patch compartment are generated between embryonic days E12-E16, followed by cells, born from E18 thru the first postnatal week, which form the matrix compartment. We employed a buoyant-density sedimentation protocol using Percoll, to separate proliferating/undifferentiated neural cells from the differentiated neurons of the striatal anlage. The striatum was dissected from E19 fetuses so that the early born - patch compartment cells could be expected to have already differentiated, and would, therefore, accumulate in the high buoyancy band of the gradient. In contrast, we predicted that cells proliferating on E19 would accumulate in the low buoyancy band, and represent - matrix precursor cells. Primary cell cultures were established for the collected fractions, and immunohistochemistry for maturational and compartment-specific markers was carried out to confirm this hypothesis, and to test whether formation of the pattern is dependent upon intrinsic or extrinsic inductive influences. The high buoyancy cells were mostly post-mitotic and expressed markers consistent with their identification as patch cells. The low buoyancy cells, as predicted, were mostly proliferating and undifferentiated at the time of dissection, and by 7 days *in vitro* began to exhibit matrix phenotype markers. These results suggest that the matrix phenotype is specified by an intrinsic program, operating independent of inductive extrinsic factors. Supported by Israel Acad. Sci

484.10

EARLY SPECIFICATION OF THE HIPPOCAMPAL CA FIELDS S. Tole*, C. Christian and E. A. Grove, University of Chicago, Chicago, IL 60637

When and how the developing cerebral cortex is divided into different areas are questions of great current interest. We address these questions in the mouse hippocampus, asking (1) how early the hippocampus is patterned into the two major fields CA1 and CA3, and (2) whether local cues in the hippocampus are sufficient for normal specification of CA field identity, or if extrinsic signals are required from elsewhere in the brain.

Classical studies in the rodent show clear morphological and connectional differences between fields CA1 and CA3 by the end of the first postnatal week. We find, however, that differences in gene expression arise much earlier. CA1 neurons express the POU-domain gene SCIP from embryonic day (E) 15.5, and CA3 neurons express KA1, a glutamate receptor subunit, from E14.5. Initially, only neurons at each end of the hippocampal plate express SCIP or KA1, while a stretch of neurons in between expresses general neuronal markers but not those specific to CA1 or CA3. SCIP and KA1 expression progresses into this region so that by birth (E19.5), CA1 and CA3 type neurons mingle in the narrow transitional field, CA2. Other field-specific features mature postnatally, such as the expression of the receptor protein tyrosine kinase Tyro3 in CA1, and the antigen Py1 in CA3. Acquisition of the mature CA1 or CA3 molecular phenotype thus requires at least 3 weeks.

To test if extrinsic signals from other brain structures are necessary for the development of CA1 and CA3 field identity, we prepared organotypic slice cultures of E17.5 hippocampus. CA1 and CA3 develop normally in these cultures with respect to the progression of SCIP and KA1 expression. Next, we isolated CA1 and CA3 fields in sub dissected explants of E17.5 hippocampus, confirming their identity by expression of SCIP or KA1 respectively. Strikingly, isolated explants, like whole slices, develop new, late-appearing features of CA1 or CA3 *in vitro*. Thus, our results suggest that by E17.5, the CA1 and CA3 fields can develop their regional identity in the absence of extrinsic signals.

Funding provided by the University of Chicago, and the Brain Research Foundation.

484.11

ARCUATE PLAN OF NEUROGENESIS IN AVIAN MIDBRAIN. T.A. Sanders* and C.W. Ragsdale. Committee on Neurobiology, Univ. Chicago, Chicago IL 60637.

The ventral midbrain of chick embryos is transiently patterned into longitudinal zones, called arcs, identified by acetylcholinesterase staining and ventricular surface ridges. The arcs in addition constitute domains for selective expression of developmental control genes, and may be a patterning mechanism by which midbrain neurons are allocated to nuclear fates. The extent to which midbrain arcs form territories controlling cell proliferation and the kinetics of neuronal differentiation remains, however, unexplored.

We have employed whole-mount *in situ* hybridization histochemistry to assess whether midbrain neurogenesis also follows an arcuate plan. Antisense riboprobes were prepared to neuronal differentiation markers (class III β-tubulin, middle-molecular weight neurofilament) and candidate vertebrate neurogenic genes (*C-Notch*-1, *C-Delta*-1, *C-Serrate*-1; Myat *et al.* (1996), *Dev. Biol.* **174**:233).

β-tubulin mRNA expression, an early marker of neuronal differentiation, identified a rostrocaudal maturation gradient in ventral midbrain, which was most advanced at the midbrain-forebrain junction. Superimposed upon this gradient was a series of longitudinal stripes of dense β-tubulin expression. These stripes appeared in a strict, progressive schedule, from HH stage 17 through stage 27, suggesting that neurogenesis in ventral midbrain is controlled at least in part by arc patterning mechanisms. By embryonic day 7 (E7), β-tubulin positive cells were more uniformly distributed, establishing that both arc and interarc territories are rich in neurons. Interestingly, even as late as stage 32, neurofilament message expression remained low or undetectable in large expanses of ventral midbrain tissue.

Chick homologues of *Drosophila* neurogenic control genes were also expressed in arcuate stripes in ventral midbrain at least from E3 through E7. *C-Notch*-1 was expressed densely but heterogeneously throughout the ventral midbrain, with clearly discernible arcuate domains. By contrast, the putative *Notch* ligands, *C-Delta*-1 and *C-Serrate*-1, were distributed in arcuate stripes that had well-defined borders and were separated from one another by districts almost free of labeled cells.

Funding provided by the Brain Research Foundation.

484.12

LAYER FORMATION IN THE CHICK OPTIC TECTUM: A POSSIBLE ROLE FOR AGGRECAN. N.P. Zilakos, I. Zhizhin, N. Sakellaridis*, N.B. Schwartz and D. Mangoura. Peds, The Univ. of Chicago Sch. of Med, Chicago, IL 60637.

Aggrecan, a large chondroitin sulfate proteoglycan is developmentally regulated in the chick embryo CNS. The time table of this transient, strictly embryonic expression differs among various CNS regions. To better understand how this correlates with morphogenetic events and elucidate the function of aggrecan in brain morphogenesis, we have undertaken topography studies of aggrecan expression in the developing optic tectum, using immunofluorescence with mAb S103L against the aggrecan core protein. Double staining with nuclear stains allows concomitant identification of the tectum layers. Aggrecan- (S103L-) immunoreactivity was the strongest at E12 or stage 38, a critical period in the formation and segregation of the outer layers of the tectum. While most neurons were immunopositive, different layers exhibited different features. High immunoreactivity was observed in medium size neurons in layers I and II (tectal ventricle surface). Strong staining was also seen in the lower half of layer VI, and in XII, the future stratum opticum, where retinal axons first ingrow to form synapses. Overall at E12 somata of medium to small size, radially oriented neurons, transversing plexiform or cell-dense layers expressed the highest, and large, horizontally disposed neurons the least immunoreactivity. These data suggest that aggrecan has an important in neuronal positioning and layer formation in the optic tectum. (HD 09402 and BRF).

484.13

CELL SURFACE MARKERS OF THE RAT FLOOR PLATE. Z. Kaprielian*, Q. Zhu and D. Kapitula. Departments of Pathology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Region-specific cell surface molecules are thought to play important roles in the morphogenesis of the developing central nervous system (CNS). We previously used the cyclophosphamide immunosuppression method to generate a panel of monoclonal antibodies (mAbs), designated CARO 1-CARO 5(for CAudalROstral), that define anterior-posterior (AP) and dorsoventral (DV) position in the developing rat neural tube. Here we demonstrate that mAbs CARO 2 and CARO 5 preferentially label the floor plate in E12 -E18 rat embryos. In contrast to the distribution of other known floor plate markers, the expression of the CARO 2 and CARO 5 epitopes in the developing spinal cord is mediolaterally restricted. mAbs CARO 2 and CARO 5 label the surfaces of the human embryonal carcinoma cell line, NT2/D1, and dissociated embryonic rat spinal cord cells. An immunoblot analysis shows that both mabs recognize a 27 kD protein in detergent soluble membrane fractions derived from NT2/D1 cells and embryonic rat spinal cord. However, mAb CARO 2 immunoprecipitates a 60 kD doublet from NT2/D1 cell extracts, while mAb CARO 5 immunoprecipitates a 20 kD protein from both NT2/D1 and E16 rat spinal cord extracts. Deglycosylation experiments suggest that mAbs CARO 2 and CARO 5 do not recognize carbohydrate epitopes. Immuno- screening of a NT2/D1 cDNA library using mAb CARO 2 has yielded several positive clones that are currently being sequenced. These findings suggest that the CARO 2 and CARO 5 cell surface antigens are novel floor plate markers that may participate in neural tube patterning and/or axon guidance.

Supported by Research Grant #95-17 from the Whitehall Foundation.

484.14

ACTIVELY PROLIFERATING STEM CELLS IN THE ADULT ZEBRAFISH BRAIN IDENTIFIED BY THE EXPRESSION OF A POU GENE, *TAICHI* Song Huang and Sheryl Sato*, Genetics and Biochemistry Branch, NIDDK, NIH, Bethesda, MD 20892-1766

Unlike epidermal or hematopoietic cells, neuronal cells constitute a group of stable and long living cells in the adult brain of higher vertebrates. However, some evidence suggests that the neurogenic process is different in lower vertebrates such as teleosts. The number of neurons in the adult fish brain increases constantly throughout adult life. Using zebrafish as a model, we hope to elucidate the cellular and molecular basis of this phenomenon. We have identified a new and important population of actively proliferating neural stem cells in the adult zebrafish brain. These stem cells have several salient features. They are located in the subependymal region throughout the brain, the probable zone of proliferation, and they are capable of incorporating BrdU. In accordance with their undifferentiated morphology, these cells do not express either a neural differentiation marker, acetylated tubulin or a glial-specific marker, GFAP. However, these cells do express a *Brain 1*-related POU gene, *taichi*. The expression of *taichi* in the adult brain is consistent with its expression in proliferating neural cell populations in the developing embryo. Moreover, the neural progenitors within the neural tube of a prim-5 embryo that are *taichi*-positive are in G1 phase. During early zebrafish neurogenesis, we observed that neural precursors follow a unique pattern of migration. The existence of an actively proliferating stem cell population can explain why there is continuous growth of the adult fish brain.

FORMATION AND SPECIFICITY OF SYNAPSES IV

485.1

REGULATION OF SYNAPSE STRUCTURE AND FUNCTION BY THE *DROSOPHILA* TUMOR SUPPRESSOR GENE DISCS-LARGE. V. Budnik*, Y.H. Kho, and M. Gorczyca Dept. of Biology, Univ. of Massachusetts, Amherst, MA 01003.

Recent studies show that the mammalian synaptic protein PSD-95/SAP-90 interact directly with NMDA receptors, suggesting its involvement in functional and structural plasticity. In flies a PSD-95/SAP-90 homolog is encoded by *discs-large* (*dlg*). Our previous studies show that mutations in *dlg* lead to abnormal development of glutamatergic postsynaptic structures [Lahey et al. Neuron 13:823 (1994), Current Biol., 1996, in press]. However, DLG protein is expressed at both pre- and postsynaptic membranes. To understand the contribution of pre- or post-synaptic DLG to the establishement of normal synapse structure we used Gal-4 enhancer traps to selectively target *dlg* expression at motorneurons or muscles. In addition, to determine if *dlg* is involved in functional aspects of Type I synapses, we used voltage clamp techniques to study synaptic transmission in *dlg* mutants. We found that by selectively targeting DLG to postsynaptic cells, we were able to substantially rescue the reduced postsynaptic structure in the mutants. In wild type, postsynaptic overexpression of DLG resulted in over development of the postsynaptic specialization. Electrophysiological analysis demonstrated that synaptic currents were 100% larger in amplitude in the mutants. Quantal analysis suggested that this phenotype resulted from a presynaptic defect, which was rescued by presynaptic targeting of DLG. These results suggest that *dlg* functions in the regulation of neurotransmitter release and post synaptic structure. We propose that *dlg* may provide a mechanism by which changes in activity regulate synapse structure during development and plasticity.

485.2

POSTSYNAPTIC REGULATION OF SILENT-SYNAPSE FORMATION IN COCULTURE OF HETERO-AGED NEURONS. K. Kiyosue*, M. Kasai†, T. Taguchi, Dept. of Organic Materials, Osaka Nat'l. Res. Inst., AIST, Ikeda 563, Japan, †Dept. of Biophys. Eng., Osaka Univ., Toyonaka 560, Japan

Major excitatory synaptic signals in the central nervous system (CNS) are mediated by glutamate at dual component synapses that are composed of N-methyl-D-aspartate receptors (NMDARs) and non-NMDA receptors (non-NMDARs). This type of synapse has a potential to cause the plastic change, such as long-term potentiation (LTP) and the refinement of neural connections. Although the ratio of these receptors are thought to be critical for the plastic change, the mechanism determining the ratio in synaptic sites has not yet been known. To determine whether the ratio of receptors in the sites is controlled by the developmental stage of postsynaptic neurons or not, dual whole cell recording was applied to cocultured neurons prepared from dissociated chick cerebral neurons of different age. Results indicate that the formation of synapse containing the both receptors requires maturing of postsynaptic neurons. In the early developmental stage of postsynaptic neuron, NMDARs were also shown to selectively exist at the synaptic sites prior to non-NMDARs, even though both receptors expressed in functional form in neuronal membranes.

This work was supported by grant from MITI.

485.3

MATURATION OF A CENTRAL GLUTAMATERGIC SYNAPSE AND THE ROLE OF CaMKII G-Y Wu, R. Malinow and H. Cline*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY11724

Whole-cell recording from optic tectal neurons in a whole-brain preparation of Xenopus tadpole were used to study the physiological maturation of a central synapse. Unitary excitatory postsynaptic currents in response to single fiber stimulation of optic tract and spontaneous miniature excitatory postsynaptic currents(mEPSCs) in the presence of TTX were recorded from tectal neurons at different development stages along the rostral-caudal axis, corresponding to different timepoints after they receive retinal input. As development progresses, the amplitude of AMPA component of the evoked EPSC is increased, and the failure rate at hyperpolarized potentials is decreased, however there is no significant change the amplitude of NMDA component of transmission nor the failure rate at depolarized potentials. As a consequence, the NMDA/AMPA ratio and the fraction of pure NMDA responses are decreased. Furthermore, the amplitude and frequency of the mEPSCs at hyperpolarized potentials are increased during development. In some immature cells recorded spontaneous and evoked events are apparent at depolarized potentials but completely absent at hyperpolarized potentials. These results indicate that the initial glutamatergic transmission is mediated only by NMDA type receptors and is silent at hyperpolarized potentials. As synapses mature they acquire a component mediated by AMPA type receptors that transmit information at hyperpolarized potentials.

In order to investigate the intracellular signal transduction pathways mediating synaptic maturation we have used the Vaccinia virus to deliver the gene for a constitutively active form calcium/calmodulin-protein kinase II(CaMKII) into tectal neurons in vivo. The results show that this synaptic maturational program is mimicked by postsynaptic expression of CaMKII. We propose that initial, randomly formed synapses are postsynaptically silent unless sufficient depolarization is provided by coincident activity. Such activity could activate postsynaptic CaMKII leading to the functional appearance of AMPA responses.

Supported by the NSF.

485.4

ACTIVITY DEPENDENT DYNAMIC CHANGES IN TECTAL DENDRITIC ARBOR MORPHOLOGY I.Rajan*, B. J. Burbach and H. T. Cline. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

Retinal ganglion cell arbors have dramatic rates of branch additions and retractions within short intervals of time ranging between 30 minutes to 8 hours. Since these axons synapse onto tectal cells we hypothesized that similar activity dependent dynamic changes may occur in the dendritic arbors of the tectal cells. Single cells in the tectum of tadpoles between stages 44-48 were selectively labeled using iontophoresis of DiI. Labeled tectal cells with at least 5 dendritic branches were selected for imaging. These cells receive retinal input and are therefore likely to be influenced by synaptic activity. Individual cells were imaged using time lapse confocal microscopy with a periodicity of either 30 minutes over two hours or 2 hours over an eight hour interval. Three dimensional reconstruction techniques were used to obtain complete information on the rates and positions of branch additions and retractions. We have begun to use pharmacological agents to manipulate the activity dependent events of the arbor dynamics.

Supported by NSF.

485.5

GENE EXPRESSION IN DEVELOPING MOUSE BRAIN IN THE ABSENCE OF NMDAR1 Roderick A. Corriveau*, Sandra A. Wiese, and Carla J. Shatz, HHMI and Dept. of Molecular and Cell Biology, University of California, Berkeley, 94720

Patterned activity plays a critical role in the development of appropriate synaptic connections in the mammalian CNS, but little is known about the molecular mechanisms underlying activity-dependent synaptic rearrangements. To test the hypothesis that activity of ligand-gated ion channels may contribute to regulation of mRNAs encoding synaptically relevant proteins we have examined mRNA levels in normal and transgenic mice lacking functional NMDAR1 protein because: 1) the NMDA receptor is widely expressed in the CNS, and 2) a number of studies have demonstrated a role for the NMDA receptor in both adult and developmental synaptic plasticity. Levels of mRNAs encoding over 20 different synaptic proteins were compared between P0 mouse brains ± NMDAR1 by northern blot analysis. The largest difference observed to date was a modest (less than two-fold) decrease in the level of SNAP-25 mRNA in NMDAR1 knockout brains. In the majority of cases little if any change was observed (for example: agrin, n-sec1, synaptotagmin (p65), VAMP-2, GluR1, GluR2, KA1, KA2, GluR Delta1, GluR Delta2). The results suggest that, at this particular time in development, the NMDA receptor may not play a large role in regulating global brain levels of synaptic proteins encoded by the mRNAs examined. (The present results do not rule out highly local NMDAR1-mediated changes in regulation in gene expression.) Alternatively, compensatory mechanisms may substitute for the lack of NMDA receptor function.

We thank S. Tonegawa and Y. Li for the NMDAR1 knockout mice, and S. Heinemann, J. Boulter, R. Scheller, M. Bennett, and P.Worley for providing cDNA clones. Supported by NSF IBN9315390 and NIH MH48018 to CJS.

485.6

EVIDENCE FOR ECTOPIC CLIMBING FIBER SYNAPSES ON CEREBELLAR PURKINJE CELLS IN GluRδ2 MUTANT MICE. K. Hashimoto[1], K. Sakimura[2], M. Mishina[3] and M. Kano[4]*. [1]Dept. Physiol., Jichi Med. Sch., Tochigi 329-04, Japan, [2]Dept. Neuropharmacol., Brain Res. Inst., Niigata Univ., Niigata 951, Japan, [3]Dept. Pharmacol. Univ. Tokyo, Tokyo 113, Japan and [4]Lab. Neuronal Signal Transduction, Frontier Res. Program, RIKEN, Saitama 351-01, Japan.

The glutamate receptor δ2 subunit (GluRδ2) is selectively localized in cerebellar Purkinje cells (PCs). The null mutant mice deficient in GluRδ2 have impairment in motor coordination and persistent multiple climbing fiber (CF) innervation of PCs (Kashiwabuchi et al., Cell 81, 245-252, 1995). The present study was undertaken to further examine the nature of CF-mediated excitatory postsynaptic currents (CF-EPSCs) of PCs in the GluRδ2 mutant mice. Cerebellar slices (250μm thick) were prepared from mature mice (PN 22-70), and whole-cell recording was made from the PC somata. We found that CF-EPSCs had multiple steps in 69.3% (88/122) of PCs in the mutant mice, whereas only 12.2% (20/164) had multiple steps in the wild type mice. The 10 to 90% rise time of CF-EPSCs in the mutant mice displayed bimodal distribution with mean values of 0.48ms (±0.15ms S.D., n=80) and 1.81ms (±0.59ms S.D., n=127). In contrast, the rise time in the wild type mice was shorter than 1ms (0.46±0.11ms, mean±S.D., n=106). The passive properties of PC membrane in the mutant mice were similar to those in the wild type mice. These results suggest that, in the GluRδ2 mutant mice, CFs form ectopic synapses on PC dendrites which are electrotonically distant from the soma where CFs do not form synapses in the wild type mice.

Supported by grants from The Japanese Ministry of Education, Science, Sports and Culture and Frontier Research Program of RIKEN.

485.7

DISRUPTION OF THE ACTIN CYTOSKELETON LEADS TO A BREAKDOWN OF GluR1 LABELED DENDRITIC SPINES D.W. Allison[1], V.I. Gelfand[1], I. Spector[2] and A.M. Craig*[1]. [1]Dept. of Cell and Structural Biology, University of Illinois, Urbana, IL 61801. [2]Dept. of Anatomical Sciences, HSC, SUNY, Stony Brook, NY 11794.

Dendritic spines are the predominant sites of excitatory input on mammalian neurons, however their function and structural composition is still largely unknown. We studied the role of the actin cytoskeleton in the organization of GluR1 receptors in dendritic spines of cultured embryonic rat hippocampal neurons. Rhodamine phalloidin staining showed high concentrations of F-actin, which colocalized with synaptic clusters of the excitatory glutamate receptor subunit GluR1 at dendritic spines. Two F-actin perturbing drugs were used in an attempt to disrupt the actin cytoskeleton within the spines. Cytochalasin D, at concentrations of up to 10 μg/ml for 24 hours, could not eliminate the intense rhodamine phalloidin staining that colocalized with spines. Although the remainder of the F-actin throughout the rest of the neuron had been disrupted, the actin at the spines was unaffected, suggesting that the actin meshwork at spines is stabilized and less susceptible to the effects of cytochalasin D. While cytochalsin D has multiple activities on actin, latrunculin A, a compound isolated from the Red Sea sponge *Negombata* (previously known as*Latrunculia magnifica*), sequesters monomeric actin and depolymerizes actin filaments (Spector, et.al., Cell Motil. Cytoskel. 13,127-144). Exposure of mature neurons to latrunculin A at 5 μM for 24 hours resulted in nearly a complete reduction of rhodamine phalloidin staining, along with a marked reduction in the number of GluR1 labeled dendritic spines. These observations point to a role for actin microfilaments in the maintenance of dendritic spines and may provide an important tool for the study of the function of spines. Supported by NIH and the Markey Trust.

485.8

SAP102, A NOVEL POSTSYNAPTIC PROTEIN THAT INTERACTS WITH THE CYTOPLASMIC TAIL OF THE NMDA RECEPTOR SUBUNIT NR2B. S. Kindler[1*], B. M. Müller[1], U. Kistner[1], W. J. Chung[2], S. Kuhlendahl[2], L.-F. Lau[3], R. W. Veh[1], R. L. Huganir[3], E. D. Gundelfinger[1], C. C. Garner[2]. [1]ZMNH, Univ. of Hamburg, Germany; [2]NRC, Univ. of Alabama at Birmingham, AL 35213-0021; [3]Depart. of Neurosci., John Hopkins Univ., Baltimore, MD 21205-2185

Recently, a novel family of synapse-associated proteins (SAPs) has been identified. In the mammalian central nervous system, SAPs are constituents of the cortical cytoskeleton associated with the pre- and postsynaptic plasmalemma. SAPs are structurally related to DlgA in *Drosophila*. In the fly, DlgA is essential for the correct assembly of septate junctions and neuromuscular junctions. Here we present a novel SAP comprising at least three isoforms with apparent molecular weights of about 102 kDa, collectively called SAP102. In rat hippocampus, SAP102 is situated in dendritic spines and at PSDs of type 1 asymmetric synapses and is enriched in preparations of synaptic junctions. As with SAP90, SAP97, and DlgA, SAP102 is composed of multiple domains. Amino-terminal are three 90 amino acid repeats, termed PDZ domains. These are followed by an SH3 region and a putative guanylate kinase domain. Interestingly, SAP102 can be co-immuno-precipitated with NMDA receptor complexes solubilized from rat brain synaptosomes. Recombinant proteins containing the COOH-terminal tail of the modulatory NMDA receptor subunit NR2B bind SAP102 from rat brain homogenates. In addition, all three PDZ domains in SAP102 interact with the cytoplasmic tail domain of NR2B *in vitro* with different relative affinities. Taken together, these data suggest that *in vivo* at excitatory type 1 synapses SAP102 links NMDA receptors in the postsynaptic plasmalemma with the underlying cortical cytoskeleton associated with PSDs.

Supported by NIH P50 HD32901 and the German BMBF.

486.1

ONTOGENY OF GLUCOCORTICOID RECEPTORS IN THE EMBRYONIC CHICK BRAIN. L. Bordone, L.M.Schrott, and S.B. Sparber*. Dept. Pharmacology, Univ. of MN, Minneapolis, MN 55455.

Developmental effects of drugs, including those with abuse liability, have been studied in a chick embryo model. CNS glucocorticoids and their receptors (GR) are important for organizational and functional maturation. Drugs can have direct and indirect action upon the developing neuroendocrine axis, which may lead to long term postnatal dysfunction. We characterized the developmental pattern of GR in two different functional regions which develop at different rates prior to initiating drug studies. The optic tectum (OT), an early developing structure, was compared to the later developing hippocampus (HIPP) on embryonic (E) day 11, 15, 18 and on hatching day (HD). Cytosolic GR were measured using Sephadex LH20 after incubation with 20nM [3H-corticosterone (CORT)], with and without 200-fold excess cold CORT. [GR] showed age-related significant reductions between E15 and E18, with further reductions between E18 and HD. The data are consistent with the idea that a functional neuroendocrine axis, vis-a-vis feedback control of blood [CORT], is established at E14-E15. Embryos maybe more vulnerable to develop-mental insult after this stage. Since [GR] were measured in brains with adrenals intact, current studies will determine if chemical "adrenalectomy" will alter the observed developmental pattern.

	OT#	OT†	HIPP#	HIPP†
E11	35.0±5.0	181.3±15.0	42.5±7.3	187.5±27.0
E15	31.2±5.9	164.8±14.7	42.0±3.9	223.5±18.1
E18	10.6±1.5*	89.2±10.6*	16.4±2.5*	160.4±25.5
HD	4.7±0.7^	39.4±2.6^	3.5±0.1^	45.6±5.2^

#fmol/mg protein; †fmol/region; *p<0.05 vs E11, E15; ^p<0.05 vs E18; M±SE (n=4). Supported in part by US PHS grant DA 04979.

486.2

ADRENALECTOMY DOES NOT RESULT IN TYPICAL APOPTOSIS IN THE GRANULE CELLS OF THE RAT HIPPOCAMPUS. Z. Hu, H. Ozawa, and M. Kawata*. Dept. of Anat. & Neurobiol., Kyoto Pref. Univ. of Med., Kawaramachi-Hirokoji, Kyoto 602, Japan.

To study the effect of glucocorticoids on the granule cell of the hippocampal dentate gyrus, light and electron microscopy were used to define the detailed morphological change of degenerating granule cells in adrenalectomized rat hippocampus. Electron microscopy revealed that early in the process of cell degeneration the degenerating neurons did exhibit coalescing of chromatin against nuclear membrane and compaction of cytoplasm. However, subsequently, large amounts of lysosomes appeared within the degenerating cells to digest the condensed cytoplasm and nucleus. No obvious apoptotic budding-off of cytoplasm was observed and no typical membrane-bound apoptotic bodies were found within granule cell layer of the dentate gyrus. The remaining degenerating debris were finally circled by glial cells. Under light microscopy, the degenerating debris did not exhibit typical round apoptotic bodies like that in other apoptotic models. These results suggest that loss of glucocorticoids activates a lysosome system to trigger a quickly autolytic procedure resulting in granule cell degeneration. Morphologically, this kind of cell degeneration does not resemble the typical apoptotic cell death.

This research was supported by Ministry of Education, Culture and Science of Japanese Government and Sasakawa Research Foundation.

486.3

NEURONAL RESISTANCE OF A GLUCOCORTICOID-SENSITIVE MURINE STRAIN TO DEXAMETHASONE-INDUCED APOPTOSIS DURING FETAL DEVELOPMENT. B.B. Turner*, S.H. Rapier, R.R. Holson. Dept. of Physiology, College of Medicine, East Tenn. State Univ., Johnson City, TN 37614 and National Center for Toxicology Research, Jefferson, AR, 72079.

Maternal stress or exposure to exogenous glucocorticoids during gestation is associated with neonatal reductions in brain weight. During fetal development, mouse strains differ in sensitivity to glucocorticoids. The A/J strain is glucocorticoid and cleft palate sensitive, whereas, the CD-1 strain is relatively non-sensitive. Using these strains, we have shown that at E13.5 the A/J liver, but none of 8 neural regions, had an increased level of glucocorticoid receptor (GR) mRNA and GRir relative to the CD-1 strain. The A/J strain showed greater dexamethasone (Dex)-induced down-regulation of GR mRNA and protein. Dex injection at E10.5 resulted in greater down-regulation of GR mRNA and GRir in the sensitive A/J strain for both liver and brain regions. Thus, neural tissue in the A/J strain would appear relatively protected against Dex insult. Here, we asked if Dex-induced reductions in brain weight were due to increased apoptosis. Using the same strain and treatment parameters as previously, apoptotic cells were identified in 8µ sagittal sections by a TUNEL procedure which labeled 3'-OH ends of fragmented DNA. In control sections, only a few, scattered apoptotic cells were seen in the entire brain, other than at sites of developmentally regulated cell death. Dex treatment induced a only a slight increase in the total number of apoptotic cells in brain. No significant strain difference was observed. However, in liver Dex induced a striking increase in the extent of apoptosis in both strains, with the effect being greater in the A/J strain. These results suggest that Dex-induced reductions in brain weight are due primarily to premature terminal differentiation rather than to apoptosis. Supported in part by a ETSU Research Development grant.

486.4

EFFECTS OF STRESS DURING PREGNANCY ON MATERNAL AND FETAL MEDIAL BASAL HYPOTHALAMIC (MBH) 5α-REDUCTASE AND AROMATASE ACTIVITY. R.W. Rhees*, S.W. Davis, N.A. Jacobson, D.R. Ladle, and E.D. Lephart. Dept. Zoology, Cellular Biology Division, Brigham Young University, Provo, UT. 84602.

The conversion of testosterone to its major metabolites plays an important role in sexual differentiation during development. The presence of the aromatase enzyme in brain is thought to be responsible for the establishment of sexual dimorphic patterns in diencephalic and limbic structures during perinatal development. There is also evidence that brain 5α-reductase (via 5α-reduced metabolites) is important in modulating neuroendocrine function and neural development. The present study was designed to examine the effects of prenatal stress (heat-light-restraint) during the last week of gestation on 5α-reductase and aromatase activity in maternal and fetal MBH. Prenatal stress significantly reduced fetal body weight and fetal anogenital distance. Prenatal stress did not significantly alter MBH 5α-reductase activity (control, 11.9 ± 3.1 vs. stress, 10.1 ± 2.7 pmol/hr/mg protein), whereas, MBH aromatase activity was significantly reduced in stressed (61) vs. control levels (90.1 fmol/hr/mg protein) in maternal animals around the time of birth. In the MBH tissue site, 5α-reductase was not significantly altered by prenatal stress in perinatal animals and there was no significant difference between males vs. females. However, fetal MBH aromatase activity was significantly reduced in prenatally stressed animals compared to controls during the prenatal and perinatal interval, as previously reported. These results indicate that 5α-reductase activity in maternal or fetal brain is not significantly altered by prenatal stress, whereas, aromatase activity is significantly reduced in the maternal and fetal brain sites. Supported, in part, by grants from NSF and BYU Development Award.

486.5

MATERNAL BEHAVIOR ALTERS HYPOTHALAMIC-PITUITARY-ADRENAL RESPONSES TO ACUTE STRESS IN ADULT RATS. D. Liu*, B. Tannenbaum, D. Francis, C. Caldji, A. Freedman, S. Sharma and M.J. Meaney. Developmental Neuroendocrinology Lab, Douglas Hosp. Res. Ctr., Depts. of Psychiatry and Neurology, McGill University, Montréal, Canada H4H 1R3.

Postnatal handling permanently alters endocrine and behavioral responses to stress. However, the critical features for the handling effects are not clearly understood. In several papers Levine (e.g., Levine In "Stress & Society" 1975) has proposed that handling alters the behavior of the mother towards the pups. Handling is known to increase maternal licking and grooming of pups and nursing behavior. The hypothesis is that such changes in mother-pup interactions underlie the effect of handling. In this study we observed the maternal behavior of mothers of unmanipulated pups including time on pups, licking and grooming, nest building, nursing postures, etc. (see Meyers, Dev Psychobiol. 1989). As adults, the offspring of mothers which licked and groomed pups more frequently showed significantly lower plasma ACTH and corticosterone responses to acute stress, an effect that is comparable to that of postnatal handling. The frequency of licking and grooming was significantly correlated with the area under the curve measure for both hormonal responses to stress (r~.60). No other measure of maternal behavior was correlated with HPA responses to stress. These findings support the Levine hypothesis and argue for the importance of maternal licking and grooming in mediating the handling effect. (Supported by MRCC)

486.6

THE EFFECT OF 5HT ON HIPPOCAMPAL GLUCOCORTICOID RECEPTOR EXPRESSION: POSSIBLE MEDIATION VIA A 5HT7 RECEPTOR. P. Laplante*, J. Diorio, and M.J. Meaney. Developmental Neuroendocrinology lab, Douglas Hospital Research Center, Depts of Psychiatry and Neurology, McGill University, Montreal, Canada, H4H 1R3

Glucocorticoid receptor (GR) expression in the hippocampus is increased by 5HT, an effect that appears to mediated by a cAMP-dependent cascade (Mitchell et al., J. Neuroscience, 1990; Neuroscience, 1992). In vitro, the 5HT effect on GR is associated with an increase in cAMP levels and is blocked with protein kinase A (PKA) inhibitors. In this study we examined the 5HT receptor subtype underlying these effects on GR expression and activation of cAMP-inducible transcription factors. Primary hippocampal cell cultures were treated with 10μM 8 bromo-cAMP, 50nM 5-Carboxamidotryptamine (5CT), and 100nM 5HT for two hours and seven days to measure transcription factors and GR levels, respectively. Each of these treatments resulted in an increase in GR expression compared to control. There was also an increase in NGFI-A expression. In each case these effects were not affected by treatment with the 5HT1A receptor antagonist, pindolol. These results suggest that the effects of 5HT on GR expression and cAMP-sensitive transcription factors are mediated by a 5HT7 receptor. (Supported by MRCC)

486.7

POSTNATAL HANDLING INDUCES CHANGES IN THE HIPPOCAMPAL EXPRESSION OF CYCLIC NUCLEOTIDE-DEPENDENT RESPONSE ELEMENT BINDING PROTEINS IN THE RAT. J. Diorio*, D. Francis, M. Walker, A. Steverman and M.J. Meaney. Developmental Neuroendocrinology Lab, Douglas Hosp. Res. Ctr., Depts. of Psychiatry and Neurology, McGill University, Montréal, Canada H4H 1R3.

Postnatal handling induces long-term alterations in hippocampal glucocorticoid receptor expression, an effect that is mediated by 5-HT induced changes in cAMP levels and protein kinase A activity. In recent studies we examined the effect of handling on the expression of transcription factors that are known to be sensitive to dynamic changes in cAMP. Neonates were handled on each of the first seven days of life and then sacrificed at various times following the last episode of handling. Handling induced a significant increase in cAMP levels and in protein kinase A activity. Brain sections from separate animals were processed for immunocytohistochemistry using CREB, phosphoCREB and CREM antibodies. Handling produced no changes in CREB expression and this was confirmed on Western blots; there were high levels of basal CREB expression in neonates throughout the hippocampus. There was a modest increase in phosphoCREB expression immediately following handling. In contrast, there was a significant decrease in CREM-ir that was apparent at 30 minutes following handling and persisted for at least 240 minutes. At this point it is not evident which of the several CREM isoforms are affected by handling. However, since most CREM-related proteins serve as inhibitors of CREB action on transcription, these data suggest that handling might serve to increase CREB-regulated transcriptional processes via alterations in CREM expression. (Supported by MRCC)

486.8

POSTNATAL HANDLING INDUCES CHANGES IN THE HIPPOCAMPAL EXPRESSION OF NGFI-A AND AP-2 TRANSCRIPTION FACTORS IN THE RAT: EFFECTS OF THYROID HORMONES AND SEROTONIN.

D. Francis[1]*, J. Diorio[1], S. Sharma[1], J.R. Seckl[2] and M.J. Meaney[1].
[1]Developmental Neuroendocrinology Lab, Douglas Hosp. Res. Ctr., Depts. of Psychiatry and Neurology, McGill University, Montréal, Canada H4H 1R3 and [2]Molecular Endocrinology Lab, Western Gen. Hosp. , University of Edinburgh, Edinburgh

Postnatal handling permanently alters hypothamlamic-pituitary-adrenal responsivity to stress, an effect that is mediated by handling-induced increases in hippocampal glucocorticoid receptor (GR) expression. The effects of handling on GR expression are mediated by thyroid hormones which, in turn, increase 5HT activity at the level of the hippocampus. Previous data from primary hippocampal cell cultures suggest that the effect of 5HT on GR expression is mediated by an increase in cAMP levels. cAMP regulation of gene transcription is mediated by a host of transcription factors, two of which are activator protein-2 (AP-2) and NGFI-A. In the present studies neonates were handled on each of the first seven days of life and then sacrificed following the last episode of handling. We found that 1) handling-induced increases in cAMP are blocked by concurrent treatment with a thyroid hormone synthesis inhibitor, propylthyrouracil (PTU), or with ketanserin; 2) using Western blotting we found that handling produces an increase in the expression of the cAMP-inducible transcription factors NGFI-A and AP-2; 3) these effects were confirmed using immunocytohistochemistry and confirmed that the effect was apparent throughout the hippocampus; 4) the effect of handling on NGFI-A and AP-2 expression is blocked by concurrent treatment with either PTU or ketanserin. The GR gene promoter contains putative binding sites for these proteins suggesting that it is ultimately transcription factor binding to the promoter which may underlie the observed handling effects on GR expression. (MRCC)

486.9

TIMING OF GROWTH IN EXERCISING HAMSTERS: EXPRESSION OF IGF-I and GH receptor/BP mRNA.

K.T. Borer*, J.Kennedy, J.Kaplan, K.Stevens, H.Mehta and M.Drellis. Division of Kinesiology, University of Michigan, Ann Arbor,MI 48109.

Growth is an episodic process. To determine the timing of growth induction and circadian occurrence of growth in exercising mature hamsters, we quantified mRNA for IGF-I and GH receptor/binding protein in total nucleic acids and in RNA extracted from liver and quadriceps muscle, respectively. In the induction experiment, groups of 7 EX (and 6 SED) hamsters were killed after 2, 4, and 8 days of exercise. For the circadian experiment, groups of 6 EX (and 6 SED) hamsters were killed every 4 h over a 24 h period after exposure to 4 weeks of running. As little as two days of exercise was sufficient to induce the expression of IGF-I mRNA in the muscle and liver of exercising hamsters. Expression of IGF-I and GHR/BP mRNA was increased during the first four hours of darkness,the period of increased level of voluntary running. Thus, in mature hamsters, exercise induces expression of growth after two days of running and growth factor gene transcription is highest at the onset of darkness.

Support: NSF IBN-9002118 and UM OVPR.

486.10

IGFBP-6 mRNA EXPRESSION IN THE RAT BRAIN.

A. Grewal and J. Pintar*, Dept. Anat. & Cell Biol., P&S, Columbia Univ., New York, NY 10032 and Dept. Neurosci. & Cell Biol., UMDNJ/RW Johnson Med. Sch., Piscataway, NJ 08854.

The IGFs have previously been implicated in neural development. However, the IGFs, their cell surface receptors and the extracellular IGF binding proteins (IGFBPs) are also present in the adult brain suggesting a larger role in neural function. Here we report the mRNA expression of IGFBP-6 in the adult rat brain using in situ hybridization. The IGFBP-6 mRNA expression pattern is distinct from that of another predominant CNS IGF binding protein, IGFBP-2, which is synthesized largely by the choroid plexus epithelium, as well as IGFBPs -4 and -5 which have previously been characterized in more rostral brain regions.

IGFBP-6 antisense RNA hybridizes most intensely to the brain meninges and major cerebral arteries. In adult rat forebrain, the IGFBP-6 gene is expressed by isolated cells in all six layers of the cerebral cortex and is particularly high in specific brain structures such as the mitral layer of the olfactory bulb, the anterior olfactory nuclei and a superficial layer(s) in the anterior cingulate cortex. In the diencephalon, highest mRNA expression is observed in the subthalamic nucleus, the pineal body and the magnocellular portion of the red nucleus. In the hindbrain, IGFBP-6 mRNA is expressed at high levels in the pontine nuclei, the reticulotegmental nucleus, the reticular formation and by cerebellar Purkinje cells. In comparison, regions expressing highest levels of IGFBP-6 at postnatal day 11 (P11) such as the meninges, the mitral layer of the olfactory bulb and the pontine nuclei correspond to a subset of the structures expressing IGFBP-6 mRNA in the adult. Thus discrete high levels of expression are at this early age are more likely related to cell-specific neural function than to development-specific function.

In general, the data suggest that IGFBP-6 produced by the meninges may be secreted into cerebrospinal fluid to modulate IGF bioactivity more globally in the nervous system while high IGFBP-6 production elsewhere within the brain is consistent with more limited paracrine or autocrine functions. (Supported by NIH 21970.)

486.11

LONGITUDINAL SEX STEROID AND MEMORY CHANGES IN HEALTHY ELDERLY MEN AND WOMEN. Linda E. Carlson* and Barbara B. Sherwin. Department of Psychology, McGill University, Montreal, Quebec, Canada. H3A-1B1.

This study investigated the associations, over time, between sex steroids and memory in healthy elderly people. 31 Men (M), 17 female estrogen users (E), and 39 female estrogen non-users (noE), matched for age (x=72.2), were tested on a battery of verbal and visual-spatial memory tests (Time 1 - T1). A blood sample to determine plasma levels of estradiol (E_2) and free testosterone (T) was collected at the time of testing. 1.5 years later (Time 2 - T2), 23 of the M, 23 noE women, and 7 E women were re-tested on the same test battery and another blood sample drawn. The E-users scored higher than the M and noE groups on Delayed Paragraph Recall at T1, coincident with their higher plasma E_2 levels at that time. At T2, E_2 levels had significantly decreased in the M group, and both E-users and M had higher E_2 levels than the noE group. At T1 and T2 the M group had higher T levels than the E and noE groups, both of whom performed better than the M group on Category Retrieval at T1, suggesting that higher T levels may hamper performance on this test. Scores on Paired Associates and Category Retrieval decreased over time in the noE group but remained stable in the E group, indicating that E users may be protected from age-related cognitive declines when compared to non-users. T was positively correlated to Visual Paired Associate scores in the men at T2. There were no correlations between E_2 and test performance within any of the groups, perhaps due to the limited range of E_2 levels in the M and noE groups and/or the small sample size of the E group.

Funded by Operating Grant #MT-11623 of the Medical Research Council of Canada.

486.12

THYROID HORMONE REGULATED GENES IN THE DEVELOPING RAT BRAIN. A.L.S. Dowling, J. Leonard, and R.T. Zoeller*. Dept Biol., UMass, Amherst, MA 01003, Dept. Med., UMass, Worcester, MA 01655.

Thyroid hormone is an important regulator of vertebrate growth and development, having profound effects on brain development. Despite the wide recognition that thyroid hormone receptors (T_3Rs) are ligand-regulated transcription factors, no thyroid hormone-regulated genes have been identified during mammalian development. We have initiated a study focused on the developing neocortex prior to the onset of fetal thyroid function. Adult female Sprague-Dawley rats were surgically thyroidectomized two weeks prior to mating. Fifteen days after mating, females were given thyroxine (25 µg/kg) or saline in two injections (half-dose each) at 0900h and 1500h. Fetuses were removed from the dam at 0900h on gestational day 16; blood was collected from the dam and T_4 levels were measured by RIA. Fetuses from half of each litter were snap-frozen, while neocortex was dissected from remaining fetuses and total RNA was extracted. RNA from pups obtained from dams exhibiting "high" and "low" serum T_4 were subjected to differential display RT-PCR (ddRT-PCR). Gene fragments appearing either up- or down-regulated by T_4 were extracted from the gel, reamplified, and cloned. *In situ* hybridization of cryo-sectioned littermates was used as an initial screen for expression in T_3R-containing brain areas. Several gene fragments exhibit neural-specific or neural-plus-liver patterns of expression and have been sequenced. Further characterization of these genes awaits correlation of their expression level and tissue-levels of thyroid hormone during development. Funded in part by UMass Research Council to RTZ.

486.13

DISTRIBUTION AND ENDOCRINE REGULATION OF THYROTROPIN-RELEASING HORMONE mRNA IN THE DEVELOPING AMPHIBIAN BRAIN. **C. Mitchell-Herpolsheimer* and S.K. Boyd. Dept. of Biological Sciences, Univ. of Notre Dame, IN 46556.**

The pituitary-thyroid axis is of key importance in amphibian metamorphosis, but the role of hypothalamic thyrotropin-releasing hormone (TRH) in this system is unclear. We used *in situ* hybridization histochemistry (ISHH) to examine TRH gene expression in *Xenopus laevis* during normal and hormone-induced metamorphosis. For distribution studies, tadpoles were raised to representative premetamorphic, prometamorphic and climax stages, brains were cryo-sectioned at 20 μm, and ISHH was performed using a 48-base probe complementary to the first TRH coding region of *Xenopus* prepro-TRH (provided by R.T. Zoeller, U. Mass, Amherst). Seven cell populations, including 3 hypothalamic nuclei, were identified which had measurable mRNA levels at all developmental stages tested. Temporal pattern of expression varied with developmental stage for each brain region. To identify potential feedback effects on TRH gene expression, hormone treatments were administered daily to stage-selected prometamorphic tadpoles for 7 days. Treatments included TRH, cyclo-(his-pro) (CHP), and prolactin (PRL), all administered intraperitoneally (IP), and thyroxine (TH), administered via absorption from tank water. Controls received amphibian Ringer's solution IP. TH treatment induced premature metamorphosis, and significantly increased hypothalamic TRH mRNA on experimental days 2 and 4, but decreased levels on day 7. PRL induced tadpole growth without differentiation, and reduced TRH mRNA levels by 25%. TRH and CHP mimicked PRL effect on external parameters, and reduced TRH mRNA to an intermediate degree. These data suggest a developmental change in TRH gene regulation. This change may account for the dichotomy in TRH action between amphibian adults and tadpoles, and between amphibians and mammals. Supported by NSF #IBN95-14305 and NIH #HD24653.

486.14

A PIGMENT-DISPERSING FACTOR (PDF) PRECURSOR IN THE LUBBER GRASSHOPPER *ROMALEA MICROPTERA*. D.C. Davis, J.P. Riehm, R. Keller[1], K.R. Rao*, J.M. Klein. Department of Biology, University of West Florida, 11000 University Parkway, Pensacola, FL 32514, USA. [1]Institut für Zoophysiologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Endenicher Allee 11-13, D-53115 Bonn, Germany.

Pigment-dispersing hormones (PDHs) in Crustacea and pigment dispersing factors (PDFs) in insects belong to the same family of amidated octa-decapeptides. Whereas PDHs play a role in the color changes, PDFs seem to be implicated in the circadian pacemaker system.

Screening a *Romalea microptera*-brain-cDNA library we isolated a clone encoding a prepropeptide which consists of a signal peptide, a PDF-precursor-related peptide (PPRP), and the octadecapeptide PDF which has been sequenced previously (Rao et al., J. Biol. Chem. 262, 2672-2675 (1987)). The sequence of the open reading frame is MTAMAVSGKLLTALVLSTYILGL ALTIQATQYEEDKYQENEVKYGRELASWLAQLAHKNEPAICAHKRNS EIINSLLGLPKLLNDAGRK. Additional clones, possibly encoding other PDF-precursors, are found and are being sequenced. The cDNA sequence reported here represents the first elucidation of an insect PDF-precursor. Except for the similarity in the general organization of the precursor (SP, PPRP, PDF), the SP and PPRP components of the precursor from *Romalea* show little or no homology to the PDH-precursors of crustaceans. The cDNA sequence from *Romalea* will serve as a tool to investigate PDF expression in cells of the nervous system of insects.

This work was supported in part by a grant of the Deutsche Forschungs-gemeinschaft (Germany) to J.M. Klein (Kl 1001/1-1).

486.15

HIGH MATERNAL INTAKE OF POLYUNSATURATED FATTY ACIDS DURING PREGNANCY ALTERS BEHAVIOR IN FEMALE OFFSPRING. L. Hilakivi-Clarke*, E. Cho, Z.Gu and M. Raygada. LCC, Georgetown University, Washington, DC 20007.

A dietary deficiency in maternal intake of polyunsaturated fatty acids (PUFA) during pregnancy is linked to permanent functional alterations in the CNS in the offspring. Diet is the only source of essential PUFA, also for the fetus, and the brain is mainly composed of lipids. We wondered whether a high maternal intake of PUFA would alter behavior in the offspring. Pregnant mice were fed with isocaloric diets containing either 43%, 31% or 16% calories derived from fat (corn oil), throughout gestation. Behavior of the female offspring, which were fed with a regular laboratory chow from birth onwards, was studied at the age of 12 months. *In utero* exposure to a high-fat diet (43% calories from fat) significantly reduced both locomotor activity in an open field ($F(2,26)=6.7$, $p<.005$) and time spent immobile in the swim test of depressive behavior ($F(2,26)=5.5$, $p<.01$), when compared with *in utero* exposure to the low-fat diet (16% calories from fat). Aggressive behavior in the resident-intruder test was increased in the high-fat offspring ($F(2,24)=4.7$, $p<.02$). No changes in voluntary alcohol intake were noted among the groups. The mothers on the high-fat diet have significantly higher serum estrogen levels than the low-fat mothers. Among the offspring, body weights, serum estrogen levels, estrus cycle, or uterine wet weights are not affected by the *in utero* dietary fat manipulations. Our findings indicate that fetal exposure to high levels of PUFA may permanently reduce depressive-like behaviors and locomotor activity, and increase aggressiveness in female offspring, suggesting masculinization of behavior. We are currently identifying the genes that exhibit an altered expression in the brains of offspring exposed to a high-fat diet through their pregnant mother, using the differential display technique.

NUTRITIONAL AND PRENATAL FACTORS: MALNUTRITION

487.1

EFFECT OF MALNUTRITION ON THE POSTNATAL DEVELOPMENT OF THE COMPOUND ACTION POTENTIAL IN THE RAT. B. Segura, A. L. Gutiérrez, J.C. Guadarrama and I. Jiménez*. Dept. of Physiology, Biophysics and Neurosciences, CINVESTAV, IPN and ENEP Iztacala, UNAM, MEXICO.

We have analyzed the effect of malnutrition on the compound action potential (CAP) of cutaneous nerves in developing animals. Malnutrition of puppy rats was evoked by reducing 50% the dairy food intake of mother rats from pregnancy to weaning. Under deep anesthesia (chloral hydrate, 6%), both sural nerves of control and malnutritioned rats at ages of 8, 16, and 30 postnatal days were dissected and placed in a recording chamber with normal Krebs saline at room temperature. Suction micropipettes were used for stimulation and recording of the nerves. Relative low intensity stimulation of the control nerves evoked a CAP which is mainly due to activation of group A fibers. This potential varied in electrical threshold, maximal amplitude and conduction velocity depending on the age of the animals. At 8 (n=6), 16 (n=6) and 30 (n=8) days old, the CAP shows a decrease in the electrical threshold (91.0±24 μA, 58±13 μA and 35±10 μA, respectively), an increase in the maximal amplitude (31.9±11.9 μV, 53.6±17.1 μV and 261±103 μV, respectively) and a increase in conduction velocity (6.7±2.1 m/s, 10.4±5.9 m/s and 27.4±10.1 m/s, respectively). The CAP's recorded in nerves of malnutritioned rats shown a similar tendency with age but higher electrical thresholds (280±80 μA n=6, 110±56 μA n=8 and 34±5.0 μA n=8, respectively), lower amplitudes (7.4±2.2 μV, 38.6±15 μV and 64.5±26 μV, respectively) and slower conduction velocities (3.35±1.1 m/s, 8.9±2.9 m/s and 13.1±3.3 m/s, respectively) than the control nerves. The above results suggests that malnutrition produce a remarkably alteration in the functional properties of the afferent fibers of cutaneous nerves. It is proposed that this effect is associated with changes in the myelination of the axons during development.

487.2

THE EFFECTS OF OMEGA-3 FATTY ACIDS ON CENTRAL CONDUCTION VELOCITY AND CORTICAL EXCITABILITY IN SUCKLING MALNOURISHED RATS. M.F. Rivera*, F. Sánchez, N.T. Urcuyo. Dept. of Physiology, National Autonomous University of Honduras, Medical School (UNAH), Tegucigalpa,Honduras.

Previous work from our laboratory (Quirk et al.,1995) showed that early malnutrition reduces the conduction velocity of corticospinal neurons. To investigate the possible role of fatty acids (a myelin component), malnourishment was produced in rats pups from birth by restricting lactating dams to half of their normal consumption of standard laboratory chow, with or without a fish oil concentrate of omega-3 fatty acid. Reductions of body weight of 65% and 57% at day 21 for malnorished and omega-3 rats, were reduced to 12% by day 70. Total brain weight at day 70 was not reduced. Close to day 75, rats were anesthetized with urethane and the response to surface electrical stimulation of motor cortex was recorded from pyramidal tract at C1. Replicating our previous findings, malnourishment reduced the conduction velocity of the fastest fibers 17% relative to controls (6 controls: 20.79 m/s; 6 malnourished rats: 17.35 m/s). Rats supplemented with omega-3 were not significantly different from controls (18.4 m/s). In addition, the amplitude of responses was reduced throughout a range of stimulus intensities by 34% and 18% in malnourished and omega-3 rats, respectively. Furthermore, at low stimulus intensities, response amplitude in omega-3 rats was normal, suggesting that the number of large corticospinal fibers was normal in this group. (Supported by UNAH).

487.3

PRENATAL PROTEIN MALNUTRITION ALTERS VIGILANCE STATE-DEPENDENT PAIRED-PULSE RESPONSE IN ADULT RATS.

J.D. BRONZINO*, J.H. BLAISE, R.J. AUSTIN-LaFRANCE and P.J. MORGANE. Dept. of Engineering and Computer Science, Trinity College, Hartford, CT 06106.

The effects of prenatal protein malnutrition on hippocampal dentate gyrus field responses to paired-pulse stimulation of the perforant pathway were examined in freely-moving adult rats. Paired-pulse stimulations were applied over a range of interpulse intervals (IPI) during the vigilance states of quiet waking (QW), slow-wave sleep (SWS) and REM sleep in animals of both the control and malnourished diet groups. Differences between the population spike amplitude of first and second evoked response were used to construct a paired-pulse index (PPI) indicative of the modulation of granule cell excitability across vigilance states. Comparison of the PPIs obtained during QW indicated no differences between the diet groups over the three primary phases of granule cell modulation, i.e., early inhibition at IPIs = 20-30 msec., facilitation at IPIs = 50-150 msec., and late inhibition at IPIs = 300-1000 msec. However, both diet groups exhibited behavioral state-dependent changes in PPI measures during SWS and REM sleep, with facilitation being absent during both vigilance states. Prenatally malnourished animals exhibited significantly greater inhibition of the second response during the late inhibitory phase than controls. This higher level of late inhibition during the sleep states suggests that prenatal protein malnutrition affects extra-hippocampal inputs to the dentate gyrus involved in sleep/waking oscillations. These inputs include afferents from the medial septum, medial and dorsal raphé, and the locus coeruleus which synapse on select hippocampal GABA-ergic interneurons. (Supported by NIH/NICHHD Grant # HD22539)

487.4

VIGILANCE STATES AND EEG POWER SPECTRA ANALYSIS BEFORE AND AFTER REM SLEEP DEPRIVATION IN RATS WITH PRENATAL PROTEIN MALNUTRITION AT 30 DAYS OF AGE.

L. Cintra[1*], P. Durán[1], I. Medina[1], A. Galván[1], and J. R. Galler[2]. [1]Centro de Neurobiología, UNAM. A.P.78228 México. 04510 and [2]Boston University School of Medicine, MA , USA.

Prenatal protein malnutrition -PM- (6% casein diet) produces anatomical and physiological alterations in the developing central nervous system (CNS). Sleep-wake cycle has been assessed in the rat in order to understand some of the functional implications of protein malnutrition, as well as the vigilance states. The objective of this study was to evaluate sleep-wake cycle and cerebral EEG activity with a power spectral analysis, using a "conflict experiment" and 24 h of REM sleep deprivation. Occipital cortex and neck muscles were recorded in PM rats of 30 days of age, 24 h before (BD) and 72 h after (RD) REMS deprivation. EEG signal was computed each four seconds and FFT analysis was employed in order to separate EEG by frequency bands as follows: 0.75-4.0 Hz; 4.0-12.0 Hz and 12.0-25.0 Hz from undistinguished rat's vigilance states, and we scored visually the vigilance states. Results revealed a significant increase in wake and a significant decrease in SWS at baseline day and also we found increases in wake in RD 2 and 3 during the rest phase. Waking and REM sleep showed a circadian phase advance, and SWS had a phase delay in PM rats. Spectral analysis showed significant decreases on 12.0-25.0 Hz band on BD and RD 3. A phase-shift was found in 0.75-4.0 Hz and 4.0-12.0 Hz during the first recovery day. Temporal pattern of distribution was disturbed after REM sleep deprivation in PM rats. PM produces a delay in the maturation of the regulatory mechanisms of vigilance states, and also produces circadian shifts in some components of EEG signal. SUPPORTED BY DGAPA-IN-208494 NIH HD-222539.

487.5

VIGILANCE STATES AND EEG POWER SPECTRAL ANALYSIS BEFORE AND AFTER REM SLEEP DEPRIVATION IN NORMAL AND CHRONIC MALNOURISHED RATS OF 30 DAYS.

P. Durán[1*], I. Medina[1], A. Galván[1], M.A. Guevara[2], and L. Cintra[1]. [1]Centro de Neurobiología, and [2]Facultad de Psicología UNAM. A.P. 70228, México, D.F. 04510.

Chronic protein malnutrition (pre and postnatal 6% casein diet) alters structures in the central nervous system (CNS), during its development, some of them involved in the sleep-wake cycle regulation. These alterations may be reflected in the vigilance states and cerebral EEG activity and its temporal pattern of distribution. In this study we analized the EEG of the vigilance states and also we used a power spectral analysis. Using selective REM sleep deprivation and inverted LD cycle ("conflict experiment"), we recorded the occipital cortex and neck muscles activity in chonic malnourished (M) rats of 30 days of age, 24 h before (BD) and 72 h after (RD) REMS deprivation. EEG signal was computed each four seconds and FFT analysis was employed in order to separate EEG by frequency bands as follows: 0.75-4.0 Hz; 4.0-12.0 Hz and 12.0-25.0 Hz from undistinguished rat's vigilance states, and we visually scored the vigilance states. Results revealed decreases in wake and REM sleep and increases in SWS during baseline day. By other hand, spectral analysis showed a significant decrease on 0.75-4.0 Hz band on RD day 2 and 3 and significant increases in 12.0-25.0 Hz band in all RD, however, 4.0-12.0 Hz band showed no significant changes during all experimental days. Temporal pattern of distribution was also disturbed after REM sleep deprivation in M rats, this was found in both, visual and spectral analyses. The increase of 12.0-25.0 Hz band may reflect the hyperactivity in our M animals and probably is a result of an alteration in the inhibitory system. SUPPORTED BY DGAPA-IN-208494.

487.6

24H EEG BANDWIDTHS ANALYSIS IN PRENATALLY MALNOURISHED MALE RATS OF 30 DAYS OLD BEFORE AND AFTER SLEEP DEPRIVATION.

A. Galván[1*], L. Cintra[1], P. Durán[1], I. Medina[1] and J.R. Galler[2]. [1]Centro de Neurobiología, UNAM. México. 04510 and [2]Boston University School of Medicine, MA , USA.

It is well known that prenatal protein malnutrition alters several neurophysiological parameters in developing rats (i.e., long-term potentiation, hippocampal kindling, the vigilance states modulation). Nothing is known at this time about how the prenatal malnutrition influences the 24h EEG activity pattern in young rats, and also what happens after sleep deprivation. In this study, we analyzed the 24h EEG pattern in prenatal malnourished rats before and after sleep deprivation. We employed two diet groups that consisted of pups born to dams fed a low (6% casein) or control (25%) diet. Pups were cross-fostered at birth to lactating dams fed the control diet. Depending from the pre-/postnatal diet, pups were denoted as 6/25 (M) or 25/25 (C). Cortical EEG recordings of 30 days old rats were obtained during a basal day-BD and three recovery days-RD (1,2,3) after 24h of total sleep deprivation by enforced locomotion (LD 12:12h). Recorded EEG signals were processed through a power spectral analysis (FFT routine). We analized the EEG bandwidths: 0.75-4.0 Hz; 4.0-7.0 Hz; 7.0-12.0 Hz and 12.0-25.0 Hz from undistinguished rats vigilance states. We found that M rats just had significantly lower and higher relative EEG power values than C animals in the 0.75-4.0 Hz and 4.0-7.0 Hz bands in middle light phase of BD respectively. Whereas in RD, M rats showed lower values in 0.75-4.0 Hz band, and also increased values in the 4.0-7.0 Hz and 7.0-12.0 Hz bands. The 24h EEG response after sleep deprivation was different between C and M groups. Thus, our results showed the impairment in the EEG activities in prenatally malnourished rats which may explain their behavioural deficits, and also highlightened the EEG activity dependence for an appropriate nutritional condition. Supported by DGAPA-IN-208494 NIH HD-222539.

487.7

SEROTONIN AND 5-HYDROXI-INDOL ACETIC ACID LEVELS IN DORSAL RAPHE (DRN) AND SUPRACHIASMATIC (SCN) NUCLEI IN NORMAL, CHRONIC MALNOURISHED AND POSTNATAL NUTRITIONAL REHABILITATED MALE RATS BEFORE AND AFTER SLEEP DEPRIVATION.

A. Alfaro[1], L. Cintra[2], L. Castilla[1] , A. Galván[2] and C. Escobar*[3]. Inst. Nal. Pediatría[1], SS, Centro de Neurobiología[2] and Inst. Fisiol. Celular[3], UNAM. México. 04510.

In our previous sleep experiments, we found that chronic malnourished rats had higher slow wave sleep percentages that controls, and also that have increased levels of cerebral biogenic amines (i.e., 5-HT, NA) throughout the animal' life. Due to the role of 5-HT in the regulatory mechanisms of the vigilance states, in this study we evaluated the 5-HT and 5-HIAA concentrations in cerebral tissue in normal, chronic malnourished and postnatal rehabilitated rats before and after sleep deprivation by enforced locomotion. Diet groups were formed with pups born from dams fed a low (6% casein) or control (25%) diet. Malnourished pups were cross-fostered at birth to lactacting dams fed to control diet, so that were designated as 25/25%, 6/25% or 6/6% based on the pre-postnatal diet. Rats of 60 days of age were sacrificed (pre or post-24h of sleep deprivation) and both SCN and DRN were dissected. Samples were analyzed by HPLC technique. Results showed that malnourished rats had increased levels of 5-HT respect to control and rehabilitated animals in both DRN and SCN before and after sleep deprivation. Also, control and rehabilitated rats had very different 5-HIAA levels before sleep deprivation. Sleep deprivation potentiated the increased 5-HT values obtained in malnourished rats. According to our results we can conclude that the synthetic and degradative indices of 5-HT of control rats was very different to indices in malnourished rats. Such finding was not found in rehabilitated rats which may indicate that postnatal nutritional rehabilitation restitutes the appropriate 5-HT enzymatic function. **Supported by DGAPA IN-208494 grant.**

487.8

EFFECTS OF PRENATAL PROTEIN MALNUTRITION ON POSTNATAL GABAergic AND APOPTOTIC-LIKE CELLS DENSITIES ON *FASCIA DENTATA*.

[1]Díaz-Cintra, S.*, [3]González-Maciel A., [3]Romero-Velázquez, R.M. [1]Aguilar, A. and [2]Morales M.A. [1]Centro de Neurobiología, and [2]Instituto de Investigaciones Biomédicas, UNAM, A.P.78228 México. 04510 [3]UISI-IMP, SS México, D.F.

GABAergic (GAD positive cells) and apoptotic-like cells' densities were analyzed on *fascia dentata* in prenatal malnourished rats. Five weeks prior to mating female rats were fed a 6% casein diet, at birth pups were cross-fostered with a normal dam (6/25); meanwhile control group was fed with a 25% casein diet (25/25). From each experimental group six 30 days-old animals were used for morphometric analysis. GAD-positive cells were identified in three sections (rostral, middle and caudal), all cells were counted in a blind manner with respect to the diet. Densities from apoptotic-like cells on *fascia dentata* were measured in semithin sections across hiliar and granular layer of the *fascia dentata*. Results showed a significant 30% increase in the number of apoptotic-like cells in prenatal malnourished rats. Also, GAD-positive cells were significantly increased in the rostral (112%) and middle (32%) levels in prenatal malnourished animals. Statistically significant decreases (94%) were found between rostral and caudal part only in prenatal malnourished rats. These cellular alterations may affect the functional inhibitory circuit in prenatal malnourished rats, which had also LTP alterations. Supported by DGAPA IN-204093 and IN-209195.

487.9

EFFECT OF PROTEIN MALNUTRITION ON THE GENERATION OF NEURONS IN THE LOCUS COERULEUS OF THE RAT. M. Ramzan*, G.J. Blatt, W.A. DeBassio, D.L. Rosene and T.L. Kemper. Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118.

Prenatal protein deprivation has been shown to result in high levels of norepinephrine in the brain at birth which persists despite rehabilitation at birth. The present study was undertaken to explore a possible mechanism of this effect by determining the effect of prenatal malnutrition on the time of neurogenesis of the noradrenergic neurons in the locus coeruleus. For 5-weeks prior to mating and during gestation, Sprague-Dawley female rats were given either a 6% casein diet (malnourished group) or a 25% casein diet (control group). Bromodeoxyuridine (BrDU), a thymidine analog that is incorporated into DNA during the synthetic phase of the cell cycle, was injected intraperitoneally into dams on embryonic days E11 and E12 at a dose of 25 µg/g body weight. Two doses were given 4-hours apart. Prior studies have shown that these neurons are generated on these two days. The pups of malnourished as well as control mothers are cross fostered to other control mothers at birth. On postnatal day 30, male rats were anesthetized and perfused with 4% paraformadehyde and the brains were processed for BrDU immunocytochemistry. The cells which stain positive for BrDU are considered to be born on the specific day. Our preliminary results suggest that there is no significant difference in the number of tagged cells in the two diet groups on E11, however there may be a difference on E12, suggesting that malnutrition may result in a perturbation in the neurogenesis of these neurons.
(Supported by the NIH-NICHD Grant No. PO1-HD 22539)

487.10

THE FREE FRACTION OF PLASMA L-TRYPTOPHAN AS AN INDICATOR OF BRAIN SEROTONIN SYNTHESIS IN MALNOURISHED NEWBORNS.
G.G. Manjarrez, J.L. Contreras, V.M. Magdaleno †, G.G. Chagoya, J. Hernández-R, Feria-Velasco A*. Laboratory of Neurontogeny, Department of Physiology, Biophysics and Neurosciences, Centro de Investigacion y de Estudios Avanzados del IPN *Laboratory of Neurochemistry, Coordinacion de Investigación Biomedica del Centro Medico Nacional and Gynecology-Obstetrics Hospital. No.4, "Castelazo Ayala" Mexican Institute of Social Security. México, D.F. († In memoriam).

We have reported evidence showing that in rats malnourished during gestation or lactation there is an acceleration in the biosynthesis of brain serotonin, starting in the fetal period, coincident with an elevation of both, the concentration of brain L-tryptophan and the activity of tryptophan-5-hydroxylase. These conditions seem to be also present in newborn humans with gestational malnutrition. Indeed, we described an elevation of the free fraction of plasma tryptophan, which suggests an increased transport of this amino acid to the brain with a possible enhancement of serotonin synthesis. Therefore we proposed the hypothesis that in preterm humans with gestational malnutrition, the free fraction of plasma L-tryptophan should be increased as related to controls, up to the lactation period.

A prospective, longitudinal and observational study was performed. Twenty five preterm infants were included. Two groups were formed gestational age calculated by the date of last menstruation and the Ballard assessment and were 33 to 36 weeks. The malnourished group was formed by 11 neonates of both sexes with body weight below the 10th percentile of intrauterine growth curve. Fourteen subjects with weight between percentiles 10 and 90 of the same curves were considered the control group. At 1, 30, 60 and 90 days after birth free fraction and total plasmatic L-Trp were determined, as well as the concentrations blood serotonin and albumin.

In the babies with intrauterine malnutrition the results were as follow: a) the free fraction of plasma L-Trp was significantly elevated, b) the bound fraction of L-Trp to albumin and the plasma albumin, were significantly lower and 3) Plasma serotonin was significantly lower from day 60, as compared to the same parameters in normally grown-age babies.

The present work confirms our observations in a former study made in plasma of newborns with intrauterine malnutrition during the first month of life and extend them to the lactational period. The free fraction of L-tryptophan is significantly elevated up to three months of age. According to previous results showing that L-tryptophan and serotonin synthesis are increased in the brain of gestationally malnourished rats, the present data in malnourished humans strongly suggest that the elevation of the free fraction of L-tryptophan in plasma may provide an increased amount of this precursor molecule to the brain, and may activate the synthesis of brain serotonin. We propose free fraction of plasma L-tryptophan an indicator of specific changes of brain serotonin synthesis and function in patients with early nutritional changes.

GLIA AND OTHER NON-NEURONAL CELLS II

488.1

INFILTRATION OF DYE-LABELLED CELLS FROM THE PERIPHERAL CIRCULATION TO SITES OF INJURY IN THE CNS. R.J.Allore, Cheng Li, P.M. Richardson*. Division of Neurosurgery, McGill University and Montreal General Hospital, Montreal, Quebec, Canada H3G 1A4

After injury in the PNS and CNS macrophages accumulate in diverse sites where they may contribute to the support of neural repair. Our studies have focussed on the question of the timing and the extent of infiltration of peripheral blood monocytes to sites of injury in the CNS. In these experiments host Lewis rats were injected intracranially with LPS or C.parvum and subsequently, injected I.V. with dye-labelled peripheral blood mononuclear cells from another Lewis rat. One to two days after I.V. injection of labelled monocytes, host rats were sacrificed and cells from the vicinity of the CNS injection site were partially purified and examined by flow cytometry. Preliminary data obtained using this protocol indicate that dye-labelled cells originating in the peripheral circulation can be purified from the lesioned side of the brain and thus that significant numbers of monocytes can be induced to infiltrate injury sites in the CNS.
(RJA is supported by the Rick Hansen Foundation)

488.2

GENE EXPRESSION IN MAMMALIAN BRAIN CELLS INVOLVED IN THE RESPONSE TO NEURAL INJURY J.K Krady, D. Giulian‡ and R.J. Milner*
Penn State Coll. of Med., Hershey, PA 17033 and ‡Baylor Coll. of Med., Houston, TX 77030

To gain a better understanding of the response of the mammalian CNS to neuronal damage we have employed molecular approaches to analyse gene expression patterns within cell populations that play key roles in the injury response. We have generated cDNA libraries from mRNAs extracted from purified populations of rat microglia, microglia activated with GM-CSF, astroglia and peripheral macrophages. Subtractive hybridization procedures were used to generate additional, subtracted libraries that are enriched in clones of mRNAs specific to one cell type; particular cDNAs from these libraries will be characterized further by sequence analysis. In parallel, we have used differential display techniques to identify and characterize specific gene expression patterns within these cell populations. Initial studies have focused on the expression of the family of genes encoding protein tyrosine kinases (PTKs), which have been implicated in signal transduction and cellular differentiation pathways. Degenerate oligonucleotide primers were made to conserved regions within the PTK sequence and used in PCR with DNA from the subtracted or unsubtracted libraries as template. Labeled PCR products were visualized on denaturing gels and fragments of unique size that showed a differential expression pattern from one or more of the cDNA libraries were isolated and subjected to sequence analysis. By this approach, we have identified PCR products encoding sixteen different PTK sequences that are differentially expressed across the cell populations. Three of these sequences appear to represent novel members of the PTK gene family. These experiments demonstrate the power of combining subtractive cloning and differential display approaches and provide the starting material for further analysis of the identified PTKs and their expression in models of neural injury. In addition to providing potential markers for the various cell populations, these studies indicate the diversity of the signaling pathways utilized by these cell populations in their response to injury. Supported by grants from the NIH.

488.3

GLIAL CELL RESPONSE AND CLUSTERIN UPREGULATION FOLLOWING RUBROSPINAL TRACT LESION. L. Liu[1], M. Svensson*[2] and H. Aldskogius.[1] Dept. of Anatomy, Biomedical Center, Uppsala University, Uppsala[1], and Dept. of Neurosurgery, Karolinska Hospital, Stockholm[2] Sweden

The rubrospinal tract in adult, anesthetized rats was transected unilaterally at the level of the C3 segment. One day - three months after lesion, the animals were reanesthetized, and sections from the red nucleus, C3 and C8 segments were processed to examine the glial reactions as well as possible expression of complement and clusterin. In the axotomized red nucleus a small increase in immunoreactivity (IR) with antibody OX-42 (microglial marker) was found, but no change in glial fibrillary acidic protein (GFAP) - or transferrin (oligodendrocyte marker) - IR. No profiles IR for the phagocytic marker ED1 or for the cell proliferation marker bromodeoxyuridine (BU) were found. No IR for complement C3, C3d and C9 were observed. Some of the neurons showed marked increase in clusterin-IR. In the lateral funiculus of the C8 segment on the operated side, similar findings were made with the exception that ED1- and clusterin-IR were strong, and a few BU-IR profiles were present. In the lesion area at C3 prominent IR for OX-42, ED1 and clusterin was observed from two days and throughout the entire postlesion period. There were numerous BU-labelled profiles, which could be labelled with OX-42 as well, indicating that they were all microglia/macrophages. GFAP-IR was increased two and four weeks postlesion. These findings indicate the following: i) the non-neuronal response by intrinsic CNS neurons is much weaker compared to the response in the vicinity of the perikarya of peripherally injured neurons, ii) white matter degeneration is not associated with complement activation, in contrast to Wallerian degeneration in peripheral nerves, iii) clusterin upregulation is a feature of central as well as peripheral axotomy, and also of the response at the site of direct damage of CNS white matter, suggesting the possibility that clusterin may be associated with some aspects of neuronal degeneration.
Supported by the Swedish Medical Research Council.

488.4

UNILATERAL HYPOTHALAMIC LESION RESULTS IN INCREASED GLIAL IMMUNOREACTIVITY FOR A TYROSINE PHOSPHATASE IN THE POSTERIOR PITUITARY. D. I. Lurie*, D. K. Kosena, J. A. Watt, and C.M. Paden. Pharm. Sci, U. of Montana, Missoula MT 59813 and Biology, Montana State U., Bozeman, MT, 59717.

Studies of the glial response to injury have historically focused on glial proliferation and hypertrophy. Using such methods, it has been difficult to identify the specific cellular responses to injury. We suggest that a reasonable approach to identifying the different roles that glial cells play following CNS damage is to concentrate on the regulatory signals that may initiate selected molecular cascades within these cells. The balance between protein phosphorylation and dephosphorylation modulates many cellular functions and tyrosine phosphorylation has been implicated in both glial proliferation and differentiation. We have found a population of cells within the rat posterior pituitary that become immunopositive for the tyrosine phosphatase PTP1C following partial denervation of the neural lobe by a unilateral hypothalamic lesion. PTP1C is an SH2 containing tyrosine phosphatase which is expressed in high levels in hematopoietic cells and is thought to play a role in the signaling cascade triggered by activated receptor protein tyrosine kinases. Rats received a unilateral hypothalamic lesion known to cause axonal degeneration and compensatory collateral sprouting within the neurohypophysis and were allowed to survive for either 5 or 10 days. Brain and pituitary tissue was processed for immunocytochemistry and stained with a polyclonal antibody generated against a PTP1C peptide. By 5 days following the lesion, there was an increase in PTP1C immunoreactivity in cells that resembled microglia. This was correlated with an increase in microglial proliferation and activation associated with phagocytosis of degenerating axons. By 10 days following the lesion, the PTP1C immunoreactivity had largely disappeared indicating that PTP1C is transiently expressed in the posterior pituitary following hypothalamic lesion. These results indicate that a PTPase may be involved in the microglial response to injury in the rat posterior pituitary. Supported in part by NIH NS32507.

488.5

TRIMETHYLTIN EXPOSURE UPREGULATES CYTOKINE LEVELS IN MOUSE HIPPOCAMPUS. A. Bruccoleri, H. Brown and G.J. Harry*. Neurotoxicology Group, NIEHS, RTP, NC, 27709.

Trimethyltin (TMT) is a prototypic neurotoxicant that produces severe neuronal necrosis in the hippocampus. In mice, the target neuronal population is the dentate granule cells of the hippocampus. In immature male mice, 17 day old CD1 (Charles River, Raleigh, NC), the onset of neuronal necrosis is accompanied by an astrogliotic response. Within 24 hours of TMT administration (2.5 mg/kg body wt, ip), there is induction of neuronal necrosis in the dentate with an accompaning increase in glial fibrillary acidic protein (GFAP) immunoreactive astrocytes and microglia. By 3 days, the neuronal necrosis in the dentate is more pronounced and sparse neuronal necrosis is evident in the CA1 pyramidal cell region. The astrogliotic response is relatively uniform throughout the entire hippocampus while the microglia response is localized to the regions of neuronal necrosis. Our lab has previously reported a temporal association between microglia and early expression of pro-inflammatory cytokines in the rat hippocampus following TMT exposure. We now present data on the upregulation of cytokine mRNAs in the mouse hippocampus following TMT. At /various time points from 1 hour to 2 weeks following a single injection of TMT, changes in cytokine mRNA levels were assessed by RT-PCR. Within 12 hours, TNF-α, TNF-β, IL-1α, and MIP-1α mRNA levels were significantly elevated. IFN-γ was elevated as early as 1 hour, possibly serving as an early "priming" signal for subsequent inflammatory responses. By 72 hours, TNFα, TNF-β and IL-1α mRNA levels began to decrease possibly due to the upregulation of an inhibitory factor. While IL-4 and IL-10 mRNA remained undetected at any time point, TGF-β1 was elevated at 72 hours suggesting its role in downregulation of the pro-inflammatory cytokines. IL-6, IL-1β and GM-CSF remained unaltered by TMT exposure. These results suggest a modulatory role for the cytokine cascade in the manifestation of chemical induced neurodegeneration.

488.6

EXPRESSION OF INTERLEUKIN-15 RECEPTOR α CHAIN IN HUMAN GLIA IN CULTURE. Y.B. Lee* and S.U. Kim. Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, Canada.

Interleukin-15 (IL-15) is a novel cytokine that has recently been cloned and exhibits T cell stimulating activity similar to interleukin-2 (IL-2). IL-15 mRNA is detectable in a wide range of cell types including fibroblasts, epithelial cells, and monocytes, but not in T cells. Recently, we demonstrated that human astrocytes and microglia produce IL-15 constitutively and that IL-15 expression is regulated by proinflammatory cytokines such as interleukin-1β (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) (Lee et al., *Soc. Neurosci. Abstr.* **21**:885, 1995). Recently, a human IL-15 receptor α subunit (IL15Rα) was cloned and showed a high affinity for IL-15. To investigate the biological role of IL-15 in the human central nervous system (CNS), mRNA levels of IL-15Rα in human astrocytes, microglia, and oligodendrocytes were determined by reverse transcription-polymerase chain reaction (RT-PCR). Purified populations of astrocytes and microglia were prepared from fetal brains of 12-20 weeks' gestation, and oligodendrocytes were prepared from adult human brain tissues obtained during epilepsy surgery. IL-15Rα mRNA was detected in both astrocytes and microglia but not in oligodendrocytes. In addition, upregulation of IL-15Rα in astrocytes was observed after treatment with IL-1β, IFN-γ, or TNF-α and in microglia by lipopolysaccharide (LPS), IFN-γ, or granulocyte-macrophage colony-stimulating factor (GM-CSF). These results suggest that IL-15 produced by astrocytes and microglia has a role in immuno-modulation in the human CNS in a paracrine or autocrine manner. (Supported by the Medical Research Council of Canada and the MS Society of Canada.)

488.7

EFFECT OF 4-AMINOPYRIDINE ON LPS-INDUCED MICROGLIAL ACTIVATION.
H.K. Pyo*[1], ILo Jou[1], S.K. Chung[2], E.H. Joe[1]. [1]Dept. of Pharmacol. Ajou Univ. Sch. of Med. Suwon, Korea; [2]Dept. of Physiol. Chung-Ang Univ. Sch. of Med. Seoul, Korea.

Microglia, brain's immune cells, are activated around injured areas and aggravate the injury through release of nitric oxide(NO). The activation of microglia correlates well with induction of outward K^+ channels. However, the exact role of K^+ channels during the activation of microglia remains to be delineated.

We prepared microglia from the neonatal rat brains and cultured for two weeks. Lipopolysaccharide(LPS), a bacterial endotoxin, was used to activate microglia. Microglia exposed for 2 days to LPS showed marked expression of K^+ channels revealed by electrophysiology and immunohistochemistry using a polyclonal antibody to Kv1.5(one of voltage-gated K^+ channels). The LPS-treated microglia underwent some morphological changes from small and/or ramified cell bodies to large and flat ones, secreted NO, lost mitotic activity, and reduced phagocytosis. To study the function of K^+ channels in activation of microglia, microglia were treated with LPS in the presence of 4-aminopyridine(4-AP) which blocks up to 90% of the outward K^+ channels(Norenberg et al., 1994, J. Physiol. 475: 15). LPS-induced morphological changes and NO secretion were reduced by 4-AP in a dose-dependent manner, but proliferation and phagocytosis were not influenced. However, tetraethylammonium(TEA) which has less effect on outward K^+ channels did not block either morphological changes or NO release. These results provide that the LPS-induced expression of outward K^+ channels are required for microglial activation, particularly morphological changes and NO secretion.

Supported by Ajou Univ. Research Fund to ILo Jou and E.H. Joe.

488.8

ETHANOL INHIBITS THE PROLIFERATION AND SURVIVAL OF MICROGLIA IN CULTURE. G.J. Brown*, W. Chen, Z.Q. Yang, N.S. Vettiyadan and C.J.M. Kane. Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Fetal ethanol exposure is the leading known cause of mental retardation affecting >1 of 1000 infants a year. The damaging effects of ethanol on the development of neurons and macroglia have been studied. However, the impact of ethanol on the development of the principal immune cell in the CNS, the microglial cell, is unknown. Microglia were purified from primary cultures of mixed glia established from the cerebral cortex of one-day old Charles River CD rats. Microglia were exposed to ethanol in the culture medium at socially and clinically realistic levels (0.05, 0.1, 0.2, 0.5, 1.0% w/v) with evaporation restricted. The impact of ethanol on microglial survival was determined by MTT assay of cell viability. Ethanol decreased microglial survival significantly in a dose-dependent manner with 35–90% cytotoxicity observed following 4 days of exposure. Proliferation of the surviving cells was quantified by [^{3}H]thymidine or bromodeoxy-uridine incorporation into replicated DNA. Quantitation of [^{3}H]thymidine in cell lysates revealed a significant 10–25% inhibition of proliferation in cultures exposed to ethanol for 4 days. Enumeration of BrdU-labeled microglia by microscopy revealed that ethanol inhibited microglial proliferation 10–25% after 1 day, and 25–40% after 4 days, of ethanol exposure when compared to untreated controls. These results suggest that microglia may be a direct target of ethanol *in vivo*. A significant decrease in microglial genesis or survival could produce long-term consequences on microglial function in the brain. Since microglial cells directly affect the function and survival of neurons, ethanol damage to the microglial population during CNS development may also contribute to the neuronal pathology associated with fetal ethanol exposure. (UAMS Foundation).

488.9

ETHANOL BLOCKS MICROGLIAL MATURATION IN THE RAT CEREBELLUM. N.S. Vettiyadan*†, K.E. Light+ and C.J.M. Kane†. †Dept. of Anatomy, College of Medicine, and +College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The neuropathology of fetal ethanol exposure includes deficits, malformations and dysfunction in neurons. Direct ethanol damage to astrocytes and oligodendrocytes is also well documented in patients and animal models. However the impact of ethanol on the principal immune cell in the brain, the microglial cell, is unstudied. This study was designed to document quantitatively microglial pathology in a rat model of fetal ethanol exposure. Charles River CD rats were administered ethanol (3 g/kg/dose) or the isocaloric equivalent as dextrose via intragastric intubation at 8 am and 4 pm on postnatal days 2 through 10 inclusive. Each litter of 10 culled pups contained 3 treatment groups: ethanol intubated, dextrose-control intubated and non-intubated suckle controls. Animals were weighed, anesthetised and perfused at 8 am on postnatal day 11. Microglia in 50 μm mid-sagittal sections of the cerebellar vermis were visualized by *Griffonia simplicifolia* lectin I-B4 binding and the peroxidase reaction. The perimeter of the stained soma and processes of individual microglial cells in the molecular layer of lobule I was measured by computer-aided image analysis. At least a 20% reduction in the cell perimeter was observed in ethanol-treated rats compared to intubated controls ($p \leq 0.05$), reflecting impaired process elaboration as a result of ethanol exposure. These results suggest that microglial maturation in the brain is damaged by moderate concentrations of ethanol during the critical period of microglial genesis and maturation. Thus, microglial damage by fetal ethanol exposure may impair the ability of microglia to defend the brain against disease. (UAMS Foundation and UAMS Graduate Student Research Fund).

488.10

GLIAL RESPONSE TO ICV LIPOPOLYSACCHARIDE INJECTIONS IN MICE OF DIFFERENT AGES. A.N. Kalchua*, F. Mangiarotti[1], J. Hengemihle, D.L. Speer, D.K. Ingram and M.G. DeSimoni[1]. GRC, NIA, NIH, Baltimore, MD 21224, and [1]Mario Negri Institute of Pharmacol. Res., 20157 Milan, Italy.

Chronic inflammation has been implicated in age-related neurodegeneration as Alzheimer's disease (AD). Cytokines, such as IL-6 and TNFα, have been found in senile plaques associated with activated microglia and hypertrophic astrocytes in AD brains. To develop a model of CNS inflammation, we have conducted ICV injections of the endotoxin, lipopolysaccharide (LPS) to produce glial cell activation in mouse brains.

LPS (2.5 μg/μl or 10 μg/μl; 1 μl) was injected into the third ventricles of 6-mo (adult), 19-mo (middle-aged) and 24-mo (aged) male C57bl/6J mice. Beginning 1.5 hours after treatment, mice were perfused with 4% paraformaldehyde buffered with 0.01M PBS, or sacrificed by decapitation for biochemical analysis. Astrocytes were identified using a polyclonal antibody to GFAP, and microglia with the B_4 isolectin from *Griffonia simplicifolia*.

LPS produced localized hippocampal glial activation within the genu of area CA3 at 2.5 μg/μl in all animals, however, serum and brain levels of TNFα, as well as serum levels of IL-6 were elevated in aged animals compared to adults. In contrast, following 10 μg/μl LPS, activated glia and macrophages were present throughout the hippocampus, cortex and thalamus of all animals, with strongly elevated serum and brain levels of both IL-6 and TNFα in aged vs. adult animals. Lastly, aged animals were also more susceptible to LPS-induced mortality associated with marked tonic-clonic seizures, than adults.

488.11

MICROGLIA AND T-LYMPHOCYTES INTERACT TO PRODUCE TNF-α: REDUCTION BY β-INTERFERON. Chabot S., Stüve O.*, and Yong V.W. Montreal Neurological Institute, Department of Neuroimmunology, McGill University, Montreal, Canada, H3A 2B4.

Clinical trials have demonstrated that β-interferon (β-IFN) is effective in the treatment of relapsing-remitting multiple sclerosis (MS), although the mechanisms of its efficacy remain unclear. In view of the observations that the migration of T-lymphocytes into the CNS is a crucial event in the pathogenesis of MS, and because the cytokine TNF-α can induce apoptotic death to oligodendrocytes, we examined the effect of β-IFN on the level of TNF-α production following the interaction between T-lymphocytes and microglia; microglial cells are known to be a prominent source of TNF-α in the CNS. Cultured adult human microglia were exposed to activated T-lymphocytes for 24h and the culture medium was then collected for ELISA measurements of TNF-α. In co-cultures of microglia and activated T-lymphocytes, the level of TNF-α was greatly enhanced (300-400pg/ml) compared to control (microglia alone), in which no TNF-α was detected. When T-lymphocytes were pretreated with IFN-β (ranging from 0.1U/ml to 1000U/ml), and were then exposed to microglia, the production of TNF-α was reduced in a dose-dependent manner. Pretreatment of microglia with β-IFN had a less marked effect on the subsequent generation of TNF-α in T-lymphocyte/microglia co-cultures. Ongoing experiments are being conducted to assess the mechanism by which β-IFN reduces the production of TNF-α. We suggest that the significant reduction by β-IFN of levels of oligodendrocyte-toxic cytokine, TNF-α, may contribute to the mechanisms of efficacy of β-IFN in MS. *(Supported by Berlex Laboratories)*

488.12

MICROGLIA IN HUMAN CNS DEVELOPMENT. A.Andjelkovic[1], A.Milosevic[2], B.Nikolic[2] and N.Zecevic[1]*, [1]University of Connecticut, Farmington, CT 06030 and [2]Institute for Biological Research, Belgrade 11000, Yugoslavia.

The development of microglia in the human brain has increasing importance because these cells are a target for fetal HIV infection. To establish the time of their appearance, we examined 9 human embryos and fetuses ranging in age from 4 to 13.5 gestational weeks (g.w.) using antibodies for two specific markers, Ricinus communis agglutinin 1 (RCA-1) and *Lycopersicon esculentum* (tomato) lectin. At 4 g.w. both markers labeled round, ameboid cells with 1-2 short processes, in the marginal zone of the neuronal tube, in the nearby connective tissue, and aligned on the outer pial surface. Primitive blood vessels were observed only in the caudal region. At 5-6 g.w., in addition to amoeboid cells, poorly ramified microglia were found in the marginal and ventricular zones of the entire CNS. At 7.5 g.w., in the cortical anlage only a few poorly ramified cells were present in the cortical plate, whereas the majority of microglia were found above and below it, following the vascularization pattern. At 13 g.w. adult-like ramified microglia were present in all areas of the CNS and in all developmental zones of the cortex. The appearance of microglia at 4 g.w., when only a few primitive blood vessels were present in caudal regions of the neuronal tube, suggests a possible dual origin for microglia in the CNS. Supported by NIH grant MH53945 (NZ).

488.13

INFLAMMATORY AGENTS INDUCE MICROGLIAL NEUROTROPHIN-3 EXPRESSION WHICH ATTENUATE MICROGLIAL TOXICITY ON NEURONS. S. Elkabes* and I. B. Black. Neurosci. and Cell Biol. Robert W. Johnson Med. Sch., UMDNJ, Piscataway, NJ 08854

We previously reported that microglia express neurotrophins *in vitro* and *in vivo*[1]. These results suggested that microglia, which have been implicated in neurotoxin production in inflammation, also support neurons and glia. To determine whether inflammatory agents regulate microglial trophin expression, we analyzed the effects of lipopolysaccharide (LPS) and interferon-γ (INF-γ) on the expression of NGF, NT-3 and NT-4 in microglia. Exposure of microglia to LPS and INF-γ for 6 or 24 hours induced a 7-fold increase in NGF, a 2-fold increase in NT-3 and no change in NT-4 mRNAs. These results suggested that microglia support or protect neurons by increasing trophic factor production in response to inflammatory agents. One way of protecting neurons in inflammation is to reduce toxic activities produced by microglia. Since our previous studies indicated that NT-3 regulates microglial proliferation and phagocytosis[1], we assessed whether NT-3 also regulates microglial toxic activities. We studied the effect of microglial conditioned medium on cortical neurons after exposure of microglia to NT-3. Conditioned medium obtained from high density control microglial cultures induced a significant decrease in the number of cortical neurons *in vitro*. Treatment of microglia with NT-3 abolished the toxic effects of microglial conditioned medium on neurons. These results raise the possibility that NT-3 protect neurons by attenuating elaboration of toxins by microglia. (NIH Grant POI HD 23315-06A1 and Trophix Pharma. Inc.) 1.Elkabes et al., 1996, J. Neurosci., 16, 2508.

488.14

DIFFERENTIAL GENE EXPRESSION IN ACTIVATED MICROGLIA. S. Thakker-Varia*, S. Elkabes, A.C. Sherwood and I.B. Black. Dept. of Neuroscience and Cell Biology, UMDNJ - Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Microglia are immunoreactive cells in the brain that have been implicated in inflammatory defense mechanisms in response to illness and injury. To understand molecular mechanisms leading to microglial activation, we used differential mRNA display, a highly sensitive, rapid differential cloning technique. This method utilizes reverse transcription and PCR to amplify partial cDNA sequences from subsets of mRNA. Display of cDNA from lipopolysaccharide (LPS)-activated microglia and controls revealed that LPS elicited unique patterns of gene expression. Eleven of these LPS-induced bands were isolated from the display, reamplified by PCR and cloned. Partial sequence analysis of one of these bands indicated that the cDNA showed homology to the squamous cell carcinoma antigen gene, a member of the serine protease inhibitor family. Using this approach, the profile of gene regulation in the recently established rat microglial cell line, MK.1, was compared to that of the primary microglia. When the same set of primers were used to display RNA from primary cultures and rat MK.1 cell line, an almost identical cell-specific cDNA pattern was observed. These results confirm that the genes expressed in cell line reflect patterns of genes in primary culture, thus validating the use of rat MK.1 for further investigations. Additional characterization of the differentially expressed genes and their relationship to microglia activation will be explored. (Supported by Trophix Pharmaceuticals Inc.)

488.15

BASIC FIBROBLAST GROWTH FACTOR INCREASES PHAGOCYTIC ACTIVITY IN A CELL LINE DERIVED FROM PRIMARY MICROGLIAL CULTURES. **A.C. Sherwood*, I.B. Black, and S. Elkabes. Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.**

Within the central nervous system, microglia respond to neuronal damage by altering morphology, proliferating, increasing phagocytic activity, elaborating toxins and trophins, and by increasing expression of markers of antigen presenting cells. Basic fibroblast growth factor (bFGF) is elevated in lesions where microglia are present, following trauma, in experimental acute encephalomyelinitis (EAE), and in Alzheimer's disease. Microglia increase expression of high-affinity bFGF receptors after trauma and in EAE. To further understand the role of bFGF in mediating functions such as phagocytosis, we have used a partially characterized microglial cell line, MK.1, that we have recently developed.

MK.1 cells were derived from primary cultures of P2 rat, cortical microglia transfected with the oncogene, p53val 135. MK.1 cells express the microglial specific markers, ED-1 and OX-42. Under normal growing conditions these cells are sombrero-like or crescent shaped and 15 % phagocytize latex beads. They make and respond to neurotrophins. When treated with bFGF (10 ng/ml), MK.1 cells increase proliferation, become elongated and process-bearing, and 50% of the cells phagocytize latex beads. We are employing MK.1 in conjunction with primary microglia to characterize mechanisms through which bFGF contributes to disease pathogenesis. Supported by Trophix Pharmaceuticals.

488.16

THE POSSIBLE ROLE OF EPIDERMAL GROWTH FACTOR IN MICROGLIAL CELL ACTIVATION IN VITRO C. Nolte* and H. Kettenmann Cell. Neurosciences, Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Str.10, D-13125 Berlin, FRG.

Epidermal growth factor (EGF) and its receptor (EGF-R) are present in the central nervous system (CNS) and evidence is accumulating that EGF regulates a variety of CNS functions[1]. Acute and chronic pathological processes of the CNS are often accompanied by an increase in EGF-R expression[1]. Microglial cells which are always activated by insults to the CNS are recruited at the lesion sites by chemotactic movement and proliferation. Since there is evidence for an action of EGF on membrane currents in microglial cells[2], we asked if EGF might also stimulate microglial chemotaxis and proliferation.

Using primary microglial cells from newborn mouse cortex and a multiwell microchemotaxis assay we demonstrate a dose-dependent effect of EGF (Sigma E-4127; Coll. Biomed. Res. 40010) on microglial chemotaxis. The maximal chemotactic response is achieved by 10µg/ml EGF in the lower compartment. It reaches 300% (± 35%) of the unstimulated control and is about half as high as induced by 10nM C5a, a potent microglial chemoattractant. Checkerboard-analysis shows that the effect of EGF is not purely chemotactic. As demonstrated by BrdU-incorporation and a colorimetric cell-ELISA, EGF (0.01 - 10ng/ml for up to 36h) is not a mitotic signal for microglia.

Our data strongly indicate that microglial cells possess binding sites for EGF. EGF specifically activates microglial chemotaxis but not proliferation and thereby might serve to direct microglial cells to the site of brain damage.

References: (1) Plata-Salaman (1991) *Peptides* **12**:653-63; (2) Ilschner et al. (1996) *Neuroscience* in press. Work was supported by DFG KE 329/8-2.

488.17

DIFFERENTIALLY EXPRESSED GENES POTENTIALLY SERVING TO PROTECT AGAINST NITRIC OXIDE-ASSOCIATED OXIDATIVE STRESS IN ACTIVATED MICROGLIA. K. Sugaya*, M. Chouinard and M. McKinney. Dept. of Neuropharmacology, Mayo Clinic Jacksonville, Jacksonville, FL 32224.

Nitric oxide (NO) generated by NO synthase (NOS) can cause oxidative stress in some cell models. However, NOS-positive cells appear to be resistant to the effects of Alzheimer's disease, a condition in which oxidative stress is known to exist. Since microglia are known to express inducible NOS (i-NOS) and produce copious NO when activated, they appear to have efficient protection mechanism(s) against NO toxicity. In this study, activated microglia were resistant to a concentration of NO which elicited DNA fragmentation in inactivated microglia. This indicates that microglia express protection mechanisms after they are activated. In order to identify those biochemical mechanisms against oxidative stress, we used "mRNA differential display" to clone sequences from genes that are preferentially expressed in activated microglial cells (line BV-2). When checking sequences for homology against the GenBank data base, many genes were found to be differentially expressed in activated BV-2 microglia. The identifiable genes included class II histocompatibility antigens, i-NOS, IL1 and certain cell surface antigens. Among these, we obtained evidence that genes related to lipid transport, anti-apoptosis and NO metabolism may play important roles in protecting these cells from oxidative damage. Such findings may in part explain why NOS-positive cells are resistant to oxidative stress imposed from self-generated free radicals.

488.18

CLEARANCE OF APOPTOTIC CELLS IN THE CNS. R. Parnaik, C. Neophytou*, J. Scholes, M. C. Raff, MRC LMCB, University College London, London, England.

It has been known for some time that many neurons and glia die by programmed cell death during neurogenesis, but what phagocytoses and clears these cells from the Central Nervous System (CNS) is much less well understood.

Electron micrographic evidence shows that all pyknotic cells in the cerebellum have already been engulfed by another cell, implying that cells which have not been engulfed by microglia (the resident macrophages and professional phagocytes of the CNS) have been engulfed by another cell type.

Using a combination of immunohistochemistry and electron microscopy we have shown that several cell types are involved in the scavenging process. In the External Granule Layer (EGL) of the cerebellum a number of neurons die but there are no microglia present in this region. Both Bergmann glia (the radial glia of the cerebellum) and other granule neurons are responsible for the clearance of pyknotic cells in the EGL. In the optic nerve however, where the cells that die are oligodendrocytes, it is apparent that virtually all of the pyknoses are engulfed by microglia. This may mean that certain cell types preferentially elicit phagocytic responses from particular other cell types, - oligodendrocytes and microglia for example. Alternatively, the situation in the optic nerve may represent a more advanced stage of development than the cerebellum at this age and in the CNS the phagocytic role is taken up progressively by the microglia as a region matures.

488.19

RELATIONSHIP BETWEEN MICROGLIAL ACTIVATION AND THE ONSET OF WALLERIAN DEGENERATION IN THE DENERVATED NEUROPIL OF THE HIPPOCAMPUS. P.E. Schauwecker* and O. Steward. Dept. of Neuroscience, Univ. of Virginia, Charlottesville, VA 22908.

Reactive sprouting following neuronal injury has been suggested to reflect an interplay of cellular signals involving microglia, astrocytes, and neurons. To understand the mechanisms governing neuronal survival and regeneration, it is essential to know more about the time course of cellular responses. This study addresses the temporal relationship between the activation of microglia in denervated neuropil and the onset of terminal degeneration as revealed by selective silver staining in normal mice and in mice carrying a mutation that delays the onset of Wallerian degeneration (Wld^s). We used the unilateral entorhinal cortex (EC) lesion as our experimental model to define degeneration and microglial activation within the denervated molecular layer of the dentate gyrus. The onset of degeneration was assessed using the Fink-Heimer method while microglial activation was assessed using immunocytochemistry for Mac-1, an antibody that recognizes the complement component receptor $C3_{bi}$. C57BL/6 and Wld^s mice were killed at 1, 2, 4, 6, 8, 12, 15, and 20 days following an EC lesion. In C57BL/6 mice, microglial induction was present as early as 1 day postlesion with peak activation occurring between 2-6 days postlesion. In contrast, microglial activation was delayed by 6-8 days in Wld^s mice and the delay in microglial activation temporally coincided with a delay in degeneration. These data suggest that the response of microglia in denervated neuropil zones is triggered by degenerative changes in axons undergoing Wallerian degeneration. Supported by NS29875. P.E.S. was the recipient of NRSA NS09972.

MOTOR SYSTEMS: DEVELOPMENT AND REGENERATION II

489.1

NMDA RECEPTOR 2A/B SUBUNIT EXPRESSION IN TRIGEMINAL MOTONEURONS, INTERNEURONS, AND MESENCEPHALIC TRIGEMINAL NEURONS DURING EARLY POSTNATAL DEVELOPMENT. J.E. Turman, Jr.*, J.Ajdari, B.Cole, and S.H. Chandler. University of Southern California, Dept. of Biokinesiology and Physical Therapy, Los Angeles, CA 90033; and University of California, Los Angeles, Dept. of Physiological Science, Los Angeles, CA 90095.

There are little data at the cellular level that explains the neural mechanisms underlying the transition from suckling to chewing. Trigeminal motoneurons (MotV), mesencephalic trigeminal neurons (MesV), supratrigeminal (SuV), and intertrigeminal (InV) neurons are important constituents of the neural circuitry responsible for these oral-motor behaviors. Insight regarding oral-motor development will be gained by understanding the development of neurotransmitter receptor complexes utilized to modulate the activity of these neurons. This study used receptor immunohistochemistry techniques to describe changes in the expression of NMDA receptor 2A/B subunits in MotV, MesV, SuV, and InV neurons during early postnatal development. Neonatal rats at postnatal day (P) 1, 3, 8, 15, and 22 were used. An affinity purified polyclonal antibody against the NMDA 2A/B subunits (Chemicon, Inc.) was used to describe the neuronal and/or neuropilar distribution of these subunits. In MoV neurons, expression was neuronal at P(1) and transitioned to primarily neuropilar labeling by P(22). Neuronal expression in MesV neurons emerged at P(3) and remained neuronal through P(22). Receptor subunit expression in SuV and InV neurons emerged at P(8). Immunoreactivity in these regions became neuropilar between P(15) and P(22). These results demonstrate that the cellular localization and temporal expression patterns of NMDA receptor 2A/B subunits differ among MotV, MesV, SuV, and InV neurons during early postnatal development. Funded by NIH-NIDR DE06193.

489.2

AN *IN VITRO* STUDY OF EARLY BRAINSTEM-SPINAL CORD PROJECTIONS IN THE RAT H.J. ten Donkelaar* and R. de Boer-van Huizen. Dept. Anatomy/Embryology, Univ. of Nijmegen, 6500 HB Nijmegen, Netherlands

So far, the early development of brainstem-spinal cord projections in mammals, apart from marsupials, could be studied only by application of DiI to the spinal cord of fixed embryos. In a first series of DiI experiments data by Auclair et al. (1993) were confirmed for rat embryos fixed on embryonic day 13 (E13) or 14 (E14). Cells of origin of interstitiospinal (from the interstitial nucleus of Cajal), reticulospinal and vestibulospinal projections were labeled. The main disadvantages of the DiI technique are the long diffusion time required (at least two months) and difficulties inherent in analyzing fluorescence labeled material. Therefore, an *in vitro* approach was applied. Precisely timed pregnant Wistar rats were used to provide embryos 10-14 gestational days of age. At the proper time, the rats were anesthetized and the embryos taken out by cesarian. The isolated embryos were immersed in an iced Ringer solution that had been oxygenated with carbogen (95% O_2, 5% CO_2). Subsequently, biotinylated dextran amine (3kD BDA, Molecular Probes, D-7135) was applied to the rostral spinal cord. After the application the embryos were placed in a perfusion chamber and superfused overnight with freshly oxygenated Ringer solution at room temperature. The next day, the embryos were fixed with 4% paraformaldehyde in phosphate buffer (pH 7.4), embedded in polyacrylamide or in Epon if treated as wholemounts, and cut coronally or sagittally on a vibratome. In the 40 μm sections, BDA was visualized with an avidin-biotin complex. In E12-embryos, the interstitial nucleus of Cajal, various parts of the reticular formation and the vestibular nucleus complex were labeled. Preliminary data in E10-embryos indicate that already in this stage a few neurons in the interstitial nucleus of Cajal and many neurons in the reticular formation innervate the spinal cord. The present study shows that an *isolated brain preparation* is well-suited for tracing studies in mammalian embryos.

489.3

MOTONEURONE SENSITIVITY TO GLUTAMATE RECEPTOR AGONISTS IS INCREASED DURING THE EARLY POSTNATAL PERIOD. J. Palecek*, G. Abdrachmanova, L. Vyklicky Jr. Institute of Physiology, Czech Academy of Sciences, Videnska 1083, Prague, 142 20 Czech Republic.

Imunohistochemical and *in situ* hybridization studies have shown that changes in the density and subunit composition of glutamate receptors in motoneurones take place during the early postnatal development of the rat. The aim of our experiments was to find out what is the functional correlate to these changes.

We have used electrophysiological techniques to study whole-cell membrane currents induced by selective glutamate receptor agonists NMDA (15 μM) and kainic acid (KAI 5 μM) in motoneurones the during early postnatal period of the rat. Recordings were made from 70 visually-identified retrogradely labeled motoneurones in spinal cord slices. The NMDA-evoked current responses normalised to the input resistance of the motoneurones at P2-3 and P4-5 were small. However, a four fold increase was observed between the two age groups P4-5 and P6-7. The mean values ± SEM were: -3.8 ± 0.8 (P2-3), -4.5 ± 0.8 (P4-5), -18.5 ± 4.7 (P6-7), -32.1 ± 11.1 (P8-9), -18.5 ± 7.5 (P10-11) and -25.6 ± 9.9 (P12-13) pA/MΩ. In contrast the normalised KAI-evoked currents gradually increased from -5.4 ± 2.5 pA/MΩ at P2-3 to -22.4 ± 6.9 pA/MΩ at P12-13.

Our results suggest that the sensitivity of motoneurones to glutamate receptor agonist is developmentally regulated.

Supported by GACR-305/96/0680, HHMI-75195 and CIPA-930268.

489.4

CHARACTERIZATION OF FACIAL AND HYPOGLOSSAL MOTONEURON PROJECTIONS IN THE DEVELOPING BRAZILIAN OPOSSUM

J.J. Swanson*, M.C. Kuehl-Kovarik, and C.D. Jacobson, Department of Veterinary Anatomy and Neuroscience Program, Iowa State University, Ames, Iowa 50011

The Brazilian opossum, *Monodelphis domestica*, is a small marsupial whose young are born in an extremely immature state with a protracted postnatal period of neurogenesis. We have previously shown that facial motoneurons take up peripherally injected cholera toxin subunit B in pups from the day of birth (1 PN) onwards (Swanson et al., Soc. Neurosci. Abs. 21:1039, 1995). These results suggest that the facial motoneuron projections already extend into the region of their target muscles at 1 PN. Facial motoneuron cell bodies were localized near the developing genu at 1 PN and migrated to the facial motor nucleus from 2 to 5 PN. To extend these results we utilized immunohistochemistry for Choline Acetyltransferase-like immunoreactivity (ChAT-IR) to examine developing facial and hypoglossal motoneurons.

Facial and hypoglossal motoneurons and nerves were ChAT-IR positive from the day of birth. At 1PN, the facial nerve, labeled with ChAT-IR, was observed entering the periphery, extending rostrally toward, and branching in its target muscles. Branches of the hypoglossal nerve, labeled at 1 PN with ChAT-IR, were observed throughout the tongue. We localized presynaptic terminals in muscles innervated by facial or hypoglossal motoneurons using synaptotagmin-like immunoreactivity. These data suggest that facial motoneurons already innervate their target muscles when their cell bodies are still migrating to their final destination in the facial motor nucleus.

Sources of support: The Whitehall Foundation and NSF.

489.5

AN ELECTRON MICROSCOPIC STUDY OF DEVELOPING SYNAPSES IN FACIAL AND HYPOGLOSSAL MOTOR NUCLEI OF THE BRAZILIAN OPOSSUM.

T.M. Pepper, J.J. Swanson, M.C. Kuehl-Kovarik, and C.D. Jacobson*, Department of Veterinary Anatomy, Iowa State University, Ames, IA, 50011.

Previously, we have shown the apparent development of synapses utilizing immunohistochemistry (IHC) for synapse-associated proteins in the facial and hypoglossal motor nuclei of the Brazilian opossum (J.J. Swanson et al., J. Comp. Neurol, in press). This study suggests that synaptogenesis is delayed in the facial motor nucleus (FMN) as compared to the hypoglossal motor nucleus (HMN). In the present study we plan to confirm and extend these findings at the electron microscopic (EM) level. We have examined the ultrastructure of the developing FMN from animals 5 to 25 days of postnatal age (PN). The specific nuclei were identified using methylene blue stained 150μm thick vibratome cut sections (H.D. Dellmann et al., Stain Technology 58:6, 1983). The FMN and HMN were then dissected out for processing, guaranteeing that visualization at the EM level would be confined to areas specific to the nuclei of interest. Initial analysis of the FMN indicates a marked increase of detectable synapses between 5 and 15 PN. These findings are in agreement with our previous studies utilizing synapse markers and IHC. Currently, we are studying the HMN for comparison with the FMN. We expect to see more detectable synapses in the HMN than the FMN at the same ages implying that synaptogenesis in the FMN is delayed in the developing Brazilian opossum. Future studies will investigate facial muscle and facial nerve interactions in regards to this delayed pattern of synaptogenesis in the FMN.

Sources of support: The Whitehall Foundation and NSF.

489.6

SEROTONIN IMMUNOREACTIVITY IN THE VENTRAL HORN OF LUMBAR SPINAL CORD FROM NEONATAL MICE. F. Shaheen, D. Reed and M.H. Droge*. Dept. of Biology, Texas Woman's University, Denton, TX. 76204.

The objectives of this study were to charcterize the serotonin immunoreactivity of the lumbar ventral horn of mice at several stages of postnatal development, and to determine whether the medial ventral horn contains more serotonin than the lateral ventral horn. Balb/C mice aged 1 day, 14 days and adult were perfused with 4% paraformaldehyde, transverse frozen sections (20μM) were cut, and three-step immunocytochemistry was performed using anti-5-HT (provided by J. Lauder) at a 1:7000 dilution. The Vectastain ABC System was used with diaminobenzidine. Both negative and positive controls were included in each experiment. Preabsorption controls were also conducted. The 5-HT immunoreaction was quantified based on optical density obtained via image analysis. The tissue from the 14 day old mice showed the most immuoreactivity, and that labeling included many axon terminals with varacosities. While the optical density data did not reveal a difference between the day old versus adult tissue, more terminals appear to exist in the 1 day old. No difference was found between the medial and the lateral ventral horn.

Supported by NIH-MBRS Grant #GM08256.

489.7

CATECHOLAMINE CONTENT OF HUMAN FETAL MESENCEPHALON AND STRIATUM. KS Eagle*, E Kogosov, D Jacques and OV Kopyov. Neuroscience Institute, Good Samaritan Hospital, 637 S Lucas Ave, Los Angeles Ca, 90017

The new treatment of Parkinson's disease (PD), and the potential for treatment of Huntington's disease (HD), with neurotransplantation has raised the question of what levels of catecholamines exist within transplanted fetal tissue. Precursors of the substantia nigra are obtained from the fetal mesencephalon for transplantation in PD patients, while striatal precursors are obtained from fetal lateral ganglionic eminence for HD transplantation. Previous reports have studied catecholamines in the central nervous system of human embryos using immunocytochemical labeling. The present study used a sensitive radioenzymatic assay to visualize catecholamine levels within the striatal precursors of human fetal tissue. Fetal cadavers were obtained with donor consent, in accordance with NIH guidelines. The mesencephalon, lateral ganglionic eminence, and medial ganglionic eminence were isolated and processed for radioenzymatic determination of catecholamines, specifically dopamine, norepinepherine and epinepherine. In mesencephalic tissue, the greatest levels of dopamine were observed at 11 weeks gestational age. Catecholamine contents within the LGE, MGE, and mesencephalon at 10 and 11 weeks gestational age were 8 to 10 times the levels observed in control fetal tissue obtained from medulla and the peduncular area. This study provides further insight into the content of fetal tissue transplants, and aids in the continuing investigation of means to improve the clinical outcome fetal transplantation for neurodegenerative diseases. Future studies will quantify the levels of gamma-aminobutyric acid and acetycholinesterase in these regions of the fetal central nervous system.

489.8

MOTONEURON MATURATION AND PENILE REFLEX ONSET: DEVELOPMENTAL CORRELATES AND ALTERATIONS AFTER SPINAL TRANSECTION. K.A. Burke* and D.R. Sengelaub. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Dendritic and somal development of motoneurons in the spinal nucleus of the bulbocavernosus (SNB) and the dorsolateral nucleus (DLN) in male rats occurs postnatally and is mature by seven weeks of age. During this period, penile reflexes mediated by these motoneurons emerge (Sachs and Meisel, 1979). Reflex onset may reflect motoneuron maturation or developmental reductions in supraspinal inhibition; in fact, spinal transection accelerates penile reflex onset by ten days, a result that has been attributed to the removal of supraspinal inhibition (Meisel and Sachs, 1980). In this study, we examined the maturation of motoneuron morphology in relation to penile reflex onset in normal and spinally transected males.

Motoneuron morphology was examined in intact males and males who received mid-thoracic spinal transections on postnatal (P) day 25. SNB motoneurons were retrogradely labeled with cholera toxin-HRP at P28, P35, or P49, and their morphology was assessed in three dimensions (Eutectic NTS). At P35, when only 20-40% of males express penile reflexes, SNB dendritic length and somal size are approaching adult values; by P49 motoneuron morphology and penile reflex expression are fully mature. At P35, all spinally transected males express penile reflexes; SNB dendritic lengths are normal, but SNB and DLN somata are significantly smaller. Thus, maturation of SNB and DLN motoneurons correlates with penile reflex emergence, and changes in spinal motoneuron morphology (and possible alterations in motoneuron excitability) after transection may be sufficient to account for accelerated reflex onset. Supported by NIH NS24877.

489.9

POLYNEURAL INNERVATION IN THE HUMAN FETUS AND IN THE RAT
A. Gramsbergen*, J.IJkema-Paassen, P.G. Nikkels, M.Hadders-Algra
Medical Physiology and Pathology Depts, University of Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands.

It is well known from animal studies that muscle fibres are innervated polyneurally at early stages. During further development, supernumerary nerve endings are retracted (in the rat, e.g. in the 1st 2 weeks of life) and this leads to mononeural innervation. Also in the human, muscle fibres are polyneurally innervated at early stages. Preliminar results indicate that polyneural innervation in the psoas muscle is replaced by mononeural innervation around the 12th week after term age. No data on this muscle are available in the rat. The problem of the present study is how the regression of polyneural innervation in the human relates to that in the rat. These data will enable to compare neuromuscular maturation in both species.

Material of the human psoas muscle was collected from 15 fetuses and babies until 8 months of term age and in 3 adults. In rats we collected material from the 2nd day after birth (P2) until P25. Motor endplates were stained by a AcCholinesterase and silver staining. Polyneural innervation was considered to occur when the axons could be traced back to the main bifurcating point of the nerve. Numbers of axon endings were counted and sizes of endplates were measured.

Polyneural innervation in the human psoas muscle occurs until the end of the 3rd postnatal month but not thereafter. In the rat, this regression is completed between P18 and P22. The regression of polyneural innervation rats's psoas muscle is retarded compared to that e.g. in the soleus muscle by a few days. Considering that the adult type of postural control in the rat develops between P18 and P20, while in the human, adult movement patterns in the leg and the trunk only start to develop after the 1st year of life, it seems that the regression of polyneural innervation is not related to the development of mature movement patterns but rather to increases in muscle activity.

489.10

DEVELOPMENTAL CHANGES IN SEROTONERGIC MODULATION OF EMBRYONIC CHICK MOTONEURONAL EXCITABILITY IN VITRO.
T. Hayashi, K.D. Phelan, B. Mendelson, R.D. Skinner* and E. Garcia-Rill. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205

We previously reported developmental changes in intrinsic membrane properties and effects of serotonin on embryonic chick lumbosacral motoneurons using intracellular recording in a submerged slice preparation (Soc. Neuro. Abstr. 20:46, 21:1791). In the present study, we provide further characterization of the responses to various serotonergic agonists in antidromically identified E12 and E18 motoneurons. Serotonin (5-HT), 5-carboxamido-tryptamine (5-CT, a 5-HT1 agonist) or α-methyl-5HT (a 5-HT2 agonist) were applied at 50 μM for 60 sec.

Motoneurons from E12 embryos exhibited less prominent inward rectification than E18 neurons. Injection of 1 sec depolarizing current pulses frequently resulted in spike adaptation in E12 neurons, while consistently producing high frequency tonic firing in E18 neurons. The majority of E18 cells also displayed an initial high firing rate prior to reaching steady state. Interestingly, the amplitude of the after-hyperpolarization (AHP), which followed the depolarizing pulses, increased as a function of decreasing time between pulse termination and last spike generation regardless of preceding spike number. This presumably reflects a high calcium buffering capacity in embryonic chick motoneurons. Superfusion of each agonist hyperpolarized all E12 motoneurons (n=7). Restoration of membrane potential to pre-drug levels during the peak hyperpolarization revealed a significant increase (~40%) in firing frequency and a block of spike adaptation. The same agonists produced membrane depolarizations and increased firing frequency in E18 motoneurons (n=16). All three agonists decreased AHP amplitude in E12 and E18 motoneurons. These findings indicate that both 5-HT1 and 5-HT2 receptor subtypes contribute to developmental changes in serotonergic modulation of embryonic chick motoneuron excitability. Supported by USPHS Grant NS 20246.

489.11

PRENATAL SUPPRESSION OF SEROTONERGIC SYSTEM RESULTS IN MOVEMENT DISORDERS AND HYPOPLASIA OF EXTENSOR MOTONEURONS IN NEWBORN RATS. K. Nakajima, K. Matsuyama and S. Mori*. Lab. of Neurobiology, National Institute for Physiological Sciences, Myodaiji, Okazaki, 444 Japan.

With the use of para-chlorophenylalanine (PCPA), it was possible to make newborn rats in which serotonergic (5-HT) innervations of brainstem and spinal cord were prenatally reduced. To make such model animals, PCPA (300 mg/kg) was intraperitoneally administered to the Sprague-Dawley mother rats beginning on day 8 of gestation followed by a daily administration of 80 mg/kg until the delivery of pups. In the pups born from the PCPA-administered mother, postnatal development of locomotor and swimming movements was delayed in comparison to that in the normal pups born from control mother. Interlimb coordination during swimming movements was disturbed from PND 1 to PND 8. (Nakajima et al., 1995). In this study, postnatal development of fore- and hindlimb extensor motoneurons and 5-HT innervation of the spinal cord was studied. Triceps brachii (TB) and quadriceps femoris (QF) motoneurons (MNs) were retrogradely labeled by intramuscular injections of CTb-HRP (0.4%) solution in the PCPA-treated and control pups at PNDs 1, 6, 14 and 22. 5-HT fibers and terminal varicosities in cervical and lumber enlargements were also assessed by utilizing 5-HT immunohistochemistry. In the PCPA-treated pups at PNDs 1 and 6, the cell body areas of TB- and QF-MNs were smaller than those in the control pups and their dendrites were poorly developed in comparison to those in the control pups. 5-HT immunoreactive fibers and terminal varicosities were poorly distributed in the motoneuron pools in comparison to those in the control pups. These results suggest that 5-HT system contributes to the pre- and postnatal development of motoneurons in the rhythm generating spinal neuronal circuitries.

489.12

THE EFFECTS OF AGEING ON CREATINE KINASE SPECIFIC ACTIVITY IN RAT DIAPHRAGM, EXTENSOR DIGITORUM LONGUS AND SOLEUS MUSCLES. O.C. Ramírez* and E. Jiménez. Dept. of Biochemistry, Ctr. Invest. Estud. Avanzados, I.P.N. México, D.F. 07000.

Evidences obtained from aged rat diaphragm suggest that, contrary to fast and slow muscles, the maintenance of choline acetyltransferase (ChAc) activity in the motor nerve terminals is due to continuous activity of this muscle and its motor nerves. It is known that creatine kinase (CK) specific activity (S.A.) is stimulated by muscle contractile activity but depressed by inactivity. Here, it was intended to demonstrate that in male rat diaphragm, fast-extensor digitorum longus (EDL) and slow-soleus muscles, the CK S.A. could follow a pattern similar to that of ChAc. It was found that, by means of the use of muscle post-mitochondrial supernatants (27Kxg), and the NADH-coupled reactions technique for CK assay, the CK activity pattern is not similar to that shown by ChAc in EDL, soleus and diaphragm from puberty to senescence. In contrast to ChAc, CK activity from EDL and soleus did not change significantly from adulthood to senescence, whereas rat senescent diaphragm was 86% more CK-dependent than those muscles at the same age. This seems to be due to differences in the metabolic pathways in which these enzymes are involved -- within the same tissues. Worthy of mention is the fact - that, regarding the PC/CK shuttle system, male rat diaphragm showed a transition pattern from the aerobic type to the anaerobic one during the studied ages.

(Departamental support)

REGENERATION: INFLUENCE OF SUBSTRATE

490.1

REGENERATION THROUGH LONG ACELLULAR NERVE SEGMENTS DEPENDS ON NEOVASCULARISATION AND CELLULAR SUPPORT FROM THE ENVIRONMENT.
J.Sørensen, K. Fugleholm.*, H. Schmalbruch., C. Krarup. Dept. of Neurophysiology, Institute of Medical Physiology, University of Copenhagen and Dept. of Clinical Neurophysiology, Rigshospitalet, Denmark.

Axons ensheathed by an impermeable silicone tube do not regenerate through a 40-mm acellular nerve segment distal to a crush lesion. We have investigated interactions between the near-nerve environment and the acellular nerve segment during axonal regeneration. Nerve de- and regeneration were assessed electrophysiologically by implanted silicone tube electrodes, and by light and electron microscopy. The silicone tubes were made permeable to allow diffusion of solutes (pore size 0.45 μm) or migration of cells (pore size 2.0 mm). In the 0.45-μm experiment, where only oxygen and solutes could enter the nerve from the environment, the central area of the acellular segment contained only dead axons without further degeneration, and no capillaries or macrophages were present. On the contrary, in the 2,0-mm experiment, where macrophage migration and capillary ingrowth from the near-nerve environment was possible, the myelin had been removed, and regenerating nerve fibers and sprouts traversed the acellular segment in less than 2 months. Axonal growth slowed down in the central part of the segment, but regained speed distally.

The study demonstrates that longitudinaly oriented vascular and cellular support from the proximal and distal stumps are not able to secure nerve regeneration in long acellular segments. Nerve repair by long cryotreated nerve grafts requires transversally oriented vascular and cellular support from the near-nerve environment.

Sources of support: The Danish Medical Research Council, The Danish Research Academy and The Novo Foundation.

490.2

INFLAMMATORY CYTOKINES INTERACT TO MODULATE EXTRACELLULAR MATRIX PRODUCTION BY ASTROCYTES.
N.A. Di Prospero, S. Meiners, G.M. Krauthamer*, and H.M. Geller. Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Elevated levels of cytokines are found after traumatic injury and pathological alterations in the central nervous system (CNS) and are believed to contribute to the process of gliosis. Gliotic astrocytes may form a barrier to regenerating axons by either physically obstructing growth or through the generation of a growth inhibitory environment. These studies sought to examine how cytokines present after CNS injury, in particular interferon-gamma (IFNγ) and basic fibroblast growth factor (bFGF), affect the biochemical nature of the extracellular matrix (ECM) produced by astrocytes. Our laboratory has previously shown that bFGF increases the levels of the ECM molecule tenascin in cortical astrocytes over time. Purified cortical astrocyte cultures from postnatal day 1 rat pups were subcultured into culture wells. Upon reaching confluency, the astrocyte monolayers were exposed to varying concentrations of IFNγ, bFGF, or combinations of both in DMEM + 10% FCS culture media for 8 days. Protein extracts from the cells were examined by Western blotting for laminin, fibronectin, and tenascin. A dose dependent reduction in all three ECM molecules was observed with increasing concentrations of IFNγ. Similarly, IFNγ was able to antagonize the effects of bFGF by reducing tenascin levels in cultures treated with both cytokines. These data suggest that the relative types and levels of different cytokines found after injury may dictate the biochemical nature of the glial scar by altering ECM levels. Supported by NIH R01 NS-24168

490.3

SCHWANN CELLS AND FETAL TECTAL TISSUE CO-GRAFTED TO THE LESIONED OPTIC TRACT OF JUVENILE RATS: INFLUENCE ON THE REGROWTH OF RETINAL GANGLION CELL AXONS.

G.W.Plant* and A.R. Harvey. Department of Anatomy and Human Biology, University of Western Australia, WA 6907.(*Present address, The Miami Project, University of Miami.)

We have previously shown that Schwann cells (Sc's) can effect the ability of retinal ganglion cells (RGC's) to recognise their appropriate target regions in Schwann cell/fetal tectal co-grafts when transplanted to the midbrain of newborn rats (Harvey and Plant (1995), Exp Neurol:134, 179-191). We have now tested these co-grafts transplanted to 20-21 day old rats after lesions to the optic tract. This was carried out to ascertain whether older RGC axons are effected by transplanted Sc's in the same way as developing RGC axons. Control and co-grafts were analysed 19-96 days after transplantation using anterograde tracing (WGA/HRP) and enzyme histochemistry (AChE). In control (pure) tectal grafts retinal axons usually were confined to the graft surface and innervation usually selective for AChE-dense patches in grafts-that represent target sites for retinal axons. Some scatterred retinal axons were seen but few in number and not very far in distance (150μm). In contrast co-grafted (with Sc's) rats showed dense retinal growth dispersed within the co-graft neuropil away from AChE patches on the graft surfaces and associated with Hoechst 33342 labelled Sc's. RGC axons grew for distances up to 500μm from the graft surface. However, some rats showed retinal axons confined to AChE patches even though Sc's were in close proximity. It appears, as in earlier work in newborn rats that Sc's can alter the way retinal axons grow into grafted tissue. RGC axons were more scattered and occupied non-target regions of tectal grafts when transplanted to older rats. Interestingly the Sc's appear to have a similar effect whether transplanted to the target (neonatal superior colliculus) or as a bridge between the dorsal lateral geniculate nucleus and superior colliculus. Work supported by the NHMRC, Australia.

490.4

SCHWANN CELLS CULTURED IN SERUM-FREE (7F) MEDIUM: MYELINATION STUDIES IN VITRO AND IN VIVO.

M. Oudega*, C. Fernandez-Valle, P. Wood, A. Gomez, A. Weber, M. Bates, M. B. Bunge The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL 33136

Autologous rat Schwann cells (SCs) promote axonal growth and myelinate regenerating fibers in the damaged nervous system. A fast procuration of SCs would serve surgical interventions aimed at improving repair of CNS and PNS injury. SC proliferation is increased ≈5-fold by removing serum and adding heregulin (7F) to the standard medium containing 10% serum, forskolin and pituitary extract (D10/M). The differentiative abilities of 7F-SCs, however, have not been determined. SCs grown in 7F or D10/M for 3-4 passages were either co-cultured with sensory neurons in myelination-permissive medium or placed within a polymer guidance channel and implanted into adult rat spinal cord after removal of T8-11 segments. The amount of myelin produced was analyzed by Sudan Black staining, toluidine blue staining, and electron microscopy. Onset of myelination in vitro was delayed by 1 week compared with D10/M-SCs and total myelin was reduced by ≈50% of control levels at four weeks. In vivo, ≈500 myelinated fibers were observed by light microscopy in grafts with D10/M-SCs 4 weeks after implantation, whereas in 7F-SCs grafts fewer than 10 myelinated fibers were observed. Electron microscopic examination of 7F-SC grafts revealed many additional thinly myelinated axons not resolved by light microscopy. Six weeks after implantation, however, the grafts of 7F-SCs had similar numbers of myelinated axons as found in D10/M-SC grafts. The results indicate that although SC proliferation is significantly enhanced by growth in serum-free (7F) medium, differentiation is delayed both in vitro and in vivo. The possibility that long term growth of SCs in 7F medium is associated with loss of differentiative function is being examined. Supported by NIH NS28059 and NS09923 and The Miami Project. Channels were provided by P. Aebischer. MO is a Daniel Heumann International Scholar.

490.5

SCHWANN CELL MIGRATION AND ANTIGENIC PROFILE IN AN INJURED PERIPHERAL NERVE *IN VIVO*; DEPENDENCE ON AXONS

M. Bresjanac[1]*, M. Popović[2] and J. Sketeli[1] Institute of Pathophysiology[1] and Institute of Pathology[2], School of Medicine, 1000 Ljubljana, Slovenia.

It is widely believed that peripheral axons require the trophic and guiding influence of Schwann cells (SC) in order to regenerate successfully. Nonetheless, our earlier work, employing a crushed sciatic nerve of a rat, demonstrated a rapid elongation of "naked" axons through acellular nerve segments (1, 2). SC, whose migration probably depends on proliferation, followed the axons rather than leading them. Interestingly, little is known on SC behavior in an injured nerve in the absence of axons, although such information would clearly aid our understanding of their role during nerve regeneration. This study tested the hypothesis that SC rapidly divide and migrate to repopulate an acellular segment of a lesioned nerve even when unaccompanied by axons. Immunohistochemistry to S100, NGFrec, GFAP and NF was employed. Young male Wistar rats were used in accord with NIH Guidelines. In deep anesthesia, sciatic nerve was crushed and a segment distal to the crush was rendered acellular by freeze-thawing (FT). The nerve was cut 3 mm proximal to the crush and at the distal end of the FT segment. The rats were sacrificed 4 and 8 weeks later. At 4 wk, S100 and NGFrec ⊕ cells covered an average 13 mm, i.e., roughly 60% of the average length of the nerve segment. GFAP ⊕ cells were not found further than 9 mm distal to the crush, i.e., 41% of the nerve length. At 8 wk, S100 and NGFrec ⊕ cells were 17 mm distal to the crush. GFAP ⊕ cells were 7 mm behind them, i.e. 68% and 40% of the average nerve length, respectively. In control nerves, where the axons grew ahead of SC, at 4 and 8 wk, S100 and NGFrec ⊕ cells arrived as far as the axons, i.e. to the end of the nerve segment. SC seem to require axonal presence for optimal migration inside an acellular nerve, where they follow the rapidly elongating axons through the old basal lamina tubes.

Supported by a Slovene MST Research Grant.

(1) *J Neurosci Res 24: 153-162 (1989)* (2) *J Neurosci Res 24: 501-507 (1989)*

490.6

ROLE OF GLIAL DESMOSOMES IN THE REGENERATION OF THE LAMPREY SPINAL CORD

D. S. Pijak, G. P. Swain* and M. E. Selzer. Dept. of Neurology and the David Mahoney Inst. of Neurol. Sce., Univ. of Pa. Med. Ctr., Philadelphia, PA 19104-4283.

Regenerating axons of the lamprey spinal cord can navigate through a lesion in their correct orientations. Their growth cones are contacted mainly by glial processes, suggesting a role for the glial cells in guiding the regeneration. Lamprey astrocytes contain keratin intermediate filaments and are interconnected by desmosomes. This is characteristic of animals that show CNS regeneration. We have hypothesized that the desmosomes play an important role in regeneration by anchoring glial cells adjacent to the lesion in place, allowing thickened, longitudinally oriented glial processes to enter into the lesion. Regeneratng axons can then grow across the lesion along this glial "bridge". To test this hypothesis, transected spinal cords were first bathed in lamprey Ringer with 5mM EGTA added to break the calcium-dependent bonding between hemidesmosomes. In some animals, a mammalian anti-desmosomal antibody (DK80.20, Sigma) was also applied to prevent reannealing. Animals were then allowed to recover for 0 to 60 days. Immunohistochemical methods were used to label the glial processes and regenerating axons. In control animals, glial processes have begun to enter into the lesion by 7 days, with the fastest growing axons entering after 10 days post-transection. Animals treated with EGTA and DK80.20, had a delay in regeneration of approximately one week. These animals showed unusual glial migration into the lesion and many misoriented glial processes. Regenerating axons had trajectories that were more tortuous than those of controls treated with lamprey Ringer alone or animals treated with EGTA/Ringer alone or EGTA/Ringer and a nonspecific IgG. Thus desmosomal interconnections among glial cells may contribute to functional regeneration in certain primitive vertebrates. Supported by NIH Grant R01 NS25581 and NSF Grant IBN-9319702.

490.7

CHARACTERISTICS OF REGENERATING OPTIC AXONS AND THEIR GLIAL ENVIRONMENT SHORTLY AFTER NEONATAL TRANSECTION IN HAMSTERS.

Changying Ling* and Gerald E. Schneider. Dept. of Brain & Cognitive Sciences, M. I. T., Cambridge, MA 02139.

Location and morphological features of regenerating retinofugal axons shortly after transection of the brachium of the superior colliculus (SC) in hamsters on the day after birth (P1) were examined using cholera toxin subunit B (CT-B) as an anterograde tracer. Accompanying changes in their immediate glial environment were found using GFAP immunocytochemistry. In control P1 hamsters, elongating retinal axons were observed through the length of SC, where the majority of their growth cones were oriented caudally and located primarily in the caudal portion. 20h after transection, the CT-B labeled axons were restricted to the region rostral to the cut. Many of them ended with growth cone-like expansions that were aimed caudally; they were located 30-60 microns from the lesion, indicating an initial retraction. At the same time, the density of GFAP positive cells, with an immature morphology, was significantly increased, particularly in regions near the lesion. 48h later, a vigorous regrowth of retinal axons was evident; the majority of them, with large growth cones, had turned ventrally and extended into the deep layers of SC. Only a few axons, if any, were seen directly entering or penetrating the lesion site, a scar-like formation containing dense GFAP+ cells which extended into the region ventral to the lesion with decreasing density. After avoiding the dense part of the scar, axons turned dorsocaudally (within 72h), and regrew into the superficial layers of SC, their original target (within 96h). In their course caudally, the axons also showed some collateralization. Two months later, the superficial SC had been filled by dense retinal axons; the glial scar, defined by an aggregation of GFAP+ cells, remained but it had became much smaller and denser. Support: NIH grants EY 00126, EY 02621.

490.8

A TISSUE CULTURE MODEL OF REACTIVE GLIOSIS - THE EFFECTS OF ADULT REACTIVE ASTROCYTES ON AXON REGENERATION.

N. Orike[1], L. Kerecuk[1], A. Butt[2], and J. Cohen[1] SPON: Brain Research Association. Departments of [1]Developmental Neurobiology and [2]Physiology, U.M.D.S., London, SE1 9RT, UK.

Reactive astrocytes which arise as a result of injuries to the mammalian central nervous system have been implicated in the failure of axon regeneration. To characterise the phenotype and properties of adult reactive astrocytes, we have modified a tissue culture model system (Trimmer, 1993), in which explants of optic nerve from enucleated adult rats (3-4 weeks post lesion) were cultured on a laminin substratum for periods up to 28 days. After 4 - 5days in vitro, a variety of cell types, identified by immunostainning with neural cell-specific markers, were observed to migrate out of the explants. The major cell types included GFAP+ve astrocytes (~ 50%), both GD3+ve and -ve, 'reactive' oligodendrocytes (~ 30%; RIP+ve/galC+ve), macrophages and fibroblasts. Most of the astrocytes from the lesioned optic nerve explants displayed markedly increased GFAP immunoreactivity (characteristic of reactive astrocytes), and the majority (~ 80%) were also GAP-43 +ve. Additionally, when established optic nerve cultures were cocultured with purified neonatal rat retinal ganglion cells (RGCs), reactive astrocytes from lesioned optic nerve were found to be both poorly adhesive (< 50% of controls) and inhibitory for RGC neurite outgrowth (> 60% shorter neurites) compared with RGCs growing on astrocytes from either neonatal or unlesioned adult optic nerve. This inhibition appeared to be contact mediated, presumably by cell surface molecules associated with the reactive astrocytes, and is likely to contribute significantly to the failure of axon regeneration in the injured adult CNS. (Supported by the Special Trustees of Guy's Hospital and the Wolfson Foundation).

490.9

AXONAL REGENERATION FROM RESPIRATORY NEURONS THROUGH PERIPHERAL NERVE GRAFTS. N. Lammari-Barreault, R. Pallini, L. Lauretti, A. Granato (°), P. Gauthier (ç), E. Fernandez *. Dept. of Neurosurgery and Anatomy (°), Catholic University, Rome, Italy; Dept. de Physiologie et Neurophysiologie (ç), Faculté de St Jerome, Marseille, France.

In adult rats, central respiratory neurons can regenerate their axons into peripheral nerve (PN) grafts implanted into the medullary respiratory structures (Lammari-Barreault et al, Exp Brain Res 98:238-244,1993). After axonal regeneration into the graft, central respiratory neurons exhibit normal electrophysiological activity and reactivity, and can establish functional connections with phrenic motoneurons or muscolar targets. The long-term preservation of the physiological properties of the respiratory neurons depends on the establishment of functional connections. In the present work, the anatomical distribution of the neurons that projected their regenerating axons into blind-ended PN grafts was investigated by using both single and double retrograde axonal tracers. Horseradish peroxidase (Sigma VI, 8% solution, 0.2-0.5 ul) was injected into the graft. Labeled neurons were distributed throughout the brainstem up to C2 spinal level mainly ipsilaterally to the implanted graft. The highest density of labeled neurons was found in the nucleus ambiguus, nucleus solitarius, area of the lateral reticulae, vestibular spinal nucleus, locus coeruleus. After injection of the fluorescent tracer Fast Blue (Sigma, 2% solution, 0.8 ul) into the graft and Diamidino Yellow (Sigma, 2% solution, 0.2 ul) into the C2 spinal level, only single labeled neurons were seen in the brainstem. This finding indicates that axonal regeneration by respiratory neurons into the graft results from the regrowth of severed axons rather than from the collateral sprouting of respiratory efferent pathways.

This paper was partly supported by MPI

490.10

MATURING OLIGODENDROCYTES INHIBIT NEURONAL GROWTH CONE MOTILITY IN DIFFERENT WAYS. S.J. Moorman* and R.M. Gould[1], Dept. of Anat. and Cell Bio. UNT Health Science Center at Fort Worth, TX 76107; [1]NYS Inst. for Basic Res. in Devel. Dis., Staten Island, NY 10314

Among the many molecules produced by oligodendrocytes are proteins that might limit regeneration by inhibiting neuronal growth cone motility. Maturing oligodendrocytes can be divided into 5 specific stages from O2A (Stage 1) precursors to mature (Stage 5) myelinating cells based on stage-specific antigen expression and characteristic morphology. Each Stage has a predictable array of surface molecules which, for certain stages, includes inhibitory molecules. We tested whether growth cones respond to contact with each stage of maturing oligodendrocytes the way they respond to contact with the purified oligodendrocyte-specific inhibitory proteins known to be expressed at specific stages.

Cells from 3-day-old neonatal rats were grown on poly-lysine for 3 to 7 days. Oligodendrocytes were physically removed from the substratum using a patch-clamp electrode and a micromanipulator. Each 'manipulated' oligodendrocyte was then placed into contact with a neuronal growth cone for 30 minutes. Growth cone surface area was compared before and after contact with an oligodendrocyte. Surface area decrease of 50% or more = collapse.

As oligodendrocytes matured from Stage 1 to Stage 4, they developed an increasing ability to cause neuronal growth cones to collapse, peaking at 95% for Stage 4. This increasing ability to inhibit the neuronal growth cone coincides with the onset of expression of NI35 by Stage 2 oligodendrocytes. Stage 5 oligodendrocytes only induced 15% of neuronal growth cones to collapse on contact. This result is surprising considering NI35 and MAG are both expressed by Stage 5 oligodendrocytes. These results suggest that growth cone response to contact depended on the developmental state of the oligodendrocyte, independent of the presence of inhibitory molecules. Therefore, growth cone response is likely to be dependent on the context in which inhibitory molecules are presented.

Supported by the American Paralysis Association.

490.11

EFFECTS OF MICROGLIA ON THE REGENERATION OF SENSORY AXONS. C.M.F. Prewitt*, W. Chen, C.J.M. Kane and J.D. Houlé. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Neurotrophic factors bound to nitrocellulose implants can promote regeneration from injured dorsal roots. To investigate the role of non-neuronal cells associated with the implant on regeneration of injured sensory axons, activated microglial cells were seeded onto nitrocellulose membrane prior to implantation into upper quadrant lesions of adult rat spinal cords (L1 level). Fetal spinal cord tissue was transplanted on each side of the membrane and cut dorsal roots were apposed to each side of the nitrocellulose membrane; one side was coated with microglia and the other uncoated side was used as control. Animals with untreated nitrocellulose membranes were a second control. Twenty-one days later, animals were sacrificed and spinal cord sections were processed for immunohistochemical detection of calcitonin gene-related peptide (CGRP) to identify the pattern of growth of regenerated sensory axons. Results showed regeneration of sensory axons into the fetal tissue on both sides of the nitrocellulose membrane implant. However, the number of axons and the extent of axonal growth was greater when associated with microglial cell-coated nitrocellulose than the control side or untreated nitrocellulose implants. Regeneration was mainly directed along the membrane and most axons grew to the ventral edge of the membrane when microglia were present. These results indicate that microglial cells can promote regeneration of sensory axons in the abscence of exogenous trophic factors. Release of cytokines from the implanted microglial cells may be involved in the increased regenerative response.

Supported by NIH Grant NS26380.

490.12

EFFECT OF A PRE-DEGENERATED PERIPHERAL NERVE GRAFT AND A CONDITIONING LESION OF OPTIC NERVE ON AXONAL REGENERATION OF RETINAL GANGLION CELLS. S.-W. You[1], K.-F. So[1,*], H.K. Yip[1] and K.S. Bedi[2]. [1]Neuroregeneration Lab. and Developmental Neurobiology Lab., Dept. of Anatomy, The Univ. of Hong Kong, Hong Kong; [2]Dept. of Anatomical Science, The Univ. of Queensland, Australia.

A conditioning lesion performed 1 to 2 weeks before a second lesion has been shown to enhance axonal regeneration in peripheral nerves (PN) but not in optic nerves (ON) in mammals. The lack of a conditioning lesion effect in ON may be due to too long a survival time before the second lesion is inflicted since axons of retinal ganglion cells (RGCs) in rodents degenerate rapidly 7 to 14 days following axotomy. In the present study, we have shortened the survival time (1-8 days) to examine if this has any effect on axonal regeneration of RGCs into a PN graft apposed to the ON transected 1 mm from the optic disc in hamsters. The results showed no beneficial effect since the number of RGCs regenerating their axons into the PN graft 4 weeks after grafting in these animals decreased significantly (Mean=758) when compared to those animals without a conditioning lesion (Mean=1644). Other studies have shown that pre-degenerated PN grafts can enhance axonal regeneration both in the PNS and in the CNS. Thus the effect of pre-degenerated (8 days) PN grafts on axonal regeneration of the RGCs which have had a conditioning lesion (1-8 days) were examined. The number of regenerating RGCs in the groups with a pre-degenerated PN graft (n=24, mean=1290) was significantly more ($P<0.01$) than that in the groups with a normal PN graft (n=24, mean=758). These data suggest that a conditioning lesion of the optic nerve in adult hamsters resulted in a deteriorative effect on the number of RGCs regenerating axons into a PN graft but this deteriorative effect can be overcome to a certain extent when a pre-degenerated PN graft was used.

Supported by research grants from The University of Hong Kong.

490.13

SCIATIC NERVE TRANSPLANTATION INTO NORMAL AND GLIA-DEPLETED SPINAL CORDS M.B. Durgun[1], S.A. Gilmore[2] and T.J. Sims*[2], Depts. of Anatomy, [1]Hacettepe Univ., Faculty of Medicine, Ankara, Turkey, and [2]Univ. of Arkansas for Medical Sciences, Little Rock, AR. 72205

Schwann cells exert strong trophic influences on regenerating axons. Thus, many investigators have used peripheral nerves as bridges to guide regrowing CNS axons. Regenerating spinal cord axons enter and grow robustly within a grafted segment of peripheral nerve, but fail to extend through the host-graft interface in significant numbers when they reach the distal end of the graft. Although efforts have been made to enhance this regrowth into the host, surprisingly little is known about the interface that develops between host spinal cord and transplanted peripheral nerve. This study examines these interfaces in two different host-graft situations. In one situation 3-day-old fresh or frozen sciatic nerves were transplanted into the lumbar spinal cords of 23-day-old normal rats. The second situation was similar except that the glial populations within the host cords were markedly depleted by exposure to irradiation when 3 days of age (Gilmore S.A. 1963 J. Neuropath. Exp. Neurol. 22:294-301). Following a 20-day postoperative period, the interfaces between host spinal cord and sciatic nerves were examined ultrastructurally and pronounced differences were noted. A distinct astrocyte scar composed of multiple processes completely enveloped the transplanted nerve in non-irradiated host spinal cord and confined Schwann cells and fibroblasts to the area enclosed by the scar. In contrast, a complete astrocytic scar did not form around the transplanted nerve in the irradiated hosts, and Schwann cells migrated from the graft and intermingled with host tissue. Fibroblasts remained in close proximity to the graft. From these observations it seems likely that the formation of a distinct astrocyte barrier in the host must be ameliorated if the transplantation of peripheral nerves is to become a useful strategy in spinal cord repair.

Supported by NIH grant NS 04761.

490.14

REGENERATION OF MOTONEURON AXONS INTO PERIPHERAL NERVE GRAFTS INSERTED INTO THE SPINAL CORD AT VARIOUS TIMES AFTER C5 & C6 ROOT AVULSIONS IN THE ADULT RAT. T.J. Zwimpfer*, C. Tarazi, J.D. Steeves. Depts. of Surgery, Zoology & Anatomy, U.B.C., Vancouver, B.C., V6T 1Z4.

It has been demonstrated in rats, cats and primates that following spinal nerve root avulsion, injured motoneurons can extend axons into ventral roots or peripheral nerve (PN) grafts immediately inserted into the cord and result in functional reinnervation of denervated muscles. Delayed reimplantation in human cases of root avulsion has recently been reported. Due to the death of motoneurons following root avulsion, such a delay in reimplantation (ie. weeks to months after root avulsion) could result in less axonal regeneration and recovery but this has not been studied.

Following avulsion of the left C5 & C6 ventral and dorsal roots in adult rats, one end of a 3.5 cm segment of common peroneal nerve was inserted 1 to 2 mm into the ventrolateral aspect of the spinal cord at C5 & C6. Animals were divided into four groups according to the time interval between root avulsion and PN graft insertion: 1) immediate insertion, 2) 1 week, 3) 1 month , 4) 2 months. Two months after insertion, a fluorescent axonal tracer, Rhodamine-Dextran amine (RDA) was applied to the free end of the graft and RDA-labeled neurons in the ipsilateral ventral horn were counted.

Of 39 animals examined, 38 had RDA-labeled neurons. The majority of neurons were in the ipsilateral ventral horn but were also seen in the contralateral ventral horn, and the ipsilateral dorsal horn and Clarke's nucleus. Using the cell counts in the immediate insertion group (n=11animals; mean of 761 cells = 100%) as a reference for comparison, the number of RDA-labeled neurons in the 1 week group was 82% (n=10), 44% at 1 month (n=9) and 38% at 2 months (n=9). This suggests that reimplantation of PN grafts into the spinal cord of patients with root avulsions, should be done within a few weeks of injury. In future studies, the PN graft will be anastomosed to the musculocutaneous nerve to allow electrophysiological and functional studies of biceps muscle reinnervation.

Supported by the B.C. Health Research Foundation & the Vancouver Hospital.

490.15

PROTEOGLYCAN UPREGULATION FOLLOWING SPINAL CORD INJURY IS CLOSELY ASSOCIATED WITH ACTIVATED MICROGLIA / MACROPHAGES. M.T. Fitch[1], E. Theriault[2], S. Mortin-Toth[2], J. Silver[1]*. [1]Dept. of Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106 and [2]Playfair Neuroscience Unit, University of Toronto, Ontario, Canada.

The cellular responses to spinal cord injury include the production of molecules capable of modulating wound healing in the CNS. This study examines the upregulation of chondroitin sulfate proteoglycan (CSPG), a molecule present in the wound healing matrix that may inhibit axon regeneration, in several models of rat spinal injury. It is uncertain why some CNS lesions result in increases in CSPG immunostaining, while other models induce gliotic reactions without observable changes in CSPG levels in the CNS. We have determined that careful dorsal root crush does not lead to detectable increases in CSPG in the dorsal columns, while upregulation of CSPG following spinal cord contusion or penetrating injury occurs within 24 hours and persists for at least 14 days. This increase in CSPG is localized to the tissue within and adjacent to the wound focus. Interestingly, CSPG increases are consistently associated with the influx of macrophages across the blood brain barrier and/or activation of microglia. CSPG localizes with macrophage presence in the lesion cavity, within the tissue adjacent to the wound, and at the interface of cavity and surrounding gliotic astrocytes. The glial dome of the dorsal root entry zone provides an important model in which to examine the role of macrophages in CSPG associated gliosis, as variable macrophage presence in the dome of injured roots correlates precisely with increases in CSPG in this defined area. These observations suggest that macrophages, their products, or other serum components that cross a compromised blood brain barrier may provide a stimulus for changes in proteoglycans after nervous system injury. (Supported by NIH NS-25713)

490.16

INTERFERON-GAMMA-INDUCED RECRUITMENT OF MACROPHAGES INTO THE ADULT MOUSE SPINAL CORD. Shalina S. Ousman* and Samuel David. Centre for Research in Neuroscience, MGH Research Institute, and McGill University, 1650 Cedar Ave., Montreal, Quebec, H3G 1A4.

The failure of long distance axon regeneration in the adult mammalian CNS may be due, in part, to the slow removal of myelin and its associated axon growth inhibitory molecules. Slow myelin clearance results from the delayed influx of macrophages and microglial activation. Interferon-gamma (INF-γ) has been shown to promote macrophage recruitment and extravasation into the rat basal ganglia. To determine the effect of IFN-γ on macrophage recruitment and influx into the white matter, we injected IFN-γ (400 units in 1μl) into the adult mouse dorsal columns. Mac-1 and F4/80 immunohistochemistry was used to detect peripheral macrophages and microglia at 6h, 12h, 1d, 2d, and 4d after injection of INF-γ. The values given below represent the number of Mac-1$^+$ cells /10mm^2. In the needle tract, round Mac-1$^+$ cells were present as early as 6h after IFN-γ injection, increased at 12h (24.9 ± 4.7), and were not seen at 4d. in the white matter (0-220μm adjacent to the injection site), round Mac-1$^+$ cells were also seen at 12h (5.4 ± 3.4), and 1d (2.9 ± 0.2), but decreased to 1.1 ± 0.7 by 4d. In contrast, process-bearing Mac-1$^+$ cells in the parenchyma of the white matter were first detected at 1d, reached maximal levels at 2d (1.8 ± 0.3) and decreased 2-fold by 4d (0.9 ± 0.3). Animals injected with vehicle alone showed much lower numbers of Mac-1$^+$ cells in the needle tract and adjacent white matter. Therefore, in the white matter, round Mac-1$^+$ cells were seen early (6h and 12h) after IFN- γ injection, and were replaced by process-bearing Mac-1$^+$ cells at later time points (2d and 4d). Similar results were also observed for the grey matter, and F4/80 labelled sections. These results suggest that IFN-γ is capable of recruiting peripheral macrophages to the site of injection and the adjacent white matter, as well as, activating microglia. Supported by a grant from the MRC.

490.17

MOLECULAR ANALYSIS OF DIFFERENTIALLY REACTIVE ASTROCYTES J.R. Bethea*#, D. Canning and J. Silver. The Miami Project#, Univ. of Miami, Miami, FLA and Dept. of Neurosci., CWRU Sch. of Med. Clev., OH

Lack of regeneration is due, in part, to an inhibitory environment created by activated astrocytes and/or the infiltration of non-neuronal cells, a process known as gliosis or gliotic scaring. Functionally, astrogliosis can associated with variable biological outcomes. For example, following injury to the CNS complete regenerative failure can result or in some instances there is relatively robust synaptic sprouting.The factors that create these functional differences are poorly understood but are probably mediated, in part, by differential gene expression in reactive astrocytes. To investigate the molecular events in activated astrocytes that constitute an inhibitory versus a growth supportive environment for neurons, an *in vitro* model of astrogliosis that utilizes cortical astroglia on substrate bound 25-35 beta-amyloid (growth inhibitory) in comparison with astrocytes on laminin (growth permissive) was employed. To investigate the genetic differences that distinguish these two models we are utilizing RAP-PCR to identify gene products that are differentially expressed under growth supporting and growth inhibitory conditions. To date, we have identified multiple new gene products that are specifically expressed under growth inhibitory conditions. *In situ* hybridization studies demonstrated that these transcripts are specifically expressed in our *in vitro* model of inhibitory astrogliosis. These studies will allow us to better understand the cellular and molecular events that regulate different forms of reactive astrogliosis and help lead to a better understanding of the mechanisms that cause regenerative failure associated with CNS injury.
Support MS grant FA1148 -A-1

490.18

TANYCYTES SUPPORT AXONAL REGENERATION IN THE CENTRAL NERVOUS SYSTEM OF ADULT RAT. N. Chauvet, M. Prieto, A. Privat* and G. Alonso. INSERM U336, University Montpellier II, 34000 Montpellier, France.

We have recently shown that tanycytes, a particular type of glial cells of the mediobasal hypothalamus which presents morphological and biochemical similarities with radial glial cells, constitute a preferential support for the regeneration of lesioned neurohypophysial axons. The present study was designed to explore the capacity of tanycytes to support axonal outgrowth of various neurons types.

In a first step, the neurotrophic rôle of tanycytes was investigated in vitro. Tanycytes derived from the median eminence or astrocytes derived from the cerebral cortex of 10-day-old rats were cultured for 4 to 7 weeks. Cells obtained from the dissociation of 3-day-old rat mesencephalon, cortex and hypothalamus were cocultured on these glial monolayers, and the number of surviving neurons and their neurite length were quantified after 4 and 8 days. Our data showed that, when compared with astrocytes, tanycytes greatly improved both survival (6- to 10-fold higher) and neurite outgrowth (2- to 5-fold longer) of cocultured neurons whatever their origin. Conditioning medium experiments showed that diffusible factors from median eminence glial cells slightly increased survival (1.7-fold higher) of cocultured neurons, but had no significant effect on neurite outgrowth. These observations indicate (i) that aged tanycytes have the capacity to support survival and neurite outgrowth for a variety of postnatal neurons and (ii) that this effect is not exerted by secreted molecules. In a second step, the axonal outgrowth-promoting properties of tanycytes were evaluated by transplanting ME tanycytes or cortex astrocytes in extra-hypothalamic regions including the mesencephalon and the spinal cord. In both sites, tanycytes transplants were found to be preferentially penetrated by numerous regenerating sprouts of various axons types. All together these data indicate that, in the adult rat CNS, tanycytes provide a supportive substrate to regenerating outgrowth of lesioned axons. Grafts of tanycytes thus appears as a promising tool for promoting regeneration in the adult CNS. (Supported by IRME and Inversiones Cavdeca, C.A.)

490.19

Axonal Regeneration into Human Schwann Cell Grafts Placed to Span the Transected Spinal Cord of the Nude Rat. JD Guest, N Kleitman*, P Aebischer , MB Bunge,RP Bunge. The Miami Project and the Dept Neurol Surgery, Univ Miami School Med, and Centre Hospitalier Universitaire Vaudois, Lausanne.

We are systematically evaluating the ability of human Schwann cell (HSC)/ Matrigel grafts within semipermeable PAN/PVC guidance channels to influence the regeneration of axotomized CNS neurons in the nude rat. In previous studies, distally capped channels were apposed to the rostral transected stump of the spinal cord at T8. Myelinated axons were found within such grafts 30 days after transplantation. Immunopositive serotonergic and noradrenergic fibers were identified in the grafts. Fast Blue (FB) was injected into the cable 5 mm from the rostral interface. Sensory, propriospinal, motor and brainstem neurons of reticular, raphe and vestibular nuclei regenerated into the cables. Using autologous nude rat SCs, the mean numbers of all FB- labeled neurons were similar.

In a separate group of experiments, the HSC channel grafts were placed to span thoracic T8-T10 resections. The ability of injured neurons to extend processes into and beyond the grafts was evaluated using anterograde and retrograde tracing following 30 days survival. PHAL was [iontophoretically] injected 3 mm above the rostral interface (n=5). Labeled propriospinal axons entered grafts ; a few processes extended up to 2 mm into the distal host spinal cord . In other animals FB was injected 4 mm caudal, and Fluororuby (FR) 8mm rostral to the grafts. In each case (n=5), FB-labeled DRG and propriospinal but not brainstem neurons could be identified above the graft. Whereas hundreds of FR positive fibers entered HSC grafts, only a few fibers were seen up to 4mm beyond them. Immunolabeled descending monoaminergic fibers entered grafts in substantial numbers but rarely extended beyond the grafts.The corticospinal tract was anterogradely labeled with FR in 4 animals; fibers did not enter the grafts, and die-back was substantial ($\bar{x}$ = 1mm) at 40 days [post grafting]. The graft-host interface studied in sagittal sections by pre-labeling SCs with Hoechst dye and immunostaining for GFAP had morphologic similarity to the dorsal root entry zone and was immunopositive for chondroitin-sulfate proteoglycan. The nude rat is thus a favorable animal model for HSC grafting because brainstem neurons regenerate into grafts placed at the lower thoracic level and, moreover, a small proportion of sensory and propriospinal fibers are able to enter the distal host cord.
Supported by NIH grants NS09923 and NS28059 and the Hollfelder Foundation and Miami Project. JG is a Fellow of the American Association of Neurological Surgeons.

490.20

CYTOKINE-MODULATED ASTROCYTES TRANSPLANTED INTO TRANSECTED ADULT RAT OPTIC NERVE FACILITATE RECOVERY OF VISUAL RESPONSE. A. Monsonego[1], E. Hauben[1], A.S. Solomon[2], E. Yoles[1]* and M. Schwartz[1], [1]Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel, and [2]Goldschleger Eye Research Institute, Sackler School of Medicine, Tel Aviv University, Sheba Medical Center, Tel Hashomer, Israel.

The inability of the central nervous system of mammals to regenerate after injury has been attributed in part to the failure of astrocytes to acquire essential postinjury characteristics, such as migratory ability, and expression of growth factors, enzymes and cell adhesion molecules. Here we demonstrate recovery of the visual response in transected optic nerve of adult rat following transplantation of mature astrocytes into the injury site, provided that the transplanted cells were premodulated *in vitro* by interleukin-1β in combination with tumor necrosis factor-α. Of 34 animals with completely transected optic nerves and loss of visual evoked potential response, 11 showed recovery starting from 7 weeks after treatment. Recovery was confirmed by unitary action potential and retrograde labeling of retinal ganglion cells by a lipophilic dye applied distally to the lesion. We suggest that poor recruitment of macrophages to the injury site and their inappropriate activation during central nervous system wound repair result in an astrocytic reaction, which is not permissive for axonal growth. This can be overcome by astrocytes premodulated by specific inflammation-associated cytokines.

490.21

TRANSPLANTATION OF ACTIVATED MACROPHAGES OVERCOMES CENTRAL NERVOUS SYSTEM REGROWTH FAILURE. O. Lazarov-Spiegler[1], A. S. Solomon[2], A. Ben Zeev-Brann[1], D. L. Hirschberg[1], V. Lavie[1] and M. Schwartz[1]*. [1]Dept. of Neurobiology, Weizmann Institute of Science, 76100 Rehovot, Israel, and [2]Goldschleger Eye Res. Inst., Tel Aviv Univ. Sackler Sch. of Med., Sheba Medical Center, Tel Hashomer, Israel.

The involvement of macrophages, known to play a key role in healing processes, has been a long-standing debate in central nervous system (CNS) regrowth. Here we show that the failure of transected optic nerve (which, like all other CNS nerves in mammals, cannot regenerate) to regrow after injury, can be overcome by transplantation of macrophages preincubated *ex vivo* with segments of sciatic nerve (spontaneously regenerating nerve). Preincubation with sciatic nerve segments stimulated macrophage activity, using phagocytosis as a marker. In contrast, preincubation with optic nerve segments inhibited the activity. We conclude that a CNS-resident macrophage inhibitory factor may be the basis of what has been known as the CNS immune privilege. This explains the failure of the spontaneously recruited macrophages to support regrowth of transected CNS nerve, circumvented here by the transplantation of sciatic nerve-preactivated macrophages. The transplantation of suitably activated macrophages into injured nerves may globally overcome multiple malfunctioning aspects of response to CNS injury and thus may be developed into a novel, practical and multipotent therapy for CNS lesions.

AGING PROCESSES: HIPPOCAMPUS

491.1

ACUTE TESTOSTERONE AFFECTS AGED MALE RAT HIPPOCAMPAL MORPHOLOGY. L.L. Waldroup[1], C. Isgor[2], D.R. Sengelaub[2] and G. Frommer*[2]. [1]Department of Biology, [2]Department of Psychology, Indiana University, Bloomington, IN 47405.

We have demonstrated androgen-sensitive sex differences in spatial learning and hippocampal morphology in rats. Androgen levels in male rats normally decline with advancing age, and age-related regressive changes in hippocampal morphology and its behavioral correlates (e.g., spatial learning) have been shown repeatedly. To determine whether age-related declines in androgen are related to regressive changes in the hippocampus, we assessed hippocampal morphology in aged males after acute testosterone (T) treatment.

Hippocampal morphology was assessed in male Fischer 344 rats at 8 and 23 months of age. Aged males were either left intact or castrated and implanted with Silastic capsules containing crystalline T (45 mm, a length which results in plasma T levels typical of young adults) for 6 weeks (n=3 per group). After treatment, brains were extracted, frozen sections taken at 40 µm, and stained with cresylecht violet. Volumes of hippocampal CA1, CA3 fields and the dentate gyrus granule cell layer were measured at 65X; CA1 pyramidal cell soma size and density were examined at 750X. CA1 and CA3 volumes were significantly larger in aged T-treated males and similar to those of young adults. In CA1, this increase in volume was at least partly due to a significant increase in pyramidal cell soma size; no differences in soma density were observed. Because acute T treatment of aged males alters hippocampal morphology, these data suggest spatial behavior may be similarly androgen sensitive. Supported by Indiana University Undergraduate Initiative.

491.2

AGE-RELATED EFFECT OF ADRENALECTOMY ON RAT CA1 HIPPOCAMPAL Ca^{2+} CURRENTS: . H. Karst[1,2], T.R. Werkman[1], O.C. Meijer[2]* and M. Joëls[1], [1]Dept. Exp. Zoology, Univ. Amsterdam, Kruislaan 320, 1098 SM Amsterdam and [2]Div. of Medical Pharmacol., Univ. Leiden, Wassenaarse-weg 72 2300 RA Leiden, The Netherlands.

Previously it was shown that Ca^{2+} currents of rat hippocampal CA1 neurons change after adrenalectomy (ADX) of young adult rats. Preliminary observations however showed that the effect of ADX dimishes with age. To study this in more detail we recorded Ca^{2+} currents in CA1 neurons of 1, 3 or 6 months old ADX and sham operated rats. Ca^{2+} currents were recorded in hippocampal slices with the patch-clamp technique. It appeared that the low voltage activated (LVA) Ca^{2+} current decreased whereas high voltage activated (HVA) currents increased in amplitude, with age. These data indicate that Ca-currents change with age, not only in early development (<1 month) or in aged animals (Chen et al., Soc. Neurosci. 1996, 236.1), but also in early adulthood. Remarkably, HVA currents of 1 or 3 months old ADX rats were already at the level of the currents observed in 6 months old sham operated controls; currents in 6 months old ADX rats were comparable to the sham controls. The LVA currents in 6 months old ADX rats were comparable to the currents observed in the 1 month old sham group. These results were confirmed using acutely dissociated hippocampal CA1 neurons, where only sustained HVA Ca^{2+} currents are present: Ca currents increased in the 1 but not in the 6 months old rats. The data thus show that ADX of very young animals results in a selective increase of the HVA currents, while ADX of 6 months old rats is only associated with a minor increase of the LVA currents. The expression of Ca^{2+} channel subunits in tissue from the same rats is currently under investigation, with in-situ hybridization. We conclude that the ADX-induced increase of Ca-currents in CA1 hippocampal neurons is age dependent and parallels the age dependent development of these currents in adrenally intact rats. (Grant A110 Dutch Epilepsy Foundation, NEF).

491.3

CHANGES IN INTRACELLULAR AND EXTRACELLULAR pH DURING AND AFTER ANOXIA I N HIPPOCAMPAL SLICES FROM YOUNG ADULT AND AGED RATS. E.L. Roberts, Jr.[1,2]* and C.-P. Chih[2]. [1]Department of Neurology, University of Miami School of Medicine, Miami, FL 33136, [2]Geriatric Research, Education, and Clinical Center, Miami VA Medical Center, Miami, FL 33125

Decreased intracellular pH (pH_i) may be linked to hypoxic or ischemic brain damage. We examined whether aging increased intracellular and extracellular acidification during anoxia, and slowed recovery of pH_i and extracellular pH (pH_o) following anoxia, in hippocampal slices. Hippocampal slices were obtained from Fischer-344 rats aged 6-9 months (young adult) and 25-27 months (aged). pH_i was assessed in hippocampal cells with the fluorescent dye SNARF-1, while pH_o was measured with pH-sensitive microelectrodes. Slices were made anoxic by switching the gas mixture bubbled through the bicarbonate-buffered physiological solution surrounding slices from 95% O_2, 5% CO_2 to 95% N_2, 5% CO_2. Anoxic episodes were terminated one minute after onset of anoxic depolarization, as seen in a negative shift of the extracellular potential recorded from the reference barrel of the pH-sensitive microelectrode. Contrary to our expectations, the magnitude of decreases in pH_i and pH_o in young adult slices were greater than those in aged slices. Both age groups recovered pH_i and pH_o 15-20 minutes following cessation of anoxia. The degree of acidification may have been less in aged slices because anoxic depolarization occurred sooner in the aged group. Our results suggest that diminished recovery of extracellular K^+ homeostasis and synaptic transmission after anoxia in aged hippocampal slices (Roberts *et al., Brain Res. 514* (1990) 111-118) cannot be due to increased intra- or extracellular acidification. (Supported by PHS grant AG08710 (NIA), and by a Grant-In-Aid Award from the American Heart Association, Florida Affiliate, Inc.)

491.4

AGE-RELATED CHANGES IN THE RHESUS MONKEY: ON-THE-SLIDE SATURATION BINDING STUDIES OF 7 NEUROTRANSMITTER RECEPTORS. TJ Nicholson, TT Gibbs, R Rizkalla, CC Freeland* & DL Rosene, Dept of Anatomy & Neurobiology, & Dept of Pharmacology, Boston University School of Medicine, Boston MA 02118 and the Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

A number of neurotransmitter receptor systems and the medial temporal lobe have been implicated in normal aging and certain age-related disorders. Receptors from three neurotransmitter receptor systems were investigated: cholinergic [muscarinic type 1 (M1), muscarinic type 2 (M2), & the high affinity choline uptake site (HACU)], excitatory amino acid [kainate (KAI) & N-methyl-D-aspartate (NMDA)], and inhibitory amino acid [gama-amino-butyric acid typeA ($GABA_A$) & benzodiazepine (BZD)]. Saturation binding of the 7 receptors was examined in groups of 3 young (≤10) and 3 aged (>20) rhesus monkeys using on-the-slide quantitative autoradiography (H^3-pirenzipine for M1, H^3-oxotremorine for M2, H^3-hemicholinium for HACU, H^3-KAI for KAI, H^3-MK-801 for NMDA, H^3-muscimol for $GABA_A$, & H^3-flunitrazepam for BZD). Previous experiments using single concentrations of ligand showed significant decreases in binding with age in all of the receptors except for the BZD receptor which showed a significant increase in binding with age. This led us to perform the current saturation experiments. The M1 receptor shows a significant decrease in Bmax (decrease in receptor number) and the NMDA receptor shows a significant increase in Kd (decrease in receptor affnity). Preliminary results for the other receptors suggest that in most cases the changes are due to a change in receptor numbers although possible changes in affinity cannot be excluded. These changes were investigated within the hippocampus for all receptors and within the temporal lobe neocortex and amygdala when possible. (Supported by NIH grant PO1-AG00001 & RR-00165)

491.5

AGE-RELATED CHANGES IN THE RHESUS MONKEY: ELECTROPHYSIOLOGY AND MORPHOLOGY OF NEURONS IN HIPPOCAMPAL SLICES J.L Luebke and D.L. Rosene* Dept. of Anatomy and Neurobiology and Center for Behavioral Development, Boston Univ. School of Med., 80 E. Concord St., Boston, MA 02118.

Numerous studies in rodents have shown an effect of aging on the synaptic and intrinsic membrane properties of hippocampal neurons (e.g. Barnes, 94). These include a decrease in the maximal evoked presynaptic fiber volley and a concurrent greater field EPSP response for a given fiber volley amplitude in aged vs. young rats. Changes in CA1 pyramidal cell resting membrane potential, action potential kinetics and potassium currents associated with aging have also been reported. There have as yet been no published data upon the effect of aging on similar parameters in the non-human primate. However there is substantial anatomical and behavioral data to suggest that age-related alterations in electrophysiological properties of primate neurons are likely. To address this question we have used extracellular recordings of field potential responses and whole cell patch clamp recordings of the intrinsic membrane properties of neurons in hippocampal slices prepared from cognitively tested, Krebs buffer perfused, young and aged rhesus monkeys. All cells from which recordings were obtained were filled with biocytin and subsequently processed for analysis of somatic and dendritic morphology. Preliminary data has been obtained from four young (5) and two aged (29 & 30) monkeys. Initial observations indicate that field synaptic responses are somewhat attenuated in the dentate gyrus of aged as compared to young monkeys. Intrinsic membrane properties (resting membrane potential, input resistance, membrane time constant and amplitude and kinetics of potassium currents) of dentate granule cells were similar in the young and aged monkeys although firing frequency appeared to be decreased in the aged monkey. Morphological examination of filled granule cells suggests a decreased complexity of dendrites in the aged monkey. Further studies are required to determine if these effects are statistically significant. (Supported by NIH grant P01-AG00001)

491.6

RESTRICTED DISTRIBUTION OF ß-AMYLOID SUBTYPES AND COLOCALIZATION WITH APOLIPOPROTEIN E IN THE TEMPORAL CORTEX OF AGED RHESUS MACAQUES. S. G. Kohama* and H.F. Urbanski. Division of Neuroscience, Oregon Regional Primate Research Center, 505 N.W. 185th Avenue, Beaverton, Oregon, 97006.

The number of β-amyloid (βA) plaques is reportedly equivalent to (Poduri et al., Am J Pathol 144:1183) or greater than (Mufson et al., Neurobiol Aging 15:621) the number of apolipoprotein E (ApoE) plaques in the brains of aged rhesus monkeys. Recently, βA (1-40) was found to be more prevalent than βA (1-42) in aged rhesus, unlike humans (Gearing et al., Neurosci. Abstr. Vol 21:109.4). This study examines the prevalence of amyloid plaque subtypes with or without ApoE, in the aged rhesus temporal cortex. Sections of the temporal lobe of adult animals (6-29 yrs) were immunostained with 10D5, an antibody which recognizes βA 1-16, or 21F12, a βA42 specific antibody (Athena Neurosciences). A subpopulation of the oldest animals displayed βA, with the number of plaques varying between animals. In positive cases, the amygdala and temporal cortex had the highest plaque density, with few plaques seen in the hippocampus, and none in the adjacent striatum. In addition, 21F12 labelled plaques only made up a portion of all plaques visualized with the 10D5 antibody. Sections from three animals were further examined by immunofluorescence double-labelling for colocalization of βA (10D5 or 21F12) with ApoE (Chemicon Int.). Smaller, low density plaques were in some cases found to be 10D5 immunopositive/ApoE- or 21F12 immunonegative/ApoE+. However, the vast majority of plaques were double-labelled for amyloid and ApoE, especially when plaque deposits possessed a compact, high density morphology. These results suggest that βA processing in rhesus differs from that observed in humans with Alzheimer's disease and that ApoE is an important component of plaque formation.

Grant Support: NIH HD-29186 and RR00163

491.7

THE AGING OF THE NMDA RECEPTOR IN C57BL MICE INVOLVES CHANGES IN AGONIST AFFINITY. K.R. Magnusson* and Ginger Sammonds. Dept. of Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523

There is a decrease in binding density of the transmitter glutamate to the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors in the hippocampus and cerebrum during the aging process in C57Bl mice, as determined by single concentration assays. The goal of the present study was to determine whether the decreases were due to changes in affinity or total numbers of receptors. Concentration response autoradiography for NMDA-displaceable [^{3}H]glutamate binding was performed on horizontal sections from 3, 10, and 30 month old C57Bl mice. Crude synaptic membranes of cerebral cortex were also used for concentration response and glutamate inhibition studies of [^{3}H]CGP39653 binding. In the autoradiographic studies, there was an increase in the Kd for glutamate between 3 and 10 month olds in all regions, which was significant in hippocampus. The Kd in 30 month olds was maintained or further increased in the cortex. In the hippocampus, the Kd for 30 month olds was intermediate between 3 and 10 month olds. There was a significant overall effect of age on Bmax, which decreased across age in the cortex, but increased in the hippocampus of 10 month olds. In cortical membranes, there was a trend for decreasing Kd for CGP39653 and increasing Ki for glutamate with increasing age. These results suggest that some of the problems with the function of the NMDA receptor in aged individuals is due to a change in the receptors that reduces agonist affinity. Supported by NIA/NIH FIRST award AG10607 and RCDA AG00659 to KRM.

491.8

DENDRITIC STABILITY OF DENTATE GRANULE CELLS IN THE RAT HIPPOCAMPUS WITH AGING. G.K. Pyapali* and D.A. Turner. Neurosurgery and Neurobiology, Duke Univ. Med. Ctr. and Durham VAMC, Durham, NC, 27710.

Aging results in synaptic reorganization in the CNS. Our hypothesis states that this CNS reorganization involves denervation of neurons due to either cell loss or decreased axonal plasticity, resulting in reactive dendritic growth (Pyapali and Turner, 1994, 1996). We investigated dendritic alterations in healthy DG cells (3, 24 month male F344 rats) using Neurobiotin staining during *in vitro* intracellular recordings and 3D computer-based dendritic reconstructions (Neurolucida).

Total dendritic length of DG cells was not different between aged (3.38±3.29 mm; n=6) and young cells (3.19±2.76 mm; n=7); no neuronal atrophy was observed. Dendritic extent (from the granule cell layer to the fissure) was significantly longer in aged (343±25.8 μm) compared to young animals (290±30.1 μm; $p<0.01$). Sholl analysis (25 μm spheres) revealed an increase in the number of intersections ($p=0.03$) and branch lengths (at 300-400 μm from the soma; $p=0.02$) in the outer molecular layer in aged cells. Branch order analysis did not show any difference in the number of branch orders or length per branch order between the two groups. The trend suggested moderate lengthening of the molecular layer extent with age.

The results demonstrate only moderate dendritic alterations of dentate granule cells with aging, with no evidence of dendritic atrophy. In contrast, CA1 pyramidal neurons show significant increased dendritic branching throughout both apical and basilar trees in 24 month F344 rats (Pyapali and Turner, 1996; Neurobiol Aging, in press). However, DG cells show minimal dendritic sprouting in response to entorhinal cortex lesions whereas CA1 pyramidal cells demonstrate robust sprouting following kainic acid CA3 lesions. Thus, cell populations within hippocampus may show differential dendritic plasticity in response to either denervating lesions or with aging, consistent with our hypothesis that denervation induces dendritic alterations in the aged CNS. Supported by NIA (GKP, DAT) and VAMC (DAT).

491.9

AGE-RELATED DECREASE IN RAT HIPPOCAMPAL NON-PYRAMIDAL NEURONS EXPRESSING GLUTAMIC ACID DECARBOXYLASE AND CALCIUM BINDING PROTEINS. A. K. Shetty* and D. A. Turner. Neurosurgery, Neurobiology, Duke Univ. Med. Ctr., VAMC, Durham, NC 27710.

We hypothesize that age-related impairments in memory acquisition may partly be due to loss of hippocampal inhibitory non-pyramidal neurons. We investigated the number of non-pyramidal neurons positive for glutamic acid decarboxylase (GAD) and those expressing calcium binding proteins (calbindin, parvalbumin, and calretinin) in 20 μm sections from perfused 4 and 24-month male Fischer 344 rats.

Aged rats displayed variable but significant reductions of non-pyramidal neurons throughout the hippocampus. GAD neurons were significantly depleted in CA1 stratum radiatum (SR), and CA3 stratum oriens (SO) and SR (33-54% reduction, $p < 0.05$). Calbindin neurons were decreased in the dentate hilus (45%, $p < 0.05$), CA1 SO and SR (29-51%, $p < 0.05$), and CA3 SO and SR (48-57%, $p < 0.005$). Parvalbumin neurons showed significant reductions in CA1 stratum pyramidale (SP; 15%, $p < 0.05$), and CA3 SP and SR (30-32%, $p < 0.05$). Calretinin neurons were significantly depleted in the dentate hilus (49%, $p < 0.005$), and both CA1 and CA3 SO (30-34%, $p < 0.05$). However, the ratio of calbindin, parvalbumin, and calretinin neurons to the total GAD neuronal population did not change with aging, and averaged 42% in CA1 SR and 70% in CA3 SR.

These results demonstrate an age-related decrease in the number of both non-pyramidal neurons positive for GAD, and those expressing different calcium binding proteins in hippocampus. The constant relative ratio of calbindin, parvalbumin and calretinin neurons compared to GAD neurons in all regions of hippocampus with aging suggests actual neuronal loss of cells expressing calcium binding proteins with age rather than merely decreased protein expression. These subgroups of interneurons may represent a majority of all non-pyramidal neurons, suggesting that inhibitory function may be radically altered with aging. Supported by RO1 AG13165, VAMC.

491.10

DECREASED mRNA OF THE PLASMA MEMBRANE Ca^{2+}-ATPase ION TRANSPORTER IN THE HIPPOCAMPUS OF AGING RATS. PG Kaminker, KC Chen*, JP Herman, PW Landfield, Depts. Pharmacology; Anat. and Neurobiol., College of Medicine, Univ. of Kentucky, Lexington, KY 40536.

There is growing interest in the possibility that changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) play a key role in normal brain aging and Alzheimer's Disease. The plasma membrane Ca^{2+}-ATPase (PMCA) is an essential protein in $[Ca^{2+}]_i$ regulation. An aging-related decrease in the V_{max} for PMCA in brain tissue has been found (Michaelis et. al.,1989), which could contribute to a rise in $[Ca^{2+}]_i$ in aged rat neurons. In these studies we investigated the possibility that reduced gene expression underlies this decline in PMCA activity. Our initial focus has been on the second isoform (PMCA2) previously shown to be expressed predominantly in the brain. A ribonuclease protection assay was employed to measure the steady state PMCA2 mRNA levels in the hippocampal homogenates from three age groups, (3-5 m.o., 12 m.o., and 24 m.o.) of male Fischer 344 rats. The PMCA2 cRNA probe was synthesized from a 282 b.p. cDNA clone obtained from rat hippocampal RNA by RT-PCR, using PMCA2 primers spanning the 3'-end region. A cRNA probe of glyceraldehyde phosphate dehydrogenase was used as an internal control.

Our results show a decrease of about 50% ($p<.05$) in hippocampal PMCA2 mRNA in mid-aged and aged rats in comparison to young adult rats. This change did not appear to be simply developmental, as the young animals were mature adults, suggesting that alterations in PMCA2 occurred early in the brain aging process. These results are consistent with the possibility that reduced transcription of the PMCA2 gene could account, in part for the previously observed changes in PMCA2 activity and, therefore, could be a significant factor in brain neuronal Ca^{2+} dysregulation. (Supported by NIH AG10836)

491.11

EXPRESSION OF NCAM PSA IN THE HUMAN HIPPOCAMPAL DENTATE GYRUS FROM INFANCY TO OLD AGE. G.B. Fox*, C.M. Ni Dhuill, S.J. Pittock, A.W. O'Connell and C.M. Regan, Department of Pharmacology, University College, Belfield, Dublin 4, Ireland.

The polysialylated form of the neural cell adhesion molecule (NCAM-PSA) regulates adult neuroplastic events associated with learning and memory . Although perturbations of NCAM function have been related to the severity of the impaired sensory processing associated with autism and schizophrenia, few studies have examined the distribution of NCAM-PSA in neuroplastic regions of the human brain. We now describe the distribution of polysialylated NCAM in the aging human hippocampal dentate gyrus, a region synonymous with processing learning-associated sensory information. Human brain tissue was obtained, with the consent of the senior pathologist, from six major Dublin hospitals within 24 hours post-mortem. The hippocampus was isolated from 16 individuals ranging in age from 5 months to 82 years with at least one sample from each decade. The tissue was dissected from the medial temporal lobe, coated in optimal cutting temperature compound and frozen in liquid nitrogen- or dry ice-cooled n-hexane. Cryosections of the dentate area were prepared and immunostained for NCAM-PSA using a monoclonal antibody specific for the polysialic acid portion of the molecule. Immunoreactivity was localised mainly to neurons in the granule cell layer and their mossy fibre axons of the infant (5-7 months). In early childhood (1-3 years), this immunoreactivity began to decline and was markedly reduced in old age. In contrast, a population of large neurons in the hilar region became immunoreactive only during early childhood and remained in significant numbers into adulthood and old age. These results demonstrate a differential, age-dependent expression of NCAM-PSA in two discrete neuronal populations of the human hippocampal dentate gyrus. *Supported by the Health Research Board of Ireland and the EU Biotechnology Programme.*

491.12

DIFFERENTIAL EXPRESSION OF Na-K ATPase α-ISOFORM mRNAs IN AGING RAT CEREBELLUM. N.B. Chauhan*, G.J. Siegel, Molecular and Cellular Neuroscience Laboratory, Hines VA, Hines IL 60141; Departments of Neurology, Molecular and Cellular Biochemistry, Loyola University Chicago, Maywood IL 60153.

Age-dependent changes in the expression of Na,K-ATPase α1- and α3-mRNAs were analyzed in the rat cerebellum by *in situ* hybridization. In young rats, α1-mRNA showed prominent labeling in the granular layer (GL) with moderate fine distribution in the molecular layer (ML), Purkinje cell layer (PCL) and white matter (WM) but no clusters over Purkinje cells. In old rats, α1-mRNA remained unchanged in ML and PCL but declined by 43% ($p<0.0001$) in GL and increased by 624% ($p<0.0001$) in WM. α3-mRNA in young rats showed large clusters of label on stellate, basket, Golgi and Purkinje cells (PCs) and fine grains diffusely in ML, GL and WM. In old rats, α3-mRNA declined by 87% in ML, 83% in PCL, 84% per PC, and 89% in GL and increased by 111% in WM (all values $p<0.0001$) relative to young rats. PC numbers were reduced by 30% but the average area of PC profiles did not change significantly. In old rats, the specific cluster-like label related to α3-mRNA on PCs, stellate, basket and Golgi cells was lost. Immunocytochemistry of cerebellum and hippocampus showed no age-related change in the distribution and density of total catalytic polypeptide. Thus, the discordance between changes in the levels of mRNAs in neuronal layers and WM in the face of constant polypeptide levels indicates age-related changes also in polypeptide turnover. Cell- and isoform-specificity of α-isoform mRNAs in aging rat cerebellum may reflect differential regulation underlying age-related impairments in signal transduction and motor learning.

491.13

DECREASES IN TYPE 1 INSULIN-LIKE GROWTH FACTOR RECEPTORS IN CORTEX AND HIPPOCAMPUS OF AGED RATS. P. Thornton*, S. Bennett, P. Cooney, R. Ingram and W. E. Sonntag, Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1083.

IGF-1 has previously been shown to have an important role in brain function including protein synthesis, neurite outgrowth, release of acetylcholine and cortical development in rodents. In this study, we assessed whether a decline in the type 1 IGF receptor could be a contributing factor in the decrease in brain function that occurs with age. Brain sections from Fisher 344xBrown Norway male rats (10, 19 and 29 months of age) were assessed for ^{125}I-IGF-1 and ^{125}I-des[1-3]IGF-1 receptor binding by autoradiography. Type 1 IGF receptors were prominent in both layers 2 and 4 of sensorimotor cortex and in hippocampus of all animals. ^{125}I-IGF-1 binding tended to increase in layers 2 and 4 from 10 to 19 months of age and decreased significantly from 19 to 29 months in layer 2 (3.65±0.2 vs 2.76±0.2nCi/mg, $p<0.05$) and layer 4 of cortex.(3.20±.0.15 vs 2.42±0.22nCi/mg). In hippocampus, receptor density decreased steadily from 10 to 29 months (3.66±0.22 vs 2.48±0.15nCi/mg, $p<0.05$). Analysis of adjacent sections with ^{125}I-des[1-3]IGF-1 revealed similar decreases with age in layer 2 (35% decrease from 19 to 29 months) and 4 (33% decrease from 19 to 29 months) of cortex. Binding in hippocampus decreased by 25% from 10 to 29 months of age. The results of these studies demonstrate an age-related decline in type 1 IGF receptor density and suggest that these decreases may have a role in the decline in brain function with age.

Supported by NIH grant 1 P01 AG11370.

AGING PROCESSES

492.1

AGE-DEPENDENT CHANGES OF THE LEVELS OF FACTORS INVOLVED IN CHOLESTEROGENESIS OF RAT CENTRAL AND PERIPHERAL NERVOUS SYSTEMS. R. Fukuyama#, S. Fushiki* and K. Yanagisawa#. #Dept. Dementia Res., National Inst. Longevity Sci., Obu, Aichi, 474; Dept. Dynamic Pathol., Res. Inst. Neurol. Dis. Geriatrics, Kyoto Prefect. Univ. of Med., Kamigyo, Kyoto, 602 Japan.

A major risk factor for Alzheimer's disease (AD) is gene dose of ε4 allele of apolipoprotein E (ApoE), which is assumed to function as a carrier of phospholipids, cholesterol and triacylglycerol. It may be a clue to determine whether *de novo* biosynthesis or recycling system is dominant for supplying cholesterol in adult brain, in order to understand molecular pathogenesis of AD. We determined the levels of mRNA and protein of hydroxy methylglutaryl CoA-reductase (HMGR), the rate-limiting enzyme in cholesterogenesis, for evaluation of endogenous supply of cholesterol and also determined those of ApoE for evaluation of recycling cholesterol at various ages of rat brain hemisphere and sciatic nerve. Quantity of mRNA of HMGR was estimated by simplified RT-competitive PCR technique. We also measured total cholesterol with enzymatic procedure in order to relate the levels of these molecules to concentration of total cholesterol in each tissue. In all ages examined, levels of mRNA and protein for ApoE and internal control β-actin in both tissues were roughly unchanged. Level of HMGR mRNA in brain showed a peak at 3-4 weeks after birth and slightly decreased thereafter. In contrast, its mRNA level in sciatic nerve showed a dramatic decrease during first 3 weeks. While the level of total cholesterol in this tissue was steeply increased in this period and then reached to plateau level, which was roughly 2.2-fold more than that of corresponding brain. On the other hand total cholesterol in brain showed only a small increase within 3 months after birth. These results indicate that; 1) aged-rat brain utilizes both *de novo* synthesis and recycling system for supplying cholesterol; 2) aged-sciatic nerve utilizes predominantly recycling system; and 3) that age-dependent change in gene expression of HMGR in central and peripheral nervous systems is likely regulated by the level of total cholesterol in each tissue.

492.2

AGE RELATED CHANGES IN GLUTAMATE RECEPTORS IN THE CANINE BRAIN CORRELATE WITH CHANGES IN ANESTHETIC POTENCY C.D. Rinehart[1]*, C. Dunlop[2], A. Wagner[2] and K.R. Magnusson[1]. Depts. of Anatomy & Neurobiology[1] & Clinical Sciences[2]; Colorado State University, Fort Collins, CO 80523.

With increasing age, animals and humans become more sensitive to the depressant effects of general anesthesia. Antagonists of NMDA and AMPA receptors can also increase the potency of anesthetics. The goal of this study was to determine whether dogs experience a reduction in the functioning of glutamate receptors with age that could thereby impact the anesthetic requirement of the animal. Six young (2-3 years) and 6 middle aged (11 years) beagles were anesthetized with isoflurane to determine the potency (MAC value) for each animal. Following euthanasia, quantitative autoradiography for NMDA and AMPA receptors was performed. The sections were incubated with either 20nM ^{3}H-AMPA or 100nM ^{3}H-glutamate in the presence of 100μM SITS, 1μM kainate and 5μM AMPA or 10nM ^{3}H-MK801 in the presence of 10μM glutamate and glycine. The old dogs showed a significant decrease in binding, with all three ligands, in a majority of the cortical and hippocampal regions. A significant positive correlation was also found between the MAC values for isoflurane and the binding for MK801 sites and NMDA receptors in the cerebral cortex. These results suggest that there is a reduction in the functioning of these receptors with age in dogs and that this change may account for some of the increase in potency of inhalation anesthetics in aged animals. Support: MIKI Society of CSU College of Veterinary Medicine & Biomedical Sciences.

492.3

ALTERATIONS IN NMDA RECEPTORS WITH AGE. X. Xu*, S. Bennett, P.Thornton, and W.E.Sonntag Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1083.

N-methyl-D-aspartate (NMDA) receptors have an important role in synaptic plasticity and neurodegeneration. Two major subtypes of these receptors are present in brain, NMDAR1 and NMDAR2. Heterogeneity of these receptors define specific functional responses of the NMDA receptor. Previous studies indicate that receptors of excitatory amino acids (including NMDA receptors) decline in aged animals and contribute to age-related changes in neuronal function. In this study, we compared NMDAR1 and NMDAR2 levels in cortex of young (4 months of age) and old (29 months of age) male F344xBN rats using RT-PCR with receptor subtype specific primers. Our results indicate the presence of both NMDAR1 and NMDAR2 subtypes in both cortical and hippocampal areas. The NMDAR1 receptor subtype increased 25% in aged animals. However, the NMDAR2 subtype decreased by 31% in old as compared to young animals (relative densities of 31.2±2.0 vs 20.3±1.9, $p<0.05$). Administration of IGF-1 to aged animals (50ng/hr for 4 weeks) did not appear to alter either NMDAR1 or NMDAR2 mRNA in cortical areas. These data are consistent with decreases in NMDA receptor density previously reported in aged animals and suggest that alterations in receptor subtype heterogeneity may lead to functional changes in neurons in aged animals. Supported by 1 P01 AG11370.

492.4

AGE-RELATED CHANGES IN THE RHESUS MONKEY: NEUROTROPHIN RECEPTOR TRK C-LIKE IMMUNOREACTIVITY. J.H. Sandell*, L.S. Baker, Jr., T. Davidov and S. Lee. Dept. of Anatomy & Neurobiol., Boston Univ. School of Medicine, Boston, MA 02118.

The receptor tyrosine kinase TrkC is the high affinity receptor for neurotrophin 3 (NT-3). We used a polyclonal antibody against a portion of TrkC (Santa Cruz Biotechnology, used at 1:500 to 1:5000) to detect sites of TrkC-like immunoreactivity in the rhesus monkey brain. We were particularly interested in whether the distribution or density of labeling changed with age. Labeling was abolished in control experiments in which the antibody was preabsorbed with peptide antigen, but was unaffected by preabsorption with heterologous peptide. Frozen sections from 2 monkeys (9 and 26 years old) were used. Selected sections from additional animals were also examined. All monkeys showed the same distribution of labeling.

TrkC-like immunoreactivity was widespread. Numerous varicose fibers were intensely immunoreactive, with very rare labeling of cell bodies. Sparse populations of positive fibers were observed in the thalamus, hypothalamus, brainstem and cortex. Intense immunoreactivity was found in the basal forebrain, throughout the raphé complex and in the locus coeruleus. In these latter areas there was a significant reduction in the numerical density of the varicosities in the old animal.

We conclude that TrkC-like immunoreactivity in the monkey brain is greatest in some of the subcortical regions that are most vulnerable in aging, and that our preliminary studies indicate that the density of TrkC-like immunoreactive profiles in these regions decreases with age. (Supported by AG-00001.)

492.5

GDNF AUGMENTS BASAL AND STIMULUS-EVOKED RELEASE OF DA IN THE STRIATUM OF AGED F344 RATS. M.A. Hebert*, P.A. Lapchak, and G.A. Gerhardt. Psychiatry, Pharmacology and Neuroscience Training Program, University of Colorado Health Sciences Center, Denver, CO 80262.

The objective of this study was to determine if glial cell-line derived neurotrophic factor (GDNF) could produce functional changes in dopamine (DA) nerve endings in the striatum of aged Fischer 344 (F344) rats. Prior studies from our laboratory have shown that DA neurons in aged rats have reduced DA output, and intranigral administration of GDNF enhances stimulus-induced DA overflow in the striatum of young rats. It has not been determined, however, whether aged (24 mo) F344 rats are capable of responding to this factor. Three weeks prior to in vivo microdialysis studies, aged F344 rats received single intranigral injections of 10 µg GDNF (in 10µl PBS). The GDNF-treated, 24 mo F344 rats were found to have significant increases in basal DA levels (0.11 ± 0.00µM, n=21, $p<0.05$) over the vehicle-injected group (0.07 ± 0.01µM, n=21), DOPAC levels (2.45 ± 0.09µM, GDNF-treated, $p<0.01$) versus (1.99 ± 0.09µM, vehicle-treated) and HVA levels (3.39 ± 0.11, GDNF-treated, $p<0.001$) versus (2.21 ± 0.11µM, vehicle-treated). In addition, both potassium- (1.29 ± 0.16µM in GDNF vs. 0.36 ± 0.04µM in vehicle group) and d-amphetamine-induced (0.82 ± 0.16µM in GDNF vs. 0.34 ± 0.04µM in vehicle group) DA overflow, as measured by microdialysis were significantly increased (n=7, $p<0.001$). These data demonstrate that a single intranigral injection of GDNF is capable of increasing the output of DA from DA nerve endings in aged rat striatum. (Supported by grants from USPHS NS09199, AG06434 and NIH Training Grant HDO7408-02. GDNF provided by Amgen, Inc., Thousand Oaks, CA).

492.6

PARTIAL CHOLINERGIC IMMUNOLESIONS AND NGF TREATMENT: COMPARATIVE CHANGES OF CHAT, NGF AND TRANSCRIPTION FACTORS NFκB AND AP-1 IN YOUNG AND AGED RATS.
G. Wortwein, J. Yu, T.E. Toliver, D. Rassin* and J.R. Perez-Polo. Dept. HBC&G, Univ. of Texas Med. Branch, Galveston, Tx 77555, and Lab. Neuropsychiatry, Univ. Copenhagen and Rigshospitalet-6102, Copenhagen, Denmark

The cholinergic neurons of the basal forebrain (BF) are the major source of cholinergic innervation of the cortex and hippocampus. In Alzheimer's disease there is a severe loss of cholinergic neurones in the nucleus basalis of Meynert, leading to a reduction of cortical cholinergic activity, which correlates with the severity of cognitive deficits. To mimic these cholinergic deficits in experimental animals and study the potential therapeutic effects of nerve growth factor (NGF) we induced a partial lesion of the BF cholinergic neurones by intracerebroventricular (icv.) injection of 1.3µg of the immunotoxin 192IgG-saporin in groups of 3 month and 30 month old rats. The lesion was followed 14 days later by icv. injection of 5.4µg NGF. Basal levels of choline acetlytranferase (ChAT) were reduced in the BF and cortex of aged animals, but not in the hippocampus and olfactory bulb. The lesion resulted in a 40 to 60% reduction of ChAT levels in cortex and hippocampus of both, young and aged rats. NGF treatment restored ChAT to close to normal values in the cortex of aged but not young rats. Basal NFκB binding activity was higher in the hippocampus of aged rats. The lesion had no significant effect on NFκB DNA binding in either age group. However, NGF treatment increased the already higher NFκB binding activity in the aged hippocampus even further. There were no basal AP-1 binding activity differences in the hippocampus of young and aged rats and the lesion did not alter this activity in either age group. NGF treatment significantly lowered AP-1 DNA binding activity in the aged hippocampus. There were no differences among the various age and treatment groups with respect to NFκB or AP-1 binding activity in the cortex. This is publication 51A from USPHS P01-A610514 awarded by NIA. Also supported by NINDS NS18708.

492.7

AGING ASSOCIATED CHANGES IN THE REGULATION OF TRANSCRIPTION FACTOR NF-κB IN RAT BRAIN.
A. Salminen*, P. Wiitanen, M. Helenius. Department of Neurology, University of Kuopio, Kuopio, Finland.

Aging process involves an altered pattern of gene expression and probably changes in transcription factor regulation. Our aim was to study whether aging affects the DNA binding activities (EMSA methods) of NF-κB, CREB, Sp-1, AP-1, and Oct-1 transcription factors in brain samples of rats and mice. We observed a significant upregulation of nuclear NF-κB factor in 30 month old Wistar rats compared to young or 18 month old rats. Similar upregulation also occurred in NMRI mice. Cytoplasmic, inducible NF-κB binding activity was not affected by aging. Supershift assays showed that constitutive NF-κB complexes contained protein components such as p50, p52, and p65. Interestingly, the binding activities of Sp-1 and Oct-1 factors were prominently downregulated during aging *e.g.* in hippocampus. However, the age-related increase in NF-κB binding activities was considerably less in brain than in other tissues such as liver and heart, suggesting that in rat these tissues are more vulnerable to aging changes than brain.

492.8

AGE-RELATED DIFFERENCES IN PILOCARPINE-INDUCED C-FOS EXPRESSION. D.J. Bucci[1*], D.L. Rosen[2], and M. Gallagher[1,2]. [1]Curriculum in Neurobiology and [2]Department of Psychology, University of North Carolina, Chapel Hill, NC 27599.

Expression of the immediate-early gene c-*fos* can be induced in specific brain regions of young rats through administration of the cholinergic agonist pilocarpine, an effect that is blocked by the M1 cholinergic antagonist pirenzepine (Hughes & Dragunow, 1993,1994). The topography of activation in forebrain structures coincides with the terminal regions of basal forebrain cholinergic neurons. In the present study, young (6 mo.) and aged (24-26 mo.) male Long-Evans rats were treated with either saline or pilocarpine (25 mg/kg; IP), and sacrificed 90 minutes after injection. Brains were then processed for *in situ* hybridization histochemistry for c-*fos* mRNA, using an ^{35}S-labeled antisense riboprobe. In many areas of the forebrain, there was no age difference in the c-*fos* mRNA response to cholinergic agonist. However, a significant increase in this response was found in retrosplenial cortex, but not in adjacent cortical regions, e.g., parietal cortex, in aged animals. Further studies will examine levels of Fos protein within this model. (Supported by NIH grants K05-MH01149 & P01-AG09973 to MG.)

492.9

NGF IN THE RAT EXTERNAL CAROTID ARTERY IS PRIMARILY ASSOCIATED WITH NERVE FIBERS AND DECLINES WITH AGE. K.A. Crutcher*, J.A. Weingartner, J. Gavazzi# and T. Cowen#, Dept. of Neurosurgery, Univ. of Cincinnati Med. Ctr., Cincinnati, OH 45267-0515 and #Dept. of Anatomy and Developmental Biology, Royal Free Hospital School of Medicine, London, UK.

The regulation of target innervation is likely to involve neurotrophic factors and there is ample evidence that innervation of target tissues by sympathetic and sensory neurons is regulated by nerve growth factor (NGF). However, the extent to which measurements of NGF in target tissues reflect levels within producer cells or levels within the resident nerve fibers is incompletely known. Recent evidence suggests that within one target tissue, the superior mesenteric artery, the majority of NGF is associated with the nerve fibers (Liu et al., J. Neurochem., in press). This question was examined by measuring levels of NGF in the external carotid artery of both young and aged SD rats with a sensitive 2-site ELISA. The contribution of nerve fibers to the levels of NGF was determined by separating the adventitia and media as well as examining the effect of denervation 3 days prior to procurement.

NGF levels were found to be high in this vessel. The mean value for whole vessel was 33 pg/mg wet weight and 10 pg/mg wet weight for young and aged animals, respectively. When measured separately, 95% of the NGF was found to be associated with the adventitia in the young vessels and 70% in the aged vessels. The age-related loss of NGF was found to occur exclusively within the adventitia (levels in the aged adventitia being 15% of the young adventitia). Denervation resulted in a decrease in NGF in the whole ECA of both young (45%) and aged (34%) animals. However, this reduction was due exclusively to declines in the adventitia.

These results suggest that the vast majority of NGF detected in the external carotid artery in young animals is associated with nerve fibers and that the decline in vascular NGF levels with age, and following denervation, is due to reductions in the levels of NGF within such fibers and/or to a loss of NGF-containing fibers. Supported by the NIH (NS17131) and the British Heart Foundation (PG 93027).

492.10

EFFECTS OF AGE ON CEREBROVASCULAR VASOMOTION, P.M. Hutchins*, C.D. Lynch, P.T. Cooney, K.A. Curseen, W.E. Sonntag, Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine, of Wake Forest University, Winston-Salem, NC 27157.

Vasomotion is necessary to increase perfusion, reduce the capillary diffusion distances, and thereby maximize transport for a particular vascular geometry. We have developed methodology to analyze the acute spontaneous fluctuations in microvascular dimensions in a chronic cerebrovascular preparation. Intrinsic rhythmic changes in the diameter of cerebral arterioles in sedated young, middle-aged, and old Brown Norway rats were assessed *in vivo*. All diameter measurements were analyzed using a traditional graphic analysis technique. Graphic analysis of the data revealed that the vasomotion frequency was markedly reduced in the old (24-35 month) Brown Norway rat to less than half that observed in the young (5-9 month) rat, 2.19 ± 0.47 vs. 0.90 ± 0.40 cycles per minute. Furthermore, the young cerebral arterioles exhibited a significantly greater amplitude of vasomotion when compared to the old cerebral arterioles of the same diameter (3.35 ± 0.58 vs. 1.94 ± 0.29). Diameter measurements revealed that there was no difference in the size of the arterioles on the cortical surface of any of the three age groups of Brown Norway rats.

These data suggest that a fundamental defect of intrinsic tone develops in the old rat that may contribute to the age-related reduction in microvascular and cardiovascular regulation. We hypothesize that this defect affecting the short-term regulation of the microvascular system, is brought about by long-term structural regulatory alterations. Supported in part by grants 1PO1-NIA-11370, 1RO1-HL-13936 and 1T35-HL-07790.

492.11

INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) GENE EXPRESSION IN BRAIN VASCULATURE: INFLUENCE OF AGE. J.K.Brunso-Bechtold*, C.D.Lynch, T.M.McShane, P.T.Cooney, M. M.Niblock, P.M.Hutchins, and W.E.Sonntag. Departments of Physiology and Pharmacology, Neurobiology and Anatomy, and Program in Neuroscience, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1083.

IGF-1 has been shown previously to have an important role in brain function. This hormone stimulates acetylcholine release, neurite outgrowth, protein synthesis and the absence of IGF-1 or its receptor impairs brain development. Although IGF-1 is important for brain function, the source of IGF-1 is controversial. Specific brain regions are known to express IGF-1 during critical developmental stages and plasma IGF-1 may also have a role in brain function. There have been several reports that IGF-1 is expressed in peripheral vasculature during wound healing but IGF-1 gene expression in cerebral vasculature during development and/or aging has not been investigated. In this study, we assessed IGF-1 gene expression in cerebral vasculature by in situ hybridization using an antisense RNA probe. Sections from Fisher 344xBrown Norway rats of 3 ages (8-10,20 and 30 months of age) were prepared and IGF-1 gene expression in vasculature from several cortical regions were compared. Results indicate high levels of IGF-1 gene expression in vasculature from each of these areas. IGF-1 mRNA expression was 1.89 to 4.88 fold greater in vessels compared to surrounding tissues and no age-related differences were detected. Expression did not appear to be related to vessel size. Our results suggest that vasculature may be an important source of IGF-1 in brain throughout life.

Supported by 1 P01 AG11370.

492.12

AGE-RELATED DECREASES IN THE CORTICAL MICROVASCULATURE ARE RELATED TO DECREASES IN GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR-1(IGF-1). C.D.Lynch*, P.M.Hutchins, P.T.Cooney, J.K. Brunso-Bechtold, W.E.Sonntag, Dept. of Physiology/Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1083.

A reduction in cerebral blood flow is a hallmark of advancing age. In the present study, we utilized a chronic cranial window preparation in young (8 months) and old (29 months) Fisher 344xBrown Norway (F344xBN) and BN (5-11 mo. young and 24-35 mo. old) rats to investigate potential mechanisms that contribute to the decreased blood flow with age. Rats were sedated and the microvasculature of the cortical surface was mapped. Young F344/BN rats had an arteriolar endpoint density (where the vessel enters the cortical surface) of 17.7 ± 2.3 arterioles/mm^2 ($n = 15$) whereas old F344/BN rats had 12.1 ± 0.7 arterioles/mm^2 ($p < 0.05$). Similarly, young BN rats showed 14.9 ± 1.2 arterioles/mm^2 and old BN 9.4 ± 0.6 arterioles/mm^2($p < 0.05$). In a second study, we investigated whether daily injection of growth hormone (which decreases substantially in aged animals) would result in an increase in vascular density in F344xBN rats. Our results indicate that 0.25mg growth hormone/day for 32 days increases the arteriolar endpoint density in aged rats by 16%. In addition, arteriolar endpoint density was positively correlated with plasma levels of IGF-1 in BN rats. These data suggest that the age-related reduction in cerebral blood flow may be due to the observed decrease in cerebral arteriolar density, and that these alterations are associated with the age-related reduction in growth hormone and IGF-1. Supported in part by 1 PO1-AG-11370, 1RO1-HL-13936.

492.13

NEURONAL IGF-I mRNA EXPRESSION DURING DEVELOPMENT AND AGING IN FISCHER344XBROWN-NORWAY RATS M.M. Niblock*, C.D. Lynch, P.T. Cooney, T. McShane, J.K. Brunso-Bechtold, and W.E. Sonntag Depts. Neurobio./Anat. & Physio./Pharm., Prog. in Neuroscience, Bowman Gray School of Med., Wake Forest Univ., Winston-Salem, NC 27157

Previous studies have reported significant changes in the expression of insulin-like growth factor I (IGF-I) in specific cell populations of the developing rat brain. Although the expression of IGF-I has been shown to fluctuate in early postnatal life (Bondy, 1991), as of yet no study has assessed whether there are changes in the overall pattern of IGF-I expression that occur as the brain ages. The present study examined IGF-I mRNA expression in the aging Fischer 344 X Brown-Norway rat using a 376 base IGF-I specific RNA probe for *in situ* hybridization. Sagittal brain sections from seven age groups spanning from 2 months to 30 months were processed. Following hybridization, sections were coated with NTB-3 photographic emulsion (Kodak), allowed to expose for 9 days, developed, and counterstained with cresyl violet for light microscopic study. Four brain areas were the focus of computerized density analyses: the superior olivary complex, the hippocampus, the Purkinje cells of the cerebellum, and layer 2 of sensorimotor cortex. Preliminary results indicate high levels of neuronal IGF-I mRNA expression throughout the brain across the life-span. Although there appears to be a trend of decreasing IGF-I mRNA expression with age, these changes fall short of statistical significance. This finding is particularly interesting in light of the fact that plasma levels of IGF-I have been shown to drop dramatically with age. That IGF-I levels in the brain remain high in spite of the fact that plasma levels of IGF-I significantly decline suggests that IGF-I expression in the brain may be independent of systemic control. IGF-I may be playing a crucial role not only during development but also throughout adulthood, perhaps providing trophic support for neurons or maintaining dendrites and synaptic connections. Another possibility is that although IGF-I levels remain high as the brain ages, the expression of some other element of the IGF system, such as the type I IGF receptor or one of the IGF binding proteins, may change with age, producing a physiological effect. Future studies will investigate the role of IGF-I in the aging brain through the direct manipulation of the IGF system.

1 P01 AG 11370

492.14

CALORIC RESTRICTION INCREASES CORTICAL MICRO-VASCULATURE IN AGED ANIMALS. B.Poe*, C.Lynch, P.Cooney, J.K. Brunso-Bechtold, W.E.Sonntag and P.M.Hutchins. Departments of Physiology and Pharmacology, Neurobiology and Anatomy, and Program in Neuroscience, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1083.

With increasing age, there is a decline in cerebral blood flow that is associated with a decline in cortical microvasculature. Recent studies indicate that moderate caloric restriction (60% of ad libitum) decreases both functional and pathological changes with age and increases lifespan. In this study, we assessed whether acute or chronic caloric restriction ameliorates the age-related decline in cortical microvasculature. Male Brown-Norway rats were calorically restricted (n=7) or fed an ad libitum diet (n=13) from 4 months to 35 months of age. Animals were prepared with a chronic cranial window and 1-2 weeks later the cortical surface vasculature was mapped. Results indicate that the number of arteriolar penetrations into the cortical surface were maintained in calorically restricted compared to ad libitum fed animals (15.0 ± 0.9 vs 9.4 ± 0.6 arterioles/mm^2, $p < 0.05$). To study the acute effects of caloric restriction on cerebral microvasculature, ad libitum fed young (6 months) and old (29 months) animals were prepared with cranial windows and placed on a moderate caloric restricted diet for 35 days. Acute caloric restriction increased arteriolar endpoints by 28.2% in young and 12.2% in old animals. Our results indicate that moderate caloric restriction prevents the loss of cerebral arterioles with age and that acute caloric restriction is capable of increasing vasculature in both young and old animals.

Supported by P01AG11370, R01AG07752 and American Federation for Aging Research.

492.15

INSULIN-LIKE GROWTH FACTOR-1(IGF-1) INCREASES WORKING MEMORY IN AGED ANIMALS. M.Mooney, W.E.Sonntag*, M.Barra, X.Xu, S.Bennett, R.Ingram, B.Poe and A.L.Markowska. Department of Psychology, Johns Hopkins University, Baltimore, MD 21218 and Department of Physiology and Pharmacology and Program in Neuroscience, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157.

The age-related reduction in tissue protein synthesis appears to result, at least in part, from a decline in growth hormone secretion and a subsequent decline in IGF-1. IGF-1 has been implicated in differentiation of cortical neurons and regulation of acetylcholine release in hippocampal neurons. The present study was designed to assess whether behavioral deficits with age may be partially overcome by increasing levels of IGF-1. Fisher 344xBrown Norway male rats (4 months old and 32 months old) were preoperatively trained in behavioral tasks and subsequently implanted with osmotic minipumps to infuse IGF-1 (50ng/hour) or vehicle, icv. Animals were retested at 2 weeks and 4 weeks after surgery. IGF-1 improved performance in the repeated acquisition task. Mild improvements in some measures of the place discrimination task which assesses reference memory were also observed. IGF-1 had no effect on sensorimotor skills but reversed some age-related deficits in emotionality. These data indicate a potentially important role for IGF-1 in the reversal of cognitive impairments that occur in aged rodents.

Supported by NIH grants AG07735 and AG11370.

492.16

THE EFFECTS OF INCREASING AGE ON ANTISACCADIC EYE MOVEMENTS A. Olincy*, R.G. Ross, D.A. Young and R. Freedman. Schizophrenia Research Center, Univ. of Colorado Health Sci. Center, Denver, CO 80262.

As the brain ages, a decrease in frontal lobe volume with decreased cortical thickness, shrinkage of large neurons, cell loss and decreased density of synapses has been observed. Studies of aging in humans support decreased cerebral blood flow and metabolism in the frontal lobe. The antisaccadic eye movement task is known to be impaired in conditions with frontal lobe pathology. The antisaccadic task activates the frontal eye fields, supplementary motor area, thalamus, putamen and superior parietal lobe. This task measures motor, inhibitory and working memory abilities, functions that may become impaired with aging. Antisaccadic task performance decreases after the age of 60, with increasing numbers of prosaccades and increased latency of the antisaccade. There is no decrease in accuracy of the antisaccade once initiated. By examining the components of the antisaccadic task, impairment in frontal lobe functioning can be clarified.
Support: VA Medical Research Service

492.17

AGE-RELATED CHANGES IN THE RHESUS MONKEY: RECOGNITION MEMORY IN THE OLDEST OF THE OLD. M.B. Moss*, R.J. Killiany, D.L. Rosene and J. Herndon. Dept. of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118 and Yerkes Regional Primate Research Center, Emory University, Atlanta, GA. 30322.

Assessment of recognition memory was performed in a group of five rhesus monkeys 29 to 31 years of age. Based on recently established life span data of rhesus monkeys, this age range represents the "oldest of the old". Their performance was compared to that of five young adult animals (5 to 11 years of age) on two tasks of recognition memory: trial-unique delayed nonmatching to sample (DNMS), and delayed recognition span (DRST). The DNMS is a visual recognition task that requires the recognition of a novel from a familiar stimulus over a delay. The task was first administered with a 10 second interval between presentation of the sample and the recognition trial. Upon reaching learning criterion, performance was then assessed on two delay conditions with the interval between sample and recognition increased to 120 sec. and then 600 sec. The DRST is a recognition test that requires identification of a novel stimulus added to the increasing array of stimuli. The number of correct responses before committing an error constitutes the recognition span for that stimulus class. Animals were tested on two stimulus conditions, one using spatial position of identical plaques and the other using unique objects. Both a non-repeating and a repeating series of spans were used for each of the two conditions. The oldest of the old group was impaired on all conditions of the two tasks relative the young group. Relative to previous findings in a younger aged group (25-28) of monkeys, the oldest old were more impaired on acquisition of the DNMS task, but were not significantly different from this age range on other performance measures. The results point to a pattern of cognitive dysfunction toward the end of the life span that may be characterized by recognition memory impairment and a disproportionate decline in new learning. (Supported by NIH grants PO1 AG00001 and RR 00165)

492.18

AGE-RELATED CHANGES IN THE RHESUS MONKEY: PATTERNS OF COGNITIVE DECLINE. J. G. Herndon*, M. B. Moss, D. L Rosene, and R. J. Killiany. Yerkes Regional Primate Res. Ctr., Emory Univ., Atlanta, GA 30322 and Dept. of Anatomy and Neurobiology, Boston Univ. Sch. of Med., Boston, MA 02118.

We evaluated the performance of young rhesus monkeys (<15 years of age) on six measures of cognitive function to determine the prevalence of impairment among early aged (19-23 years), advanced aged (24-28 years) and oldest old (29-33 years) monkeys. Impairment was defined as performance that fell outside the range for that of young monkeys. For early aged monkeys, prevalence rates were: (1) 25% (95% Confidence Interval - CI: 10-38%) on initial acquisition of Delayed Non-Matching-to-Sample (DNMS), (2) 42% (95% CI: 21-73%) on performance of the DNMS, with 2-min delay, (3) 67% (CI: 43-91%) on the spatial condition of the Delayed Recognition Span Test (DRST), a test of short-term spatial memory, (4) 0% (CI: 0- 31%) for memory span in a non-spatial condition of the DRST, (5) 45% (CI: 24-77%) for in Spatial Reversal, a test in which a positional preference must be reversed, and (6) 27% (CI: 11-61%) for Object Reversal. For acquisition of DNMS, DRST - Non Spatial and Spatial Reversal, we detected a significant trend toward increasing prevalence of impairment in increasingly older groups of aged monkeys. Finally we used principal components analysis to derive a Cognitive Performance Index (CPI), a measure of global cognitive performance. The results confirm the multidimensional nature of age-related decline and provide the CPI as a measure representing the global cognitive capacity of the individual. The CPI was negatively correlated with age (r=-.78, n=53, p<0.001). (Supported by NIH grants PO1 AG00001, R55 AG12610 and RR 00165).

492.19

EARLY STEPWISE OPERANT TRAINING FACILITATES NOVEL LEARNING IN OLD-AGED RATS: BEHAVIORAL AND NEUROBIOLOGICAL MECHANISMS. K.Pawlik*, M.-L. Nuessgen, G.Sammer and H.-G.Thiele. Dept.s of Psychology and Internal Medicine, Univ.Hamburg, D-20146 Germany.

Earlier research by the first 2 authors showed major facilitation of novel learning in aged rats as function of raising in mo.s 1-3 after weaning. In this exp. behavioral and neurobiological mechanisms of across-age memory facilitation are studied in 150 young (4) and 240 old (15 mo.s at criterion-tests) HAN-Wistar rats raised according to a 3x2 factorial design (early-late-no enrichment; longitudinal training-no training). Criterion tests: 3 new learning tasks (simple vs. delayed-alternation bar-pressing; radial maze); spontaneous activity tests, immunology (blood) assays, EEG recordings, also across age; post-mortem histology (cortex, hippocampus) and HPLC (catechol., 4 brain regions). Results: Facilitation of novel learning due to enrichment is larger in old than in young rats, in old rats more effective if offered late. Facilitation from longitudinal training is more effective than, and additive to, enrichment.Unrelated to general activation, facilitation relates to higher (brainstem) norepinephrine levels, smaller immune assay age-decrements and higher (hippocampal) EEG (theta) power. Results are discussed within contexts of neuronal plasticity theory.

Source of support: DFG Pa 108/12-5

493.1

3D-MULTI-IMAGE-STACK-ACQUISITION-SOFTWARE (3D-MISA) FOR CONFOCAL LASER SCANNING MICROSCOPES

W. Zuschratter*[1], T.Steffen[1], K. Braun[1], B. Michaelis[2] and H. Scheich[1]
[1]Federal Inst. for Neurobiology, P. O. Box 1860, D-39008 Magdeburg, FRG
[2]Univ. Magdeburg P. O. Box 4120, 39016 Magdeburg, FRG

Confocal laser scanning microscopy is now widely used to produce image stacks from neuronal tissue with high resolution. However, image acquisition at high magnification is inevitably correlated with a limited overview of sections. To overcome this limitation we designed a software for the automatic acquisition of multiple image stacks in xy-direction (3D-MISA). The system consists of a 4 channel Leica TCS4D confocal laser scanning microscope which was equipped with a xy-motorized stage (Märzhäuser). The 3D-MISA software was linked to the microscope software (Scanware, Leica Lasertechnik). It allows storage and recall of 70 xyz-positions and the automatic 3D-scanning of image arrays between two selected xyz-coordinates. The number of images within one array is limited only by the amount of disk and/or memory available. Currently, our system provides 288 MB RAM which is sufficient to handle 1132 images with 512 x 512 pixels. In practice, 3 fluorescence channels with an array of 5 x 5 x 15 (xyz) images can be acquired during one scan process. Images are than temporally stored on a hard disk and later archived on a CD for further analysis. At the end of the scanning procedure the program provides a gallery of the extended focus images of each channel on a graphic monitor. A gallery of individual focal planes can also be calculated.

In summary, the 3D-multiscan software represents a powerful tool for confocal laser scanning microscopy because it allows image acquisition of coherent regions in combination with high resolution of the single images.

Supported by DFG INK15/A1 TP A4

493.2

SURFACE GUIDED RECONSTRUCTION OF 3D VOLUME FROM HISTOLOGICAL MATERIAL.

D. Kozinska[1*], J. Nissanov[2], O. Tretiak[1,2] and C. Ozturk[2]. [1]Dept. Elec. Comp. Eng. [2]Biomed. Eng. & Sci. Inst., Drexel Univ., Philadelphia, PA 19104

Reconstruction of brain volume from sectional material can reveal important anatomical information. However, accurate alignment of consecutive sections is typically difficult and consequently reconstruction is not done routinely. We have developed an algorithm which greatly simplifies the task. Using it, registration is guided by prior knowledge of the surface of the tissue. We have previously described a structured light technique which rapidly maps this surface before tissue sectioning (Nissanov et al., 1995, SPIE 2598:334-340) and here we report on the algorithm which employs this information to drive alignment. The algorithm consists of three stages. First, sections are aligned to each other using a 2D rigid body distance-based approach. Next, the aligned set is subjected to 3D rigid body matching to the surface (Kozinska et al., 1995, Neurosci. Abst. 21:427.10). In the subsequent step, inter–section shear is permitted to better match each section contour with the surface envelope. The last two steps are iterated until a stopping criterion is satisfied. To assess the performance of this algorithm we determined the average residual distance between each section and the surface. We found using 100-section tissue blocks from rat brains, that we obtain an average residual error of less than 4 pixels (160 microns). In addition to its utility in reconstruction of tissue sections, the algorithm, in combination with a reference atlas and 3D alignment, may also prove useful in multimodality fusion of data from different animals.

(Supported by NIH P41RR01638).

493.3

NEUROZOOM - TOPOGRAPHICAL MAPPING AND STEREOLOGICAL COUNTING, DISTRIBUTION OF DATA, AND COLLABORATIVE COMPUTING,

W.G. Young*, J.H. Morrison†, P.R. Hof†, E.A. Nimchinsky†, and F.E. Bloom. Dept of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037. Neurobiology, Mt. Sinai School of Medicine, NY, NY 10029†.

NeuroZoom is a software application for Macintosh computers, combining topographical mapping functions with stereological counting techniques to collect, in 3D, data on the distribution, density, and number of labelled profiles. The software provides unlimited anatomic structures and layering, and flexible visibility controls and graphical attributes. Stereological tools include optical disector, fractionator, rotator, and estimators for structural length, area and volume (see Hof et al, this meeting, for example).

NeuroZoom is being used to create a rat atlas (see Bloom et al, this meeting). Mapping and stereological tools then acquire precise, quantitative data to be displayed in the context of the atlas.

NeuroZoom supports data distribution and sharing in three ways: 1. Peer to peer: data are shared directly with any Internet NeuroZoom user. 2. Peer to server: local labs can install a server database. 3. Server to server: a centralized server acts as a registry for participating local servers. By devising and implementing this easy-to-use software running on any popular and low-cost personal computers, it should be possible to provide the neuroscience community shared common tools for data acquisition, storage, organization, access, distribution, and re-use in community networks.

(Supported by the Human Brain Project Consortium grant MH/DA 52154)

493.4

NEUROZOOM - NEUROANATOMIC ATLASES AND DATABASES FOR ACQUISITION AND DISPLAY OF STEREOLOGICAL DATA.

F.E. Bloom*, J.H. Morrison†, E. Battenberg†, and W.G. Young. Dept of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037. Neurobiology, Mt. Sinai School of Medicine, NY, NY 10029†.

NeuroZoom is a software application for Macintosh computers, combining topographical mapping functions with stereological counting techniques (Young et al, this meeting). With it, we have created multiple rat brain atlases with video images of microtome sectioned frozen surfaces, with multiple series of stained sections, and with high resolution MRI image sets. The basic protocol for serially sectioned prepared slides follows.

Two adult male rat brains (W. Welker, U of WI) were sectioned every 25μ in 2 adjacent series. Each adjacent section was stained either with cresyl violet or a fiber stain for a total of 166 horizontal and 500 sagittal sections. Each was imaged with a Zeiss Macroscope and captured with a Leaf digital camera at a resolution of 3400X2800, 24 bits, reduced in density to 72 dpi, and registered with previous sections. The images were opened by NeuroZoom and regions such as pial surface, gray/white matter boundaries, ventricular, and major nuclear groups, were demarcated, producing a template based, neuroanatomic structural atlas for the rat brain.

The combination of atlas templates, spatially correct precise data, and stereologically accurate counting profiles produce a usable database that supports uniform acquisition of data with NeuroZoom, and the development of probability models of the distribution of cellular profiles through regions of the brain that heretofore have been described only with subjectively acquired boundary information.

(Supported by the Human Brain Project Consortium grant MH/DA 52154)

493.5

THREE-DIMENSIONAL MORPHOLOGY OF ASTROCYTES EXPRESSING GREEN FLUORESCENT PROTEIN.

M.D. Andersen[1], R. Bizios[1], W. Shain[2], J.N. Turner[1,2], D.L. Martin[1,2*], D.H. Szarowski[3], M. Sena-Esteves[4], X.O. Breakefield[4].
[1]Rensselaer Polytechnic Institute, Troy, NY 12180, [2]Wadsworth Center and School of Public Health, Albany NY 12201-0509, [3]Wadsworth Center, Albany NY 12201-0509, [4]Massachusettes General Hospital, Harvard, Charlestown, MA 02129.

Cellular interactions in tissues are traditionally imaged using selective stains and immunohistochemistry. These methodologies use fixed tissues, precluding visualization of dynamic interactions. To overcome these difficulties astrocytes were transfected with Green Fluorescent Protein (GFP), resulting in cells expressing fluorescent protein allowing visualization of dynamic changes in cell morphology. Both LRM55 cells and astrocytes in primary cell cultures were used. In initial experiments to optimize transfection conditions, LRM55 cells were transfected with wild-type GFP and a red-shifted mutation. Expression of GFP was compared using cytomegalovirus (CMV) and phosphoglycerate kinase (PGK) promoters. The PGK promoter resulted in significantly higher expression of GFP with transient transfection. The stable transfection rate was low, <1%. More than 60 clones were screened and several of the most fluorescent lines were maintained for further experimentation. The red-shifted GFP construct was used to optimize visualization because this protein is optimally excited at ~490 nm, matching the excitation wavelength of the argon laser(488 nm). To increase the transfection rate in primary astrocyte cultures, a retroviral construct with the red-shifted GFP and the PGK promoter was used. The three-dimensional morphology of astroglia was observed following injection of varying numbers of cells into cerebellar organotypic cultures. Cerebellar cultures were prepared using 400-μm thick tissue slices from 14-day old Wistar rats and cultured for 7-14 days. In order to obtain the highest resolution, injected GFP-expressing cells were imaged using scanning confocal microscopy. Changes in cell morphology are being studied as both a function of time in three-dimensional organotypic culture and following applications of drugs that produce astrocyte stellation in monolayer culture. (This work is partially supported by NIH RR-10957.)

493.6

3-D RECONSTRUCTION OF GASTRIC EFFERENT NEURONS AND ASSOCIATED NEUROPEPTIDES AND RECEPTORS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS IN THE RAT.

L. A. Ladic and A. M. J. Buchan*. Dept. of Physiology, Univ. of B.C., Vancouver, B.C. V6T 1Z3 Canada.

Retrograde tracing, multi-label fluorescence immunohistochemistry, confocal microscopy and 3-D reconstruction techniques were combined to examine the spatial relationship of immunoreactive nerve terminals containing calcitonin gene-related polypeptide (CGRP) and substance P (SP) to identified gastric efferent neurons in the dorsal motor nucleus of the vagus in the brainstem of the rat. The availability of an antibody to the receptor for SP (NK-1r) also permitted observation of the association of peptide and receptor. In 3-D reconstructions of selected retrogradely-labelled gastric efferent neurons, CGRP-immunoreactivity (IR) was visualized in the form of continuous fibres that surrounded the cell body and primary projections of the neurons. Unlike CGRP, SP-IR was observed as punctate labelling of varicosities that surrounded all surfaces of the identified neurons. NK-1r-IR was localized to the cell membrane of the efferents as well as to a subpopulation of the surrounding fibres. SP-IR fibres were observed in close association with the NK-1r-IR on the surface of identified neurons and surrounding fibres. Although no discrete synaptic structures were observed, the close proximity of neuropeptides to the identified gastric efferents and, in the case of SP, to its receptor, would suggest the possibility for neural communication via volume transmission. The combination of techniques described permit detailed visualization and analysis of the 3-D spatial arrangement and location of peptide containing nerve terminals and receptors with respect to all surfaces of identified neurons.

NSERC (Canada)

493.7

STEREOLOGY AND COUNTING: ESTIMATION OF OBJECT HEIGHT IN SECTIONS; THIS CONVERTS THE LINDERSTRØM-LANG /ABERCROMBIE EQUATION ("ABERCROMBIE CORRECTION") TO THE EMPIRICAL METHOD EQUATION. J.C. Hedreen*. New England Medical Center, Boston, MA, USA.

Accurate estimation of object height in sections, in the axis normal to the plane of section, is difficult. This estimation is required, for example, in the Linderstrøm-Lang/Abercrombie (LLA) method for converting counts of profiles to object number. Gaule and Lewin [1896; see Hedreen and Vonsattel historical poster] pioneered a method for this conversion where they first, in a pilot study of 50 serially-sectioned neurons, found the mean number of profiles per cell (k), then obtained a total profile count (n), and finally divided k into n to reach an unbiased estimate for total neuron number (N). Thus, N=n/k. Coggeshall and colleagues [1984, 1990; Pover and Coggeshall, 1991] recently reintroduced and perfected this method under the name 'empirical method'. (Both empirical and LLA methods are now superseded by the disector method.) Consideration of the problem of calculating object height, h, from k shows that mean h=t(k−1), where t=section thickness. Thus preliminary estimation of k in serial sections allows estimation of object height whether h is >t or <t. It is, of course, necessary to estimate t, and also to attempt to correct k for lost caps. One could set out to use the LLA method, calculating h in this way. But substituting this formula for h into the LLA equation converts the latter into the empirical method equation: $N = n\frac{t}{t+h} = n\frac{t}{t+t(k-1)} = n/k$. Having done the serial section study, one would therefore be better off to forget about LLA and continue with the empirical method. (Supported by NIH-NS29484)

493.8

NEW CALIBRATION TECHNIQUE FOR CELL COUNTING METHODS APPLICABLE TO NEUROSCIENCE C. N. R. Henderson* and Qiang Zhang. Palmer Chiropractic University, 741 Brady Street, Davenport Iowa 52803

The ability to make accurate cell counts is essential in neuroscience; providing critical information in studies of the development and normal internal organization of the nervous system, as well as evaluation of the nervous system's response to toxins, hormones, metabolites, and physical trauma. Previous studies have been hampered by cell counting approaches that utilized correction factors to reconcile profile and cell counts (e.g., the methods of Abercrombie and Konigsmark). Unfortunately, the simplifying assumptions underlying these correction factors have been proven incorrect, casting doubt on the conclusions of many seminal neuroscience studies. Stereological cell counting methods, such as the physical and optical dissector methods, are unbiased by cell size or orientation and do not rely upon correction factors. Fundamental tenets regarding neurogenesis and branching of dorsal root ganglion cell processes have recently been challenged because of observations made with these new counting techniques. Coggeshall demonstrated that neuroscientists must calibrate their application of cell counting techniques. However, the present calibration method, serial reconstruction, is tedious and requires a great deal of time. In addition, serial reconstructions are not readily applied to calibrate the optical dissector approach. We developed a calibration standard that is inexpensive and easily produced in the laboratory. Moreover, this standard may be used to calibrate all of the new stereological cell counting methods. Our standard, 'Latex Particle Calibration Blocks,' was produced by embedding a known number and size distribution (3μm, 6μm, and 10μm) of red latex beads in JB-4 PLUS resin blocks. Constant sonication maintained the beads in suspension during the early phase of the embedding process. Latex bead populations of known counts were dispersed within 10 resin blocks without clumping, and the blocks were sectioned and counted using physical or optical dissector methods. Bead counts were validated by serially reconstructing three of the calibration blocks. There was excellent agreement between the serial reconstructions and the stereological bead counts in the 10 calibration blocks (coefficient of variation = 1.1 to 1.5%).

PRESYNAPTIC MECHANISMS: CALCIUM AND RELEASE

494.1

INTERACTION OF N-TYPE CALCIUM CHANNEL WITH THE SYNAPTIC CORE COMPLEX IS REQUIRED FOR NEUROTRANSMITTER RELEASE. S. Mochida*[1], Z.-H. Sheng[2], C. Baker[2], H. Kobayashi[1] and W.A. Catterall[2]. [1]Dept. of Physiology Tokyo Med. Coll., Tokyo 160, Japan and [2]Dept. of Pharmacology, Univ. Washington, Seattle, Washington 98195, U.S.A.

To examine the functional significance of the interaction of the synaptic core complex (Syntaxin, SNAP25 and VAMP/Synaptobrevin) with N-type Ca^{2+} channels in neurotransmission, we studied the effects of fusion proteins containing the synaptic protein interaction site of the N-type Ca^{2+} channel on synaptic transmission at cholinergic synapses formed between sympathetic neurons in culture. Block of the interaction between presynaptic Ca^{2+} channels and the synaptic core complex by the fusion proteins substantially reduced the efficiency of synaptic transmission. No effect was observed with related control fusion proteins, and no effect on N-type Ca^{2+} channel activity was observed at the site of injection in the cell body, consistent with the conclusion that the synaptic protein interaction site peptides reduce neurotransmitter release without alternation of Ca^{2+} channel function. These results indicate that interaction of presynaptic Ca^{2+} channels with the synaptic core complex plays an important role in neurotransmitter release and is required for efficient synaptic transmission.

494.2

PRESYNAPTIC 5-HT_3 RECEPTORS ARE PERMEANT TO Ca^{2+} UNDER PHYSIOLOGICAL CONDITIONS: DIRECT OBSERVATION USING CONFOCAL MICROSCOPY. P. Rondé and R.A. Nichols* Departments of Pharmacology and Anatomy and Neurobiology, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19129

Presynaptic regulation of neurotransmitter release by 5-HT_3 serotonin receptors has been inferred in several neuropharmacological studies. To directly observe responses to presynaptic receptor activation, we have recently utilized laser scanning confocal microscopy to measure Ca^{2+} changes in individual isolated nerve terminals (synaptosomes) from rat corpus striata using the fluorescent Ca^{2+} indicator Fluo-3. Addition of 5-HT_3 receptor agonists was found to induce Ca^{2+} changes in a subset of individual striatal synaptosomes. Here, we used the same method to investigate the pathway by which Ca^{2+} enters synaptosomes on stimulation of presynaptic 5-HT_3 receptors. In confirmation of previous results, the inorganic Ca^{2+}-channel blockers Cd^{2+} plus Co^{2+} (10 μM each) effectively blocked K^+-induced changes in $[Ca^{2+}]_i$. In contrast, Co^{2+}/Cd^{2+} did not block Ca^{2+} influx in response to the addition of the 5-HT_3 receptor agonist mCPBG (100 nM). Moreover, prior depolarization of the synaptosomes with 20 mM K^+ or replacement of external Na^+ by the non-permeant ion NMDG did not affect 5-HT_3 receptor-induced Ca^{2+} influx. These results suggest that, in contrast to postsynaptic 5-HT_3 receptors which, on activation, are mainly permeant to Na and K under physiological conditions and consequently induce a large membrane depolarization, presynaptic 5-HT_3 receptors are permeant largely to Ca^{2+}, leading to Ca^{2+} influx into the nerve terminal rivaling that found on K^+-induced depolarization. (Supported by a grant from the Allegheny-Singer Research Institute).

494.3

KINETIC PROPERTIES OF LARGE CONDUCTANCE K_{Ca} (BK) CHANNEL ON THE CHICK PRESYNAPTIC NERVE TERMINAL. Sun, X.P.* and Stanley, E.F., Synaptic mechanisms Section, NINDS, NIH, Bethesda, MD 20892

Several studies have suggested that BK channels in the nerve terminal are involved in the repolarization of the action potential and the cessation of transmitter release. However, these channels are known to be only slowly (>100 ms) activated by the direct elevation of internal calcium (Ca_i) and, furthermore, they are not significantly recruited by even strong depolarizations at normal Ca_i levels (10^{-8}-10^{-7}). We have explored the function of presynaptic BK channels by *in situ* recording using the calyx nerve terminal of the chick ciliary ganglion.

We first titrated channel open probability with changes in Ca_i and membrane potential. We next confirmed that these channels exhibit a long delay between direct application of Ca_i and channel opening and also that voltage tends to increase the fraction of channels open more than delay the activation time. We then tested whether external calcium (Ca_e; 2-110 mM) could affect the channels. There was no effect on channel activation with pulses beyond E_{Ca} but a prepulse to -20 or 0 mV, enabling Ca influx, reduced the time constants of BK activation by 20-100% in both whole-cell and on-cell configurations. This finding suggests that calcium influx can have a long-lasting effect on channel kinetics. Our results suggest that BK channels may be activated by microdomains close to the clustered calcium channels and that their recruitment may require a closely spaces series of action potentials so that the calcium entering during one impulse enables channel opening during the next.

Supported by the NINDS, NIH, Intramural Research Program.

494.4

PRESYNAPTIC FUNCTION AND MODULATION OF INHIBITORY AND EXCITATORY SYNAPSES IN RAT HIPPOCAMPAL CULTURES. Rosenmund, C.*, and Auger, C. MPI biophysical Chemistry, Göttingen, Germany.

The hippocampus has a variety of modulatory inputs that may change both local and global information processing by differentially affecting release and excitability of interneurons and principal cells. We tested presynaptic function in both cells using whole-cell recordings from single autaptic cultured 'microdot' neurons. The external medium was exchanged using a fast-flow system.

$[Ca]_o$-sensitivity of release was measured by varying [Ca]o from 0.5-10 mM in presence of 4 mM Mg. The Dose-response curves of the postsynaptic currents (PSC's) showed a similar sensitivity (EC_{50}=3.29 vs. 3.31 mM) for EPSC/IPSC's respectively with a slope of 2.28 and 2.14. However, during presynaptic high frequency stimulation (10Hz, 10s, 4 mM Ca) EPSC's showed less depression than IPSC's: 38±25% vs 92±2%(n=18). In 1 mM Ca, EPSC were potentiated by 98±35%, whereas IPSC showed reduction of 52±8% (n=22).

Modulation of release by various neurotransmitter was tested. GABAb agonist baclofen (50 µM) inhibited both synapses (EPSC by 85.3±3%;n=22/IPSC by 81.6±5.1%;n=22), the metabotropic agonists DCGIV (2-5 µM) and (1S-3R) ACPD (50 µM) inhibited only EPSC's (29.9±6%,n=26). Noradrenergic receptors inhibited a subpopulation of excitatory neurons via α_2 receptors (41.7±6.5%; n=32), whereas inhibitory cells showed an enhancement or inhibition of the IPSC (161-10%). Muscarine (100 µM) showed for both synapses either inhibition or an enhancement of PSC's. Adenosine (50 µM) reversibly inhibited EPSC's (78.1±5.0%;n=21 vs. 7.8±14.2%;n=6). No modulation of vesicle pool size by modulators, as defined by the vesicle release by hyperosmotic solutions, was seen for Baclofen, (1S-3R) ACPD, or Noradrenaline (n=10). These results suggest that inhibitory and excitatory synapses have similar Ca sensitivity during low frequency stimulation, but show different dependency on activity. Also, modulation of release is highly diverse between interneurons and principal cells in our preparation. C.R. is a Helmholtz fellow; C.A. is supported by the french government.

494.5

ENDOCYTOSIS FOLLOWING SINGLE DEPOLARIZATIONS IN BOVINE ADRENAL CHROMAFFIN CELLS. K.L. Engisch* & M.C. Nowycky. Dept. Neurobiol. & Anat., Med. Coll. Penn., Philadelphia PA 19129.

In the perforated patch recording mode, capacitance changes (ΔC_m) due to exocytosis (membrane addition) and endocytosis (membrane retrieval) are highly reproducible. Here we examine endocytosis following ΔC_m jumps elicited by calcium entry during single step depolarizations. Under control conditions (5 mM $CaCl_2$), exocytosis evoked by 40 ms depolarizations was followed by endocytosis that essentially matched the amount of exocytosis in 13/31 cells (compensatory retrieval). In 18/31 cells, exocytosis evoked by a 40 ms depolarization was unaccompanied by endocytosis (no retrieval). In contrast, 320 ms depolarizations initiated endocytosis in 30/31 cells, and in 18/31 cells, the amount of endocytosis greatly exceeded the peak exocytotic increase in ΔC_m (excess retrieval[1,2]). The decay in C_m during endocytosis could be fit with a single exponential ($\tau = 4.9 \pm 0.9$ s, n = 11), or the sum of two exponentials ($\tau_1 = 508 \pm 95$ ms, $\tau_2 = 5.0 \pm 0.5$ s; n = 13; or $\tau_1 = 1.3 \pm 0.2$ s, $\tau_2 = 32.8 \pm 7.4$ s, n = 8). A strong correlation exists between the presence of excess retrieval and a $\tau_1 < 1$ s following a 320 ms depolarization (10/14 cells). The kinetics of endocytosis observed here are remarkably similar to those described in whole cell recordings of melanotrophs[1] and calf adrenal chromaffin cells[2], suggesting that the underlying mechanisms of endocytosis are highly conserved. Supported by NS22281.

[1]Thomas et al., J. Cell Biol. 124:667, 1994
[2]Artalejo et al., Neuron, 16:195, 1996

494.6

THE ROLE OF N- AND P- TYPE CALCIUM CHANNELS IN REGULATING TRANSMITTER RELEASE FROM SYMPATHETIC VARICOSITIES OF THE MOUSE VAS DEFERENS. D. K. Kim and N. A. Lavidis. Narcotics Research Laboratory. The Institute for Biomedical Research, Department of Physiology, The University of Sydney, N.S.W., Australia, 2006.

Evoked transmitter release from the sympathetic varicosities of the mouse vas deferens is highly dependent on extracellular calcium ($[Ca^{2+}]_o$). Most spontaneous transmitter release is also dependent on $[Ca^{2+}]_o$ with about 20% of the spontaneous release persisting in zero $[Ca^{2+}]_o$. Of the 5 known voltage dependent calcium channels known only N- and P- type calcium channels have been shown to affect transmitter release at the sympathetic varicosities of the guinea-pig vas deferens (Smith & Cunnane, 1996, Neuroscience, 70: 817-824). In this study the effect of various calcium channel blockers on spontaneous and evoked transmitter release has been investigated in order to identify the types of voltage-dependent calcium channels found on the presynaptic membrane of the sympathetic varicosities of the mouse vas deferens.

Sympathetic varicosities were visualised using $DiCO_2$-fluorescence before placing an extracellular electrode over small groups of varicosities to record both evoked (EJCs) and spontaneous excitatory junctional currents (SEJCs). N- type calcium channels were blocked with ω-conotoxin (ω-CTX) GVIA while P- type calcium channels were blocked ω-CTX MVIIA and L- type calcium channels with nifedipine. ω-CTX MVIIA (16nM) decreased evoked average EJC amplitude by 19.6±10.6% (n=5) and in one preparation there was no effect. ω-CTX GVIA (40nM) decreased evoked average EJC amplitude by 79±8% (n=6). Nifedipine (10μM) did not effect evoked EJC amplitude (n=3). SEJC frequency was not affected by any of the calcium channel blockers used.

These results indicate that evoked transmitter release is dependent on calcium entry predominantly via N -type calcium channels and partly P- type calcium channels. Spontaneous transmitter release, although greatly reduced in $[Ca^{2+}]_o$, is not affected by N-, P- or L- type calcium channel blockers.

(This work was supported by an NH & MRC Project Grant 950213)

494.7

CALCIUM CURRENTS IN SYNAPTIC AND NONSYNAPTIC NEURITIC VARICOSITIES OF XENOPUS NERVE-MUSCLE CO-CULTURES. Robert E. Poage* and Stephen D. Meriney, Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Perforated patch clamp recording techniques were used to study Ca^{2+} currents in the varicosities formed by *Xenopus laevis* motor neuron neurites in culture. Neurites form varicosities (2-8 μm diameter) both at sites of muscle contact (synaptic) and at isolated points along the neurite (nonsynaptic). Whole cell currents recorded in 10 mM Ca^{2+} activated at about -20 mV and reached a peak at about +10 mV in both synaptic and nonsynaptic varicosities (V_{hold} = -60 or -80). Average Ca^{2+} current densities were 45±19 pA/pF at synaptic, and 41±12 pA/pF at nonsynaptic varicosities. Steady-state inactivation in varicosity recordings was estimated by from H-infinity plots, in which 50% current inactivation was achieved with a holding potential of approximately -30 mV. *w*-Conotoxin GVIA (1-2 μM) blocked 57 ± 7% of the peak I_{Ca} in synaptic varicosities and 52 ± 14% in nonsynaptic varicosities. *w*-Aga-IVA (500 nM) had little (<10%) or no effect on varicosity I_{Ca}. Dihydropyridines had no effect on I_{Ca} in varicosities or cell somata. All *w*-conotoxin GVIA resistant currents were blocked by 200 μM Cd^{2+}. These data indicate that Ca^{2+} currents in both synaptic and nonsynaptic varicosities were similar and was carried by N-type channels, as well as others that are, as yet, uncharacterized. Since our study of nonsynaptic varicosities was restricted to neurites that made contact with target at another site (to ensure that these were cholinergic), it is possible that target contact by a neuron influences that expression of calcium current at all varicosities along the neurite -- including those that were not in direct contact with target. Supported by NIH NS 32345 and MH 18273.

494.8

PRESYNAPTIC NMDA RECEPTORS CONTROL PRESYNAPTIC CALCIUM Amanda J. Holt*, Neil E. Schwartz and Simon Alford Department. of Physiology, Northwestern University Medical School, Chicago IL 60611.

The large reticulospinal axons of the lamprey have been shown to receive glutamatergic and GABAergic depolarizing synaptic inputs recorded under whole-cell patch clamp. These inputs, whether evoked or spontaneous, are mediated by the activation of NMDA, AMPA and $GABA_A$ receptors located on the axons adjacent to their presynaptic elements. Evoked synaptic inward currents were blocked by 2-amino-5-phosphonopentanoate (AP5; 100μM), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10μM) and bicuculline (5μM). The glutamatergic component of this input showed a reversal potential close to zero (0.3±9.1mV; n=3) and the NMDA component was sensitive to the presence of Mg^{2+} in the superfusate. Inward currents were activated in the presence of tetrodotoxin (1μM) by application of AMPA or NMDA. To investigate if the synaptic activation of presynaptic glutamate receptors could alter presynaptic Ca^{2+} concentrations, the 3000 MW dextran-amine-conjugate of Calcium Green™-1 (5mM) was applied to the cut end of the spinal cord. Tissue was then maintained *in vitro* for 12 to 24 hrs to allow retrograde labeling of the reticulospinal axons. Stimulus induced Ca^{2+} transients were readily observed in the axons with a confocal microscope following microstimulation of the spinal cord (5-25 shocks at 50 Hz; 5-15μA). Risetimes to individual stimuli were faster than the sampling rate (8ms for a single line) and the response decayed to background following 5 shocks in approximately 1.5s. Washout of Mg^{2+} from the superfusate caused a significant increase in the amplitude of the evoked Ca^{2+} transient (increase to 152±7% of control responses; n=6; $p<0.05$). Addition of AP5 (100μM) to the Mg^{2+} free superfusate reduced the evoked response significantly (reduced to 60±7% $p<0.05$). The data imply that stimulation of the spinal cord evoked an NMDA receptor-mediated rise in presynaptic Ca^{2+} in these axons. The Ca^{2+} entry may be via NMDA receptors activated by synapses onto the axons or by NMDA receptors acting as autoreceptors at the output synapse of the axon. Supported by NINDS grant # NS31713.

494.9

SIMULTANEOUS FLUOROMETRIC CALCIUM AND pH MEASUREMENTS IN BOVINE CHROMAFFIN CELLS. T.H. Müller• Max-Planck-Institute for Biophysical Chemistry, Dept. of Membrane Biophysics, 37077 Göttingen, Germany.

Transient changes in intracellular Ca concentration induced by Ca influx may also lead to corresponding changes in intracellular pH, since Ca ions are known to displace protons from many binding sites. In order to investigate the relationship between intracellular Ca concentration and pH, these two parameters were measured during voltage-clamp depolarisations causing influx of Ca into bovine chromaffin cells through voltage-gated Ca channels.

The whole-cell configuration of the patch clamp technique was used to load the cells with the fluorescent indicator dyes fura-2 and BCECF, sensitive for Ca ions and protons, respectively. A polychromatic light source was used to excite the dye mixture at different wavelengths and epifluorescence signals were recorded using a CCD camera system. An extension of the classical fluorescence ratio method was developed to separate pH and Ca signals. Both excitation and emission spectra of the individual dyes and the dye mixture were recorded from loaded cells to assess possible dye interactions.

Depolarisations caused a reduction in pH as well as an increase in Ca concentration. The time course of pH changes differed from those of Ca, the latter having a faster rise and decay. These correlated changes may be due to an exchange of Ca ions and protons with cellular compartments other than the cytosol.

Supported by the DFG (Deutsche Forschungsgemeinschaft; Mu1243/1-1)

494.10

PRESYNAPTIC CALCIUM DYNAMICS AT A PLASTIC GLUTAMATERGIC SYNAPSE Neil E. Schwartz*, Amanda J. Holt and Simon Alford Department of Physiology, Northwestern University Medical School, Chicago IL 60611.

Glutamatergic synapses between vestibulospinal and reticulospinal neurons in the lamprey brainstem show long-term potentiation (LTP) following tetanic stimulation of the vestibulospinal pathway. This LTP is prevented by bath application of the mGluR antagonist (R,S)-α-methyl-4-carboxyphenylglycine (MCPG; 500 μM) and requires a postsynaptic Ca^{2+} transient during LTP initiation. Calcium dynamics were investigated at both presynaptic and postsynaptic elements of this synapse. Postsynaptic neurons of the reticulospinal and presynaptic elements of the vestibulospinal systems were loaded with Calcium Green™-1 by retrograde labeling following application of the 3000 MW dextran-amine-conjugate of the dye to the cut end of the spinal cord. During and following tetanic stimulation, a long-lasting Ca^{2+} transient was observed in both pre- and postsynaptic elements with similar decay times. The postsynaptic Ca^{2+} transient was markedly reduced by the application of the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) and the NMDA receptor antagonist 2-amino-5-phosphonopentanoate (AP5; 100μM); the presynaptic Ca^{2+} transient was also depressed but to a lesser extent (to 82±6% of control; $p<0.05$). In the presence of CNQX and AP5, MCPG (500 μM; a dose sufficient to block LTP induction) caused a significant increase in the amplitude of the presynaptic Ca^{2+} transient (to188±22% of response in CNQX and AP5; $p<0.001$). However, increasing the dose of MCPG to 1mM reversed this effect. These concentrations of MCPG had no effect on the amplitude of EPSCs recorded in reticulospinal neurons following either single shock stimulation of the vestibular tracts or repetitive stimulation. Additionally, in the lamprey spinal cord the mGluR agonists L(+)-2-amino-4-phosphonobutyric acid (L-AP4) and (1S,3R) 1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) had no effect on the amplitude of voltage-gated Ca^{2+} currents. Bath application of quisqualate (5-100μM), in the presence of CNQX (10μM) and tetrodotoxin (1μM), to activate mGluRs caused a dose-dependent increase in presynaptic Ca^{2+}. There may be at least two MCPG-sensitive presynaptic mGluRs at this synapse. Supported by NINDS grant # NS32114.

494.11

A SIMPLE MODEL OF CALCIUM DYNAMICS AND TRANSMITTER RELEASE FROM NERVE TERMINALS IN THE GUINEA PIG HIPPOCAMPUS.

P. Saggau*, L.-G. Wu and S.R. Sinha. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Most presynaptic terminals in the mammalian CNS are relatively inaccessible to direct investigation. We have overcome this limitation by using presynaptic calcium transients ($[Ca^{2+}]_{pre}$) optically recorded with either a high-affinity (fura-2) or a low-affinity (furaptra) calcium indicator. A simple model of a mammalian presynaptic terminal was used to constrain several parameters involved with transmitter release and calcium dynamics in the presynaptic terminals of the Schaffer collateral-CA1 synapse in the guinea pig hippocampal slice. The model consists of concentric spherical shells with radial diffusion only. In addition to diffusion, the dynamics of Ca^{2+} binding to intrinsic buffers and to calcium indicators is also included. Transmitter release is modelled as a power function of $[Ca^{2+}]_{pre}$ and a saturable process with a finite number of release sites.

Model parameters were adjusted to fit the decay time course of the optically-recorded transients. This procedure indicated that the predominant intrinsic calcium buffer in these terminals is relatively slow (k_{on}~10^5/M·sec). Both furaptra and fura-2 can be used to accurately measure the degree of nonlinearity between $[Ca^{2+}]_{pre}$ and transmitter release. Thus, while fura-2 may be saturated by the local high $[Ca^{2+}]$ domains responsible for transmitter release, the volume-averaged fura-2 signal still accurately reflects relative changes in these domains. This places a further constrain on the system: the I_{Ca} evoked by a single action potential must be small enough that fura-2 is not saturated and can reflect changes in I_{Ca}. Putting this constrain on the model suggests that I_{Ca} will be on the order of 1 pA, which means that very few calcium channels (<10) are opened.

Supported by NIH NS-33147 to P.S. and NRSA MH-10491 to S.R.S.

494.12

ACCOUNTING OF Ca^{2+} & Na^+ IN PRESYNAPTIC AXON VARICOSITIES

J.L. Winslow*[1,2], R.L. Cooper[2], H.L. Atwood[1,2]. [1]Biomedical Eng., [2]Physiology Dept. U. of Toronto, Toronto, Ont. M5S 1A8.

Entry of Ca^{2+} via voltage-activated (Ca_V) channels normally triggers neurotransmitter release. Reconstructed serial electron microscope sections of presynaptic varicosities from crayfish opener muscle neuromuscular junctions show 30-50 synapses which each contain 0-5 active zones (AZs) where Ca_V channels are located.

To investigate the dynamics of ion accumulation during homosynaptic frequency facilitation, we developed a computational model in which concentrations of Ca^{2+}, Na^+, K^+, and Ca^{2+} buffers can be estimated within a whole presynaptic varicosity. Freeze fracture micrographs of crayfish presynaptic active zones, AZs, show prominent intramembrane particles which are thought to represent Ca_V and Ca^{2+}-activated K^+ channels, and thus provide an estimate of their densities.

The effects of numbers of active AZs per synapse and numbers of synapses per variocosity on total $[Ca^{2+}]_i$ and immobile buffer products were investigated. In long pulse trains, we found that $[Ca^{2+}]_i$ was a linear function of action potential frequency for moderate frequencies but non-linear for higher frequencies due to the change in Ca^{2+} equilibrium potential when $[Ca^{2+}]_i$ increases, and interaction of Ca^{2+} pumps. Funded by NSERC, MRC, & NCE of Canada.

494.13

CORRELATION BETWEEN VESICULAR VOLUMES AND QUANTAL SIZE IN BOVINE CHROMAFFIN CELLS.

M.I. Glavinović*[,1], M.L. Vitale[2] and J.-M. Trifaró[2] Departments of Anaesthesia Research[1] and Physiology[1] McGill University, Montreal P.Q. H3G 1Y6 Canada and Department of Pharmacology[2] University of Ottawa, Ottawa Ont., K1H 8M5 Canada.

Attempts have been made long ago to measure the vesicular diameters from electronmicrographs of thin sections of secretory cells and to estimate the vesicular volumes and the concentration of the substances stored. Since the crossections of the vesicles do not occur exclusively over the largest diameter, but randomly, corrections were suggested to account for it (Coupland, *Nature*. **217**, 384, 1966). In this study we estimated the probability of 'true' vesicular diameters (pdt) from crossectional vesicular diameters determined morphometrically from electron micrographs using Monte Carlo simulation that assumes random and uniform sections. Probability density function (pdf) of the 'true' vesicular diameters is calculated by adding ptds corresponding to each crossectional diameter. Pdfs of crossectional diameters of both adrenaline or noradrenaline secreting cells were skewed, thus not well described by a gaussian function. Basic parameters (means, coefficients of variation and skews) were greater for 'true' than for crossectional vesicular diameters, and for noradrenaline than for adrenaline secreting cells. The relationship between quantal size (determined from the areas underneath the spontaneous current spikes measured electrochemically) and vesicular volume is positive throughout, but becomes less pronounced at higher volumes suggesting that the internal concentrations are lower the greater the vesicular size. Thus vesicular volumes alone do not determine the vesicular contents. Binding capacity of chromogranin and/or ATP - probably plays a major role in determining the amount of catecholamine stored in the vesicle.

Supported by the Medical Research Council of Canada.

494.14

EVIDENCE FOR MULTIQUANTAL RELEASE AT THE HIPPOCAMPAL MOSSY FIBER SYNAPSE

D.A. Henze* and G. Barrionuevo *Department of Neuroscience and Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, PA 15260.*

We have previously shown that the large mEPSCs (>30 pA) that are observed in whole-cell recordings from CA3 pyramidal cells arise predominantly from the mossy fiber (MF) synapse (Henze et al., submitted). The largest of these mEPSCs can exceed 400 pA. In contrast, recordings from CA1 pyramidal cells reportedly fail to show mEPSCs with amplitudes greater than 30 pA (e.g.: Malgaroli and Tsien, 1992). We hypothesize that the mechanism that gives rise to the largest MF-mediated mEPSCs is the coordinated release of multiple quanta from the multiple release sites in the large MF bouton as seen at the electron microscopic level (Hamlyn, 1962).

Whole cell recordings (V_{rest}= -80 mV) were obtained from CA3 pyramidal cells in hippocampal slices submerged in ACSF containing 1μM TTX and 10 μM bicuculline at 33°C. mEPSCs were collected in three minute epochs and analyzed using an algorithm which automated detection and amplitude measurements. Bath application of 1nM α-latrotoxin (LTX), which directly induces vesicular release, caused a 4.26 ±0.89 fold increase in mEPSC frequency while simultaneously shifting the cumulative probability histogram of amplitudes towards smaller amplitudes (n=5: $p<0.05$ K-S test). This suggests that LTX's effects occur downstream of the mechanism which leads to the large MF mEPSCs. We then investigated whether release from intracellular Ca^{2+}stores was the mechanism by which multiple vesicles could be synchronously released in a spontaneous manner. Bath application of 1μM thapsigargin (a Ca^{2+}/ATPase inhibitor) caused a shift in the cumulative probability amplitude histogram towards smaller amplitudes (n=2). Similarly, bath application of 5-15 μM cyclopiazonic acid (a Ca^{2+}/ATPase inhibitor) produced a shift of the cumulative probability amplitude histogram towards smaller amplitudes (n=4/6). We conclude that multiquantal release occurs in the absence of action potentials and that intracellular Ca^{2+} stores play a role in synchronizing the release of multiple vesicles. Supported by NS24288 and NIMH predoctoral fellowship10474.

494.15

SOURCES OF SYNAPTIC UNRELIABILITY INVESTIGATED WITH CALCIUM IMAGING IN CULTURED CORTICAL AND HIPPOCAMPAL NEURONS.

P.J. Mackenzie* and T.H. Murphy. Kinsmen Lab, Dept. of Psychiatry, University of British Columbia, Vancouver, Canada V6T 1Z3

Changes in reliability at single CNS synapses may mediate various forms of neuronal plasticity. Potential presynaptic sources of variability in synaptic transmission include action potential generation and conduction, Ca^{2+} influx at release sites and vesicle trafficking. To evaluate presynaptic sources of unreliability, we have combined current-clamp recording and Ca^{2+} imaging. Single action potentials (APs) elicited at the soma were reliably conducted (>96% of 994 events) throughout the main axon and collaterals to trigger localized Ca^{2+} influx at all axonal boutons. We confirmed that boutons were functional release sites using FM 1-43, a marker of vesicular turnover. Although conduction was reliable, we observed small fluctuations in the Ca^{2+} transient (<20%) that could impact upon release, given a power relationship between Ca^{2+} influx and release (*Neuron, 16:* 783). We have further investigated conduction reliability during AP stimulation of varied frequency by comparing the Ca^{2+} transient integral observed to that expected for successful conduction. Four APs elicited at the soma (25 Hz) were reliably conducted throughout all axonal branches. At higher frequency (4 APs at 100 Hz), conduction was still reliable despite progressive decreases in the amplitude of the action potential during the train (in fact ~10% facilitation of the Ca^{2+} transient was observed). Other sources of unreliability were apparent as differences in the magnitude of Ca^{2+} influx between release sites were observed. Preliminary data suggest a positive correlation between Ca^{2+} influx and FM 1-43 loading within a single axon. In conclusion, reliability within a single axon is not likely affected by AP conduction and may be influenced by observed fluctuations in the presynaptic Ca^{2+} transient and differences between boutons in Ca^{2+} influx or vesicle turnover.

Supported by MRC of Canada and EJLB Foundation.

494.16

PRESYNAPTIC EFFECTS ON AMPLITUDE DISTRIBUTIONS OF RESPONSES TO ASYNCHRONOUS GABA-RELEASE EVOKED FROM A SINGLE PRESYNAPTIC NEURON *IN VITRO*.

Jan C. Behrends and Gerrit ten Bruggencate*. Department of Physiology, University of Munich, 80336 München, Germany

The scope of the functional repertoire of single release sites during synaptic transmission is an unresolved matter but it has been proposed that postsynaptic responses at a given site are limited to vary between 0 and 1 quantum by either presynaptic (one vesicle-hypothesis) or postsynaptic (receptor saturation) constraints.

We have recorded inhibitory postsynaptic Cl^--currents during evoked synaptic transmission between pairs of GABAergic striatal neurons in cell culture under conditions where transmitter release was desynchronized by substituting $SrCl_2$ for $CaCl_2$ in the extracellular medium. A late ($\Delta t>50$ ms) phase of the responses consisted of a succession of clearly discernible events with waveforms indistinguishable from ("quantal") miniature postsynaptic currents. Like miniatures, the asynchronous evoked events showed wide, positively skewed amplitude distributions ranging from ~5 to >100 pA at -70 mV holding potential. This amplitude distribution was shown to be sensitive to manipulations known to affect the probability of transmitter release, such as variations in Sr^{2+}-concentration or addition of the $GABA_B$-receptor agonist baclofen (5-30 μM): in addition to the expected changes in their frequency, significant (Kolmogorov-Smirnov-test) concurrent shifts in the amplitude distribution of asynchronous events were observed.These results suggests that, at the synapses studied here, the amount of transmitter released simultaneously determines the size of elementary responses underlying evoked synaptic transmission and that this quantity may express presynaptic changes in synaptic efficacy.

Supported by the Deutsche Forschungsgemeinschaft (Grants Be-1739 and SFB 220 TP A1).

494.17

GIANT, TTX-INSENSITIVE, INHIBITORY POSTSYNAPTIC CURRENTS IN CULTURED NEURONS. C. A. Lewis* and D. S. Faber. Dept. of Neurobiology & Anatomy, Med. Coll. of Penn. & Hahnemann Univ., Philadelphia, PA 19129.

In whole-cell patch clamp studies on cultured rat embryonic spinal cord and medullary neurons, bathed in TTX, APV, and CNQX, large and long-lasting, spontaneous IPSCs (LIPSCs) were occasionally recorded. The amplitudes of these events were an order of magnitude larger than those of mIPSCs. Since these large currents had reduced amplitudes in Ca-free saline and in solutions containing glycinergic or GABAergic antagonists, we conclude that they were probably produced by large and prolonged release of glycine and/or GABA, which subsequently bind to their postsynaptic receptors.

The frequency of mIPSCs increased dramatically during the long, slow decay phase of these LIPSCs. Considering the requirement for extracellular calcium for the occurrence of these large responses, we hypothesize that this increased frequency reflected an increased intracellular [Ca^{++}] in the presynaptic terminal. Similar evidence for LIPSCs and prolonged transmitter release was observed in cell-attached patches which also exhibited the smaller, mIPSCs, suggesting that these large events are properties of single synaptic terminals.

Periodic large increases in the frequency of mIPSCs occurred in both cell-attached patches and in the whole-cell mode, and these increases were only sometimes associated with the LIPSCs. The rhythmicity in both recording configurations had similar temporal characteristics, with average interburst intervals of 5 and 12-14 s. Presumably these bursts of mIPSCs reflected periodic oscillations in the [Ca^{++}] in presynaptic terminals.

Both the probability and the frequency of occurrence of LIPSCs doubled during the 7 day period of time in culture when experiments were performed, suggesting that these large currents may play a role during development.

Supported by NIH grant NS21848 to D.S.F. and NS27016 to C.A.L.

494.18

MOTONEURON NERVE TERMINAL POTASSIUM CURRENT IS PREDOMINATELY CALCIUM-DEPENDENT. John M. Pattillo* and Stephen D. Meriney. Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

Calcium-dependent potassium channels have been localized to the active zone of adult frog neuromuscular junctions (Robitaille, et al,: Neuron, 11:645). Release of neurotransmitter following single action potentials has been shown to be potentiated by blockade of these channels with charybdotoxin, presumably due to an increase in calcium influx resulting from a widening of the presynaptic action potential (Robitaille and Charlton, J. Neuroscience, 12:297). To investigate the functional expression of calcium-dependent potassium channels in a nerve terminal, we used perforated patch techniques to measure calcium-dependent potassium current (I_{Kca}) in synaptic varicosities of *Xenopus* neuron-muscle co-cultures. I_{Kca} comprised a significant majority (60-75%) of the total potassium current in synaptic varicosities (evoked by step to 10mV, Vhold = -80mV). The activation of this current (time to 50% of peak) was approximately 1 ms. In contrast, our preliminary data suggests that I_{Kca} makes up a minority of potassium current in the somas of motoneurons which innervate co-cultured muscle cells. These data suggest that I_{Kca} is selectively expressed in nerve terminals. Further, I_{Kca} was activated quickly by depolarization. Taken together, these data are consistent with a role for I_{Kca} in regulating calcium influx and transmitter release following single action potentials.

Supported by NIH NS32345

494.19

RELEASE OF PEPTIDE AND CLASSICAL TRANSMITTERS WITH SINGLE ACTION POTENTIALS. M.D. Whim* and L.K. Kaczmarek. Dept. of Pharmacology, Yale University School of Medicine, New Haven, CT 06520-8066.

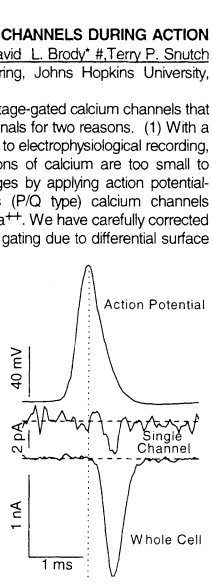
Studies in a variety of systems have led to the general formulation that the release of peptide transmitters requires high frequency firing. We have examined this question using Aplysia neuron B2 which synthesizes the small cardioactive peptides A and B (SCPs) and ACh (Lloyd et al, 1985). By culturing single B2 neurons with single pleural sensory neurons (SN) as post-synaptic detector cells, it has been possible to detect the release of the SCPs. In many experiments, a single pre-synaptic action potential was sufficient to induce a slow inward current in the SN. The inward current is likely to involve the release of the SCPs since application of exogenous SCPB to the SN evoked a current with a similar time course, conductance change and reversal potential to the synaptic current. Further, the synaptic current was not markedly affected by a cholinergic antagonist which blocked the effect of ACh on the SN. In contrast, when single B2 neurons were cultured in a soma-soma configuration with buccal neuron B3, single B2 action potentials evoked a rapid outward current in the post-synaptic B3 at a holding potential of -40mV. This outward current was blocked by application of d-tubocurare, reversed around -60mV, and was mimicked by the application of ACh but not the SCPs. The rapid outward current is therefore consistent with the release of ACh from B2. Thus the release of both classical and peptide transmitters from neuron B2 can be evoked with single action potentials. (Supported by NIH).

CALCIUM CHANNEL: STRUCTURE, FUNCTION, AND EXPRESSION II

495.1

GATING OF RECOMBINANT A-CLASS CALCIUM CHANNELS DURING ACTION POTENTIAL-SHAPED VOLTAGE WAVEFORMS. David L. Brody* #,Terry P. Snutch and David T. Yue#. #Dept. of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, 21205

Little is known about the physiological gating of voltage-gated calcium channels that trigger neurotransmitter release from presynaptic terminals for two reasons. (1) With a few exceptions, presynaptic terminals are not accessible to electrophysiological recording, and (2) unitary currents at physiological concentrations of calcium are too small to resolve clearly. We have approached these challenges by applying action potential-shaped voltage waveforms to recombinant A-Class (P/Q type) calcium channels expressed in HEK 293 cells and recording in 90 mM Ba^{++}. We have carefully corrected for liquid junction potentials, and for the shift in voltage gating due to differential surface charge screening between solutions containing 2 mM Ca^{++} and 90 mM Ba^{++} (+26 mV). Based on whole cell data there do not appear to be significant differences in gating other than this shift. Thus the single channel (middle) and whole cell (bottom) records shown in the figure are likely to accurately represent the gating of these channels to action potentials (top, similar to those recorded by Borst et.al at the Calyx of Held (*J Physiol*, **489** 825-840.)) under physiological conditions. The inward current is extremely brief even at room temperature, activating and deactivating entirely within the <1 msec repolarizing phase of the action potential. Deactivation is nearly complete before the hyperpolarizing phase. Thus the temporal precision of the action potential seems preserved or even enhanced at the level of calcium influx. Supported by a NSF PFF grant to DTY and a MSTP fellowship to DLB



495.2

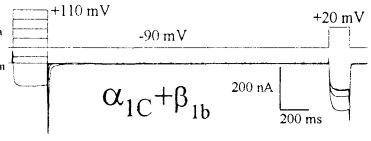
INFLUENCE OF DIFFERENT β SUBUNITS ON THE BIOPHYSICAL PROPERTIES OF THE HUMAN α_{1A-2} CONTAINING CALCIUM CHANNELS STABLY EXPRESSED IN HEK293 CELLS. A. Urrutia, M. Hans*, P. Brust, C.C. Lu, E.C. Johnson, M. Harpold and K. Stauderman. SIBIA Neurosciences, Inc., La Jolla, CA 92037.

The biophysical properties of recombinant human calcium (Ca^{2+}) channels were studied in HEK293 cells stably transfected with cDNAs encoding full-length human α_{1A-2} and $\alpha_{2b}\delta$ subunits together with the β_{1b}, β_{2e}, β_{3a} or β_{4a} subunit. Whole-cell recordings revealed that the magnitude of the voltage-activated barium (Ba^{2+}) currents varied with the coexpression of the different β subunit. The peak current amplitude was similar with β_{4a} and β_{2e} (1.5 ± 0.6 nA, n=14 and 1.3 ± 0.6 nA, n=6) and similar with β_{3a} and β_{1b} subunits (0.3 ± 0.1 nA, n=35 and 0.6 ± 0.5 nA, n=7). The voltage-dependence of channel activation was determined from tail-current analysis and could be described by a Boltzmann function with $V_{1/2}$ values varying between 9.4 mV (β_{1b}) and 19 mV (β_{3a}). The rank order for the rate of activation was $\beta_{3a}>\beta_{1b}\approx\beta_{2e}>\beta_{4a}$. The properties of current inactivation kinetics varied substantially with the different β subunits. Cells expressing β_{2e} and β_{3a} displayed bi-exponential inactivation with time constants (τ_1,τ_2) of 44 ± 13, 301 ± 153 ms and 112 ± 37, 1200 ± 750 ms, respectively, while inactivation kinetics with β_{1b} and β_{4a} were mono-exponential with time constants of 347 ± 123 and 415 ± 80 ms. The rank order for the rate of inactivation was $\beta_{3a}>\beta_{1b}>\beta_{4a}>\beta_{2e}$. The noninactivating component with β_{1b} was substantially larger (36 ± 11%) than with the other β subunits (10-14%). The steady-state inactivation (h_∞) was determined using a 20 s test pulse. The $V_{1/2}$ for h_∞ was most negative for β_{4a}: -31 ± 1 mV followed by β_{3a}: -27 ± 1 mV, β_1: -19 ± 1 mV, and β_{2e}: -16 ± 2 mV. These findings demonstrate that biophysical properties of calcium channels containing the α_{1A-2} subunit are critically dependent on the specific β subunit. The β subunits may also alter pharmacological characteristics of the channel (see Stauderman et al. this meeting).

495.3

A LONG-LASTING FACILITATION OF THE α_{1C} + β_{1b} CALCIUM CHANNEL. J. L. Costantin*[1], J. Zhou[2], L. Birnbaumer[2] and E. Stefani[2]. [1]Dept. of Neurology, [2]Dept. of Anesthesiology, UCLA School of Medicine, Los Angeles, CA 90024-1769

The α_{1C} calcium channel expressed in *Xenopus* oocytes displays prepulse facilitation (Bourinet *et al.*, EMBO J. 1994), that is dependent on the identity of the accessory β subunit that is coexpressed (Costantin *et al.*, Biophysical J. A186, 1996; Cens *et al.*, Pflugers Arch. 1996). We report here that coexpression of the β_{1b} or β_3 subunit causes a long-lasting facilitation that lasts for several seconds. The Figure illustrates currents after coexpression of the α_{1C} + β_{1b} subunit. The current during the second-pulse is elicited by a voltage step to +20 mV, and the prepulse is delivered between the holding potential (-90 mV), and +110 mV, in +50 mV increments. The current during the second-pulse is facilitated by 1.8 fold after an interval of two seconds between the first- and second-pulse. The long-lasting facilitation is not present in the α_{1C} channel when expressed alone, although a shorter-lived potentiation has been reported that decays on the order of milliseconds (Sculptoreanu *et al*, PNAS., 1993).



(Supported by N.I.H grants AR-38970 to E.S and AR-43411 to L.B.)

495.4

Cloning of a calcium channel β subunit from the jellyfish *Cyanea capillata*. Michael C. Jeziorski*, Peter A.V. Anderson, and Robert M. Greenberg. The Whitney Laboratory, University of Florida, St. Augustine, FL.

Voltage-gated calcium channels in vertebrates consist of a pore-forming α_1 subunit and several associated subunits (β, $\alpha_2\delta$, and sometimes γ). Expression of channel subunits in *Xenopus* oocytes or other heterologous systems reveals that β subunits have profound effects on the kinetics of α_1 subunits, and highly conserved regions within each subunit are involved in α_1-β interaction. Although calcium channel subunits have also been cloned from dipterans, the degree to which calcium channel structure is conserved throughout metazoans is not fully understood. Cnidarians comprise the earliest existing species to possess differentiated tissues, and voltage-gated calcium currents have been identified in neurons of the scyphozoan jellyfish *Cyanea capillata* and in neural and muscle cells of other cnidarians. We have previously cloned a full-length cDNA from *Cyanea* encoding a homologue of L-type calcium channel α_1 subunits. Using RT-PCR with degenerate oligonucleotide primers, combined with cloning techniques to extend cDNA sequences, we have now isolated a full-length clone from *Cyanea* encoding a homologue of a calcium channel β subunit. The cDNA encodes a protein of 457 amino acids that exhibits 47-54% identity to insect and vertebrate β subunits, but is most conserved within the region implicated in interaction with α_1 subunits. (Supported by NRSA grant MH 10625 to MCJ.)

495.5

ISOLATION, CHROMOSOMAL LOCALIZATION AND CHARACTERIZATION OF THE HUMAN N-TYPE CALCIUM CHANNEL α1B SUBUNIT GENE, D.S. Kim[1], H. H. Jung[1], S. H. Park[2], A. Thomas[1], and H. Chin.*[1] [1]Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892 and [2]Dept. of Anatomy, Korea University College of Medicine, Seoul, Korea.

ω-conotoxin-sensitive N-type calcium channels, unlike dihydropyridine-sensitive L-type channels, are exclusively expressed in nervous tissues. To understand the molecular basis for neuron-specific expression of the N-type channel, we have isolated genomic clones encoding human α1B subunit gene, localized to the short arm of chromosome 9 (9q34) by fluorescence *in situ* hybridization, and characterized its 5' upstream region. The promoter region of the human α1B gene is highly GC-rich, and lacks a typical TATA box but contains several consensus sequences for transcription factor binding. Primer extension experiments revealed the presence of a major transcription start site located at -147 and a minor site located at -151. Transfection experiments using luciferase fusion gene constructs demonstrate that: 1) the 4.5-kb fragment of 5' upstream region is able to drive reporter gene expression in neuronal cells but not in nonneuronal cells, consistent with the endogenous α1B gene expression, and 2) the region between -4500 and -1780 contains a repressor element(s) responsible for the neuron-specific expression of the N-type α1B subunit gene. Gold particle-mediated transfer of truncated deletion α1B - Green Fluorescent Protein fusion gene constructs into rat hippocampal slice cultures using a Biolistic apparatus further confirms the presence of important regulatory element(s) in the distal promoter region of the α1B gene.

This work is funded by the NINDS, NIH, Intramural Program.

495.6

COMPARISON OF PROPERTIES OF CLONED α1A AND α1E CALCIUM CHANNELS TRANSIENTLY EXPRESSED IN THE COS-7 CELL LINE. G.Stephens, N.Berrow, J.R.Burley, K.Page, I.Tedder, E. Fitzgerald* and A.C.Dolphin Dept of Pharmacology, Royal Free Hospital School of Medicine, LONDON NW3 2PF, UK

The rat calcium channel clones α1A and α1E were expressed in COS-7 cells together with the accessory subunits β1b and α2-δ. Transfected cells were identified by co-expression of cDNA for the reporter Green Fluorescent Protein. Voltage-dependent calcium currents were observed between 24 and 72 hours after transfection with the three calcium channel subunits. The current density for the combination α1A/α2-δ/β1b was lower than that for α1E/α2-δ/β1b and the former combination produced a more slowly inactivating current (τ_{inact} 332 ± 46ms (n=5) at +20mV and 124 ± 13ms (n=5) at -20mV respectively). Tail current deactivation at -80mV was slower for α1E than α1A (τ_{deact} 0.76 ± 0.06ms (n=4) and 1.28 ± 0.08ms (n=5) respectively). The voltage-dependence of activation and steady-state inactivation were more depolarised for α1A than for α1E by 25 and 30mV respectively, under the same conditions. Both currents were completely blocked by 100μM Cd^{2+}, whereas only α1A currents were blocked by ω-conotoxin MVIIC (up to 1μM). Dose-response curves for ω-agatoxin IVA revealed K_D values of approximately 11 and 51 nM for α1A and α1E currents respectively, but the rate of block of α1E currents was much slower. α1E current was substantially (74 ± 8 %) (n=3) inhibited by the 1,4-dihydropyridine antagonist nicardipine (1μM).

The results suggest that α1A expressed in these cells has a greater resemblance to the P type than the Q type component of calcium current in native neurons. Although α1E has some similarities with slowly inactivating central T type currents, the pharmacology and biophysical characteristics of α1E currents do not completely fit any endogenous calcium current component presently identified.

495.7

IMPORTANCE OF A β SUBUNIT FOR MEMBRANE ASSOCIATION AND EXPRESSION OF α1A AND α1E CALCIUM CHANNEL SUBUNITS. N.L. Brice, G.J. Stephens, N. Berrow and A.C. Dolphin* Dept of Pharmacology, Royal Free Hospital School of Medicine, LONDON NW3 2PF, UK

Native voltage-dependent calcium channels are composed of at least three subunits, a pore-forming α1, a largely extracellular α2-δ and a cytoplasmic β subunit. Little is known about targeting the subunits to the plasma membrane. We have transiently expressed α1A and α1E, α2-δ and β1b either alone or in combination in COS-7 cells, and examined their distribution by confocal immunocytochemistry, in parallel with electrophysiological studies. When α1A was expressed alone it showed no preferential localisation to the plasma membrane. In contrast, when α1A was co-expressed with α2-δ and β1b there was clear membrane association, and this could be mimicked by β1b alone, but not by α2-δ. Similar results were obtained for expression of other β subunits. Electrophysiological recordings parallel the immunocytochemical results, in that I_{Ba} was rarely observed in α1A or α1A/α2-δ transfected cells, but was observed more frequently (4/22 cells with α1A/β1b, or 44/90 cells with α1A/α2-δ/β1b). In contrast, when α1E was expressed alone, or together with α2-δ or β1b, a similar proportion of cells (8/14, 10/16 and 6/11, respectively) expressed I_{Ba}. When all subunits were expressed a larger proportion of cells (10/12) expressed I_{Ba}. However, the amplitude of α1E and α1E/α2-δ I_{Ba} was 10-fold smaller than current due to α1E/β1b or α1E/α2-δ/β1b. These data suggest that assembly of functional α1A channels in the plasma membrane of COS-7 cells only occurs significantly in the presence of β1b, and whereas α1E channels do assemble functionally in the membrane in the absence of a β subunit, this is significantly enhanced by β subunits.

495.8

MOLECULAR CHARACTERIZATION OF CALCIUM CHANNELS IN SMALL-CELL LUNG CANCER CELLS. Y.I. Kim, C.E. Davis, J.J. Pancrazio* and L. Mei. Departments of Biomedical Engineering, Neurology and Pharmacology, University of Virginia School of Medicine, Charlottesville, VA 22908.

To determine whether the putative P-type calcium channel is expressed in the small-cell lung cancer (SCLC) cells and its level of expression, we have attempted to characterize Ca^{2+} channel α-subunits from H146 SCLC cells using a molecular biology approach. First strand cDNA was produced from SCLC RNA to be used as a template for a PCR reaction. Primers were chosen in the region of the α-subunit conserved among P-, N-, and L-type Ca^{2+} channels; the sense primer encoding WNI(V)FDF: 5'-CGC GAA TTC TGG AA(C,T) (A,G)T(A,C,G,T) TT(C,T) GA(C,T) TT and the antisense primer encoding VIMDNF: 5'-CGC GGA TTC (A,G)AA (A,G)TT (A,G)TC CAT (A,G,T)AT (A,C,G,T)AC. The size of the PCR products was ~500 bp, as predicted for DNA fragments between the primers of previously characterized calcium channel α-subunits. Furthermore, these products hybridized with the ^{32}P-end-labeled degenerate oligonucleotide 5'-TGC CA(A,C,G,T) GC(C,T) TC(A,C,G,T) CC(A,C,G,T) GT(A,C,G,T) GC (antisense) encoding ATGEAWQ(H) derived from the amino acid residues between the two primers. Following subcloning of the PCR products, DNA sequencing showed that they encoded a fragment of polypeptide characteristic of α-subunits of calcium channels. Most significantly, all eight clones that had been analyzed by DNA sequencing appeared to encode a single polypeptide which is 91.5% homologous to the BI-2 (P-type) calcium channel (*FEBS*, **308**: 7-13, 1991). These results suggest that P-type calcium channel may be predominant in SCLC, consistent with electrophysiological properties of the calcium channels in SCLC cells (*J. Physiol.*, **488**: 303-317, 1995) (Supported by grants from NIH and MDA).

495.9

USE OF TRUNCATED AND CHIMAERIC FORMS OF THE α1 AND β SUBUNITS TO STUDY THE VOLTAGE DEPENDENT FACILITATION OF L-TYPE CA CHANNEL. P. Charnet*, T. Cens, A. Valentin, C. Dalle, and J. Nargeot CRBM CNRS UPR 9008 Montpellier France F 34033

L-type Ca currents recorded from Xenopus oocytes injected with the α_{1C} and β_{1b} subunit cDNAs can be potentiated by strong predepolarisations [1]. We have recently shown that this form of facilitation was not recorded with Ca channels encoded by the α_{1A}, α_{1B}, or α_{1E} subunits. Moreover we have demonstrated that this facilitation required the co-expression of a "permissive" β subunit (β_1, β_3 or β_4), since coexpression of the β_2 subunit did not allowed this potentiation [2]. To further identify the molecular determinants of this voltage-dependent facilitation we have constructed N- and C-termini deleted forms of the β_1, β_2 and α_{1C} subunits and tested their effects on control Ca currents and Ca currents recorded after positive predepolarisations.

Our results suggest that removal of the first 215 amino acid of the β_{1b} subunit and the last 212 amino acids on the β_2 subunit did not change their ability to induce facilitation.

[1] Bourinet *et al.* (1994) EMBO J., **13**: 5032-5039
[2] Cens *et al.*, (1996) Pflügers Arch., **431**: 771-774.

495.10

DENDRITIC T-TYPE CA^{2+} CHANNELS ON PURKINJE CELLS IN CEREBELLAR SLICE CULTURES.

D. Mouginot, J. L. Bossu and B. H. Gähwiler*. *Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland*

The presence of low-threshold-activated Ca^{2+} channels was investigated in Purkinje cells (PCs) in rat cerebellar slice cultures, using both the whole-cell and the cell-attached recording configurations of the patch-clamp technique, and Ca^{2+} ions as the charge carrier. A low-threshold fast-inactivating Ca^{2+} current could be recorded in isolation either after rundown, or pharmacological block of P/Q-type Ca^{2+} currents. The Ca^{2+} current had a threshold for activation of -60.3 ± 1.1 mV, and reached a maximal amplitude (-0.69 ± 0.08 nA) at -33 ± 1.7 mV. The decay time constant decreased from 25 ms at -60 mV to 15 ms at -30 mV, and both steady-state activation and inactivation were described with Boltzmann equations, with half activation and inactivation of -51 and -86 mV, respectively. The Ca^{2+} current was nifedipine insensitive, but its amplitude was reversibly reduced by $NiCl_2$ (19 ± 3%) and amiloride (53 ± 4%). Cell-attached recordings revealed a corresponding ensemble Ca^{2+} current insensitive to P/Q-type Ca^{2+} channel blockers. The ensemble Ca^{2+} current activated at +10 mV relative to the resting potential (RP), and reached a maximal amplitude (-7.9 ± 2.1 pA) at +43 mV relative to RP. Channels giving rise to this channel activity had a conductance of 7 pS and were preferentially located on the dendrites.

Supported by the Human Frontier Science Program (grant RG 67/92 B) and the Swiss National Science Foundation (grant 31-42174.94).

495.11

FUNCTIONALLY DISTINCT ISOFORMS OF THE Ca CHANNEL α_{1B}-SUBUNIT ARE EXPRESSED IN RAT SYMPATHETIC NEURONS. Zhixin Lin, Stephanie Haus, Edward Hawrot* & Diane Lipscombe. Depts. of Neuroscience & Mol. Pharm. & Biotech., Brown Univ., Providence, RI 02912.

We show that muitiple isoforms of the Ca channel α_{1B}-subunit, which differ in their activation and inactivation kinetics, are expressed in rat sympathetic neurons. Our cDNA analysis revealed the presence of three potential splice sites characterized by localized sites of sequence deletions or insertions (±A at pos.415, ±SFMG at 1236→, ±ET at 1559→), each of which has been reported in at least one other mammalian α_{1B} clone. In addition, ~10% of α_{1B} clones were characterized by a ~2.5 kb truncation in the 3'UTR. Restriction digest analysis of RT-PCR-derived cDNAs, obtained using several primer pairs and rat sympathetic mRNA, indicated the following composition of the cDNA pool: +A(~50%), -A(~50%); +SFMG (~30%), -SFMG (~70%); +ET (~80%), -ET (~20%). RNase protection analysis confirmed a predominance of α_{1B} sequences *lacking* SFMG (70%) in sympathetic ganglia and also showed that, in contrast, in brain the α_{1B} isoforms *containing* the SFMG site predominate. This was true for both adult and new-born brain. Heterologous expression of clones rscg$\alpha_{1B\text{-}b}$ (+SFMG/-ET) and rscg$\alpha_{1B\text{-}d}$ (-SFMG/+ET) in frog oocytes revealed statistically significant functional differences in the kinetics of channel gating. The activation and inactivation kinetics of rscg$\alpha_{1B\text{-}d}$ currents were significantly faster than those of rscg$\alpha_{1B\text{-}b}$. The time to peak, following a step to 0 mV, was 22.0 ± 1.0 ms (n=7) for rscg$\alpha_{1B\text{-}d}$ currents, compared to 57.8 ± 9.2 ms (n=6) for rscg$\alpha_{1B\text{-}b}$. Likewise, the rate of activation of rscg$\alpha_{1B\text{-}d}$ currents was 2-fold faster (2.7 ±0.4 ms, n=5) at 0 mV compared to rscg$\alpha_{1B\text{-}b}$ (5.0 ± 0.4 ms, n=4). Expression of multiple isoforms of α_{1B} could account, at least in part, for kinetically distinct N-type channel currents seen in recordings from native cells. *NIH NS2996.*

495.12

N-TYPE Ca CHANNELS MEDIATE THE EGF-INDUCED STIMULATION OF Na CURRENT DENSITY IN PITUITARY GH3 CELLS. E. Monjaraz, A. Navarrete and G. Cota*. Dept. of Physiol., Biophysics, and Neuroscience, Cinvestav, Mexico City, DF 07000.

We previously showed that chronic exposure of GH3 cells to epidermal growth factor (EGF) stimulates high-threshold Ca current density. EGF-treated cells also exhibit an enhanced level of Na current expression. Here, we provide evidence that the latter effect is secondary to the influx of Ca through newly expressed N-type Ca channels. GH3 cells were grown under standard conditions (control cells) or in the presence of 5 nM EGF, 5 μM nimodipine, 0.2 μM ω-Aga-IVA, 3 μM ω-CTx-GVIA, or a combination of EGF and a Ca channel blocker. On day 5, EGF and the Ca channel blockers were removed from the culture medium. Subsequently, after a recovery period of 60 min, the cells were subjected to whole-cell patch clamp recording to evaluate peak Na current density at +10 mV (HP -80 mV). Relative to its control value, Na current density increased by a factor of 1.8 in cells exposed to EGF alone, and decreased by half in cells treated only with nimodipine, as reported previously. The stimulatory effect of EGF was unaffected by the continuous presence of ω-Aga-IVA, but was completely blocked by ω-CTx-GVIA. Cells treated with EGF and nimodipine behaved almost as control cells. Finally, neither ω-Aga-IVA nor ω-CTx-GVIA, given alone, were able to induce a significant change in Na current density. Thus, the functional expression of Na channels in GH3 cells is normally sustained by L-type Ca channel activity, but depends on both L-type and N-type Ca channels after EGF treatment. *Supported in part by the HHMI.*

495.13

DIFFERENT SPATIAL PROFILE OF INTRACELLULAR CALCIUM TRANSIENTS AND FLUORESCENT DIHYDROPYRIDINE BINDING IN RELAY CELLS AND LOCAL INTERNEURONS FROM THE RAT DORSAL LATERAL GENICULATE NUCLEUS.

T. Budde*, T. Munsch, H.-C. Pape. Institut für Physiologie, Otto-von-Guericke Universität, D-39120 Magdeburg, Germany.

The different subtypes of voltage activated calcium (Ca^{2+}) channels (VACC) serve distinct roles in excitation and signaling of thalamic neurons. The subcellular distribution of two types of VACC, the low voltage activated (LVA) and he high voltage activated (HVA) Ca^{2+} current seems to be critical for the electrical properties of thalamic neurons. We used fluorescence imaging of Fura-2 in combinaton with the whole-cell patch clamp technique to investigate the subcellular distribution of LVA- and HVA-activated Ca^{2+} channels in relay cells and local interneurons of the dorsal lateral geniculate nucleus (dLGN). Cells were visually identified by infrared videomicroscopy. Selective activation of LVA or HVA Ca^{2+} currents by appropriate voltage protocols caused transient changes in the intracellular Ca^{2+} concentration in soma and dendrites of relay cells and local interneurons. However, the amplitudes of these Ca^{2+} transients were characteristically different in varying regions of the two cell types. In relay cells, LVA Ca^{2+} transients slightly decreased from soma (100%) to dendrites, whereas HVA transients were strongly reduced (38±19%, n=15). In contrast, the LVA transients in interneurons strongly increased from soma to dendrites (219±124%, n=8) and the HVA transients were only slightly reduced. We also used the fluorescent Ca^{2+} channel probe STBodipy-DHP (fDHP) to investigate the subcellular distribution of L-type Ca^{2+} channels. Fluorescence labelling after 1 h incubation with 25 μM fDHP was highest in the soma and the proximal dendrites. This selective labbeling could be blocked by preincubation with BAYK 8644 (50 μM). The data suggest that the subcellular distribution of low- and high-voltage activated Ca^{2+} channels is not homogeneous and different in dLGN relay cells and local interneurons, which may be important for their specific functional role.

Supported by the DFG (H.-C.P.) and the Helmholtz program (T.B.).

495.14

α_{1A}-TYPE Ca^{2+} CHANNEL mRNA AND PROTEIN ARE DIFFERENTIALLY REGULATED BY MELANOTROPE DOPAMINE RECEPTOR STIMULATION.

P. Sharma[1], S.A. Sands[1], D.M. Beatty[1], K.E. Hagler[1], W.Smith[2], S.G. Volsen[2], R. Baker*[2], S.J. Morris[1] and B.M. Chronwall[1]. School of Biological Sciences[1], University of Missouri-Kansas City, Lilly Research Centre[2], Windlesham, Surrey, UK.

Melanotopes of the rat pituitary intermediate lobe are tonically inhibited by dopamine axons emanating from hypothalamic neurons. This effect is mediated by dopamine D2 receptors. Chronic stimulation of D2 receptors decreases the rise in intracellular Ca^{2+} on K+ depolarisation in melanotopes (Beatty et al.,1995: Osipenko et al 1994). P-type Ca^{2+} channels, encoded by the α_{1A} gene have been pharmacologically identified in melanotropes (Beatty et al., 1995). Our studies in primary culture show that chronic treatments with D2 receptor antagonists reduce channel activity and α_{1A} mRNA and protein immunoreactivity. Here we report the distribution pattern and D2 receptor regulation of α_{1A} mRNA and protein in the pituitary intermediate lobe in vivo. In situ hybridisation using digoxigenin labelled oligonucleotide probes demonstrate that α_{1A} mRNA was heterogeneously distributed. By densitometry, comparable central sections of individual melanotropes ranged from 0.15-0.90 OD units, with 20-30% of the cells expressing high levels of mRNA. Likewise, immunoreactivity for the protein subunit was distinctly more intense in some melanotropes. Chronic treatment (14d) with the D2 receptor agonist bromocriptine, differentially regulated the α_{1A} channel mRNA and protein levels. Overall measurements indicated that the protein immunoreactivity decreased whereas the mRNA level increased. Possibly, in vivo down regulation of the α_{1A} channel protein triggers compensatory mechanisms which may lead to reduced degradation or upregulation of the mRNA without affecting protein translation. These results indicate that the P channel is among several melanotope proteins controlled by the D2 receptor.

495.15

THE EXPRESSION OF VOLTAGE-DEPENDENT CALCIUM CHANNEL ß SUBUNITS IN HUMAN CEREBELLAR PURKINJE CELLS.

S.G. Volsen [1], N.C. Day [2], A.L. McCormack [1], R. E. Beattie [1], P.J. Craig [1], P.Ince [2], P. J. Shaw [2], I. A Pullar* [1], M. Williams [3], M.M Harpold [3] and W. Smith [1]. Lilly Research Centre, Windlesham, Surrey UK [1]; MRC Neurochemical Pathology Unit, Newcastle Upon Tyne, UK [2]; SIBIA Neurosciences Inc, La Jolla, US [3].

Purkinje cells are of particular interest in the study of native VDCCs, since the majority of their somatic whole cell calcium current is of P type. Whilst α_{1A} remains the strongest candidate for the pore forming subunit of the P channel, the nature of the associated ß protein is not known. To help address this issue, we have prepared both class specific riboprobes and polyclonal antisera to human ß1, ß2, ß3 and ß4 subunits and used them in parallel in situ mRNA hybridisation and immunohistochemical analyses of human cerebellar Purkinje cells. Detailed serial section studies demonstrate that each class of β subunit may be found in single Purkinje cells and their subunit proteins are distributed into specific subcellular domains. For example, ß1, ß2, ß3 and ß4 proteins all produce punctate immunohistochemical staining in the Purkinje cell soma, making the identification of the ß subunit specifically associated with the P channel difficult. However, ß3 alone is expressed preferentially in the fine Purkinje cell dendritic arbor where its distribution is identical to α_{1E}.

495.16

DOPAMINERGIC REGULATION OF Ca^{2+} CHANNEL α_1 SUBUNIT GENE EXPRESSION IN THE NEUROINTERMEDIATE LOBE OF THE RAT PITUITARY. D. M. Fass*[1], K. Takimoto[2], & E. S. Levitan[1,2]. Departments of Neuroscience[1] and Pharmacology[2], University of Pittsburgh, Pittsburgh, PA 15260.

The dopaminergic input to melanotrophs of the intermediate lobe of the rat pituitary may inhibit secretion in part by supressing Ca^{2+} channel gene expression. To test this hypothesis, female rats (200-224 g) were treated with dopaminergic agents (the agonist bromocriptine or the antagonist haloperidol--each at 5 mg/kg, IP injections) to act at D2 receptors for 6 h (one injection) or 30 h (three injections at ~10 h intervals). Total RNA was isolated from neurointermediate lobes and Ca^{2+} channel α_1 subunit mRNA levels, were measured by RNase protection assays. In control animals, we detected α_{1A} and α_{1D} mRNAs. After 6 h, haloperidol increased α_{1D} mRNA levels but had no effect on α_{1A} mRNA levels (n = 2, 6 animals per group). In contrast, bromocriptine decreased α_{1D} mRNA levels (n = 2, 6 animals per group). 30 h treatments produced similar changes in α_{1D} mRNA levels (n = 1, 4 animals per group, α_{1A} mRNA levels were not tested). These data support the hypothesis that α_{1D} mRNA levels are suppressed in vivo in rat melanotrophs by dopamine.

This work was supported by a NIH grant to E. S. L. and a predoctoral fellowship to D. M. F. from the American Heart Association (Pennsylvania affiliate).

495.17

CLONING AND EXPRESSION LOCALIZATION OF CDNA FOR A RAT HOMOLOGUE OF TRP PROTEIN, A POSSIBLE STORE-OPERATED CALCIUM CHANNEL. M. Funayama, K. Goto* & H. Kondo Dept. of Anatomy, Tohoku Univ. Sch. of Med., Sendai, 980 Japan

TRP encoded by a Drosophila *trp* (transient receptor potential) gene is considered to represent the store-operated Ca^{2+} channel. We cloned 3.2kbp cDNA encoding a possible mammalian candidate for the store-operated Ca^{2+} channel because of high homology to a Drosophila *trp* (transient receptor potential) gene from a rat brain cDNA libraty. The deduced amino acid sequence was 51.8% identical to TRP and contained ankyrin motifs, a coiled-coil structure and six transmembrane segments similar to the previously identified TRP family and named as TRP-R (rat TRP). Northern blotting detected distinct hybridization signal only in the brain among various tissues of adult rats. By in situ hybridization histochemistry on embryonic day 15, no significant expression signals for TRP-R was detected in a whole body of rats. On embryonic day 20 and postnatal day 1, the expression signals were most evident in the septum, cerebral cortical plate and hippocampal neuronal layers. On postnatal day 7 and thereafter the expression in the cerebral cortex and the septum decreased progressively, and weak expression was remained only in the septum and CA1 and CA2 neuronal layers of the hippocampus in the brain on postnatal day 21 and 49. This limited spatiotemporal expression of this novel molecule suggests that this novel molecule, TRP-R, is involved in some specific functions related to the neuronal differentiation.

495.18

INOSITOL TRISPHOSPHATE AND RYANODINE RECEPTORS IN HUMAN PURKINJE CELLS DURING DEVELOPMENT. N.Zecevic and B.E.Ehrlich*, University of Connecticut, Farmington, CT 06030.

Two receptors regulate release of Ca from internal stores in the cell: inositol trisphosphate (IP3R) and ryanodine (RyR). Changes in intracellular Ca influence cellular development and synaptic plasticity in the CNS. In order to define the timing for the initial expression of these receptors in Purkinje cells of the human cerebellum, we studied 13 human fetuses from 6 to 32 gestational weeks (g.w.) using immunocytochemical methods. Anti-IP3R and anti-RyR antibodies were applied to 14µm frozen sections of fetal brains. A defuse staining was first observed at 11 g.w. for IP3R and at 13 g.w. for RyR in the intermediate zone where Purkinje cells were migrating. At 17-18 g.w. a positive reaction with both markers was found below the molecular layer, in the Purkinje cell layer which was several cells thick. At 27 and 32 g.w. immunoreactivity for both markers was present in Purkinje cells which were now aligned in a single layer with the dendritic tree expanding into the molecular layer. In this study the IP3R was observed earlier than the RyR. In human cerebellum the appearance of these receptors correlates well with cellular maturation and synaptogenesis of Purkinje cells (Zecevic and Rakic, 1976, Yachnis et al.,1993). Supported by NIH grants MH53945 (NZ) and GM51480 (BE).

POTASSIUM CHANNELS: STRUCTURE AND FUNCTION

496.1

IDENTIFICATION OF *MYSTERY*, A NOVEL PUTATIVE K CHANNEL IN MAMMALIAN BRAIN. W. J. Joiner[#+], L. Gan[#*], M. D. Tang[#!], and L. K. Kaczmarek[#]. Departments of Cellular and Molecular Physiology and Pharmacology, Yale University School of Medicine, New Haven, CT 06520.

By exploiting the rapidly expanding database of expressed sequence tags and genomic DNA fragments within Genbank we have identified more than 40 novel genes in both prokaryotes and eukaryotes that we believe are members of the K channel superfamily. Here we report the cloning of one of these, which we call the *mystery* channel, from mammalian brain. Like many other K channels, *mystery* appears to have 6 potential transmembrane domains and a conserved hydrophobic P-like domain that falls between putative transmembrane domains 5 and 6. However, *mystery* differs greatly in primary sequence from other 6 transmembrane domain K channels such as the *Kv*, *eag*, and *slo* families. The apparent paucity of charged residues within *mystery*'s presumed S4 domain, which in other 6 transmembrane domain channels is thought to serve as part of a voltage-sensing apparatus, suggests that *mystery* might serve a role distinct from the rapidly repolarizing currents generated by other K channels displaying a similar predicted topology.

supported by NIH Grant GM48851
+ supported in part by a predoctoral fellowship from HHMI
! supported in part by a NIH MSTP Grant

496.2

IDENTIFICATION OF A NOVEL KvLQT1-LIKE GENE (KL1) M. D. Tang[#+] and L. K. Kaczmarel[#*] Dept. of Cellular and Molecular Physiology and Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

A combination of traditional and computer-based screening techniques was used to identify a gene from rat brain (KL1). KL1 shares significant sequence similarity (approx. 70%) with the recently partially cloned *KvLQT1* gene, implicated in Long-QT Syndrome and highly expressed in heart and kidney. As is the case for cloned *Shaker*-type K channels, KL1 has 6 putative transmembrane domains and charged amino acid residues in the predicted S4 domain. Unlike *KvLQT1*, however, KL1 does not possess a long C-terminus.

supported by NIH Grant GM48851
+ supported in part by NIH MSTP grant

496.3

SEVEN K^+ CHANNEL FAMILIES REVEALED BY THE *C. ELEGANS* GENOME SEQUENCING PROJECT. A. Wei*, T. Jegla and L. Salkoff. Department of Anatomy and Neurobiology, Box 8108, Washington University School of Medicine, Saint Louis, MO 63110.

The wealth of accumulating data from the *Caenorhabditis elegans* genome sequencing project has rapidly accelerated the discovery of novel potassium channel genes and now places within reach the possibility of describing the total complement of potassium channels used by an individual species. Using annotated GenBank sequences, BLAST searches of unfinished sequences, and degenerate oligonucleotide PCR screens, we have identified and compiled genes for 31 *C. elegans* potassium channels and 2 cyclic nucleotide-gated cation channels, representing seven groups of conserved multigene families. Several novel families of channels were revealed, as well as conserved homologues of all known vertebrate families of potassium channels. Present were direct *C. elegans* homologues of two human potassium channels recently implicated in hereditary long QT arrhythmias. The genes are evenly distributed on all six *C. elegans* chromosomes, with two instances of gene clustering on the X chromosome. Of particular note is an exceptionally large class of at least 17 genes with a novel subunit structure having two tandem "P" domains; these channels may form dimers in contrast to all other types which form tetramers. Initial reports suggest that these *C. elegans* potassium channel genes will have mammalian homologues (Lesage, *et al.*, 1996) and may thus represent new conserved potassium channel gene families.

Supported by the NIH and the Muscular Dystrophy Association (L.S.)

496.4

CLONING AND CHARACTERIZATION OF A KIR3.1 (GIRK1) C-TERMINAL TRUNCATED SPLICE VARIANT

C.S. Nelson* and C.N. Allen CROET, Depts. Psychiatry & Physiology /Pharmacology, OHSU Portland, OR 97201.

Southern blot analysis of RT-PCR products from rat heart and hypothalamus revealed multiple products for a C-terminal region of Kir3.1. Sequencing yielded clones for wild type and a putative splice variant with a predicted translation for C-terminal truncation. A second round of RT-PCR yielded identical overlapping products from rat heart, hypothalamus and human heart. Expression in *Xenopus* oocytes of a full length construct coding for the truncation variant (Kir3.1Δ) showed loss of activation by coinjected dopamine D_2 receptors as measured using two electrode voltage clamp. When Kir3.1Δ and Kir3.1 were coinjected no significant difference with respect to high potassium (HiK) and D_2 stimulated current amplitudes, pertussis toxin sensitivity and desensitization was found compared to Kir3.1 channels alone. However, single exponential fits to the agonist stimulated portion of currents showed faster current activation times (Kir3.1=11.2±0.8 s vs. 6.9±0.6 s=Kir3.1Δ, $p < 0.0047$). Overexpression of D_2 receptors also revealed an increase in constitutive, HiK activated currents. Kir3.1Δ as a member of a Kir3.4/3.1Δ concatemer construct was compared to a Kir3.1/3.4 concatemers during voltage jump relaxations. Kir3.4/3.1Δ concatemers showed no detectable expression except when coinjected with the wild type Kir3.1/3.4 concatemer. Both constructs showed an unresolvable instantaneous current component and a slower component with similar time constants but significantly greater current component amplitude for Kir3.4/3.1Δ containing channels. Therefore, Kir3.1Δ is a channel variant with restricted capacity for channel assembly which increases G-protein responsiveness, possibly by alteration of channel gating or relief from magnesium block. (Supported by AG10794)

496.5

BIOCHEMICAL CHARACTERIZATION OF MOUSE BRAIN GIRK1-GIRK2 PROTEIN COMPLEX. A. Inanobe[1], K. Morishige[2] and Y. Kurachi[1, 2*] [1]Department of Cell Biology and Signaling, Yamagata University School of Medicine, Yamagata, 990-23, Japan, and [2]Department of Pharmacology II, Faculty of Medicine, Osaka University, Osaka, 565, Japan.

GIRK1 (Kir3.1) and GIRK2 (Kir3.2) belong to the same subfamily of inwardly rectifying K^+ channels. Because the expression pattern of GIRK1 mRNA overlapped with that of GIRK2 in brain, it is speculated that some of the brain G protein-gated muscarinic K^+ channels are heteromeric complexes composed of GIRK1 and GIRK2 proteins. No experimental evidence has been, however, provided so far. To examine this possibility with biochemical approach, antibodies against peptides corresponding to sequences of GIRK1 and GIRK2 were developed (anti-GIRK1C1 and anti-GIRK2C5, respectively). Each antibody specifically recognized a single band of membrane proteins of mouse forebrain on SDS-PAGE. The immunoprecipitant of each antibody from membrane proteins extracted with Triton X-100 were composed of several different proteins. In the immunoprecipitant of anti-GIRK2C5, GIRK1 protein was detected by anti-GIRK1C1, and vice versa. In immunohistochemistry, both GIRK1 and GIRK2 immunoreactivities were localized to the similar presynaptic regions in rat VTA. Almost only GIRK1 immunoreactivity was detected in PVN, while GIRK2 immunoreactivity was abundantly observed in the reticulum of SN. These results strongly support the notion that GIRK1 and GIRK2 physiologically form a channel complex in some specific regions of the brain.

496.6

MOLECULAR CLONING AND CHARACTERIZATION OF MURINE *GIRK2* AND ITS MULTIPLE ISOFORMS J. Wei*, M.E. Hodes, Y. Wang, Y. Feng, B. Dupree, B. Ghetti and S.R. Dlouhy. Ind. Univ. Sch. of Medicine, Indianapolis, IN 46202.

A single base change in a G-protein-gated inwardly rectifying K^+ channel, *Girk2*, is the primary defect of *weaver* (*wv*) mice. The *wv* channel loses its selectivity for K^+ and leaks Na^+. How expression of mutant *Girk2* affects a variety of target neurons and results in death of only some is not known. Although two partial cDNA sequences encoding 414-426 amino acids have been reported for *Girk2*, northern blot analysis led us to suspect that additional transcripts may exist and may be relevant to the pathology of the *wv* mouse. We have concomitantly investigated the genomic structure of the locus and isolated a number of different *Girk2* cDNA isoforms. Restriction mapping indicates that the gene is probably at least 50 kb. Genomic walking (utilizing primers based upon published cDNA sequence) indicates that the published *Girk2* cDNA is comprised of 6 exons. The largest exon (921 bp) contains the majority of the protein coding region of the channel, including the site of the *wv* mutation. 5' and 3' cDNA RACE from this exon yielded multiple fragments. To date, we have isolated an additional 4 *Girk2* cDNA isoforms that include the site of the *wv* mutation. These include 4 new exons, and we have identified the intron/exon boundaries. One isoform does not appear to be polyadenylated. These isoforms can yield proteins ranging from ca. 300 to over 420 amino acids. Northern blot and *in situ* hybridization studies indicate that some of these isoforms are differentially distributed in mouse brain and testis. (Supported by P01 NS27613 and R01 NS14426)

496.7

INTRODUCTION OF NOVEL DISULFIDE BONDS AND HIGH-AFFINITY METAL BINDING SITES INTO A K CHANNEL PORE. H.S. Krovetz, H.M.A. VanDongen, A.M.J. VanDongen.* Department of Pharmacology, Duke University Medical Center, PO Box 3813, Durham, NC 27710.

Voltage-dependent K channels consist of four identical subunits surrounding a central pore. Each subunit contains six putative transmembrane segments, S1-S6. The linker region between the S5 and S6 transmembrane segments forms a hairpin loop that lines the external vestibule and the narrow part of the pore. In order to obtain structural information on the pore region we have introduced novel disulfide bonds and high-affinity metal binding sites by cysteine substitution mutagenesis. Individual replacement of I379 and Y380 with cysteine resulted in an enhanced block by Cadmium (IC50 = 26 uM and 256 uM , resp). A potassium-selective binding site was detected that affected the apparent Cd^{2+} affinities of the I379C and Y380C mutants. The double mutant (GYGDIYP => GYGDCCP) resulted in the spontaneous formation of novel disulfide bonds, which obliterated channel function. Breaking these disulfide bonds with dithiothreitol restored function. This reduced channel displayed an extreme sensitivity to cadmium (IC50 = 20 nM). Dimeric constructs were used to demonstrate that the disulfide bond and the high-affinity metal binding site are formed by cysteines at position 379 and 380 in neighboring subunits. Taken together, these data put narrow constraints on several distances in the entry to the narrow part of the pore. The geometry is more consistent with a radial pore model than with either the eight-stranded barrel or the newer "tongue" model.

This work was supported by Grant NS31557 from N.I.N.D.S.

496.8

IDENTIFICATION AND EXPRESSION OF POTASSIUM CHANNEL β SUBUNITS IN THE EMBRYONIC AMPHIBIAN NERVOUS SYSTEM. AD Hofmann[1], MA Lazaroff[1], K Nakahira[2], JS Trimmer[2], AB Ribera[1]* [1]Dept Physiology C-240, UCHSC, Denver, CO 80262, [2]Dept Biochemistry and Cell Biology, SUNY Stony Brook, NY, 11794.

Differentiation of voltage dependent potassium current in amphibian spinal neurons is delayed with respect to the maturation of calcium current, regulating action potential duration. Maturation of potassium current is pivotal for action potential differentiation, and thus studies have focused on this ion channel. Previous molecular analyses concerned the Kvα potassium channel subunits. However, auxiliary Kvβ subunits also influence function. Thus, the roles of Kvβ subunits in Kv potassium current maturation are being investigated.

Three *Xenopus* Kvβ subunit cDNA clones have been isolated using rat Kvβ1 and Kvβ2 subunit cDNAs as probes. One of these clones contains an entire open reading frame and predicts a protein with 98% amino acid identity to the rat Kvβ2 subunit. The other 2 clones are partial and most similar to rat Kvβ1. Whole mount *in situ* hybridization indicates that *Xenopus* Kvβ genes have a ubiquitous expression pattern in the spinal cord during the period of potassium current maturation. In contrast, Kv1.1α subunit mRNA is restricted to dorsal sensory neurons. Coexpression of *Xenopus* Kv1.1α and Kvβ2 subunits in a heterologous system has not yet provided information regarding the potential functional role of this auxiliary subunit. Given that Kv β subunits are thought to interact with Kv1 α subunits, the *in situ* hybridization results suggest that additional Kv1 α genes are expressed in neurons that reside in non-dorsal regions of the developing spinal cord. Supported by NIH NS34383 (JST) and NS25217 (ABR).

496.9

IDENTIFICATION OF PROTEINS ASSOCIATED WITH THE Kv2.1 VOLTAGE-GATED K^+ CHANNEL. K. Nakahira*, H. Murakoshi, R. Scannevin and J. S. Trimmer, Dept. Biochem. and Cell Biol., SUNY at Stony Brook, Stony Brook, NY 11794.

Recent studies have shown that the highly divergent cytoplasmic domains of voltage-gated K^+ channel α-subunits from the *Shaker* (Kv1) subfamily can mediate diverse protein-protein interactions. N-terminal cytoplasmic domains mediate both α/α- and α/β-subunit interaction, while C-terminal cytoplasmic domains interact with PSD-95 family members. Each of these interactions is restricted to members of the Kv1 subfamily, suggesting that members of other subfamilies of K^+ channel α-subunits may have similar associated proteins. Indeed, previously we have shown that Kv2.1, a member of the *Shab* (Kv2) subfamily of voltage-gated K^+ channels, is found in tight association with a 38 kD protein in rat brain (*PNAS*, 88:10764. 1991).

We therefore searched for rat brain proteins that interact with the Kv2.1 polypeptide using the yeast two-hybrid system. We screened 3.4 x 10^6 colonies of a rat brain cDNA library using the N-terminal cytoplasmic domain of Kv2.1 as the bait. We obtained 40 positive colonies, which by sequence analysis were shown to represent 17 independent cDNAs. Some of the cDNAs corresponded to known genes, however, none were similar to known Kvα or Kvβ family members. A representative of each cDNA class was then epitope-tagged and co-expressed in COS-1 cells with full length Kv2.1. Some of the expressed polypeptides were found to associate with Kv2.1 in this cell line, as determined by co-immunoprecipitation experiments. Immunofluorescence staining revealed that these same polypeptides also exhibited clear co-localization with Kv2.1. These data indicate that the proteins encoded by the rat brain cDNAs identified in this two hybrid screen can tightly associate with Kv2.1 in mammalian cells, and lead us to further investigate their association with Kv2.1 in mammalian brain and its physiological consequences.

Supported by NIH grant NS34383 (to JST).

496.10

POSTSYNAPTIC CLUSTERING OF KV4.2 POTASSIUM CHANNELS IN RAT CENTRAL NEURONES, H. Widmer, G. Alonso and M. Sheng*. URA1197 and INSERM U336, Universite Montpellier II, Montpellier, France.

Ion channels play a key role in determining both neuronal membrane excitability and specificity. Neuronal specificity is achieved by several mecanisms, including influence of afferent network and expression of cell-to-cell specific sets of ion channels. Recently, demonstration of subcellular segregation in central neurons has suggested an additionnal way of modulating neuronal properties. Potassium channels, which form numerous subtypes expressing a wide range of electro-physiological properties when expressed in *Xenopus* oocyte, have been shown to segregate both a the cellular and subcellular level in various brain regions. However, owing to the limitations of light microscopy, the precise localization of the channels on pre- or post-synaptic membrane has not been assessed by now. We used confocal and immunoelectron microscopy to determine in the hypothalamus the localization of Kv4.2 subtype of potassium channel. The Kv4.2 antibody was kindly given by Dr. Sheng (Sheng *et al.* (1992) *Neuron* **9**, 271). We showed that intense immunostaining was localized to the supraoptic nucleus in which it often formed rings outlining the somata of magnocellular neurones. The even distribution of the staining throughout the nucleus suggests that Kv4.2 channel is present in both vasopressin and oxytocin neurons. Electron microscopy further revealed that staining was essentially associated with the plasma membrane limitating the somata or the dendrites of these neurones and that it was particularly concentrated at the site of synaptic contacts. The specific localisation of the Kv4.2 channel indicate that this channel may play a role in signal integration at the synapse. Any identification of Kv4.2 channels with ion currents described in supraoptic neurons requires further knowledge of the subunit composition of the channel *in situ*.

Drs H. Widmer and G. Alonso were supported respectively by CNRS and INSERM grants (FR).

496.11

WITHDRAWN

496.12

Diversity of voltage-gated potassium channels in a cnidarian. Peter A.V. Anderson*, Alisa E. Shaw, and Michael C. Jeziorski. The Whitney Laboratory, University of Florida, St. Augustine, FL.

The heterogeneity of potassium currents in neurons and other excitable cells is accounted for in part by the Shaker family of voltage-gated potassium channels, which is comprised of four subfamilies (*Shaker*, *Shab*, *Shaw*, and *Shal*). These subfamilies, fully represented in species ranging from *Drosophila* to human, are thought to have arisen from an ancestral potassium channel gene. Members of the phylum Cnidaria (jellyfish, anemones, and corals) represent the earliest existing species with differentiated neural and muscle tissues. The recent discovery of homologues of the *Shaker* subfamily in a hydrozoan jellyfish (Jegla *et al.*, *J. Neurosci.*, **15**:7989, 1995) suggests that the four channel subfamilies diverged before the appearance of the first nervous systems. We have used degenerate oligonucleotide primers designed against conserved regions of the voltage-gated potassium channel family to isolate cDNA clones from the sea anemone *Aiptasia pallida*. RT-PCR, coupled with standard cloning techniques, has led to the identification of three full-length cDNA sequences encoding homologues of the *Shaker*, *Shaw*, and *Shal* subfamilies. Each *Aiptasia* sequence is more closely related to its counterpart in higher vertebrates (45-67% identity within the core region spanning T1-S6) than to the other two anemone sequences (36-50% identity). Expression of the anemone *Shaker* homologue in *Xenopus* oocytes produces a voltage-activated, 4-aminopyridine-sensitive current that exhibits slow inactivation (τ = 375±26 ms). We plan to use heterologous expression to characterize the remaining channel clones. (Supported by NRSA grant MH 10625 to MCJ and NSF grant IBN-9222803.)

496.13

MOLECULAR CLONING OF VERTEBRATE POTASSIUM CHANNEL cDNA INTERRUPTED BY INSERTION OF TRANSPOSABLE ELEMENT. R. B. Puchalski*, J. Kang and J. H. Teeter. Monell Chemical Senses Center, Philadelphia, PA 19104

We have discovered transposase sequences in a partial length cDNA clone encoding a delayed rectifier K^+ channel isolated from channel catfish. The cDNA was cloned by PCR with degenerate oligonucleotide primers corresponding to highly conserved amino acid sequence of different subfamilies of the delayed rectifier K^+ channels. The cDNA was synthesized by reverse transcription of total RNA isolated from catfish maxillary barbels and used as a template for PCR. DNA sequence analysis reveals that the clone of 942bp consists a novel sequence of 439bp flanked by a 5' sequence of 360bp similar to transposases and by a 3' sequence of 107bp similar to delayed rectifier K^+ channels. The 360bp sequence is 67% similar to a Tc1-like transposon family, which moves in the genome through a DNA intermediate via a cut-and-paste mechanism. The novel sequence might encode the transposon's 3' inverted repeat. Interruption of the coding segment of the putative transposase gene by stop codons in all three reading frames indicates that the transposon is not autonomous and that the 3' sequences of the K^+ channel are not translated.

Supported by Monell Institutional Fund (R.B.P.), NIH 2T32 DC00014 (J.K.) and DC01838 (J.H.T.).

496.14

ALPHA-DENDROTOXIN AND DELTA-DENDROTOXIN INTERACT WITH DIFFERENT AMINO ACIDS AT THE PORE REGION OF Kv1.1. R.G. Sorensen' and A. Matlapudi. Dept. Pathology, Anatomy and Cell Biology, Jefferson Medical College, Philadelphia, PA 19107.

The block of the dendrotoxin-sensitive K channel, Kv1.1, by several dendrotoxin homologues purified from green mamba venom was studied. Two homologues, α-dendrotoxin and δ-dendrotoxin, are potent blockers of Kv1.1, with Kd of 6.5 ± 1.4 and 0.062 ± 0.038 nM, respectively. The difference in potencies reflects the dissociation rates of the toxins; α-dendrotoxin is readily reversible whereas δ-dendrotoxin has a much slower, largely irreversible, off rate.

The dendrotoxin molecules possess a net positive charge. Alanine substitutions of the negatively-charged amino acids found near the ion conduction pathway of Kv1.1, E348A, E350A, E351A, E353A, D361A and D377A, were made to screen for potential electrostatic interactions between these residues and the positively-charged dendrotoxin molecules. The results suggest that α-dendrotoxin and δ-dendrotoxin make electrostatic contact with different amino acids of Kv1.1. For example, the mutation E353A causes a 20-fold decrease in the Kd for block by α-dendrotoxin while having little effect on the affinity for block by δ-dendrotoxin, whereas E350A causes a 5-fold decrease in the Kd for δ-dendrotoxin while having little effect on the affinity for block by α-dendrotoxin. Additional amino acid substitutions are being made to confirm the electrostatic nature of these dendrotoxin/Kv1.1 interactions. These results will be discussed with respect to the geometry for the binding of the dendrotoxins to Kv1.1. Supported by NIH grant NS31670.

496.15

CLONING OF G-PROTEIN-COUPLED, INWARD RECTIFIER K^+ (GIRK) CHANNELS FROM THE RAT ANTERIOR PITUITARY. K.A. Gregerson*, M. Anderson, O. Lauring, P.A. Welling, Depts. of Pediatrics and Physiology, University of Maryland at Baltimore, Baltimore, MD 21201.

Dopamine (DA) is a physiological regulator of prolactin (PRL) secretion, exerting tonic inhibitory control. DA activates an inward-rectifier K^+ (IRK) channel in rat lactotropes causing membrane hyperpolarization and inhibition of Ca^{2+}-dependent action potentials. Both the activation of this effector K^+ channel and the inhibition of PRL release are mediated by D_2 receptor activation and PTx-sensitive G proteins. To study the molecular basis of this channel, a homology-based PCR approach was employed to identify members of the IRK channel family which are expressed in pituitary and may comprise this D_2-activated, G-protein-gated channel.

Non-degenerate primers corresponding to regions specific for IRK channels known to be G-protein-activated were synthesized and used in the polymerase chain reaction with reverse-transcribed female rat anterior pituitary (AP) mRNA as the template. PCR products of predicted sizes for GIRK1, GIRK2, and CIR (GIRK4) were consistently observed by ethidium bromide staining after 18 amplification cycles. No products were observed in reactions using mRNA rather than first strand cDNA as the template, ruling out spurious genomic amplification. Identities of the products were confirmed by subcloning and sequencing. Sequences were identical to the corresponding regions published for GIRK1, GIRK2, and CIR. Expression of each of these gene products in AP was confirmed by Northern blot analysis.

In conclusion, GIRK1, GIRK2, and CIR are excellent candidate subunits for the D_2-activated, G-protein-gated channel in pituitary lactotropes where they may play a critical role in excitation-secretion coupling.

Supported by DK-40336 (to KAG) and DK-48271 (to PAW).

496.16

VOLTAGE SENSING IN A JELLYFISH SHAKER POTASSIUM CHANNEL (JSHAK1). N. G. Grigoriev, J. D. Spafford, W. J. Gallin and A. N. Spencer* Dept. of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9 and Bamfield Marine Station, Bamfield, B.C., Canada, V0R 1B0.

In all known *Shaker* K^+ channels, except *jShak1*, the putative voltage sensor (S4 segment) has seven, conserved, positively charged amino-acids positioned at every third residue. However, in a primitive, *Shaker*-like potassium channel (*jShak1*) from the hydrozoan jellyfish, *Polyorchis penicillatus* there are only six positively charged residues in the S4 segment. When compared with other *Shaker* currents, *jShak1* currents exhibited a strong positive shift (+33mV) in the mid-point of activation. We examined the possibility that 'normal' *Shaker* electrical properties could be restored by adding a seventh charge 'in phase' with the charged motifs already present. Mutations of the S4 region of *jShak1* involved insertion of motifs, and rewriting. Electrophysiological analyses showed that none of the constructs expressed currents that had properties similar to other *Shakers*, despite the seven-charge pattern being restored. Adding charge to the N-terminus of S4 by rewriting does not change substantially any voltage-dependent properties. However, mutation by insertion of positively charged motifs in the C-terminal portion of S4 caused significant changes in voltage and kinetic properties. On the basis of this, and other studies, we suggest a synthetic, molecular model of voltage sensing in *Shaker* channels. Supported by NSERC Canada.

496.17

AMINO-ACID CHANGES IN THE N-TERMINAL SEQUENCE OF KALIOTOXIN RESULT IN PEPTIDES WHICH DISCRIMINATE AMONG KV1 α-SUBUNITS. M. Crest ¥, V. Frémont#, M. Gola¥, J.Y. Barthe*, R. Romi#, H. Rochat#, M.F. Martin-Eauclaire# and L. van Rietschoten#. ¥Lab. de Neurobiologie, UPR CNRS 9024, *Neurobiologie et Mouvements, UPR CNRS 9011, Ch. J. Aiguier 13402 Marseille, France; #Lab. d'Ingéniérie des Proteines, URA CNRS 1455, Faculté de Médecine, 13326, Marseille, France.

Scorpion toxins have been used to reveal the Kv channel structure. However, the reasons of their selectivity among Kv channels remained to be determined. To solve this problem we modified the kaliotoxin (KTX) sequence whithout changing the toxin residues facing the channel pore: i) punctual substitutions in the Gly1 to Lys 7 region of KTX increased up to 100-fold the peptide affinity to Kv1.3; ii) the natural KTX isoform, KTX3, which differed from KTX by 5 residues in the N-terminal region had a 50-fold higher affinity to Kv1.3 than KTX; iii) various peptides mutated in the KTX α-helix (Ser11 to Gly22) had affinities up to 200-fold higher than KTX to Kv1.1. Substitution in the N-terminal and α-helix regions of scorpion toxins may provide a suitable approach to construct peptides blocking with a high ratio of selectivity a given Kv1 α-subunit.

This work was supported by the Centre National de La Recherche Scientifique (CNRS).

496.18

ALTERNATIVELY SPLICED PORE LINING REGIONS IN *PANULIRUS SHAKER*. M.Kim, D.J.Baro, C.L.Cole, M.Doshi, H.S.Moskowitz, T.Podleski*, R.M.Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

Potassium channels play a critical role in the electrophysiological behavior of pyloric neurons in the stomatogastric ganglion (STG) of *Panulirus interruptus*. By screening cDNA libraries in combination with RACE (rapid amplification of cDNA ends), we have obtained alternatively spliced full-length *shaker* clones. As in *Drosophila*, multiple alternative exons occur at the amino and carboxy termini in *Panulirus shaker*. However unlike *Drosophila*, alternative splicing was found in the pore-forming region between the S5 and S6 transmembrane spanning domains of *Panulirus shaker*. Three different pore forming regions were found (0, I, II). Forms I and II are homologous to *Drosophila* and differ in only four amino acids. However, form I has a T residue at the outer mouth of the pore, corresponding to T449 in *Drosophila shaker* while form II has a positively charged R residue at this site. Structure/function studies in *Drosophila* suggest that this will alter C-type inactivation, drug resistance, and ion permeability. Form 0 lacks a pore forming exon altogether. Expression studies in *Xenopus* oocytes of form II with a short amino terminus exon produced currents with hypothesized rapid C-type inactivation. Expression studies of form 0 produced no functional channel and did not function as a dominant negative. Supported by NIH NS25915.

496.19

SUBUNIT BASIS FOR ION PERMEATION AND GATING SUGGESTED BY SUBCONDUCTANCE LEVELS IN K CHANNELS. M.L. Chapman, D.S. Roane*, H.M.A. VanDongen, A.M.J. VanDongen, Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710 and *Div. of Pharmacology, Northeast La. University, Monroe, LA 71209.

Channel opening and permeation are two essential properties of ion channels, the molecular bases of which are poorly understood. Analysis of single channel behavior of a voltage dependent K channel suggested that intermediate current levels ("sublevels") appeared to be associated with transitions between the open and closed conformations. Since K channels are formed by four identical subunits, it was hypothesized that sublevels corresponded to channel states with heteromeric pore conformations. This subunit-subconductance hypothesis predicts that sublevel behavior should be more abundant when activation is incomplete. Experimental evidence supported this prediction as sublevels were increased tenfold in recordings near the activation threshold. To further test the hypothesis, a mutation was introduced in the amino-terminal region of the voltage sensor of the channel. The resulting channel (drk1-LS) was found to have a slight reduction in the voltage-dependence of open probability but the voltage-dependence of the activation time constant was reduced nearly twenty-fold. Additionally, drk1-LS exhibited sublevel activity over a broadened voltage range. The subunit-subconductance hypothesis predicts just such behavior if the rate of activation is slow, relative to the rate of channel opening. Based on these results a subunit basis is proposed for channel opening and ion permeation.

Supported by Grant NS31557 from NINDS to AMJVD.

497.1

Kv2.1 SUBUNIT EXPRESSION IN RAT HIPPOCAMPAL INTERNEURONS AND ITS KNOCKOUT BY ANTISENSE OLIGONUCLEOTIDES IN CULTURED HIPPOCAMPAL SLICES. J. Du*, S. Keros & C.J. McBain. Lab. of Cellular & Molecular Neurophysiology, NICHD, NIH, Bethesda MD, 20892.

The potassium channel subunit, Kv2.1, is widely expressed throughout the rat CNS and is thought to have a role in normal physiological function (Maletic-Savatic et al. J. Neurosci. 15, 1995). In the hippocampus, Kv 2.1 is expressed not only in both pyramidal and granule cells, but also in presumed inhibitory interneurons. Experiments were designed to determine whether Kv2.1 was expressed in defined populations of interneurons using double immunostaining with anti-Kv2.1 and markers for identified interneuron subpopulations. Double staining of anti-Kv2.1 and anti-parvalbumin (PV) antibodies show that > 90% of the PV positive basket cells are Kv2.1 positive. Experiments are in progress to determine the distribution of Kv2.1 in other interneuron populations. To determine the functional role of channels formed by Kv2.1 in pyramidal neurons, cultured hippocampal slices were incubated in antisense oligonucleotides directed against Kv2.1 to block subunit expression. Western blot analysis revealed that total Kv2.1protein content decreased ~30% after a 5-day treatment with antisense (4.5μM) compared with nonsense oligos. Consistent with this reduction, whole-cell delayed rectifier currents from antisense treated CA1 pyramidal neurons were significantly reduced (~26%) when compared to nonsense or control treated cells. Channel density in outside out patches from antisense treated cells was 52 ± 12 pA/pF compared to 100 ± 25 pA/pF in control or nonsense treated cells. Under conditions of elevated $[K^+]o$ (8.5mM), action potential duration in antisense treated cells was increased 317% of that seen in control or nonsense treated cells. All other action potential parameters were unchanged. Thus functional channels comprising Kv2.1 subunits are expressed in both excitatory and inhibitory cells of the hippocampus and the delayed rectifier currents through these channels selectively contribute to action potential repolarization. This work was supported by the NIH.

497.2

ARACHIDONIC ACID BLOCKS THE TRANSIENT POTASSIUM CURRENT IN CA1 PYRAMIDAL NEURONS IN ORGANOTYPIC CULTURE. S. Keros, J. Du, and C. J. McBain*. Lab. of Cellular & Molecular Neurophysiology, NICHD, NIH, Bethesda MD, 20892.

Arachidonic acid (AA) strongly modulates both neuronal excitability and synaptic transmission. Recent evidence suggests that AA selectively blocks transient "A-type" outward potassium currents through Kv4.2 homomeric channels in oocytes (Villarroel and Schwarz J. Neurosci. 16, 1996), while currents through homomeric channels formed by members of the Kv1, 2 or 3 subfamilies were largely resistant. Currents through Kv4.2 channels have been proposed to underlie the principal transient current in the mammalian CNS (Serodio et al. J. Neurophys 72, 1994) consistent with the strong Kv4.2 expression in hippocampal pyramidal neurons (Maletic-Savatic et al. J. Neurosci. 15, 1995). To determine whether hippocampal transient currents are through Kv4.2 channels, we have investigated the effects of AA on currents in outside-out patches excised from CA1 pyramidal neurons in organotypic hippocampal cultures prepared from P6-8 rats. AA dose-dependently blocked the transient current in CA1 pyramidal neurons; IC_{50} = 0.1μM. At a test potential of +40mV, AA (10μM) blocked 70% of the transient current charge transfer. In addition a more rapid time constant of current inactivation was observed in the presence of AA (20ms in control versus 5ms in AA). The effects of AA were mimicked by eicosatetraynoic acid (ETYA), a non-metabolizable AA analog. ETYA (10μM) blocked 75% of the transient current charge transfer and like AA, accelerated the rate of current inactivation. Previously we have shown that the transient current in inhibitory basket cells (but not pyramidal neurons) was selectively inhibited (~40%) when perfused in 0mM $[K^+]_o$, consistent with currents through channels predominantly formed by Kv1.4 subunits. Future experiments will determine whether the remaining current fraction in basket cells is sensitive to AA. These data suggest that the transient current in pyramidal neurons is predominantly through channels formed by Kv4.2. This work was supported by the NIH.

497.3

CORTICOTROPIN RELEASING HORMONE MODULATION OF A HYPERPOLARIZATION-ACTIVATED CURRENT (I_H). Y. A. Kuryshev[1], G. V. Childs[2] and A. K. Ritchie[1]*. Depts. of Physiology & Biophysics[1] and Anatomy & Neurosciences[2], Univ. Texas Medical Branch, Galveston, TX 77555.

Corticotropin releasing hormone (CRH) increases firing frequency and plays an important role in stimulation of ACTH secretion from corticotropes. In this work on highly enriched cultured rat corticotropes, we used the amphotericin perforated patch technique to study CRH modulation of a hyperpolarization-activated, sodium-potassium current (I_H). The compositions of the recording solutions were (in mM): pipette - 60 K_2SO_4, 30 KCl, 10 NaCl, 1 $MgCl_2$, 1 EGTA, 10 HEPES, pH 7.2, 300 μg/ml amphotericin B; bath - 50 KCl, 95 NaCl, 10 TEACl, 0.05 $CaCl_2$, 1 $MgCl_2$, 5 4-aminopyridine, 10 HEPES, 10 glucose, 30 sucrose, 0.05 $LaCl_3$, 0.25 $BaCl_2$, 0.001 TTX, pH 7.4 (36°C). I_H activated at potentials negative to -40 mV, was non-inactivating and exhibited slow activation kinetics (τ_{act}=507±82 ms at -100 mV, N=15). The activation curve was well fit by the Boltzmann relation with half-maximal activation at -75.5 mV and a slope factor of 8.0 mV (N=9). In physiological saline the reversal potential of I_H obtained by instantaneous current-voltage relations was -46.5 mV (N=4). The reversal potential with changing $[Na^+]_o/[K^+]_o$ was described by the Goldman Equation with pNa/pK = 0.161. I_H was insensitive to Ba^{2+} (2mM), Cd^{2+} (1 mM) and TEA (25 mM), was slightly attenuated (17%, N=3) with 5 mM 4-aminopyridine, and was blocked in a voltage-dependent manner with external Cs^+ (K_d = 83 μM at -100 mV, N=3). CRH (20 nM) did not change the amplitude or voltage-dependence of I_H, but significantly decreased τ_{act} by 35% (N=5). These results suggest that I_H current could contribute to the resting membrane potential of corticotropes and modulation of I_H by CRH could enhance spike frequency by increasing the rate of depolarization during the interspike interval. Supported by DK 44363 and DK 39553.

497.4

MODULATION OF ELECTRICAL DEVELOPMENT OF *XENOPUS* EMBRYONIC MYOCYTES BY FIBROBLAST GROWTH FACTOR. A.E. Spruce, E. Prabhakar and I.M. Stanford*. Dept. Pharmacology, Univ. of Birmingham, Birmingham. B15 2TT.

The amount of sodium current (I_{Na}) in *Xenopus* embryonic myocytes is increased by co-culture with dissociated neurons and the absence of synaptic contacts implies an effect of diffusible factors (Prabhakar et al. 1996. Neurosci. Lett. 207:1-3). Fibroblast growth factor (FGF) is known to promote maturation of the neuromuscular junction and here we assess its ability to modulate the developmental expression of voltage-dependent currents in muscle. Myocytes were removed from *Xenopus* embryos at Stages 15-18 and cultured for 18-30 hr in the absence (control) or presence of acidic FGF (aFGF) at 10 ng/ml. Whole-cell patch clamp recordings were made using a pipette solution containing (in mM): 90 K.aspartate, 10 NaCl, 2 $MgCl_2$, 2 EGTA, 10 HEPES, 2 Na_2ATP, 0.1 cAMP; pH 7.4.

aFGF significantly increased mean I_{Na} density in current-expressing myocytes: 24.8 ± 5.6 pA/pF (n = 16; control); 57.5 ± 16.8 pA/pF (n = 16; aFGF); $p < 0.04$ (t-test). In addition, the proportion of cells containing I_{Na} was increased: 16/25 (control); 16/17 (aFGF); $p < 0.05$ (Fisher's exact test). In contrast to the effects on I_{Na}, aFGF caused downregulation of the expression of inward rectifier potassium current (I_{IR}). I_{IR} density was 18.8 ± 2.2 pA/pF (n = 13) in control and 4.3 ± 1.1 pA/pF (n = 12) in aFGF ($p < 0.0001$). Although myocyte capacitance is significantly increased by aFGF (47.5 ± 2.4 pF; n = 35; control vs. 56.4 ± 2.3 pF; n =. 38; aFGF; $p < 0.003$), this can not explain the four-fold decrease in I_{IR} density. Mean steady-state outward potassium current was not affected by aFGF, however: 12.2 ± 2.8 pA/pF (n = 17; control) vs. 14.2 ± 2.4 pA/pF (n = 17; aFGF). Preiiminary results suggest a similar effect of bFGF. Therefore, FGF-induced modulation of current expression is predicted to increase the electrical excitability of embryonic myocytes.

The Wellcome Trust is gratefully acknowledged for providing financial support.

497.5

NONSYNAPTIC MODULATION OF VOLTAGE-ACTIVATED POTASSIUM CHANNELS. S.C. Baraban*, M.C. Bellingham, A.J. Berger and P.A. Schwartzkroin. Depts. of Neurological Surgery & Physiology/Biophysics, University of Washington, Seattle WA 98195.

Nonsynaptic influences, such as the activity-dependent fluctuation in extracellular osmolarity, play key roles in regulation of neuronal excitability. Utilizing whole-cell and single-channel recording techniques in conjunction with infrared differential interference contrast video microscopy, we examined the effect of hypo-osmolar external solutions on voltage-activated K^+ channel function. Hypo-osmolar solutions (osmolarity reduced 10% to 267 mOsm) produced a significant and reversible potentiation of whole-cell K^+ current from lacunosum/moleculare (L/M) interneurons (+43%; n =10). Although cell swelling was induced by this treatment in both interneurons and CA1 pyramidal cells, no potentiation was observed for CA1 neurons (n = 8). Hypo-osmolar solutions did not produce a modulation of hyperpolarization-activated current on either type of neuron. In single-channel outside-out patch recordings from L/M interneurons (n = 7) hypo-osmolar solutions increased the mean open time and the probability of channel opening; no effect was observed on CA1 neurons (n = 3). Ensemble K^+ current amplitude was increased during application of hypo-osmolar solutions only for L/M interneurons. Osmotically-induced modulation of K^+ channel function was not associated with a change in the threshold for activation or the amplitudes of single-channel currents. These data suggest that osmotic stress may play a critical role in regulating neuronal excitation during both normal and pathophysiological conditions. This neuron-specific finding has important implications for understanding nonsynaptic mechanisms which modulate excitability in the central nervous system. Support: American Epilepsy Society, Parker Family Foundation and NIH.

497.6

MODULATION OF SINGLE INWARD RECTIFIER POTASSIUM CHANNELS IN *XENOPUS* EMBRYONIC MYOCYTES. R. Chauhan-Patel and A.E. Spruce*. Dept. of Pharmacology, Univ. of Birmingham, Birmingham, B15 2TT. UK

Current rectification through cloned inward rectifier potassium (IRK) channels is attributed to diffusible blockers (Lopatin et al. 1994. Nature. 372:366). We have investigated whether this mechanism also applies to native embryonic skeletal muscle IRK channels. We have also studied the effect of various potential modulators of channel activity to assess their role in regulating the functional expression of IRK current during development. Myocytes were isolated from *Xenopus laevis* embryos (Stage 15-20) and cultured for up to 30 hr. The activity of single IRK channels was recorded in cell-attached and excised, inside-out patches (Patel et al. 1995. J.Physiol. 489.P:73P). The pipette and bath solutions contained 120 mM K^+. Test solutions were applied from an adjacent electrode.

In control solution, IRK channel activity at -60 mV disappears "instantaneously" on application of a voltage step to +60 mV. Perfusion of the excised patch with Mg^{2+}-free (containing 1 mM EDTA) solution did not alter the rectification (n=17). However, when Mg^{2+}-free solution at pH 9.1 (CHES substituted for HEPES) was applied to the patch, channel activity at +60 mV was observed and could be correlated with IRK channel openings at -60 mV (3/4 patches). This result is consistent with the hypothesis that polyamines cause rectification in native IRK channels, since reduction in their state of ionisation at high pH will diminish their binding affinity.

We have also tested the ability of various chemicals to modulate IRK channel activity. Exposure of the cytoplasmic face of excised patches to 1 mM ATP or 10 μM GTP-γS did not affect the open probability of IRK channels. However, in cell-attached patch recordings, perfusion of aFGF onto the cell substantially reduces IRK channel activity within 10 min (3/3).

The financial support of the Wellcome Trust is gratefully acknowledged.

497.7

MODULATION OF VOLTAGE-DEPENDENT K^+ CURRENTS VIA cAMP CASCADE IN CULTURED EMBRYONIC NEURONS OF *DROSOPHILA*: ALTERATIONS IN MEMORY MUTANTS. M.-L. Zhao* and C.-F. Wu. Dept. of Biol. Sci., Univ. of Iowa, Iowa City, IA 52242, USA

Ion channel modulation by the cAMP cascade has been suggested to be an important mechanism underlying neuronal plasticity in the nervous system. In *Drosophila*, *dunce* (*dnc*) and *rutabaga* (*rut*) mutants are defective in cAMP metabolism, causing memory deficiencies in adult flies. We have previously observed in subsets of cultured mutant neurons altered frequency coding of firing evoked by step current injection, and aberrant spike activities in response to multiple stimulation paradigms. In order to examine if voltage-dependent K^+ currents are targets of the cAMP cascade, we performed whole-cell voltage-clamp recordings on embryonic neurons from *dnc* and *rut* mutants. A majority of cells could be divided into two categories based upon different inactivation time constants of the transient K^+ currents. We found that a variety of properties of voltage-dependent K^+ currents were affected in neurons of *dnc* and *rut*, likely due to chronic changes in cAMP levels. Acute application or short-term incubation with membrane-permeable cAMP analogues modified K^+ currents in amplitude, kinetics, and voltage-dependence, which were altered in mutants. Furthermore, we examined, in *dnc* and *rut* background, effects of cAMP cascade on heat shock-inducible, identifiable K^+ currents mediated by the ShD subunit expressed in transgenic flies. Our results suggest that neuronal voltage-activated K^+ currents are potential targets for modulation by the cAMP cascade. Altered modulation in *dnc* and *rut* mutants may contribute to their learning deficiencies. [Supported by NIH grants NS 26528, HD 18577]

497.8

CANNABINOID AGONISTS MODULATE TWO SUBTYPES OF POTASSIUM A-CURRENT (I_A) IN CULTURED RAT HIPPOCAMPAL NEURONS. Shou-Yuan Zhuang*, Jian Mu, Josef T.J. Kittler, Zoe L. Sinclair, M. Todd Kirby, Robert E. Hampson, and Sam A. Deadwyler, Department of Physiology and Pharmacology and Center for the Neurobiological Investigation of Drug Abuse, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

Cannabinoid receptor agonists modulate potassium A-currents (I_A) via a cAMP second messenger pathway (Deadwyler *et al.* 1996, JPET; Hampson *et al.* 1996, Life Sci.). Investigation of the final link in this cascade from protein kinase to ion channel (see J. Mu, *et al.*, this meeting) revealed differential effects on steady-state and transient inactivation of I_A recorded via whole cell patch clamp in cultured hippocampal neurons. The potent cannabinoid agonist, WIN 55,212-2, enhances I_A via a positive shift in voltage dependence of steady-state inactivation. WIN 55,212-2 also markedly reduced the time constant (*tau*) of I_A transient inactivation from 50 ms to 25 ms. Inhibitors of cAMP-dependent protein kinase (PKA) also reduced *tau*. Stimulation of PKA or elevation of cAMP had no effect. Cannabinoid effects on *tau* were blocked by the cannabinoid receptor antagonist SR141716A (Sanofi).

These effects suggest that I_A actually reflects a combination of potassium channel subtypes. Under control conditions, I_A may consist of currents with 50 ms *tau* such as those produced by Kv4.2 homomeric channels or Kv1.2/1.4 heteromeric channels. In the presence of cannabinoids acting through cAMP, I_A resembles homomeric Kv1.2 currents. RT-PCR and fluorescent antibody labelling have revealed that mRNA and channel proteins for Kv1.2, Kv1.4 and Kv4.2 were present in these cells. *[Supported by NIDA grants DA03502, DA07625 & DA00119 to S.A.D.]*

497.9

CANNABINOIDS ALTER MEMBRANE EXCITABILITY AND SYNAPTIC TRANSMISSION IN CA1 PYRAMIDAL CELLS OF THE RAT HIPPOCAMPUS. M.T.Kirby*, R.E. Hampson, and S.A. Deadwyler, Neuroscience Program, and Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC, 27157-1093.

The effects of the cannabinoid agonist WIN 55,212-2 (5μM, RBI) on CA1 pyramidal cells were investigated using the hippocampal slice preparation and sharp electrode intracellular recording. Bath-applied WIN 55,212-2 was found to alter input resistance to both hyperpolarizing and depolarizing current injections (n=24). WIN 55,212-2 enhanced a hyperpolarization-activated inward current (I_Q). The increase was effectively blocked by the cannabinoid antagonist SR141716A (300nM, Sanofi) or by intracellular injection of QX-314 (100mM, Astra), yet remained potentiated in 2mM Ba^{2+}. WIN 55,212-2 also enhanced a depolarization-activated current that was sensitive to 5mM TEA, such that the combined effect of the drug was to shallow the slope of the overall I-V relationship. Evidence of a presynaptic effect of cannabinoids was supported by a bicuculline-sensitive (50μM) hyperpolarization in the presence of WIN 55,212-2 that was attenuated by TTX (1μM) or Ca^{2+}-free media.

Constructed input-output curves revealed decreased EPSP amplitudes (via stimulation of the Schaffer collaterals) via WIN 55,212-2. The decrease persisted in bicuculline, and results will be discussed in terms of the above postsynaptic changes in membrane resistance. Collectively, these data illustrate that cannabinoids mediate changes in cellular excitability of hippocampal neurons through at least one presynaptic and two different postsynaptic conductance changes. *[Supported by NIDA Grants DA07625, DA03502 and DA00119 to S.A.D. and Training Grant DA07246.]*

497.10

ROLE OF PROTEIN PHOSPHORYLATION IN THE CANNABINOID MODULATION OF POTASSIUM A-CURRENT (I_A) IN CULTURED RAT HIPPOCAMPAL NEURONS. Jian Mu*, Shou-Yuan Zhuang, Robert E. Hampson, M. Todd Kirby, and Sam A. Deadwyler, Department of Physiology and Pharmacology and Center for the Neurobiological Investigation of Drug Abuse, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

The potent cannabinoid agonist, WIN 55,212-2, modulates potassium A-current (I_A) in rat hippocampal neurons via cAMP-dependent protein kinase (Deadwyler *et al.* 1995, JPET; Hampson *et al.* 1995, Life Sciences). The likely mechanism for this modulation is via phosphorylation and dephosphorylation of the I_A channel protein. Whole cell currents were recorded from cultured E-18 fetal rat hippocampal neurons, 7-15 days in culture, with TTX 1μM, 4-AP 500μM and TEA 30mM in the bathing medium to block currents other than I_A. WIN 55,212-2 (RBI) was applied extracellularly via pressure pipette (2μM). IP-20 (10μM, Sigma), PKA catalytic subunit (PKAc; 500 units/ml, Sigma), and okadaic acid (2μM, RBI) were administered via the intracellular recording pipette.

The positive shift in steady-state inactivation of I_A and decreased time constant (transient inactivation, *tau*) produced by WIN 55,212-2 were reversed when PKAc was included in the patch clamp recording pipette. Cannabinoid effects were also blocked by the phosphatase inhibitor, okadaic acid. Addition of the protein kinase inhibitor IP-20 (10μM) with PKAc produced a positive shift in I_A and reduction in *tau* similar to WIN 55,212-2. Together these data suggest that cannabinoid effects on I_A are mediated by phosphorylation and dephosphorylation of ion channel proteins.

[Supported by NIDA grants DA03502, DA07625 & DA00119 to S.A.D.]

497.11

A-CURRENT DEVELOPMENT IN HIPPOCAMPAL NEURONS: LINKS TO KINASE ACTIVITY AND MEMBRANE INSERTION. Rui-Lin Wu* and Michael E. Barish. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

A-current appears early during development of mouse hippocampal neurons, being present in acutely-dissociated pyramidal neurons as early as embryonic day 15-16. Nevertheless, A-current amplitude in neurons of dissociated cell cultures prepared from E15-E16 hippocampi depends on the extent of contact with co-cultured astroglial cells, an observation suggesting that mRNA(s) related to A-current may be synthesized early, and that regulation of A-current involves other downstream process. In these experiments, we sought to identify processes internal to neurons that limit the appearance of A-current. Neurons were grown in both serum-containing and serum-free media, drugs were added 1-10 hr after cell dissociation and plating, and amplitudes of voltage-gated potassium currents (A-, D- and K-currents) were assayed after 4-7 days. Two main observations have emerged. First, the appearance of A-current, but not D- or K-currents, is linked to neurite outgrowth. Pyramidal neurons grown in serum-containing medium and not in contact with astroglial cells are smaller and have lower A-current amplitudes and densities than neurons with more extensive neurites grown under serum-free conditions that reduce numbers of non-neuronal cells. Second, the sensitivity of A-current development to kinase inhibitors is similar to that of membrane transporters regulated by vesicle insertion. Thus development of A-current, but not D- or K-currents, is reduced by continuous exposure to staurosporine (2-20 pM), K252a (1 µM), KT5926 (10 µM) and wortmannin (0.1-2 µM), but is not sensitive to calphostin C (0.1-0.5 µM), K252b (1 µM), KN-62 (10 µM) or bisindolmaleimide I (1 µM). This pattern suggests phosphatidylinositol 3-kinase and/or myosin light chain kinase as possible rate limiting enzyme(s). Supported by NIH R01 NS23857.

497.12

C_2-CERAMIDE ATTENUATES POTASSIUM CONDUCTANCES INDIRECTLY VIA RAS IN CULTURED OLIGODENDROCYTES. H. Hida, M. Takeda and B. Soliven*. Dept. of Neurology, The Brain Research Institute, The Univ. of Chicago, Chicago, IL 60637.

Ceramide is generated from sphingomyelin hydrolysis and has been implicated in TNF-α signaling and stress-induced apoptosis. We have previously shown that TNF-α inhibits K^+ currents in cultured oligodendrocytes (OLGs) (Soliven et al., J. Memb. Biol. 124: 127-137, 1993). To investigate further the signaling mechanisms of TNF-α on OLGs, we studied the effect of C_2-ceramide (Cer) on inwardly rectifying (I_{Kir}) and outward K^+ currents (I_{Ko}) in cultured neonatal OLGs ($Galc^+$ cells) and progenitors ($O4^+Galc^-$ cells) using the whole-cell patch-clamp technique. Cer (10 µM) decreased the amplitudes of I_{Ko} and I_{Kir} to 64.2 ± 6.5 % (n=13) and 75.3 ± 6.9 % (n=13) of the initial current at 5 min after perfusion, respectively. The effect was concentration-dependent and was observed with concentration as low as 1 µM. The inhibitory action of Cer on K^+ currents was attenuated by the addition of neutralizing antibody against Ras-p21 in the pipette solution (92.4 ± 2.1 % and 103.1 ± 3.1% of the initial current for I_{Ko} and I_{Kir}, n= 11) or by pretreatment with H-8 (30 µM) for 1hr (89.1 ± 2.9 % and 96.4 ± 4.8 % of the initial current for I_{Ko} and I_{Kir}, n= 7). Pretreatment with quinacrine (10 µM), inhibitor of phospholipase A_2, for 1 hr did not block the effect of Cer on K^+ currents. We conclude that Cer-induced inhibition of K^+ conductances is due to Ras-mediated channel phosphorylation.

Supported by Nat'l MS Society Gr. RG2195-B3 and Spinal Cord Research Foundation, #1240-03.

497.13

ELECTROPHYSIOLOGICAL STUDIES IN CULTURED HUMAN MICROGLIA J.G. McLarnon*, R. Xu, Y.B. Lee[1] and S.U. Kim[1] Dept. of Pharmacology & Therapeutics, and [1] Div. of Neurology, Dept. of Medicine, The Univ. of British Columbia, Vancouver, B.C. V6T1Z3

Patch clamp studies have been carried out on microglia isolated from fetal human brains. An inwardly-rectifying K^+ current, but no outward K^+ current, was expressed within two days of cell adherence. After 5 days of cell attachment to substrate, an outward K^+ current was evident in addition to the inward K^+ current. The outward K^+ current was composed of two components with different kinetic behavior and with different pharmacology. In early adherent cells (day 1 or 2 after plating) treatments with IFN-γ (200 U/ml for 12 h) led to expression of the outward K^+ current which was not observed in the absence of the cytokine treatment. Two high conductance channels were expressed in inside-out patches including a high conductance BK-type of K(Ca) channel (unitary conductance of 106 pS with physiological levels of K^+ across the patch) and an anion channel (unitary conductance of 280 pS). For K(Ca), with the patch potential held at 0 mV, the open probability was 0.5 with internal Ca^{2+} at a concentration of 7 μM. Recordings from cell-attached patches also provided evidence for a unitary current which was activated by suction applied to the patch pipette. This channel had a conductance of 30 pS and was non-selective for different cations. The properties and expressions of the different currents in the cultured human microglia may be correlated with functions of these cells in the CNS when activated by a variety of stimuli.

NSERC - CANADA

497.14

ACTIVATION OF INWARD RECTIFYING POTASSIUM CHANNELS BY CANNABINOID CB1 AND CB2 RECEPTORS. Y. Uezono*, Y. Ueda, K. Pleyte, J.T. Eells, F. Izumi and B.Y. Ho. Univ. of Occup. & Environ. Health, School of Medicine, Kitakyushu 807, Japan, and Dept. Pharmacol. Medical College of Wisconsin, Milwaukee, WI 53226, USA

The ability of the cannabinoid receptors to activate G protein-coupled inward rectifying potassium channels (GIRKs) were studied by expressing the brain (CB1) or peripheral (CB2) cannabinoid receptor with two GIRKs (GIRK1 and CIR) in *Xenopus* oocytes. The cRNAs from various clones were synthesized by in vitro transcription and microinjected into oocytes. Recordings were performed 3-6 days after injection. When the CB1 or CB2 receptor was co-expressed with GIRK1, the cannabinoid receptor agonist CP55940 (1 μM) elicited 51 ± 10 and 12 ± 7 nA current, respectively, at a holding potential of -80mV. When either receptor was co-expressed with both GIRK1 and CIR, the currents elicited by CP55940 were significantly increased to 251 ± 47.5 (CB1 receptor) and 120 ± 41 nA (CB2 receptor). Another agonist WIN55212-2 (10 μM) also elicited 272 ± 69 and 118 ± 32 nA currents, respectively, for the CB1 and CB2 receptors. The EC_{50} of CP55940 and WIN55212-2 were estimated to be 150 and 440 nM when the CB1 receptor was co-expressed with GIRK1 and CIR. These findings suggest that both the CB1 and CB2 cannabinoid receptors are able to couple to GIRK. Since co-expression of GIRK1 and CIR resulted in much greater currents than expression of GIRK1, GIRKs may be expressed as heteromers.

Supported by Human Frontiers Scientific Program, American Heart Association, American Cancer Society

POTASSIUM CHANNELS: MODULATION II

498.1

THE *DROSOPHILA ETHER-A-GO-GO* (Eag) POTASSIUM CHANNEL SUBUNIT IS A SUBSTRATE FOR CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II (CaMKII)
Z. WANG* and L.C. GRIFFITH Department of Biology and Center for Complex Systems, Brandeis University, Waltham, MA 02254

Behavioral, physiological and biochemical interactions of CaMKII and Eag in *Drosophila Melanogaster* suggest that an important component of neural plasticity may be mediated through modulation of Eag function by CaMKII (Griffith *et al*, PNAS, 1994). To determine whether Eag was a substrate for CaMKII, partial Eag sequences were expressed in *E. coli* as glutathione-S-transferase (GST) fusion proteins. Two separate fusions corresponding to Eag N-terminal residues 44-210 and to C-terminal residues 556-802 were examined *in vitro*, as both fragments were presumed to be cytoplasmic. Only the C-terminal fragment was actually phosphorylated by purified CaMKII. In this fragment, one of the CaMKII consensus phosphorylation sites (Thr^{655}) falls within the putative Eag cyclic nucleotide-binding domain. Phosphorylation of Thr^{655} by CaMKII could potentially alter the ability of cyclic nucleotides to regulate potassium currents. A phosphospecific polyclonal antibody was raised against a phospho-Thr^{655}-containing peptide. The purified antibody recognized only the phosphorylated form of the C-terminal fusion protein but not the unphosphorylated form, unambiguously identifying Thr^{655} as an *in vitro* phosphorylation site of CaMKII. However, a corresponding T655A mutant fusion protein was also phosphorylated by CaMKII, indicating the presence of other potential CaMKII phosphorylation site(s). The Thr^{655} phosphospecific antibody did not recognize the phosphorylated form of the mutant fusion protein. Whether Thr^{655} is an *in vivo* target for CaMKII is under investigation. (Supported by NIH)

498.2

MODULATION OF POTASSIUM CHANNEL Kv1.4 BY THE m1 MUSCARINIC RECEPTOR. T. Rossignol and S.V.P. Jones*. Department of Psychiatry, University of Vermont College of Medicine, Burlington, VT 05405.

The effects of muscarinic receptor-induced modulation of a transient potassium channel (hKv1.4) were investigated by co-expression of hKv1.4 with the m1 muscarinic receptor in TSA cells. Using the whole-cell patch clamp technique, large outward transient potassium currents were obtained in 80-90% of the cells transfected. Cells were voltage clamped at -60 mV, hyperpolarized to -100 mV for a 1.5 s prepulse, and stepped to a variety of potentials from -110 mV to +50 mV for 500 msec. Current-voltage activation curves were constructed from the peak of the outward current response. To obtain the inactivation curve the cells were held at -60 mV, then stepped to a variety of prepulse potentials, from -110 mV to -50 mV for 1.5 s then depolarized to +50 mV for 500 ms. Current-voltage inactivation curves were constructed from the peak of the outward current at the +50 mV depolarization step. Stimulation of the m1 muscarinic receptor by application of 100 μM acetylcholine, via a pressure ejection pipette, had no significant effect on the peak current amplitude, and produced no shift in the I-V curves. However, pre-incubation with 100 ng/ml of pertussis toxin (overnight) resulted in a reduction of the peak current amplitude upon application of acetylcholine (n=30). Peak current amplitudes were reduced from 4.15 ± 1.49 nA (n=7) to 3.37 ± 1.24 nA (n=7) in the presence of 100 μM acetylcholine, a decrease of 19%. Current amplitudes returned to control levels of 3.96 ± 1.39 nA (n=7) after washout of the acetylcholine. To investigate the mechanism of action of acetylcholine, a variety of second messengers were applied to the cells. Stimulation of protein kinase C by phorbol esters and activation of cAMP-dependent protein kinase had no effect on Kv1.4, however 5-10μM arachidonic acid induced a dose-dependent reduction in Kv1.4 current amplitude.

498.3

TYROSINE KINASE INHIBITION DECREASES THE LEVEL OF PHOSPHOTYROSINE IN HEK 293 CELLS EXPRESSING Kv3.1 K^+ CHANNELS. S.D. Critz*, K.P. Wade, and B.A. Wible@. Rammelkamp Ctr. for Research, Case Western Reserve, Cleveland, OH@ and Dept. of Structural & Cell Biology, Univ. of South Alabama, Mobile, AL. 36688.

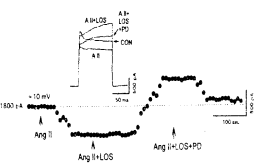
Although the regulation of K^+ channels by serine/threonine kinases is well known, much less is known about the effects of tyrosine kinase-mediated phosphorylation of these channels. We found previously that tyrosine kinase inhibitors block K^+ channels stably expressed in HEK 293 cells (*Soc. Neurosci. Abstr.* 19:711, 1993).

This effect was examined further biochemically by analyzing the effects of tyrosine kinase inhibition in the cells. Kv3.1 K^+ channel transfects were incubated 30 min in depolarizing saline at 10, 30, 100, and 300 μM doses of the tyrosine kinase inhibitor genistein. The cells were lysed and solubilized proteins were processed by SDS-PAGE and Western transfer for immunodetection of phosphorylated tyrosine using mouse anti-phosphotyrosine antibody. We find that the phosphorylation of two proteins (22 kD and 18 kD) are decreased in a dose-dependent manner by tyrosine kinase inhibition. We also determined that the proteins were associated with the particulate fraction. These results suggest that the phosphorylation of tyrosine residues on these proteins may play a role in regulating K^+ channel activity. (Alzheimer's Assoc. PRG-90).

498.4

ELECTROPHYSIOLOGICAL CO-LOCALIZATION OF ANGIOTENSIN II TYPE 1 (AT_1) AND TYPE 2 (AT_2) RECEPTORS IN CULTURED NEURONS FROM RAT BRAIN. C.H. Gelband, M. Zhu, R.L. Casto*, P. Posner, and C. Sumners. Dept. of Physiology, Univ. of Florida, Gainesville, FL 32610.

We previously demonstrated that angiotensin II (Ang II) either inhibits or stimulates, via AT_1 and AT_2 receptors respectively, the delayed rectifier K^+ current (I_K) in neurons cultured from newborn rat hypothalamus and brainstem (Kang *et al.*, 1993; Sumners *et al.*, 1996). Using standard whole-cell patch clamp techniques, we have investigated whether these responses occur on the same or different populations of neurons. Four distinct electrophysiological responses to Ang II (100 nM) were observed. First, application of Ang II caused a losartan (1 μM) sensitive, AT_1 receptor mediated decrease in I_K (n=10). Second, application of Ang II caused a PD 123319 (1 μM) sensitive, AT_2 receptor mediated increase in I_K (n=10). Third, application of Ang II caused a biphasic effect on I_K. Ang II elicited a losartan sensitive, AT_1 receptor-mediated decrease in I_K followed by a PD 123319 sensitive, AT_2 receptor-mediated increase in I_K (see Figure, n=6). Fourth, the application of Ang II caused a PD 123319 sensitive, AT_2 receptor-mediated increase in I_K followed by a losartan sensitive, AT_1 receptor-mediated decrease in I_K (n=3). This indicates the presence of a population of neurons that contain both AT_1 and AT_2 receptors, and other populations that express only a single type of Ang II receptor. (Supported by HL-49130 and an AHA-FL Affiliate II Award).



498.5

SIALIDATION MODIFIES THE FUNCTION OF KV1.1 POTASSIUM CHANNELS. W.B. Thornhill and J.F. Margiotta*, Dept. Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029.

Exo- and endo-sialidase sensitivity of brain Kv1.1 potassium (K) channels suggests that the tetramer contains 150-200 negatively-charged sialic acids, some of which are in an unusual tandem-linked structure. To examine the role of sialidation in K channel function, CHO cells deficient in glycosylation (Lec mutants) were transfected with rat brain Kv1.1 cDNA. Patch-clamping techniques determined that functional Kv1.1 channels were expressed in all cell lines but the voltage dependence of activation ($V_{1/2}$) was shifted to more positive voltages (up to 23mV) and the activation kinetics were slower in the Lec mutants compared with controls. A similar shift in $V_{1/2}$ to positive voltages was recorded in control cells expressing Kv1.1 following treatment with sialidase or by raising extracellular calcium. In contrast, these treatments had little or no effect on the Lec mutants, indicating that channel sialic acids appear to be the negative charges sensitive to screening by calcium. The data suggest that sialic acid addition modifies Kv1.1 function, possibly by influencing the local electric field detected by its voltage-sensor, but that these carbohydrates are not required for cell surface expression. Supported by NIH grants to WB Thornhill (NS29633) and JF Margiotta (NS24417).

498.6

THE *DROSOPHILA SLOWPOKE (dSlo)* CALCIUM-DEPENDENT POTASSIUM CHANNEL IS PHOSPHORYLATED AT SERINE 942. J. Wang* and I. B. Levitan. Department of Biochemistry and Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254.

We have shown previously that the cloned calcium-dependent potassium channel, *dSlo*, can be phosphorylated at Serine 942 (S942) by various protein kinases *in vitro* (Wang and Levitan, Soc. for Neurosci. Abstr. 21, 1829, 1995). To investigate if S942 is phosphorylated *in vivo*, we generated two polyclonal antibodies against *dSlo*. One antibody, against a phosphorylated peptide corresponding to the *dSlo* sequence flanking S942, recognizes *dSlo* on a western blot only when it is phosphorylated at S942. The other antibody, raised against the carboxy-terminal 58 amino acids of *dSlo*, is used for immunoprecipitation as well as western blotting. Immunoprecipitates of *dSlo*, expressed transiently in the mammalian cell line tsA201, were analyzed on western blots with the phosphorylation-specific antibody. The results indicate that a fraction of *dSlo* protein is phosphorylated at S942 with basal kinase activity. Treatment of cells with forskolin dramatically increases the phosphorylation, implying that cAMP-dependent protein kinase (PKA) can phosphorylate *dSlo* at S942 *in vivo*. Furthermore, *dSlo* protein immunoprecipitated from *Drosophila* head lysates is phosphorylated at S942. Thus, the native channel protein is indeed a substrate for protein kinase(s), and S942 is phosphorylated under physiological conditions. Supported by a research grant from NIH.

498.7

DIRECT ASSOCIATION OF SRC TYROSINE KINASE WITH HUMAN Kv1.5 POTASSIUM CHANNEL MEDIATED BY AN SH3 DOMAIN INTERACTION. T.C. Holmes*, D.A. Fadool, R. Ren† and I. B. Levitan. Department of Biochemistry and Volen Center for Complex Systems, †Department of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02254.

Tyrosine phosphorylation modulates the properties of potassium channels, but the mechanism(s) that mediate channel/kinase association are unknown. The human isoform of the Kv1.5 potassium channel (hKv1.5) contains proline-rich sequences that are identical to sequences characterized previously as Src homology 3 (SH3) domain binding sites. Additionally, this channel contains potential tyrosine phosphorylation sites. The association of hKv1.5 and Src tyrosine kinase (Src) was examined in HEK 293 cells transfected transiently with cDNA constructs encoding both proteins. hKv1.5 and Src are associated in lysates from these cells, as shown by immunoprecipitation with specific antibodies for hKv1.5 or Src followed by western blot. hHv1.5 also binds to a fusion protein containing the Src SH3 domain, as shown by precipitation and filter binding assay. In contrast, the closely related rat Kv1.5 and Kv1.3 channels, which lack the Src SH3 domain binding site, do not associate strongly with Src kinase. Furthermore, hKv1.5 is tyrosine phosphorylated and functionally modulated by Src kinase. These results provide direct biochemical evidence for a potassium channel-protein kinase signalling complex. Supported by research and training grants from NIH.

498.8

MUTATIONS IN THE S4-S5 LOOP OF THE CALCIUM-DEPENDENT POTASSIUM CHANNEL, *mSlo*, DECREASE *SHAKER* BALL PEPTIDE BLOCK AND SHIFT GATING PROPERTIES. M. H. Holmqvist*, D. A. Sullivan and I. B. Levitan. Department of Biochemistry and Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254.

The *Shaker* inactivating peptide blocks the mouse large conductance calcium-dependent potassium channel, *mSlo*. In the *Shaker* channel, the intracellular loop between membrane spanning regions 4 and 5 has been suggested to form part of the receptor for the *Shaker* peptide (Isacoff et al., Nature 353:86-90, 1991). To test if the peptide also interacts with this loop in *mSlo*, we mutated three conserved residues in this region to alanines (T229A, S232A, L235A). This triple mutation decreases the number of blocking events by the peptide on reconstituted *mSlo* channels in artificial bilayers. Furthermore, this mutant is 50 times less calcium sensitive than the wild type channel, as measured with macroscopic currents in inside-out patches from HEK 293 cells transfected with *mSlo*. At a given calcium concentration, the activation curve of the mutant channel is shifted by 80 mV to more positive potentials. Using constructs with only one amino acid mutated, we found that L235A and T229A *mSlo* have properties intermediate between wild type and triple mutant channels. Thus the S4-S5 intracellular loop of *mSlo* plays a role in both *Shaker* ball peptide binding and the calcium and voltage-dependent gating of these channels. Supported by a research grant from NIH.

498.9

TYROSINE PHOSPHORYLATION OF TWO CLONED CALCIUM-DEPENDENT POTASSIUM CHANNELS. Y. Zhou*, J. Wang and I. B. Levitan. Department of Biochemistry and Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254.

It has been shown recently that tyrosine phosphorylation modulates the properties of voltage-dependent potassium channels. To determine whether calcium-dependent potassium channels are targets for tyrosine kinases, two large-conductance calcium-dependent potassium channels cloned from *Drosophila* (*dSlo*) and mouse (*mSlo*) were expressed in the mammalian cell line tsA201 using calcium phosphate-mediated transfection. The tyrosine phosphorylation of the channels was assessed by immunoprecipitation and Western blot analysis, using a phosphotyrosine-specific antibody and antibodies that recognize specifically either *dSlo* or *mSlo*. Both *dSlo* and *mSlo* channels are tyrosine phosphorylated to a limited extent under basal conditions. The tyrosine phosphorylation of both *dSlo* and *mSlo* is increased when channel-transfected cells are treated with the membrane-permeant tyrosine phosphatase inhibitor pervanadate (200μM, 10-30 min). Co-expression of the constitutively active tyrosine kinase, v-src, with the channel proteins, also causes a large increase in tyrosine phosphorylation of both *dSlo* and *mSlo*. Thus cloned calcium-dependent potassium channels are substrates for endogenous and co-expressed tyrosine kinases in cells. Supported by a research grant from NIH.

498.10

SRC TYROSINE KINASE HAS MULTIPLE FUNCTIONAL EFFECTS ON THE CLONED VOLTAGE-GATED K^+ CHANNEL, Kv1.3. D.A. Fadool*, T.C. Holmes, and I.B. Levitan. Department of Biochemistry and Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254.

We are studying the regulation of ion channels by protein tyrosine kinases (TKs) using a cloned voltage-dependent potassium channel, Kv1.3, as a model. Kv1.3 channels are studied by transient overexpression of their cDNAs in human embryonic kidney (HEK 293) cells using lipofectamine transfection. We have demonstrated previously that Kv1.3 tyrosine phosphorylation is increased and current is suppressed, following channel co-expression with the constitutively active cellular TK, v-src. Putative tyrosine phosphorylation sites were changed to phenylalanine (Y to F mutations). These mutagenesis experiments have shown that tyrosine residues 137 and 449 are required for TK induced current suppression, residues 111-113 and 449 are required for increasing the channel deactivation kinetics, and residues 111-113, 137, and 479 are required for decreasing the rate of C-type inactivation of the channel. Thus v-Src can have different effects on Kv1.3 by phosphorylating multiple residues. Whole-cell outward currents in rat olfactory bulb neurons (OBNs), which express Kv1.3 channels, are suppressed following internal application of c-Src^{pp60}. Hence tyrosine phosphorylation of Kv1.3 and related channels may be involved in modulation of the activity of OBNs. Supported by research and training grants from NIH.

498.11

***SLOB*, A NOVEL PROTEIN THAT INTERACTS WITH THE *DROSOPHILA SLOWPOKE (dSlo)* CALCIUM-DEPENDENT POTASSIUM CHANNEL.** W.M. Schopperle, J.Wang and I.B. Levitan*. Department of Biochemistry and Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Protein-protein interaction is a fundamental mechanism for cellular regulation of protein function. The cloned calcium-dependent potassium channel, *dSlo*, was used in the yeast two-hybrid system, a genetic tool for studying protein-protein interactions, to identify *dSlo*-interacting proteins. Putative intracellular portions of the *dSlo* channel were screened against a *Drosophila* head cDNA library and six clones were identified as potential *dSlo*-interacting proteins. Four of the clones contained partial sequences of the same cDNA. DNA probes were used to clone the full length cDNA from a cDNA library. The sequence encodes a 483 amino acid protein. Searches for sequence homology with known proteins reveal no specific matches suggesting that the protein, named *Slob* (*Slo*-*b*inding), is novel. *Slob* and *dSlo* interact *in vivo*, as shown by transiently co-expressing *dSlo* and HA-epitope-tagged *Slob* in the mammalian cell line tsA201, immunoprecipitating *dSlo,* and detecting *Slob* in the immunoprecipitate by western blotting with anti-HA antibodies. Work is in progess to identify the function of *Slob*. Supported by research and training grants from NIH.

498.12

ATP ACTIVATION OF BK-CHANNELS IN GH3 CELLS INVOLVES PLA_2. D. D. Denson and D. C. Eaton*. Depts of Anesthesiology and Physiology and The Center for Cell and Molecular Signaling, Emory Univ. Sch. of Med., Atlanta, GA 30322.

BK-channels are activated by both Mg-ATP and ATP-γ-S in excised patches and in lipid bilayers. It has been suggested that this activation is due to a direct PKC mediated phosphorylation of the channel by ATP. Since PLA_2 production of arachidonic acid is also a potent activator of BK-channels in excised patches and since PLA_2 can be activated by a PKC mediated phosphorylation it is possible that ATP activation of these channels involves phosphorylation of PLA_2. To test this hypothesis, we first compared the effects of Mg-ATP and ATP-γ-S on BK-channel activation in excised patches. We then examined the effects of Mg-ATP on BK-channels exposed to the PLA_2 inhibitor, aristolochic acid. Single channel recording techniques in excised patches were used for these experiments. Solutions containing 150 mM KCl were used in both the pipette and bath. Ca_i^{2+} in the bath was buffered with 5mM K_2EGTA to 0.1 μM for experiments without aristolochic acid and to 1.0μM for experiments with aristolochic acid. Mg-ATP (1mM; N=6) significantly increased the open probability of BK-channels (P_o) 39.1 ± 14.1% ($p < 0.001$) while ATP-γ-S (200μM; N=6) significantly increased the P_o by 151.8 ± 57.4% ($p < 0.001$). As expected, the increase in P_o with the poorly hydrolyzable substrate, ATP-γ-S was significantly greater than with Mg-ATP ($p < 0.001$). Channel activation with both substrates implies the presence of a functional protein kinase (probably PKC) in excised patches. Inhibition of PLA_2 with aristolochic acid (400μM; N=6) significantly reduced the P_o by 89.6 ± 6.8% ($p < 0.0001$). Subsequent treatment of aristolochic-acid-treated patches with Mg-ATP increased P_o 6.0 ± 14.8% which was not significant. This increase was, however, significantly less than the increase in P_o noted above for control cells treated with Mg-ATP ($p < 0.005$). The lack of significant Mg-ATP activation of BK-channels pretreated with aristolochic acid supports our hypothesis that PLA_2 is involved in channel activation by ATP. Since the results of these studies did show a small increase in P_o, we cannot rule out the possibility that a small portion of the channel activation with ATP arises by direct phosphorylation of the channel.

498.13

SUBSTANCE P - INVOKED SENSITIZATION OF (BK-) K^+-CHANNELS TO ANESTHETIC INHIBITION IS MEDIATED BY AN INCREASE IN PHOSPHORYLATION. W.H. Stapelfeldt*, J.M. Oleszewski. Department of Anesthesiology & CCM, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, and VA Medical Center, Pittsburgh, PA 15240.

We recently demonstrated that the general anesthetic agents propofol (PRO) and ketamine augment substance P (SP)-evoked inward current responses of guinea-pig inferior mesenteric ganglion (IMG) neurons through synergistic interaction with a SP-invoked signal transduction mechanism, producing selective inhibition of a charybdotoxin-sensitive (BK-)$G_{K(Ca)}$-conductance which normally restrains SP excitatory responses and which also contributes to the afterspike hyperpolarization (ASH) of these neurons. Aim of the present study was to determine the possible role of a SP-invoked change in phosphorylation in mediating this sensitization. Single-electrode (manual) voltage clamp recordings were obtained from isolated perfused IMGs. Study parameters included baseline ASH amplitudes, SP-evoked inward current responses, and *associated changes in ASH amplitudes* and *peak amplitudes* of intermittently (0.5 Hz) evoked electrotonic action potentials (APs) *before and during SP responses*. Exogenous SP was applied by pressure microejection from nearby micropipets (Picospritzer). Propofol (100 μM) alone had no effect on baseline AP parameters, but caused a significant (44%, $p<0.05$) increase in the amplitude of SP inward current responses which was related in amplitude ($p<0.01$) with a decrease in peak ASH amplitudes and selectively *occluded* by charybdotoxin (CTX, 10 nM). In the presence of the kinase inhibitor staurosporine (100 nM), the SP-invoked effect of PRO, but not the effect of CTX, was abolished. In contrast, in the presence of the phosphatase inhibitor okadaic acid (50 nM), the addition of PRO alone, even in the absence of SP, produced inhibition of ASH amplitudes ($p<0.01$). These findings suggest that the *anesthetic sensitivity* of the underlying CTX-sensitive $G_{K(Ca)}$-conductance to inhibition by propofol is not static but *subject to physiologic neuromodulation through a net change in phosphorylation.*

Support: VA MERIT REVIEW, FAER & Syntex Laboratories (Young Investig. Award)

498.14

MODULATION OF "MAXI" POTASSIUM (K^+) CHANNEL FROM NONPIGMENTED CILIARY EPITHELIAL CELLS BY DIBUTYRYL CYCLIC AMP. B. J. Bhattacharyya*, D. Krupin, P. E. Hockberger and T. Krupin. Depts. of Ophthalmology and Physiology, Northwestern University Medical School, Chicago, IL 60611.

Pigmented and nonpigmented epithlelial (NPE) cells in the ciliary body are responsible for secretion of the aqueous humor in mammalian eyes. The ionic conductance of epithelial cell membranes regulates the water and ionic content of the secretion and thereby influences intraocular pressure. As a first step towards understanding ciliary epithlelial secretion, we have examined a calcium-dependent "maxi" K^+ channel in NPE cells from enzymatically dissociated (trypsin, 0.5 mg/ml, in Ca/Mg-free, low Cl saline) ciliary bodies from adult rabbits. The channel was detected in 30% of inside-out patches (n = 50) in symmetrical KCl solutions (150 mM) with elevated bath calcium (1.2 mM). The single-channel current displayed a linear I-V relationship with an average slope conductance of approximately 130 pS. The channel open probability was markedly increased by bath application of dibutryrl-cyclic AMP (20 μM), and the conductance was blocked by barium (2 mM) or quinidine (1 mM). These results suggest that a cyclic AMP-dependent mechanism is involved in activation of the "maxi" K^+ channel in NPE cells.

This research was supported in part by the NIH (EY-05240).

498.15

PHOSPHATASE-KINASE BALANCE REGULATING THE HIPPOCAMPAL Ca^{2+}-ACTIVATED K^+ CURRENT I_{AHP} IN BASAL CONDITIONS.

P. Pedarzani[1]*, W. Stühmer[1] and J.F. Storm[2], [1]*MPI for Experimental Medicine, Göttingen, Germany, and* [2]*Inst. of Neurophysiology, Univ. of Oslo, Norway.*

The slow Ca^{2+}-activated K^+ current I_{AHP} underlying spike frequency adaptation in hippocampal pyramidal neurons is regulated by different neurotransmitters (NT) via multiple second-messenger systems and kinases. Previous data (Pedarzani & Storm, Neuron 11, 1023-35, 1993) suggested the presence of a basal phosphorylation-dephosphorylation turnover, affecting the I_{AHP} in the absence of NT stimulation. By intracellular application of kinase and phosphatase inhibitors, we have found evidence for opposing actions of protein phosphatase 1 (PP1) or 2A (PP2A), but not of calcineurin (PP2B), and of PKA in this basal modulation of the I_{AHP}.

Whole-cell recordings were obtained from 45 CA1 pyramidal cells in slices from young rats. Inclusion of the PP1-PP2A inhibitors microcystin (5-50 μM; n=6), cantharydic acid (10 μM; n=3) and calyculin A (5-10 μM; n=3) in the pipette solution invariably caused a substantial run-down in the I_{AHP} amplitude, never seen in controls of comparable durations (n=7). Since these inhibitors block both PP1, PP2A and most likely also PP2B at the concentrations we used, we are currently performing experiments with a specific peptide inhibitor of PP1 to try to define which phosphatase is affecting the I_{AHP} in basal conditions. Furthermore, we tested the involvement of PP2B by applying intracellularly the specific PP2B inhibitor FK506 (10-200 μM; n=3; generous gift of Fujisawa). Intracellular application of FK506 had no effect on the I_{AHP} amplitude, suggesting a lack of involvement of PP2B in the basal modulation of this current. Further experiments with a specific PP2B peptide inhibitor will be performed to rule out a possible wash-out of the immunophilin FK-binding protein as a reason for the lack of effect of FK506. Finally, when we co-applied intracellularly microcystin (10 μM) and the PKA inhibitor Rp-cAMPS (500 μM; n=7), we did no longer observe a run-down in the I_{AHP} amplitude, suggesting a role for PKA in counterbalancing the effect of PP1 or PP2A on this current in basal conditions.

[Supported by the Max-Planck Gesellschaft and NFR]

498.16

LPS RAPIDLY INCREASES BK CHANNEL OPENING IN INSIDE-OUT PATCHES OF RAT CEREBROVASCULAR SMOOTH MUSCLE CELLS. **L. M. Hoang, D. M. Mathers* Dept. of Physiology, Univ. of British Columbia, B.C., V6T-1Z3.**

Lipopolysaccharide (LPS) activates the expression of inducible NO synthase (iNOS) in both cerebral and peripheral blood vessels, leading to an overproduction of NO and potentiation of vasorelaxation. This NO-mediated relaxation most likely involves the L-arginine-NO-cGMP pathway. Elevation of cGMP activates the cGMP-dependent protein kinase G (PKG) to phosphorylate large-conductance Ca^{2+}-dependent K^+ channels (BK channels) which hyperpolarize the smooth muscle membrane, resulting in vasorelaxation. We here demonstrate a very rapid increase in the probability of BK channel opening (P_o) in response to acute application of LPS. Using inside-out patches excised from dissociated primary cultures of rat cerebrovascular smooth muscle cells (CVSMCs), BK channel activity was monitored with the cytoplasmic face exposed to saline containing $[Ca^{2+}]_i=1$ μM. Upon application of 100 μg/ml LPS to the culture, P_o rapidly and reversibly increased without changing the amplitude of currents flowing in single channels. This enhancement of P_o was recorded 22.4±3.4 sec after exposure to LPS and averaged a 4.96 fold increase (n=11). However, following a 4 h period (22°C) pre-incubation of CVSMC cultures with 1 mM of the NOS inhibitor, L-NAME, application of 100 μg/ml LPS resulted in P_o values not significantly different from values obtained prior to application of the endotoxin. When 0.3 mM cGMP was applied to the cytoplasmic face of the patch, P_o also increased by a factor of 3.34±0.88 over control values. These results suggest that the rapid increase in P_o on acute application of LPS is apparently mediated by the L-arginin-NO-cGMP-PKG-BK channel pathway.

498.17

ACTIVATION OF CALCIUM-DEPENDENT POTASSIUM CHANNELS IN NEURONS BY NEUROTROPHINS. Ninna R. Holm[1,2], Palle Christophersen[2], Søren-P. Olesen[2], Jørn Hounsgaard[3]* and Steen Gammeltoft[1]. [1]Department of Clinical Biochemistry, Glostrup Hospital; [2]NeuroSearch A/S, Smedeland 26B, 2600 Glostrup; [3]Department of Medical Physiology, University of Copenhagen, Denmark

The neurotrophins are signaling factors essential for survival and differentiation of distinct neuronal populations during development and regeneration of the nervous system. The long-term effects of neurotrophins have been studied in detail, but little is known about their acute effects on neuronal activity. Here we use permeabilized whole-cell patch clamp to demonstrate that neurotrophin-3 (NT-3) and nerve growth factor (NGF) activate calcium-dependent, paxilline-sensitive potassium channels (BK channels) in cortical neurons. Application of NT-3 or NGF produced a rapid and gradual rise in BK current that was sustained for 30-50 min; brain-derived neurotrophic factor and insulin-like growth factor-1 were without significant effect. The response to NT-3 was blocked by inhibitors of Trk tyrosine kinases, phospholipase C and serine/threonine protein phosphatase 1 and 2a. Omission of Ca^{2+} from the extracellular medium prevented the NT-3 effect. Activation of protein kinase C decreased the BK current and inhibited the NT-3 response. Our results indicate that NT-3 stimulates BK channel activity in cortical neurons through a signaling pathway that involves protein dephosphorylation. Activation of BK channels may be a major mechanism by which neurotrophins acutely regulate neuronal activity.

498.18

Transient absence of an inward rectifier that governs a window of spontaneous activity during muscle cell differentiation is mediated by modulation of a single channel type. A. K. Davis and W. J. Moody*. Department of Zoology, University of Washington, Seattle, WA 98195.

Coordinate expression of functional ion channels is required for normal cellular differentiation of ascidian muscle (Dallman et al, 1994). We study this organism because it has pigmented muscle cells allowing us to identify muscle precursors at all stages of development. An inwardly rectifying potassium channel (I_{IR}) is present at constant density in muscle-lineage cells at all stages of development from the oocyte to mature muscle (36 hours later) except for a brief period of 2-4 hours near neurulation when its functional expression is down-regulated by >80%. I_{IR} is the sole resting conductance of these cells, therefore its absence destabilizes the resting potential. The down-regulation of I_{IR} is temporally coordinated with the first appearance of lineage specific calcium (I_{CA}) and slowly activating outward potassium currents (I_{KV}) that govern the waveform of the action potential. The coordinate regulation of these ion channels (I_{IR}, I_{CA}, I_{KV}) creates a window of time during which spontaneous activity occurs. Activity (calcium entry) during this window is essential for the normal differentiation of a calcium-dependent potassium current that is expressed in mature muscle and E-C coupling (Dallman et al, 1994).

The transient absence of I_{IR} is a critical factor controlling the expression of spontaneous activity in the muscle precursor cells. To address the mechanism of I_{IR} transient down-regulation we examined I_{IR} single channel properties before and after the window of spontaneous activity. I_{IR} has the same single channel conductance before its disappearance (10.35 ± 0.52 pS) as after the window (11.55 ± 1.59 pS) ($p=.163$) suggesting that I_{IR} before and after the window are products of the same gene. We are currently pursuing studies to determine whether I_{IR} reappearance requires mRNA synthesis.

This work was supported by a NIH grant to WJM (H. D. 17486).

ACETYCHOLINE: STRUCTURE/FUNCTION II

499.1

PRESERVATION OF SPIKE TIMING AND INCREASED EXCITABILITY UNDER CHOLINERGIC MODULATION IN NEOCORTICAL NEURONS Akaysha C. Tang*, and Terrence J. Sejnowski. The Salk Institute, Computational Neurobiology Lab, La Jolla, CA 92037.

Acetylcholine (ACh) enhances neuronal responsiveness of cortical neurons. Associated with this enhancement is a reduction in spike frequency adaptation: interspike intervals increase with time when a constant depolarizing current is injected. As a result, ACh appears to not only increase the firing rate but also to alter the temporal structure of the spike train. This cholinergic modification of spike timing would lead to a failure in faithful transmission of the input signal if a neural code based on spike timing were used by the brain. We have examined the effects of ACh on spike timing in rat neocortical neurons using the whole cell patch clamp technique (Science 95, Vol268:1503–1506). When physiologically realistic fluctuating inputs are used, ACh preserves the timing of action potentials in the spike train, while at the same time increasing the firing rate by inserting spikes between existing spikes. These two contrasting effects of ACh suggest that under in vivo conditions, cholinergic modulation may be more protean and less limited than we had previously envisaged—permiting the preservation of spike timing information, and at the same time, enhancing the output signal by increasing the firing rate.

Supported by the Howard Huges Medical Institute.

499.2

THE CONTRIBUTION OF AFTERHYPERPOLARIZATION CURRENTS TO CHOLINERGIC ENHANCEMENT OF EXCITABILITY AND PRESERVATION OF SPIKE TIMING IN A NEOCORTICAL NEURON MODEL. A.M. Bartels, M.J. McKeown, A.C. Tang, T.J. Sejnowski*
Computational Neurobiology Lab., Salk Institute for Biological Studies, La Jolla, CA 92037-1099

Recent physiological studies have shown that the effects of cholinergic modulation can be different when physiologically more realistic **fluctuating** rather than **constant** depolarizing currents are injected into a neocortical neuron (see companion poster, Tang-Sejnowski, 1996). In a modified version of a 10 compartment neocortical neuron model (Bush-Sejnowski, 93), we show that the effect of cholinergic modulation is a stimulus–dependent simultaneous preservation of spike timing and enhancement of neuronal excitability. We focus on the hypothesis that although playing a dominant role in cholinergic enhancement of excitability under **constant** depolarizing input I_{AHP} is less important in the modulation of the firing rate under the physiologically more realistic **fluctuating** inputs. We first constrained the model to reproduce the phenomenon of spike frequency adaptation (SFA) and a reduction in SFA due to cholinergic modulation (modeled as a decrease in maximum conductance, g_{KCa}.) Next, by varying the fluctuation amplitude and mean intensity of the current injection, we compute a spike count–based index, D, for the contribution of I_{AHP} to enhanced excitability. We found that D can decrease as the amplitude of current fluctuation increases. This suggests that the contribution of I_{AHP} to cholinergic enhancement of neuronal excitability will be more prominent for physiologically unrealistic stimuli than that observed under *in vivo* conditions.

Research supported by the Howard Hughes Medical Institute.

499.3

ROLE OF SEROTONIN$_3$ RECEPTORS IN ACETYLCHOLINE RELEASE IN THE RAT FRONTAL CORTEX: AN *IN VIVO* BRAIN MICRODIALYSIS STUDY.

H. Hirano*, M. Hirata, M. Yamada and Y. Matsuda. Department of Neuropsychiatry, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan.

The role of serotonin (5-HT)$_3$ receptors in the serotonergic regulation of cortically projecting cholinergic neurons was studied using *in vivo* microdialysis to measure extracellular acetylcholine (ACh) concentrations in the frontal cortex of freely moving rats.

Neither systemically administered selective 5-HT$_3$ receptor antagonist MDL 72222 (0.02, 0.2 mg/kg, s.c.) nor ondansetron (0.02, 0.6 mg/kg, s.c.) influenced the ACh output in the frontal cortex compared with distilled water. Systemic administration of fenfluramine (10 mg/kg, i.p.) significantly increased cortical ACh release. Pretreatment (60 min) with ondansetron (0.6 mg/kg) did not impact the effect of fenfluramine on ACh release. Local application of the 5-HT$_3$ agonist 2-methyl-5-HT (1 μM for 60 min) in the frontal cortex *via* reverse dialysis failed to affect the basal ACh output.

These results suggests that the 5-HT$_3$ receptors do not play an important role in the serotonergic regulation of ACh release in the frontal cortex.

(supported by Jpn. Pharmacopsychiat. Res. Found.)

499.4

ENHANCEMENT OF THE EXPRESSION OF CHOLINE ACETYLTRANSFERASE mRNA IN HUMAN T-LYMPHOCYTES BY IMMUNOLOGICAL STIMULATION.

T. Fujii, S. Yamada, H. Misawa[1], S. Tajima, K. Fujimoto, T. Kasahara[2] and K. Kawashima*. Depts. of Pharmacology and [2]Biochemistry, Kyoritsu College of Pharmacy, Tokyo 105, Japan and [1]Department of Neurology, Tokyo Metropolitan Institute for Neuroscience, Fuchu City, Tokyo 183, Japan.

Acetylcholine (ACh), a neurotransmitter in the nervous systems, has been suggested to play an important role in the regulation of interaction between the immune and nervous systems. We have previously reported that ACh is present in the blood of various mammals including humans, and that its synthesis is catalyzed by choline acetyltransferase (ChAT), which is known to catalyze ACh synthesis in the nervous system. In the present study, we used detection of ChAT mRNA to examine whether ACh synthesis in T-lymphocytes is stimulated during immune responses. Total RNA was obtained from human peripheral blood mononuclear leukocytes (MNLs) cultured in the presence or absence of T-cell activator, phytohemagglutinin (PHA), and human leukemic cell lines derived from peripheral blood cells. The expression of ChAT mRNA was analyzed by reverse transcription-polymerase chain reaction (RT-PCR) using specific order and reverse primers for human brain ChAT cDNA. A single transcript of RT-PCR product was detected on agarose gel in samples obtained from PHA-stimulated MNLs and T-cell lines (CEM, HSB-2 and MOLT-3), but not in unstimulated MNLs or a B-cell line (Daudi). The position of RT-PCR product obtained from PHA-stimulated MNLs and three T-cell lines was identical with that in the human brain sample used as a positive control. These results suggest that the expression of mRNA for ChAT in T-lymphocytes is enhanced during the immune responses, and indicate that ACh synthesis by ChAT in T-lymphocytes is initiated by immune stimulation.

499.5

SPECIFIC CHOLINERGIC IMMUNOTOXINS IN MICE. J. Berger-Sweeney[1], U. V. Berger[2*], D. A. Lappi[3], C. F Hohmann[4], A. Ewusi[1], K. M. Frick[1]. [1]Wellesley College, Wellesley, MA; [2]Brigham and Women's Hospital, Boston, MA 02115, [3]Advanced Targeting Systems, San Diego, CA 92191, and [4]Morgan State University, Baltimore, MD 21239.

We have shown previously that 192 IgG-saporin, a p75 (NGF receptor)-specific antibody linked to the ribosomal inactivator saporin, is an effective lesioning agent for cholinergic basal forebrain neurons in rats (*J. Neurosci.* 14:4507-4519). The 192 IgG antibody, however, does not crossreact with the mouse NGF receptor, making it unsuitable for studies in mice. The goal of these experiments was to create an effective cholinergic immunotoxin for mice. Monoclonal (gift of D. Anderson) and polyclonal (gift of M. Chao) antibodies to the mouse p75-low affinity NGF receptor were coupled to saporin. The two immunotoxins, uncoupled antibody and saporin, and saline were injected into the medial septal area or the lateral ventricle of mice (n= 4 - 6 per group). Brains were processed for choline acetyltransferase (ChAT) neurochemistry or for immunocytochemistry. ChAT activity levels were reduced by 49% and 30% (relative to controls) in the hippocampi following the monoclonal and polyclonal immunotoxin injection into the medial septum, respectively. Monoclonal immunotoxin injections into the ventricle led to 42% and 60% decreases (relative to controls) in cortex and hippocampus respectively; polyclonal immunotoxin injections led to similar but not quite as dramatic reductions. Immunocytochemical analysis using an antibody to parvalbumin (putative GABAergic neurons) revealed no significant reductions in staining of cells in the medial septal area after direct monoclonal and polyclonal immunotoxin lesions. These data suggest that we created specific cholinergic immunotoxins in mice. Further experiments will be required to verify specificity and optimal dose ranges. (Supported by NIH/SBIR 1R43 NS34591-01)

499.6

GABAERGIC MODULATION OF CORTICAL ACETYLCHOLINE RELEASE IN VIVO. M. Giorgetti, L. Bacciotini, M.G. Giovannini & P. Blandina*. Dipartimento di Farmacologia Preclinica e Clinica, Universitá di Firenze, 50125 Firenze, Italy.

GABA is the major inhibitory transmitter in the vertebrate CNS, however, there are reports that it facilitated release of ACh from in-vitro preparations of cortex and hippocampus. There are no studies on the effect of GABA on cortical ACh release in vivo. Yet this approach may yield important information about the physiological role of GABA receptors in the modulation of the cortical cholinergic system. We used the microdialysis technique to study the effects of GABAergic agents on cortical ACh release from cortex of freely moving rats. Perfusion flow rate was 3 µl/min. Twenty-four hours after implantation with dialysis fiber, cortex of male Wistar rats (250 g) released spontaneously 2.8±0.1 pmol/10 min ACh (N=25), measured by HPLC with electrochemical detection. Introduction of bicuculline (1-25 µM), a GABAA antagonist, into the perfusion medium increased spontaneous ACh release. The effect was concentration-dependent, and the maximal increase was about 150%. Spontaneous ACh release returned rapidly to basal values upon perfusion with antagonist free medium. The GABAA agonist muscimol (5-25 µM) alone did not alter spontaneous ACh release, but it (25 µM) antagonized the effect of bicuculline (10 µM). Thus, these results suggest that GABA tonically inhibits cortical ACh release through activation of GABAA receptors. Supported by M.U.R.S.T- Universitá di Firenze (Italy)

499.7

Peripheral Anionic Site Residues Involved in the Interaction of Toxogonin with Carbaryl-Inhibited Acetylcholinesterase. G. Cohen, E. Cohen#, R. Adani, Z. Radic▲, P. Taylor▲ and G. Amitai*. IIBR, Israel, #Hebrew University Agricultural Faculty, Israel and ▲University of California, San Diego, La Jolla, Ca. 92093.

The inhibition rate of acetylcholinesterase (AChE) by 1-naphthyl N-methyl carbamate (carbaryl, CAR) is markedly enhanced by certain quaternary oximes such as toxogonin (TOX). Accordingly, the LD_{50} of CAR in the presence of TOX decreases by 2-4 fold in mice. We have examined the involvement of peripheral anionic site (PAS) residues of AChE in the interaction of oximes with the carbamylation process. Treatment of mice with the specific PAS ligand propidium (PR) (0.8mg/kg, iv) together with TOX (12.6 mg/kg, im) and CAR (14 mg/kg, iv, $0.7LD_{50}$) virtually abolished the TOX-induced increase in CAR toxicity observed without PR (4 out of 5 survivors with PR vs. no survivors without PR). SAD-128, a bisquaternary pyridinium ligand devoid of oxime groups did not alter CAR toxicity in mice (100% survival at 14 mg/kg, iv). These in vivo data indicate the involvement of the PAS of AChE in the toxicity enhancement induced by TOX and that the oxime group is probably required for this effect. Inhibition kinetics of recombinant mouse AChE (mAChE) and its PAS mutants by CAR was measured in the presence and absence of TOX and 1-(2-pyridine aldoxime) 1'-(3-quinuclidinone) dimethylether dichloride (AB-8). Inhibition kinetics of mAChE by CAR (0.1-1µM) in the presence of TOX (0.1mM) displayed 10 fold lower value of K_I and 10 fold larger k_{obs} value than without TOX. Inhibition of the mutant W286R by CAR in the presence of TOX displayed a 2.5 fold lower carbamylation rate constant (k') as compared to the wild type enzyme ($k' = 0.088\ min^{-1}$ vs. $0.22\ min^{-1}$, respectively, $p<0.001$). No enhancement by TOX was observed with the triple mutant Y72N/Y124Q/W286R. Similar inhibition rate of wt mAChE was obtained in the presence and absence of AB-8. In accordance with these in vitro data, administration of AB-8 did not increase CAR toxicity in mice and survival was observed. Our data indicate that TOX enhancement of inhibition rate of AChE by CAR is influenced by PAS residues of AChE.

499.8

THE INFLUENCE OF OCCLUSAL AND MASTICATORY FANCTION ON CHOLINERGIC OR CALBINDIN-D28k-IMMUNOREACTIVE NEURONS IN AGING RATS. Hidero TERASAWA[1], Takafumi NINOMIYA[2], Toshihiro HIRAI[1], Yasuhiro IKEDA[1],Shin NAKAGAWA*[3], Dept. of Removable Prosthodontics, Health Sciences University of Hokkaido, School of Dentistry, Toubetsu, 061-02 Japan[1]. Dept. of Anatomy, Sapporo Medical University School of Medicine, Sapporo, 060 Japan[2]. Dept. of Anatomy, Faculty of Medicine Hokkaido University, Sapporo, 060 Japan[3].

The relation between occlusal and masticatory alteration and degeneration of cholinergic or calbindin-D28k immunoreactive neurons in rat brain was investigated using immunohistochemical technique. Eighteen male rats (25 weeks old) were divided into three groups, a control group (fed with a solid diet), a soft diet group (fed with a powder diet containing the same components as the solid one) and a molarless group (all molars were removed at 25th weeks and then fed with a powder diet). After 15 weeks (40 weeks old), their brains were fixed with 4% paraformaldehyde, and cut into 50 µm sections which were processed free-floating. These sections were incubated 2 days with rabbit polyclonal anti-choline acetyltransferase (CAT) or mouse monoclonal anti-calbindin-D28k (CB) further processed with the SAB-technique. The number of CAT- in basal forebrain, trigeminal motor nucleus, and CB- in nucleus solitarius positive neurons of the molarless group was the smallest. The number of CAT- and CB-positive neurons in the soft diet group was significantly decreased compaired to the control group. These results define that neuronal degeneration in basal forebrain, trigeminal motor nucleus, and nucleus solitarius likely progress by removal of molars and soft diet.

499.9

BASAL FOREBRAIN CHOLINERGIC SYSTEM: A KNOWLEDGE BASE INSTALLED ON THE WORLD WIDE WEB. L. Zaborszky* and Z. Nadasdy. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

A database is designed for the acquisition, storage and analysis of neuroanatomical, neurophysiological, molecular pharmacological and behavioral data related to the basal forebrain, in particular the basal forebrain cholinergic (BFC) projection system. An important component of this database is a 3-dimensional representation of the basal forebrain neurons, including their dendritic and axonal organization, input-output relationships and other neurochemical markers. Data acquisition is provided by a commercial neuroscience computer system (Neurolucida, MicroBrightField, Inc.) followed by conversion of data into a WWW (HTTP) based knowledge system. The database is divided into two components, the hypertext pages and the 3-D database. This latter component includes 3-D databases of the reconstructed basal forebrain neurons with different complexities, implemented as virtual reality environments and supported by the VRML protocol. The hypertext pages are edited as standard platform-independent HTML documents. The documents contain either text or images (graphs, diagrams, microscope images, computer graphics). A 3-D reference atlas will ensure relational links among different types of data obtained from different brains at the cellular level. As compared to other neuroanatomy databases the advantages of this database are the following 1) operation system independent, 2) supports links to external databases via the Internet, 3) supports 3-D real time navigations, 4) moves from macro-mapping to cellular and subcellular level of the mapped neural systems.
Supported by USPHS Grant NS23945.

499.10

DISTRIBUTION OF NEURONS CONTAINING DIFFERENT TYPE OF CALCIUM BINDING PROTEINS IN THE CHOLINERGIC BASAL FOREBRAIN. S. Poobalasingham, K. Pang* and L. Zaborszky. Rutgers University, Newark, NJ 07102 and Bowling Green State University, OH 43403.

The basal forebrain cholinergic (BFC) projection system has recently attracted considerable clinical interest due its role in arousal, learning, memory and its compromise in neurodegenerative disorders, including Alzheimer's (AD) and Parkinson's diseases. Several recent studies, however, suggest that non-cholinergic, mostly GABA-containing neurons may be important constituents of the basal forebrain (Zaborszky et al., 1986; Gritti et al., 1993). GABAergic neurons represent a heterogeneous class of inhibitory neurons and have been subdivided in other brain areas into different subclasses based upon the presence of different calcium binding proteins. The present study was conducted to quantitatively analyze parvalbumin (PV), calretinin (CR) and Calbindin-D28k (CB) neurons in basal forebrain areas rich in BFC neurons. Alternate basal forebrain sections were immunostained for PV, CR, CB and choline acetyltransferase (ChAT) and immunostained neurons were mapped using a computerized 3-D reconstruction program (Neurolucida). Each cytochemically characterized neuronal population show distinct morphology and distribution pattern. Taken together these non-overlapping population of GABAergic neurons, the ratio of cholinergic/GABAergic neurons is 1:4, however in some basal forebrain areas it is as high as 1:10. It is expected that defining the precise input-output relations of these neurons in combination with their functional characterization will contribute to a better understanding of specific basal forebrain regulatory mechanisms that are compromised in AD and related disorders.
Supported by USPHS Grant NS23945.

499.11

BASAL FOREBRAIN CORTICOPETAL SYSTEM: 3-D COMPUTER GRAPHIC RECONSTRUCTION. B. Lynch, D. Orosz* and L. Zaborszky. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

The basal forebrain has recently attracted considerable clinical interest, especially since it has become apparent that some of its cell populations, including the cholinergic cells are severely affected in a number of neurologic and psychiatric disorders. The cholinergic cells, which are widely dispersed in the basal forebrain, provide the major part of the acetylcholine found in the cerebral cortex. Although several studies investigated the efferent projections of this system, there is no consensus to the principles which determine this projection (Woolf et al., 1984; Rye et al., 1984; Baskerville et al., 1993). Several recent studies, however, suggest that non-cholinergic, mostly GABA neurons may be important constituents of the basal forebrain. These GABA containing neurons along with cholinergic neurons project to the cortical mantle, may represent interneurons and/or give rise to projections to the thalamic reticular nucleus and the caudal diencephalon. No systematic study addressed the precise distribution and contribution of the non-cholinergic cells to the cortical projection. The retrograde tracer Fluoro-Gold (1.0 ul) was injected into different parts of the somatosensory and auditory cortex of rats. Forebrain sections were stained for the presence of choline acetyltransferase and parvalbumin (PV), a marker for GABAergic cells. Our preliminary studies suggest a surprisingly high proportion (52-65%) of non-cholinergic projection to these cortical areas. Studies are underway to investigate whether other cortical areas receive such a strong non-cholinergic projection and whether PV is colocalized in these neurons. It is expected that using a 3-D reconstruction program, additional clues to the organization of the basal forebrain corticopetal system will emerge.

Supported by USPHS Grant NS23945.

ACETYLCHOLINE RECEPTORS: MUSCARINIC—STRUCTURE/FUNCTION I

500.1

CYSTEINE SCANNING MUTATGENESIS OF TRANSMEMBRANE DOMAIN V OF THE M1 MUSCARINIC RECEPTOR. K. Allman, K.M. Page and E.C. Hulme. SPON: Brain Research Association. MRC National Institute for Medical Research. Mill Hill, London, NW7 1AA, U.K.

The aim of this study is to identify amino acids in the m1 muscarinic acetylcholine receptor (mAChR) which contact the acetylcholine sidechain. The pricipal approach was cysteine substitution mutagenesis followed by chemical reaction. Consecutive residues (I188- A196) in transmembrane domain V of the m1 mAChR were mutated to cysteine, and alanine or glycine. Agonist binding studies of these mutants were consistent with α- helicity of this sequence. ACh binding affinity was not affected by mutation of residues predicted to face outwards towards the lipid bilayer, whilst mutation of residues predicted to face inwards towards a ligand binding pocket decreased binding affinity, the largest effects being given by I188C, T192C and A196C. In antagonist binding studies, only the mutation of residue A193 caused an effect. The agonist analogue bromoacetylcholine has the potential to alkylate accessible cysteines. Its ability to react with the cysteine mutants was investigated, and it was found to react specifically and irreversibly with T192C. This implies that residue T192 is likely to be a principal component of the binding pocket for the sidechain methyl group of ACh.

Support: Medical Research Council, U.K.

500.2

ASP122 AND TYR124 IN THE M1 MUSCARINIC RECEPTOR ARE CRITICAL FOR RECEPTOR FOLDING BUT NOT FOR SIGNALING. Z. Lu , P.G.Jones, C.A.M. Curtis and E.C. Hulme. SPON: Brain Research Association. MRC National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

An AspArgTyr triad occurs in a majority of rhodopsin-like G protein-coupled receptors. The fully-conserved Arg is critical for G protein activation, but the function of the flanking residues is not well understood. We have expressed m1 muscarinic receptors mutated at Asp122 and Tyr124 in COS7 cells. Excepting conservative aromatic substitutions of Tyr124, mutations at either position attenuated or abolished expression of binding sites, even though immunocytochemistry using a C-terminally-directed antibody showed full-length translation. Mutants which bound ligands retained virtually normal abilities to mediate an ACh-induced phosphoinositide response. Expression was substantially rescued by deletion of 129 amino acids from the third intracellular loop. We propose that Asp122 and Tyr124 make inter-domain contacts which are critical for receptor folding, but only exert a secondary effect on signaling. Their role is completely distinct from that of Arg123, whose mutation abolishes signaling, but without diminishing receptor expression.

Support: Medical Research Council, U.K. and Wellcome Trust.

500.3

ISOLATION AND CHARACTERIZATION OF DIFFERENT m1-TOXINS J Carsi-Gabrenas* and LT Potter, Molecular and Cellular Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101.

It is now clear that the venom of the green mamba (*Dendroaspis angusticeps*) contains a number of toxins that are highly selective antagonists of m1 muscarinic receptors. Since the first such toxin was m1-toxin (Max *et al*, J Neurosci 13, 4293,1993), we call this series: m1-toxin, m1-toxin2, m1-toxin3 etc. These toxins are time-consuming to purify because of their low abundance (~100 μg of each/g dry venom) and similar size (65-66 amino acids), sequence, charge (very weakly cationic) and hydrophobicity. They are also slow to characterize, since their identification requires sequencing, and assays of their specificity require pure cloned m1-m5 receptors. These toxins have been isolated by various combinations of gel filtration on Sephadex G-50 (which separates m1-toxins from toxins of similar size, possibly according to their aromaticity), cation-exchange chromatography and reversed-phase HPLC. The different properties of these toxins are interesting, and some selection of toxins is possible for particular purposes. (1) Their highly conserved amino acid sequences show the structural requirements for anti-m1 and antagonist activity. (2) Their differences from m4-toxin (which is m4-selective) and MT1 (an agonist with m1≈m4 affinity) provide clues as to the residues required for selective anti-m4 and agonist activity. (3) One toxin shows almost perfect selectivity for m1 receptors, and is therefore optimal for physiological studies. (4) Some bind irreversibly at 37°C, facilitating studies of m1 receptors *in vivo*. (5) Some are very stable to biotinylation, which permits direct anatomical and biochemical assays, and the rapid purification of toxin-receptor complexes by affinity chromatography. (6) Some can be radioiodinated. [Supported by AG-06170].

500.4

CLONING AND EXPRESSION OF THE cDNA FOR m1-TOXIN3 JL Krajewski*, IM Dickerson and LT Potter, Pharmacology and Physiology, University of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101.

Mamba venoms contain toxins that are the only specific ligands known for any of the subtypes of muscarinic receptors. The first such toxins to be isolated, MT1 and MT2 (Adem *et al*, 968, 340, 1988), have nearly equal affinity for m1 and m4 receptors, and MT1 can act as an agonist (Kornisuik *et al*, Toxicon 33, 11, 1995). In contrast, m1-toxin binds pseudoirreversibly to m1 receptors with m1 > > > m4 affinity (Max et al, J Neurosci 13, 4293, 1993), m4-toxin binds reversibly with m4 > > m1 affinity, and both are antagonists (Max *et al*, Neurosci Abstr 19, 462, 1993; Liang *et al*, Toxicon, in press). We have chosen to express the cDNA for another m1-toxin for four reasons: (1) m1-toxin3 has the highest specificity for m1 receptors of any of the anti-muscarinic toxins; (2) to obtain much larger amounts of an anti-m1 toxin than can be obtained by purification (~100 μg/g dry venom); (3) to permit studies by site-directed mutagenesis of the amino acid residues that confer high toxin affinity and specificity, and that confer antagonist *vs.* agonist activity; and (4) to facilitate radiolabeling. Messenger RNA was obtained from the venom glands of a green mamba (*Dendroaspis angusticeps*). Degenerate primers to regions of the desired protein sequence were made, and RT-PCR was used to prepare cDNA products. RACE techniques were then used to isolate the full length cDNA for m1-toxin3. This cDNA establishes the mature toxin sequence: LTCVKSNSIWFPTSEDCP-DGQNLCFKRWQYISPRMYDFTRGCAATCPKAEYRDVINCCGTDKCNK, which differs from that of m1-toxin only in glutamine-29. This cDNA was used to transfect COS-1 cells, which expressed active toxin. This cDNA is now being used to produce m1-toxin3 in stable cell lines. [Supported by AG-12976]

500.5

DISTINGUISHING BETWEEN TWO-STATE ALLOSTERIC AND CONVENTIONAL SINGLE STATE RECEPTOR MODELS. C.-M. Staschen [*§], L.D. Homer[§§] and S.T. Ahlers[§]. Naval Medical Institute, Kopperpahler Allee 120, 24119 Kronshagen, Germany; [§§]Legacy Research, Emanuel Hospital, 2801 North Gantenbein Ave, Portland, Oregon 97227, USA; Naval Medical Research Institute, Bethesda, MD 20889-5607, USA.

There are situations with agonist receptor binding that do not fit a conventional single state receptor model. This has renewed interest in the use of a two-state allosteric receptor model that better fits and explains experimental outcomes. The goal of the present study was to determine which experimental design best distinguishes between these models using competitive interactions in radioligand binding studies. Computer simulations were used to generate data from a two-state allosteric model. Then the two-state allosteric model and the conventional single state model were fitted to the computer generated data. Simulated transient data were obtained with Runge-Kutta-Fehlberg solutions of standard kinetic equations. Parameters were selected which allowed simulated data to resemble data from real equilibrium and transient kinetic experiments. A 4% random error was added to the simulated curves. Using these computer generated data, parameters were estimated by weighted least-squares with simultaneous analysis of pooled equilibrium and kinetic data for both the two-state model and the conventional model. Since the conventional model is a special case of the two-state model, the statistical significance of the restricted fit can be evaluated with a partial F-test. Successful statistical discrimination between the two models was obtained with a design consisting of equilibrium, association and dissociation experiments performed with several different radioligand and several different inhibitor concentrations. In contrast, equilibrium experiments alone failed to distinguish between the two different models.

500.6

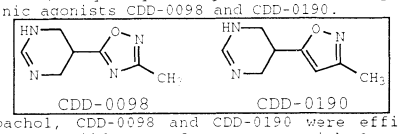
MOLECULAR DETERMINANTS INVOLVED IN DIFFERENTIAL COUPLING EFFICIENCIES OF m1 AND m3 MUSCARINIC RECEPTORS. N.S.M. Geoghagen, R.T. Cline and N.H. Lee*. Department of Molecular and Cellular Biology. The Institute for Genomic Research, Rockville, MD 20850.

The m1 and m3 muscarinic acetylcholine receptors (mAChRs) are members of the G-protein coupled seven transmembrane domain receptor family. Both the m1 and m3 mAChRs elicit dose-dependent increases in the hydrolysis of phosphoinositides in transfected Chinese hamster ovary (CHO) cells. However, the maximal increase in total inositol phosphates elicited with the m1 mAChR is approximately 2-fold greater than in cells expressing similar densities of m3 mAChR. The amino- and carboxyl-terminal portions of the third intracellular loop (i3-loop) of both receptors are required for efficient signal transduction. With the exception of two amino acids, the terminal portions of the i3-loop are well conserved between the two receptors. In the m1 mAChR, the amino- and carboxyl-terminal portions correspond to the sequences RIYRETENR and SLVKEKKAAR (underlined amino acids are not conserved between the m1 and m3 sequences), respectively. In the m3 mAChR, the amino- and carboxyl-terminal sequences are RIYKETEKR and SLVKEKKAAQ, respectively. We have recently demonstrated that the KKAAR motif in the carboxyl-terminal portion of the m1 mAChR is critical for agonist-mediated signal transduction. To determine whether the two non-conserved amino acids are responsible for potential signaling differences between the m1 and m3 mAChRs, we have mutated these residues and expressed the mutant receptors in CHO cells. (supported by TIGR)

500.7

ROLE OF THREONINE AND ASPARAGINE RESIDUES IN AGONIST INTERACTIONS WITH m3 MUSCARINIC RECEPTORS. W.S. Messer, Jr.*, M.A.N. Edgar, J.L. Freed and X.-P. Huang Department of Medicinal & Biological Chemistry, Center for Drug Design & Development, College of Pharmacy, The University of Toledo, Toledo, OH 43606

Conserved amino acids in transmembrane domains V and VI of the rat m3 muscarinic receptor may be important for hydrogen bond interactions with muscarinic ligands (Wess, et al., 1992; Bluml, et al., 1994). Mutant rat m3 receptors, replacing Thr234 or Asn507 with Ala, were stably expressed in CHO cells to test functional activation (PI hydrolysis) by the structurally-related muscarinic agonists CDD-0098 and CDD-0190.



CDD-0098 CDD-0190

Carbachol, CDD-0098 and CDD-0190 were efficacious agonists for wild-type m3 receptors with S_{max} levels greater than 400 % above basal, yet carbachol and CDD-0098 were much more potent than CDD-0190. Each ligand exhibited lower potency and efficacy in stimulating PI hydrolysis via Rm3 (TA) receptors. Carbachol and CDD-0098 exhibited modest agonist activity at the Rm3(NA) receptors, yet CDD-0190 was essentially inactive. The data are consistent with a major role for Thr234 in agonist potency, and a lesser (though important) role for Asn507 in agonist activation of m3 receptors.

This work was supported by NS 01493 and NS 31173.

500.8

m2-TOXIN, A SELECTIVE LIGAND FOR m2 MUSCARINIC RECEPTORS HH Valentine*, J Carsi-Gabrenas and LT Potter, Department of Molecular and Cellular Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101.

The only selective antagonists known for m1 and m4 muscarinic receptors are weakly cationic toxins (Max *et al*, J Neurosci 13, 4293, 1993; Max *et al*, Neurosci Abstracts 19, 462, 1993; Liang *et al*, Toxicon, in press). There are no comparably selective ligands available for m2, m3 or m5 receptors, but they are widely needed as research tools. Since the de-salted venom of the green mamba (*Dendroaspis angusticeps*) has been shown to inhibit the binding of ^{3}H-N-methylscopolamine to m2 muscarinic receptors (Liang *et al*, Toxicon, in press), we have pursued the isolation of a toxin with m2-selective activity. During gel filtration on Sephadex G-50, the NMS-blocking activity of mamba venom on m2 receptors moved with the predominant monomeric toxins of the venom that have molecular masses of about 7000 Daltons. Chromatography of these peptides on Bio-Rex 70 revealed two peaks of NMS-blocking activity, and indicated at least two strongly cationic toxins. Cation-exchange HPLC of the more active of these components yielded one active peak. This "m2-toxin" inhibits the binding of ^{3}H-NMS to pure m2, but not m1, m3, m4 or m5 receptors in CHO cells. Inhibition curves obtained with m2 receptors from the rat brainstem have a Hill coefficient≈1.0, showing that m2-toxin competes with NMS for m2 receptors, even though it probably binds to an allosteric receptor site, like m1-toxin (Max *et al*, Mol Pharmacol 44, 1171, 1993). Studies are in progress to finish the purification of m2-toxin, to determine its amino acid sequence, and to establish whether it is an agonist, like MT1 (Jerusalinsky *et al*, Toxicon 33, 389, 1995), or an antagonist, like m1-toxin and m4-toxin. [Supported by AG 12976]

500.9

REGULATION OF m2 MUSCARINIC ACETYLCHOLINE RECEPTOR FUNCTION BY G-PROTEIN COUPLED RECEPTOR KINASE-2 AND ARRESTIN-2. M. L. Schlador* and N. M. Nathanson. Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195.

Muscarinic acetylcholine receptors (mAChRs) couple to intracellular effector molecules through the actions of heterotrimeric G-proteins and are phosphorylated by a number of G-protein coupled receptor kinases (GRKs). In order to test the ability of specific GRKs and arrestins to regulate mAChR function in intact cells, we have chosen to use a transient transfection assay which couples muscarinic receptor activation to changes in expression of a cAMP-responsive reporter gene.

When overexpressed in JEG-3 choriocarcinoma cells, the m2 mAChR undergoes phosphorylation, sequestration, and a modest functional desensitization upon exposure to agonist. Cotransfection of a cDNA encoding GRK2 with the m2 receptor enhances agonist-induced desensitization as well as receptor phosphorylation. Cotransfection of arrestin2 has little effect on m2 receptor signaling but potentiates the effects of GRK2 on m2 receptor desensitization. The actions of GRK2 and arrestin2 on receptor signaling are blocked by deletion of the third intracellular loop of the m2 receptor (amino acids 217-380), which contains multiple GRK phosphorylation sites. In addition, we have found that cotransfection with either GRK2 or arrestin2 cDNAs enhances both the rate and extent of m2 receptor sequestration. Coexpression of both GRK2 and arrestin2 with the m2 mAChR leads to an enhancement of sequestration that is greater than that seen with either construct alone. These data support the hypothesis that GRK2 and arrestin2 have multiple actions on m2 mAChR function. (Supported by the National Institutes of Health).

500.10

XANOMELINE, AN M-1 MUSCARINIC AGONIST, SELECTIVELY INHIBITS THE ACTIVITY OF A10 DOPAMINE NEURONS K. Rasmussen* and M. E. Stockton, Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285.

Cholinergic and dopaminergic neurons are known to have complex interactions. Recently, the Ch5 and Ch6 cholinergic cell groups have been hypothesized to play an important role in the etiology of schizophrenia by affecting the activity of dopamine cells. In order to explore the interactions of the cholinergic and dopaminergic systems we examined the effects of xanomeline, an M-1 muscarinic agonist, on the single unit activity of dopamine cells in the substantia nigra (A9) and ventral tegmental area (A10) in chloral hydrate anesthetized male rats. Administration of xanomeline inhibited the activity of A10, but not A9, dopamine cells (ED50=3.0 μg/kg, iv). Xanomeline also reduced the number of spontaneously active A10, but not A9, dopamine cells after both acute (1, 10 mg/kg, sc) and chronic (1, 10 mg/kg/day X 21 days, via osmotic minipump) administration. The effects of xanomeline on the number of spontaneously active dopamine cells was reversed by the administration of the muscarinic antagonist scopolamine. These results support the hypothesis that the cholinergic system has a strong influence on the dopaminergic system and may help to explain some of the effects of xanomeline seen in man. In recent clinical trials with Alzheimer's Disease patients, xanomeline, in addition to enhancing cognition, showed particularly good efficacy in treating certain problematic behaviors including vocal outbursts, suspiciousness, delusions, agitation, and hallucinations. The effects of xanomeline on dopamine neuronal activity described here may play an important role in its reduction of these problematic behaviors in Alzheimer's Disease patients.

500.11

L-758,527: A NOVEL M_1-M_2 SELECTIVE MUSCARINIC COMPOUND WITH AGONIST ACTIVITY. R.J. Bendesky, C.M. Harrell, P. Kling, A. Kuo, J. Williams, P. Mallorga, W. Thompson, I. Bell and R. Ransom* Merck Research Labs, West Point, PA 19486

We have identified a novel structural class of compounds that possess significantly higher receptor binding affinity at M_1, M_2 and M_4 vs. M_3 muscarinic receptors. While most of the compounds in the series exhibited antagonist activity, a carbamate, L-758,527, was found to have agonist properties. L-758,527 inhibited [^{3}H]NMS binding to cloned human M_1, M_2, M_3 and M_4 receptors with K_i values (nM) of 17, 1, 2014 and 3, respectively. This selectivity profile was also observed in binding assays with endogenously expressed M_1 (cerebral cortex), M_2 (heart) and M_3 (salivary gland) receptors. In rabbit vas deferens, L-758,527 completely inhibited field stimulated contractions with an EC_{50} of 0.6 μM. The compound similarly inhibited inotropic responses to field stimulation in guinea pig left atria. The effects of L-758,527 in both tissues were atropine sensitive. At M_3 receptors in guinea pig ileum longitudinal muscle, L-758,527 was without agonist activity at high μM concentrations. In a pithed rat preparation, the compound produced a sustained, dose-dependent (ED_{50} = 0.1 mgKg^{-1}) bradycardia that was reversed by atropine. The compound was also found to be as efficacious as carbachol in functional assays ([^{35}S]GTPγS binding, adenylyl cyclase) with the cloned human M_2 receptor but slowed minimal efficacy in PI assays with M_1 receptor cells and was an antagonist in assays with M_3 (PI) and M_4([^{35}S]GTPγS) cells.

500.12

IgG-192 SAPORIN LESIONS PRODUCE UNCOUPLING OF THE M1 MUSCARINIC RECEPTOR P.E.Potter*, C.Gaughan, and Y.Assoulinc, Departments of Anesthesiology and Neurological Surgery, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY 10467

Injection of the immunotoxin IgG-192 saporin (SAP) into the medial septum causes a selective lesion of cholinergic neurons projecting from the septum to the hippocampus. To determine the effect of cholinergic lesions on the function of postsynaptic cholinergic receptors, the ability of the M1 muscarinic receptor agonist McN-A-343 to modulate the release of radiolabelled norepinephrine (NE) was studied in hippocampal slices. McN-A-343 did not induce spontaneous release of NE, but enhanced the release evoked by low-frequency electrical stimulation. The effect of McN-A-343 was significantly reduced in hippocampal slices from rats that had been lesioned with SAP. Binding studies using ^{3}H-pirenzepine indicated no changes in either receptor number or affinity after the lesion. Curves for displacement of pirenzepine by oxotremorine-M were constructed in the presence and absence of the non-hydrolyzable GTP analogue GppNHp, to assess the degree of coupling of the M1 receptor to G-proteins. In hippocampal P2 membranes from sham-operated animals, addition of 0.2 mM GppNHp caused a nearly 10-fold shift to the right of the displacement curve. In contrast, the curve was already shifted to the right in hippocampal membranes from SAP-lesioned rats, and addition of GppNHp had little effect. These results suggest that the M1 muscarinic receptor becomes uncoupled from G-proteins after lesion of cholinergic inputs with SAP, and thus less responsive to agonists. Uncoupling of the M1 receptor has also been reported to occur in Alzheimer's disease, and SAP lesions may provide a model in which this phenomenon can be examined. Supported by NIH grant AG11384.

500.13

REGULATION OF THE CHICK M2 PROMOTER BY THE GATA FAMILY OF TRANSCRIPTION FACTORS AND BY CYTOKINES. Marc L. Rosoff*[1], John B. E. Burch[2], Neil M. Nathanson[1], [1]Dept. Pharmacol., Univ. Washington, Seattle, WA 98195; [2] Fox Chase Cancer Inst., Philadelphia, PA 19111.

We have isolated genomic regions containing the putative chick m2 (cm2) promoter and show regulation of cm2 promoter constructs by the cytokines leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF). The cm2 promoter is also regulated by the GATA family of transcription factors.

Transfection of constructs containing the cm2 promoter driving the expression of the firefly luciferase gene into a human choriocarcinoma cell line (JEG-3) results in luciferase expression levels similar to those seen with vector alone. Co-transfection of the cm2 firefly reporter constructs with an expression vector containing chick GATA-6 results in a dramatic increase in luciferase activity. Currently we are determining the specificity of the GATA factor(s) involved in this regulation. The proximal promoter region of the cm2 gene contains several potential GATA factor binding sites. We are investigating if chick GATA factors will bind these sites *in vitro*.

Using chimeric receptors containing the extracellular region of the GCSF receptor fused to the intracellular region of gp130, the common signaling subunit shared by the LIF and CNTF receptor, we are mapping cytoplasmic regions of gp130 involved with induction of the cm2 promtoer by cytokines. We are also defining region(s) of the cm2 promoter necessary for cytokine regulation.

Supported by the American Heart Assoc. of Washington and NIH.

500.14

EVIDENCE FROM DIRECT INFUSION MICRODIALYSIS THAT THE SUBTYPE IDENTITY OF THE PRIMARY MUSCARINIC AUTORECEPTOR IS m2 FOR BOTH RAT STRIATUM AND HIPPOCAMPUS. W. Billard, H. Binch III, G. Crosby, and R.D. McQuade* Schering-Plough Research Institute, Kenilworth, NJ 07033

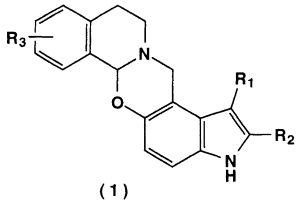
Recently, our laboratory compared the relative binding affinities of several standard muscarinic antagonists at each of 4 muscarinic receptor subtypes (m1, m2, m3, m4) with their corresponding relative abilities to elevate basal acetylcholine (ACh) levels in vivo in rat striatum following direct infusion through a microdialysis probe (J. Pharmacol. Exp. Ther. 273: 273-279, 1995). The conclusion from this study was that the antagonists' ACh-elevating effects (a presumed autoreceptor response) were best correlated with their relative binding affinities for the m2 subtype. In the current study the identical correlative approach was used to ascertain muscarinic autoreceptor identity in rat hippocampus. For the 4 antagonists evaluated, i.e. scopolamine, himbacine, AF-DX116, and pirenzepine, potencies for elevating hippocampal ACh (relative to scopolamine) were 1, 3.3, 10 and 33, respectively. Corresponding binding affinities for the same antagonists at the cloned human m2 receptor (0.88, 4.4, 32.4 and 212 nM) provided a much better relative match to the ACh-increasing effects than either m1, m3 or m4 binding data. While the *relative* ACh-elevating potencies of the antagonists were comparable for both brain regions, infusion of a higher concentration of each in hippocampus (3 to 10-fold) was necessary to elevate ACh levels. This likely reflects the much lower cholinergic tone observed in this region, which may require a higher percent occupancy of autoreceptor sites by antagonist before an ACh-releasing response is triggered. In conclusion, these results support an m2 autoreceptor identity for both striatum and hippocampus and reinforce the utility of direct infusion microdialysis as a useful tool in reaching this determination.

Funding by Schering-Plough Research Institute

500.15

BENZOXAZINE ISOQUINOLINES AS POTENTIAL m4 SELECTIVE MUSCARINIC ANTAGONISTS. C.E. Augelli-Szafran[1]*, D.W. Moreland[1], C. Nelson[2], J.R. Penvose-Yi[1], R.D. Schwarz[2], and J.C. Jaen[1], Departments of Medicinal Chemistry[1] and Pharmacology[2], Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.

Selective m4 muscarinic antagonists may be useful in the treatment of Parkinson's Disease. The synthesis of compounds of general structure **(1)** has led to the identification of several analogs that appear to be m4 selective antagonists. The affinity of these compounds for the five human receptor subtypes (m1-m5) was determined by [^{3}H]-NMS binding using membranes from transfected Chinese Hamster Ovarian (CHO) cells. Functional data (PI hydrolysis and cAMP accumulation) on the most potent compound confirmed its muscarinic antagonist properties. The biological activity and structure-activity relationships (SAR) of these compounds will be discussed.



(1)

(Supported by Warner-Lambert)

500.16

PHARMACOLOGICAL CHARACTERIZATION OF PD102807: An m4 SELECTIVE MUSCARINIC ANTAGONIST. C. B. Nelson[1], C.E. Augelli-Szafran[1], R.D. Schwarz[1]*, J.R. Penvose[1], J.C. Jaen[1], J. Wiley[1], K.A. Frey[2], 1Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co. 2Neurosciences Laboratory, The University of Michigan, Ann Arbor, MI 48105.

The anti-muscarinic agent Artane (trihexyphenydil) has been used clinically to suppress tremor and relieve rigidity associated with the early stages of Parkinson's disease (PD). However, motor benefits have been accompanied by cognitive decline. In situ hybridization and antibody studies have found that the m4 subtype of muscarinic receptors predominates over m1 in the striatum, while the reverse is true for the hippocampus and cerebral cortex. Thus an antagonist specific for the m4 over m1 subtype could be efficacious against PD motor symptoms without deleterious cognitive side effects.

In the search for selective antagonists, membrane preparations from CHO cell lines transfected with each of the human muscarinic subtypes were utilized in [^{3}H]-NMS competition binding experiments. The compound PD102807 was found to have m4 (IC_{50}=90.7nM) selectivity of 72-fold against m1 (IC_{50}=6569nM), 38-fold against m2 (IC_{50}=3440nM), 10-fold against m3 (IC_{50}=951nM), and 82-fold against m5 (IC_{50}=7412nM). Artane, on the other hand, although found to have considerable m4 affinity, was most potent at the m1 subtype (IC_{50} values; 1.3nM for m1, 18.6nM for m2, 26.3nM for m3, 6.7nM for m4 and 10.3nM for m5). Measurement of functional activity (PI hydrolysis for m1, m3, and m5 and cAMP accumulation for m2 and m4 receptors) correlated with this profile, as did an autoradiographic study in rat brain where PD102807 bound with highest affinity in the striatum. In a murine *in vivo* model, PD102807 inhibited spontaneous locomotor activity after i.c.v. administration, however showed little effect following oral dosing. Thus PD102807 may serve as a prototype for the development of an m4-selective antagonist to treat early PD motor symptoms.

(Supported by Warner-Lambert)

500.17

LOCALIZATION OF m2-MUSCARINIC RECEPTORS IN ACETYLCHOLINESTERASE-RICH INFRACORTICAL NEURONS OF THE HUMAN AND MONKEY CEREBRAL CORTEX. S.B. Schueler*[1], J.F. Smiley[1], A.I. Levey[2], F. Morrell[3], and M.-M. Mesulam[1]. [1]Northwestern University, Chicago, IL, [2]Emory University, Atlanta, GA and [3]Rush Presbyterian-S.t. Luke's Medical Center, Chicago, IL

Numerous polymorphic AChE-rich neurons are found in Layer VI and the adjacent infracortical white matter of the primate cerebral cortex. These neurons are thought to be cholinoceptive, but this has not been confirmed. We investigated the expression of the m2-muscarinic receptor subtype in these neurons, using simultaneous immunocytochemistry and AChE histochemistry. These experiments demonstrated a close correspondence between m2-immunoreactive and AChE-rich cells in all cortical areas that were examined. At least 77-90% of AChE-rich infracortical neurons were clearly m2-immunoreactive. Conversely, at least 75-89% of the m2-positive cells were clearly AChE-rich. Comparable results were found in the cerebral cortex of the monkey and in human anterior temporal cortex, which was obtained at surgery. Human infracortical m2-immunoreactive cells were like those of monkey in morphology, density, and AChE reactivity. These findings demonstrate that the vast majority of AChE-rich infracortical neurons in the primate brain express the m2-muscarinic receptors, and support the hypothesis that these neurons are cholinoceptive.

Supported by the Alzheimer's Association

500.18

EFFECT OF GINKGO BILOBA EXTRACT ON MUSCARINIC BINDING SITES IN RAT BRAIN. E. Moysé, N. Ravel*, F. Boilet, C. Lioger, M.T. Droy-Lefaix (1). IPSEN, F-75781 Paris (1); Lab. de Physiologie Neurosensorielle, Univ. Lyon1-CNRS, F-69622 Villeurbanne

The Ginkgo biloba extract (EGb 761, IPSEN, Tanakan) is a well defined drug prepared from green leaves of Ginkgo biloba. Egb761 is mainly indicated for cognitive deficits affecting elderly patients. Experimental behaving data have suggested that some therapeutic effects of EGb761 might be mediated through the central cholinergic system. Thus, chronic treatment of rats by EGb761 prevented scopolamine-induced blockade of odor retention (Ravel et al 94), suggesting that EGb761 might induce an increase of brain muscarinic receptor density. To test this hypothesis, we assess here effects of the same experimental paradigm in rat upon affinity (Kd) and concentration (Bmax) of muscarinic receptors in various brain structures.

Rats chronically administered per os with EGb761 (50 or 100 mg/kg/day) or water were trained in an olfactory delayed matching to sample. At the end of treatment (total duration 6 weeks), rats were sacrificed and their brains frozen and serially sectioned for saturation assay of ^{3}H-QNB binding by quantitative film autoradiography. EGb761 treatment resulted into a significant Bmax increase specific to the glomerular layer of olfactory bulb, significant Bmax decrease in hippocampal CA1 (-69%) and dentate gyrus (-80%), and no change in other olfactory areas (anterior olfactory nucleus, olfactory tubercle and piriform cortex). These effects were more important with a 50 mg/kg dose of EGb761 than with 100 mg/kg, as already reported in behavioral studies.

These data indicate that EGb761 can modulate characteristics of cholinergic muscarinic receptors in specific areas of rat brain. Considering the previously reported interaction between EGb761 and scopolamine on olfactory retention, the hypothesis of a selective decrease of M2 muscarinic autoreceptors in the hippocampus is currently investigated.

This work was funded and supported by IPSEN.

500.19

ACETYLCHOLINE AUTOEXCITES RELEASE OF PROOPIO-MELANOCORTIN-DERIVED PEPTIDES FROM MELANOTROPE CELLS OF *XENOPUS LAEVIS* VIA AN M_1 MUSCARINIC RECEPTOR

F.J.C. van Strien[1], E.W. Roubos[1], H. Vaudry*[2], B.G. Jenks[1]. [1]Department of Cellular Animal Physiology, Nijmegen Institute for Neurosciences, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands, [2]European Institute for Peptide Research, Laboratory of Cellular and Molecular Neuroendocrinology, INSERM U 413, University of Rouen, 76821 Mt St Aignan, France.

The release of proopiomelanocortin (POMC)-derived peptides from the melanotrope cells in the pituitary pars intermedia of *Xenopus laevis* is regulated by multiple inhibitory and stimulatory neurochemical messengers. In this study we examined the role of ACh in regulating melanotrope cell activity. *In vitro* biosynthesis experiments demonstrated that dissociated melanotropes can synthesize ACh. Oscillations in $[Ca^{2+}]_i$ are thought to drive the secretion of POMC-derived peptides from *Xenopus* melanotrope cells. A technique was developed to study simultaneously calcium oscillations and peptide secretion from single melanotropes. Dynamic confocal imaging of $[Ca^{2+}]_i$ revealed that ACh increases both the frequency and the amplitude of calcium oscillations in such cells as well as their release of POMC-derived peptides, in a concentration-dependent way. The actions of ACh on the cells was mimicked by muscarine but not by nicotine, indicating the exclusive presence of a muscarinic cholinergic receptor. Selective muscarinic receptor antagonists showed that ACh acts through an M_1-subtype muscarinic receptor. Immunofluorescence cytochemistry in combination with confocal laserscanning microscopy visualized muscarinic receptors on the surface of melanotrope cells. It is concluded that ACh stimulates the release of POMC-derived peptides from the *Xenopus* melanotrope in an autocrine way, via a muscarinic M_1 receptor, acting on the intracellular free calcium concentration.

Supported by the Netherlands Organization for Scientific Research (NWO).

500.20

TRANSIENT HEAT PRODUCTION ASSOCIATED WITH ACTIVATION OF MUSCARINIC (M1) RECEPTOR EXPRESSED IN CHO CELL. M. Ikeda[1], O. Zoher[2], D. L. Alkon[2], H. Nakamura[1], H. Shinagawa[1], H. Inoue[1] and T. Yoshioka[1]*. [1]Dept of Mol. Neurobiol. Waseda Univ, Tokyo JAPAN, [2]NIH-NINDS, Md, USA.

In order to develop novel technique to know localization of transmitter receptors, we set up thermal imaging system using thermo-sensitive fluorescent dye, EuTTA which has λex = 345 nm and λem = 612 nm, as a probe. CHO and muscarinic receptor expressed CHO (m-CHO) cells cultured in a small dish were incubated in 50 μ M EuTTA solution for 30 min to stain and submitted to measurements. Fluorescence intensity of the dye in living cell membrane was found to be highly temperature sensitive (10^{-1}/°C). Interestingly this dye was also sensitive to pH changes. When 500 μ M Ach (5 μ L) was applied with a glass micropipette for 100 msec, thermal response was observed in m-CHO- but not in CHO cells. Ach responsive cells were identified by Ca imaging. Receptor expression was also confirmed by immunohistochemistry using anti-m1 receptor antibody. Time course of heat production was faster than that of elevation of intracellular Ca. Furthermore atropine, antagonist for m1 receptor, also produced transient heat. Therefore it can be concluded that heat is produced when agonist or antagonist binds to its receptor.

ACETYLCHOLINE RECEPTORS: NICOTINIC—MOLECULAR BIOLOGY AND KNOCK-OUT

501.1

TRYPTOPHAN SUBSTITUTIONS AT THE M4 TRANSMEMBRANE SEGMENT OF THE *TORPEDO*, AND NEURONAL ACHRs INCREASE OPEN CHANNEL PROBABILITY. S. Tamamizu*, J.A. Lasalde, and M. McNamee, Mol. And Cell. Biology, U.C. Davis, Davis, CA 95616

We introduced tryptophan substitutions at different positions of the postulated lipid exposed segment M4 of the *Torpedo* and neuronal α4 and β2 subunits in order to examine functional consequences at the single channel level. At αG421 both phenylalanine and tryptophan produced a substantial increase in the open time constant for the *Torpedo* receptor. A lack of response from a tyrosine substitution at the αG421 suggests that the side chain volume is not the main structural element responsible for the effect of tryptophan on the stabilization of the open state of the channel. Three multiple mutants, αC418WG421A, αC418WG421W, and αC418WβC447W were constructed in order to find a correlation between the number of lipid exposed tryptophans and the channel open time constant. The αC418WG421A double mutant demonstrated that when both previous mutations are combined the open time constant was increased 1.5-fold relative to the αC418W. When the two mutants (αC418W and αG421W) were combined in a single mutant a functional receptor was expressed and the open time constant of the new double mutant was increased to 33.4 ms.

Estimation of free energy from the rate constants for the opening transition suggests that each tryptophan contributes to the stabilization of the open state of the channel by about 0.8 kcal/mol. This suggests that each subunit contributes independently to the energy barrier between the open and closed state. The similar side chain volumes occupied by Tyr, Phe and Trp suggest that the indole group could be a possible candidate to produce a difference between the Phe and Trp free energy barrier. Preliminary data on neuronal subunits α4 and β2 suggest that neuronal AchRs also display a similar effect of M4 mutations as *Torpedo* and mouse receptors. (Supported by NIH Grant NS22941)

501.2

DETERMINANTS OF SPECIFICITY FOR α-CONOTOXIN MII ON α3β2 NEURONAL NICOTINIC RECEPTORS. S.C. Harvey*, J.M. McIntosh[1,2], G.E. Cartier[1], F.N. Maddox, C.W. Luetje. Department of Molecular and Cellular Pharmacology, University of Miami, Miami, FL 33101 and Departments of [1]Biology and [2]Psychiatry, University of Utah, Salt Lake City, Utah 84112.

α-conotoxin MII selectively blocks α3β2 neuronal nicotinic receptors (Cartier et al., 1995, JBC 271:7522-7528). We have now identified lysine 185 and isoleucine 188 of α3, and threonine 59 of β2, as determinants of sensitivity to this toxin. Important sequence segments were first identified using chimeric subunits. Chimeras of the α3 and α4 subunits, and the α3 and α2 subunits, were expressed in combination with β2. Chimeras of the β2 and β4 subunits were expressed in combination with α3. Toxin sensitivity was determined using a 5 min. incubation with 50 nM toxin, since this blocks α3β2 receptors (1.8 ± 0.5 % of control) while having no effect on α2β2, α4β2 or α3β4. We identified sequence segment 181-195 of α3 as containing the major α subunit determinant of MII sensitivity. We changed individual residues within this region from what occurs in α3 to what occurs in α2. Only the K185Y and I188K mutations had a significant effect on toxin sensitivity (19.3 ± 3.1% and 42.0 ± 4.9 % of control, respectively). We also identified several sequence segments of β2 that contain determinants of toxin sensitivity (1-54, 54-63, 63-80), with segment 54-63 being the most important. When individual residues in this region were changed from what occurs in β2 to what occurs in β4, only the T59K mutation had a significant effect on toxin sensitivity (19.4 ± 10.0 % of control).

[Support: NIH DA08102 and AHA-FL to CWL, NIH K20 MH00929 to JMM]

501.3

PHARMACOLOGY OFα3/β2 RECEPTORS AFTER MUTATION IN M2 CHANNEL LINING DOMAIN. L. M. Colquhoun* and J. W. Patrick
Division of Neuroscience, Baylor College of Medicine, Houston, Tx 77030.

Neuronal nicotinic receptors expressed in *Xenopus* oocytes are distinguishable by their pharmacologies. Cytisine is a potent agonist at α3β4 receptors but has primarily antagonist action at α3β2 receptors. The difference in sensitivity between subunits to cytisine has been mapped to differences in their N-terminal amino acid sequences. Mutations in the M2 domain of the α7 subunit have also been shown to alter pharmacology of that expressed homooligomer. Mutations were made in the M2 region of α3 and β2 subunits. The *Xenopus* oocyte expression system was used to examine the electrophysiology of receptors composed of combinations of wild type and mutated α3 and β2 subunits.

Cytisine blocked α3/β2 receptors but, when β2L251C was substituted for wild type β2, cytisine potently activated the receptor. The same mutation on α3 subunit when paired with β2 wild type, did not change the cytisine pharmacology. Cytisine was an agonist on receptors containing a mixture of wild type β2 and β2L251C. The current elicited by acetylcholine from oocytes injected with α3/β2L251C desensitized slower than wild type. There was no shift in the dose-response curve to acetylcholine. D-tubocurarine, dihydro-β-erythroidine and hexamethonium,were tested for agonist activity on α3/β2L251C receptors. None of the three elicited a current from oocytes injected with this combination.

Mutation of L251 in β2 subunit alters the pharmacology of a receptor containing this mutated subunit. Mutation of the homologous residue in α7 allows antagonists at this receptor to act as agonists. Unlike α7 where 5 mutated subunits are coexpressed, here 3, or fewer, of the subunits composing the receptor contained the mutation. This was sufficient to allow cytisine, but not other antagonists to gate receptors with this mutation on the β2 subunit.

(Supported by NIH NS13546)

501.4

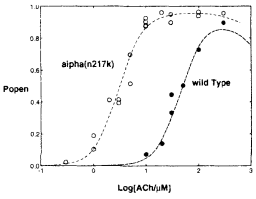
IDENTIFICATION OF RESIDUES THAT DETERMINE CURARE SELECTIVITY IN THE ADULT ACETYLCHOLINE RECEPTOR N.D. Bren and S.M. Sine* Department of Physiology and Biophysics, Mayo Foundation, Rochester, MN 55905.

The adult muscle acetylcholine receptor is a pentamer of homologous subunits with composition $\alpha_2\beta\varepsilon\delta$. The two ligand binding sites, formed by αε and αδ subunit pairs, exhibit 100-fold different affinities for the competitive antagonist dimethyl-*d*-tubocurarine (DMT). We previously showed that Y117 of the γ subunt confers high affinity to the αγ site of the fetal AChR, whereas the equivalent residue T118 of the δ subunit confers low affinity to the αδ site. Here we show that in the adult AChR, the mutation εS117R decreases DMT affinity, but the aromatic mutations, εS117Y and εS117W, show no change in affinity. Thus, although near the site of DMT binding, residue 117 is not the source of high affinity in the ε subunit. To identify residues that confer high affinity in the ε subunit, we made ε–δ chimeras, coexpressed them with complementary subunits in 293 HEK cells, and measured DMT binding by competition against the initial rate of ^{125}I-α-bungarotoxin binding. When we introduce ε sequence into the N-terminal 65 positions of the δ subunit, the resulting binding site shows a 10-fold increase in affinity for DMT. Conversely, when we introduce δ sequence between positions 44 and 65 of the ε subunit, the resulting binding site shows a 10-fold decrease in affinity. Thus, DMT selectivity is determined by one or more of the eight residue differences in this segment. We conclude that alternative segments of the ε, δ, and γ subunits confer DMT selectivity to the two binding sites. Supported by NIH grant NS31744.

501.5

MUTATION IN THE M1 DOMAIN OF THE ACETYLCHOLINE RECEPTOR α SUBUNIT DECREASES BOTH THE RATE OF AGONIST DISSOCIATION AND RATE CONSTANTS FOR CHANNEL GATING. H.-L. Wang*, N. Bren, A. Auerbach, A. G. Engel, and S. M. Sine Mayo Foundation, Rochester, MN 55905

We previously reported that a point mutation in the M1 domain of the α subunit (αN217K) of the acetylcholine receptor (AChR) caused a slow channel congenital myasthenic syndrome (SCCMS) (Ohno et al. Neurology 46: A214, 1996). When expressed in 293 HEK cells, AChR containing αN217K exhibits prolonged activation episodes strikingly similar to those observed at the SCCMS muscle end plates. Here we use single channel kinetic analysis to show that αN217K markedly decreases the rate of ACh dissociation, as well as decreases both the opening and closing rate constants. The kinetic parameters also describe the concentration dependence of the open probability (see Figure), revealing a 20-fold shift in the EC_{50} compared to wild-type (αN217K: 3μM; wild-type: 50μM). These results indicate that this mutation in the M1 domain of the α subunit affects both ligand binding and channel gating. Mutating the equivalent asparagine in the other subunits (β,δ,ε) does not affect single channel kinetics nor agonist binding affinity. The mutation αN217E decreases the open probability, whereas αN217Q has no effect. These results indicate that the effects of αN217K are specific to the α subunit and are related to substitution of a charged residue at this position.



Supported by NIH grant NS 31744.

501.6

Generation of mice deficient in the alpha7 neuronal nicotinic receptor gene by targeted recombination

Avi Orr-Urtreger, Finn Goldner, Jim Patrick* and Art Beaudet
Department of Molecular and Human Genetics, The Howard Hughes Medical Institute, and the Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030

The alpha7 neuronal nicotinic acetylcholine receptor subunit forms homo-oligomeric nicotine gated ion channels when expressed in oocytes, has a substantial permeability to calcium, is blocked by the snake toxin α-bungarotoxin and is widely expressed in the brain. To better understand the functional role of this receptor subunit we generated a mouse in which exons eight through ten, which encode transmembrane domains two through four of the alpha7 protein, have been deleted by homologous recombination with a vector in which these sequences were replaced with those encoding a selectable marker. The resulting mouse no longer synthesizes immunoreactive material that can be purified by affinity chromatography on α-cobratoxin columns from detergent extracts of these brains. The brains of these mutant mice appear normal on routine histological examination, show no differences when examined for acetylcholinesterase activity and, at age eight weeks, show normal barrel fields in the somatosensory cortex.

This work was supported by grants from NIDA, NINDS, the HHMI and a fellowship from Fogarty International.

501.7

LEVELS OF CHOLINERGIC LIGAND BINDING IN MUTANT MICE LACKING THE β2 SUBUNIT OF THE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) M. Zoli*, J.-P. Changeux, and M.R. Picciotto Lab of Molecular Neurobiology, Pasteur Institute, Paris, France, 75724.

We have generated a line of mice lacking the β2 subunit of the nAChR using homologous recombination. Regional binding of several nicotinic ligands was evaluated by quantitative receptor autoradiography in mutant and wild type mouse brain. Mutant mice completely lack high affinity nicotine binding in the brain, indicating that nicotine cannot bind with high affinity to nAChRs that do not contain the β2 subunit. Other nicotinic ligands do show residual binding in brain areas expressing the β4 subunit (such as the habenulo-interpeduncular (MHb/IPN) system), however. Rank order of binding for these ligands in the MHb/IPN of mutant mice was: epibatidine>cytisine>>methylcarbamylcholine=ACh (in the presence of atropine) >nicotine (which was undetectable). We have also used α-bungarotoxin binding to determine whether the α7 family of nAChRs is affected by the absence of the β2 subunit of the nAChR. A significant increase (50%) in α-bungarotoxin binding was seen selectively in the CA regions of the hippocampus in β2 mutant mice, indicating that these two families of nicotinic receptor subunits may interact in wild type animals. We also examined the levels of M1 and M2 binding in mutant and wild type mouse brain in order to determine whether other cholinergic markers are perturbed in the absence of the high affinity receptor for nicotine. No significant change in either M1 or M2 receptor binding was observed in any region studied.

Funding for this work was provided by the College de France, the CNRS., the Association Francaise contre la Myopathie a grant from the Human Frontiers Science Program and a Fogarty/CNRS cooperative Fellowship to M.R.P.

501.8

GAMMA-PKC NULL MUTANT MICE HAVE REDUCED SUSCEPTIBILITY TO NICOTINE-INDUCED SEIZURES, E.H. Owen*, J.E. Carter, A. Abeliovich, S. Tonegawa, J.M. Wehner, A.C. Collins. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309-0447.

PKC-γ null mutant mice are differentially sensitive to ethanol but not barbiturates or benzodiazepines. The behavioral effects of each of these drugs result from actions on the GABA receptor. Brain nicotinic receptors contain consensus sites for PKC phosphorylation. Consequently, effects of nicotine on open field activity, body temperature, and seizures were measured in wild type (+/+), heterozygotes (+/-), and null mutant (-/-) mice. Dose-response curves were constructed for each measure. No differences were observed across genotypes for the open field and body temperature tests. However, the (-/-) mice were more sensitive to nicotine-induced seizures than were the (+/+) mice while the (+/-) mice showed intermediate sensitivity. Current evidence argues that nicotine's effects on locomotor activity and temperature are regulated by α4-containing nicotinic receptors whereas nicotine-induced seizures are influenced by α7-containing receptors. The result with null mutants argues that phosphorylation of the α7-type nicotinic receptor may modulate its activity. Supported by DA-10156, DA-00197, and AA-00141.

501.9

CHARACTERIZATION OF THE RAT NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR α7 SUBUNIT GENE. U. Nagavarapu* and R.T. Boyd. Department of Pharmacology, The Ohio State University College of Medicine, Columbus, Ohio, 43210.

nAChRs binding the snake venom toxin, α-bungarotoxin (αBgt), are expressed in the CNS as well as in autonomic neurons and function as ligand-gated cation channels. Recent evidence supports a role for these αBgt-AChRs in neurite outgrowth and presynaptic control of neurotransmitter release. Many of these αBgt-AChRs contain the α7 subunit. The α7 gene is also expressed in developing skeletal muscle indicating that nAChRs containing α7 subunits may play a role in muscle development.

We are interested in isolating and characterizing the rat α7 gene and genetic elements which control cell-specific expression. These elements will probably be complex with distinct elements controlling expression in muscle during development and other sequences controlling expression in specific neuronal populations.

A rat genomic library was screened using a rat α7 cDNA as a probe. Nine genomic clones containing the α7 gene were isolated. The genomic clones were mapped and the exon-intron boundaries localized. The 5' α7 cDNA probe was used to isolate genomic clones containing the 5' flanking region of the α7 nicotinic AChR gene. Restriction fragments from these clones were subcloned and sequenced to identify the promoter region of the gene. Further characterization and expression of these subclones is currently in progress.

This work supported by AHA (Ohio Affiliate), Bremer Foundation (Columbus, Ohio), and NIH Grant NS29746.

501.10

CLONING AN ALPHA-LIKE NICOTINIC ACETYLCHOLINE RECEPTOR (AChR) SUBUNIT FROM THE PARASITIC NEMATODE *ASCARIS SUUM*. H.L. Brooks [1], R.C. Foreman [1], J.F. Burke [2], N.C Sangster [3], L. Holden-Dye [1] & R.J. Walker *[1]. Department of Physiology and Pharmacology, School of Medical Sciences, University of Southampton, SO16 7PX,[2] Department of Biochemistry, University of Sussex, BN1 9QG,[3] Department of Veterinary Pathology, University of Sydney, NSW 2006.

The nicotinic receptor mediating muscle contraction in the parasitic nematode *Ascaris suum* has been well characterized in terms of its agonist, antagonist and toxin selectivity. This receptor is the target for some commonly used anthelmintics, including levamisole, for which resistant strains are now appearing the parasitic nematodes. In order to facilitate rational anthelmintic design we have set out to establish the structural basis for the pharmacological properties of the *Ascaris* nicotinic receptor by cloning the nicotinic receptor subunit genes. We have screened an *A. suum* larval cDNA library at low stringency with PCR fragments, obtained using degenerate probes to the conserved regions of other invertebrate AChR genes (Neurosci. Abst., 334.1:832,1995), and a genomic fragment which encodes the conserved second transmembrane region from the nAChR alpha-like subunit of *Trichostrongylus colubriformis*. We have cloned the cDNAs of 8 putative nAChR subunits, one of these, As38, is an alpha-like subunit and shares 54% amino acid identity with the *Drosophila* alpha-like nAChR subunit and 49% amino acid identity to the chick alpha-2 neuronal subunit. Functional characterization of this subunit will be achieved by using the *Xenopus* oocyte expression system.

Supported by the BBSRC

501.11

TRANSCRIPTIONAL CONTROL OF AN ACETYLCHOLINE RECEPTOR SUBUNIT GENE. Y. He, L.C. Yang, J. G. Ma, S. Houser and T. Schmidt-Glenewinkel*. Department of Biological Sciences, Hunter College and Graduate Center of CUNY, New York, NY 10021

The *ard* gene encodes a non-α-like subunit of one of the two subtypes of neuronal nicotinic acetylcholine receptors from *Drosophila*. We have been studying the cis-acting elements required for temporal and spatial expression of the gene encoding this receptor subunit. We have previously shown that a 325 bp fragment of the 5' flanking sequence was sufficient to direct β-galactosidase expression in the nervous tissue during development with a pattern very similar to the distribution of transcripts of the corresponding *ard* gene. DNase I footprinting has been carried out to precisely locate the essential regulatory sequences. Site-specific mutagenesis is performed based on the results of DNase I footprinting. P-element mediated transformation with the mutagenized promoter elements will establish which regulatory sequences are required for temporal and spatial expression of this subunit gene *in vivo*.

Supported by PSC-CUNY Grant 665150 and RCMI Award RR-03037, NIH

501.12

FUNCTION OF TRANSGENIC NICOTINIC ACETYLCHOLINE RECEPTORS EXPRESSED IN HUMAN CELLS STABLY TRANSFECTED WITH α7 SUBUNITS. R.J. Lukas*, L. Lucero, C.M. Eisenhour and E. Puchacz. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013 USA.

We have continued studies of stably transfected cells of the SH-SY5Y human neuroblastoma or other clonal lines that express functional nicotinic acetylcholine receptors (nAChR) as homooligomers of transgenic α7 subunits. cDNA inserts containing wild-type or mutant forms of chick α7 subunits kindly provided by Jean-Pierre Changeux and Jean-Luc Galzi were subcloned in sense or anti-sense orientations into the pCEP4 expression vector. Following electroporation to introduce transgenes into cells and selection of stable transfectants in hygromycin B, many more cells carrying anti-sense transgenes survived than did cells carrying sense transgenes coding for mutant subunits predicted and shown here to combine to form non-desensitizing, Ca^{2+}-permeable nAChR. Inclusion of 100 nM methyllycaconitine (MLA) in culture medium spared sense orientation transfectants from cell death, reflecting MLA's actions as a potent, competitive antagonist of mutant α7-nAChR function as assessed by isotopic ion flux assays. These assays also show that di-hydro-β-erythroidine (DHβE) is a potent agonist at mutant α7-nAChR and that EC_{50} values are in the nM range for nicotine activation of mutant α7-nAChR function. Abilities of DHβE, nicotine, and MLA to inhibit specific 125-I-labeled α-bungarotoxin binding to transgenic α7-nAChR do not differ for receptors containing wild-type or mutant subunits. These studies indicate utility of stably transfected cells expressing α7 subunits as models for elucidating basic principles of ligand-gated ion channel structure and function. Funded by NIDA, ADCRC and STRC.

501.13

IDENTIFICATION OF CALCIUM BINDING SITES WHICH REGULATE POTENTIATION OF A NEURONAL ACHR. D. Bertrand*, J.L. Galzi, P.J. Corringer, S. Bertrand and J.P. Changeux University of Geneva, 1211 Geneva, Switzerland, Institut Pasteur 75734 Paris Cedex France.

The divalent cation calcium potentiates the physiological response of neuronal nAChRs by enhancing ionic current amplitudes, apparent agonist affinity and cooperativity. The potentiation observed on the α7-5HT_3 chimera and its absence on the wild type 5HT_3 receptor indicates that this allosteric modulation depends on the N-terminal domain of the protein. Furthermore, sequence analysis of this protein segment suggests the existence of five putative calcium binding sites. Here, we report that mutations of these putative calcium binding loops alter calcium potentiation of the α7-5HT_3 chimera. Moreover, "grafting" of the most important loop in the 5HT_3 receptor results in a serotonin receptor which can be potentiated by extracellular calcium. In addition calcium inhibits terbium binding to a synthetic peptide exhibiting the amino acid sequence of this main loop and mutations within this peptide alter terbium binding.

Work supported by HFSP, the Swiss National Foundation, OFES and the EEC.

502.1

NNC 90-0270 - A NOVEL NICOTINE RECEPTOR MODULATOR: IN VITRO CHARACTERISTICS. K. Rimvall*, K. Eskesen, M. Sheardown, J. Egebjerg, P.H. Olesen, J.E. Tønder, M.E. Judge, T. Rasmussen and M.D.B. Swedberg. Health Care Discovery, Novo Nordisk, Novo Nordisk Park, DK-2760 Malov, Denmark.

Modulators of nicotinic receptors in the CNS are of potential benefit in a variety of CNS disorders. We have characterized the novel, nicotinic compound NNC 90-0270 in several in vitro tests and compared its effects to those of standard compounds like nicotine, ABT418, lobeline and methylcarbachol (MCC).

NNC 90-0270 inhibits [^{3}H]MCC binding to cortical and hippocampal rat brain membranes and to Sf9 cells expressing the $\alpha4\beta2$- or the $\alpha3\beta2$-subunit combinations with Ki values between 0.9 and 2.3 nM. It is selective for nicotine receptors, compared to muscarinic receptors, with a MCC/oxotremorine IC_{50}-ratio of 320. NNC 90-0270 induces the release of [^{3}H]dopamine from prelabeled rat striatal slices with an EC_{50} of 0.61 nM and with an efficacy, at 1 µM, of 60% relative to 1 µM nicotine. NNC 90-0270 evokes spreading depression in the chick retina (MEC 1 µM). The values for nicotine and ABT418 were 10 and 23 µM, respectively. NNC 90-0270 acts as an agonist on Xenopus oocytes expressing $\alpha3\beta2$- and $\alpha4\beta2$-subunit combinations with EC_{50} values of 4.2 and 0.31 µM. At the $\alpha4\beta2$-subunit combination, both MCC and NNC 90-0270 are more efficacious than nicotine giving responses of 230% and 300% at a concentration of 1 µM.

Funded by Novo Nordisk.

Acknowledgements: The generous gifts of nicotine clones by Dr. J. Patrick and of non-labeled MCC by Dr. Abood are gratefully acknowledged.

502.2

IDENTIFICATION OF A NOVEL (ISOXAZOLE)METHYLENE-1-AZABICYCLIC COMPOUND WITH HIGH AFFINITY FOR THE CENTRAL NICOTINIC ACh RECEPTOR: NNC 90-0270. P.H Olesen, M.D.B. Swedberg, K. Rimvall, K. Eskesen, M.E. Judge, J.Egebjerg, J.B. Hansen, J.E.Tønder, T. Rasmussen and M.J. Sheardown*. Health Care Discovery, Novo Nordisk, Novo Nordisk Park, DK-2760 Malov, Denmark.

New ligands which selectively activate subtypes of the nicotinic acetylcholine receptor (nAchR) may have therapeutic potential for the treatment of central nervous system (CNS) disorders.

Here we report on a novel series of (isoxazole)methylene-1-azabicyclic compounds which possess nanomolar affinity and high selectivity for brain nAChRs. The compounds have different affinity for the various subtype combinations of neuronal nAChRs in transfected cells. The synthesis and structure-activity relationships for *in vitro* binding assays, *in vitro* functional assays and *in vivo* functional assays for the leading members of the series are described. Our major lead compound, NNC 90-0270, has a 3.0 nM affinity for rat brain [^{3}H]-MCC binding sites and demonstrates a 400 % efficacy, compared to nicotine, with regards to stimulation of the current response in Xenopus oocyte expressed with the human $\alpha4\beta2$ nAChR subtype.

Funded by Novo Nordisk.

502.3

NNC 90-0270: IN VIVO CHARACTERIZATION OF A NOVEL CENTRALLY ACTING NICOTINIC AGONIST. M. D. B. Swedberg*, T. Rasmussen, M. E. Judge, K. Rimvall, K. Eskesen, J. Egbjerg, M. J. Sheardown, J. B. Hansen and P. H. Olesen. Health Care Discovery, Novo Nordisk, Novo Nordisk Park, DK-2760 Malov, Denmark.

NNC 90-0270 is a novel nicotinic agonist with nanomolar affinity for brain nicotinic acetylcholine receptors (nAChR; see Olesen et al., this meeting). In vitro, NNC 90-0270 was more effective than nicotine at the $\alpha4\beta2$ subunit in the Xenopus Oocytes (see Rimvall et al., this meeting). In a rat nicotine drug discrimination assay (nicotine ED_{50}: 0.01 mg/kg), NNC 90-0270 (ED_{50}: 0.1 mg/kg), like lobeline (ED_{50}: >5.4 mg/kg) and ABT-418 (ED_{50}: 0.2 mg/kg), produced partial nicotinic responses, whereas cytisine (ED_{50}: 0.7 mg/kg) produced full effect. When comparing oral to s.c. ED_{50}'s in the drug discriminaton test, NNC 90-0270 had a ratio of 5 and ABT-418 a ratio of 10. NNC 90-0270, lobeline and ABT-418 all produced dose-dependent response rate decreases, whereas nicotine and cytisine did not reduce rates at the doses tested. Locomotor activity in the mouse was equally reduced by NNC 90-0270 (ED_{50}: 0.4 mg/kg) and nicotine (ED_{50}: 0.3 mg/kg) and less so by ABT-418 (ED_{50}: 10.0 mg/kg). NNC 90-0270 dose-dependently reduced body weights in rats more efficaciously than nicotine at equimolar doses when given via osmotic minipumps for two weeks. NNC 90-0270 and ABT-418 both appear not to cause dose-dependent intravenous self-administration in naive mice, whereas nicotine produced a characteristic inverted U-shaped dose-response curve.

Funded by Novo Nordisk

502.4

PRELIMINARY CHARACTERIZATION OF ETHYL (3-QUINUCLIDINYL)ACETATE AS A NOVEL ACETYLCHOLINE RECEPTOR LIGAND. D. Canney [2], J. Buccafusco [3], R. Aronstam [4], M. Gattu [3], M. Zhang[2], G. Sterling[1]*, P. Doukas [2]. Temple Univ., Schools of Medicine[1] and Pharmacy[2], 3307 N. Broad St. Phila., PA 19140; Medical College of Georgia[3], Dept. of Pharmacol.and Toxicol., Augusta, GA 30912, Guthrie Research Institute[4], Sayre, PA, 18840.

Recent studies suggest that neuronal nicotinic acetylcholine receptors (nAChR) may play a role in several CNS disorders including Alzheimer's Disease. Molecular cloning experiments indicate that multiple subtypes of the nAChR exist in brain. Recent work in our laboratories show that 3-substituted quinuclidines exhibit potent CNS activity when administered i.p. to mice. In particular, ethyl (3-quinuclidinyl) acetate (EQA) was shown to induce seizures which could be prevented by pretreatment with mecamylamine. The objective of the present study was to evaluate EQA as a potential cholinergic ligand. EQA blocked the vasodilatory response to ACh on blood pressure, and blocked ACh-induced contraction of ileal smooth muscle. Binding studies ([^{3}H]cytisine, [^{3}H]perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX), scopolamine, pirenzepine, α-bungarotoxin) show that EQA has µM affinity for both muscarinic and nicotinic receptors. Behavioral studies following i.c.v. injections of EQA and methylcarbamylcholine suggest that EQA is a nicotinic antagonist. These data indicate that EQA has varying degrees of affinity/activity for muscarinic and nicotinic receptors in the periphery and in the CNS. Especially interesting is the potential development of EQA-based compounds as neuronal nAChR antagonists.

Supported by Med Research Service, Veterans Affairs & Temple Univ.

502.5

WALGERIN-1 BLOCKS THE EMBRYONIC MUSCLE-TYPE ACETYLCHOLINE RECEPTOR (ACHR). J.P. Dilger and J.J. McArdle*. Dept Anesthesiology, SUNY, Stony Brook 11794 and Dept Pharm & Phys, NJ Med School (UMDNJ), Newark, NJ 07103.

We previously demonstrated that the lethal effect of Waglerin-1 (WTX) in adult mice is associated with block of the ACHR at the neuromuscular junction. Conversely, the lack of lethality in neonatal mice is associated with resistance of the immature neuromuscular junction to WTX. To explore the role of the embryonic muscle type ACHR in resistance to WTX, we examined the effect of WTX on the embryonic mouse muscle-type ACHR from BC3H-1 cells. Multi-channel currents were activated by rapid perfusion of 100 μM ACh to outside-out patches. WTX had two effects on the currents. The first was to reduce peak current amplitude (before desensitization); the IC50 was 0.75 μM WTX. The second effect was to decrease the rate of rise of the current. The effects occurred within 30 sec of WTX perfusion. Recovery from the effects after washout of WTX required tens of minutes and was usually incomplete. The potency and time course of action of WTX at the nicotinic ACHR of the BC3H-1 cell is similar to that described at the mature neuromuscular junction. This would suggest that the ACHR at the end-plate of neonatal mouse skeletal muscle is not particularly resistant to WTX. Similar studies with cells expressing the mature form of the ACHR are in progress. (Supported by NIH grants GM42095 and NS31040).

502.6

THE NICOTINIC AGONIST (±)EPIBATIDINE RELEASES [^{3}H]NOREPINEPHRINE BUT NOT ADENOSINE FROM RAT HIPPOCAMPAL SLICES. T.D. White* and K. Semba. Departments of Pharmacology and Anatomy & Neurobiology, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

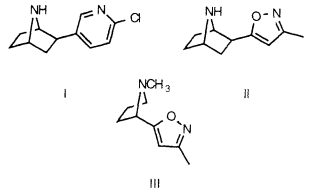
(±)Epibatidine is a natural toxin from frog skin that exhibits potent analgesic activity unrelated to opioid receptor activation. It has been shown to be an agonist at neuronal nicotinic receptors and to release catecholamines from brain slices. Significantly, epibatidine's analgesic actions in the hot plate test are blocked by adenosine receptor antagonists such as caffeine and theophylline, suggesting that the analgesia may involve extracellular adenosine (Gunther et al., Soc. Neurosci., 21, 247.11, 1995). We determined if epibatidine releases adenosine, which might then act at specific adenosine receptors to produce analgesia. We also compared epibatidine's ability to release adenosine with its ability to release [^{3}H]NE from slices of rat brain and contrasted these effects with the releasing effects of NMDA. In either the presence or absence of extracellular Mg^{2+}, (±)epibatidine (0.1µM) released [^{3}H]NE but **not** adenosine from hippocampal slices. In contrast, NMDA (300µM) released both [^{3}H]NE and adenosine from hippocampal slices, incubated in the absence of extracellular Mg^{2+} to relieve its block of NMDA receptors. (±)Epibatidine (0.1µM) also did not release adenosine from slices of rat parietal cortex, in contrast with NMDA (300µM) which, in the absence of extracellular Mg^{2+}, readily released adenosine. A very high concentration of (±)epibatidine (40µM) failed to release adenosine. These findings indicate that (±)epibatidine does not release adenosine from slices of either rat hippocampus or cortex. However, it is possible, if somewhat unlikely, that epibatidine somehow specifically releases adenosine only from those limbic and spinal regions involved in nociception.

Supported by grants from the MRC of Canada to T.D.W. and to K.S., and from the Scottish Rite Foundation to K.S.

502.7

EPIBOXIDINE; AN ANALOG OF EPIBATIDINE WITH ALTERED SELECTIVITY AS A NICOTINIC AGONIST. H.M. Garraffo, C. Plummer, J.W. Daly and B. Badio*. Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD 20892–0820.

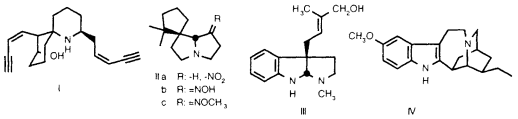
Epibatidine (I), a very potent nicotinic agonist selective for $\alpha_3\beta_{4(5)}$- and $\alpha_4\beta_2$–subtypes of nicotinic receptor–channels, is a potent antinociceptive agent (*Mol. Pharmacol., 45*, 563, 1994). Epiboxidine (II), an analog of epibatidine in which the chloropyridyl moiety is replaced by a methylisoxazolyl moiety, has been synthesized. The methylisoxazolyl moiety has precedent in ABT 418 (III), a nicotine analog in which the pyridyl moiety of nicotine has been replaced by the methylisoxazolyl moiety. Epiboxidine is about ten–fold less potent than epibatidine at central–type $\alpha_4\beta_2$–nicotinic receptors, and about five–fold less potent than epibatidine at neuromuscular–type $\alpha_1\beta_1\gamma\delta$-nicotinic receptors, while being nearly as potent as epibatidine at ganglionic–type $\alpha_3\beta_{4(5)}$-nicotinic receptors.



502.8

FURTHER CLASSES OF ALKALOIDS ACTIVE AS NONCOMPETITIVE NICOTINIC ANTAGONISTS; SPIROPYRROLIZIDINES, PSEUDOPHRYNAMINES AND IBOGAINE. B. Badio, W.L. Padgett, H.M. Garraffo and J.W. Daly*. Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD 20892–0820.

A variety of alkaloids have proven to be potent and useful noncompetitive blockers of nicotinic receptor–channels. Foremost among such alkaloids is histrionicotoxin (I) and various analogs, in which the presence of side chains with varying degrees of unsaturation, markedly affect both potency and selectivity as blockers of different subtypes of nicotinic receptors. Two unique new structural classes of alkaloids acting as blockers are the relatively polar spiropyrrolizidines (IIabc) and the physostigmine–like pseudophrynaminol (III). The spiropyrrolizidines are selective for ganglionic–type $\alpha_3\beta_{4(5)}$-nicotinic receptor–channels, while III is very potent and non–selective. In addition to these "amphibian alkaloids", the plant alkaloid ibogaine (IV), now widely studied as an antiaddictive agent, is a very potent and selective blocker of ganglionic–type nicotinic receptors with an IC_{50} in PC12 cells of about 20 nM.



502.9

ABT-089: DISCRIMINATIVE STIMULUS PROPERTIES AND ELECTROPHYSIOLOGICAL ACTIONS. D.J.B. Kim*, M.S. Brodie, M.W. Decker, S. P. Arneric and J.D. Brioni. Neuroscience Discovery, Abbott Laboratories, Abbott Park, IL. 60064 and University of Illinois, Chicago, IL. 60612-7342.

ABT-089, [2-methyl-3-2(2-(S)-pyrrolidinylmethoxy)pyridine dihydrochloride], is a novel cholinergic channel modulator (ChCM) that has subtype selective efficacy to modulate neuronal nicotinic acetycholine receptor (nAChR) function. It displaced [3H]-cytisine binding to the human $\alpha4\beta2$ subunit combination stably expressed in the K177 cell line with a 16 nM potency while (-)-nicotine exhibited a Ki=1 nM. In contrast, ABT-089 was 500-fold less potent to displace [125I]-α-Bgt binding (Ki=10,000 nM) from rat brain membranes (Sullivan, et. al. This meeting).

A series of experiments was conducted to determine the discriminative stimulus properties of ABT-089 in comparison to (-)-nicotine. Rats were able to discriminate (-)-nicotine 1.9 µmol/kg s.c. from saline in 43 days, but they were not able to discriminate 19 or 62 µmol/kg of ABT-089 from a saline solution after 64 days of training. In rats trained to discriminate 1.9 µmol/kg (-)-nicotine from saline, ABT-089 induced a reduced generalization (6.2-190 µmol/kg, s.c.) and was 100 times less potent than (-)-nicotine to induce the cue. A-94224.3, the enantiomer of ABT-089, induced a saline response at 62 µmol/kg. The effects of (-)-nicotine and ABT-089 were completely blocked by the cholinergic channel blocker, mecamylamine (15 µmol/kg). ABT-089 exhibited comparable effects in (-)-nicotine-trained rats after oral administration. In vitro electrophysiological studies in dopamine-containing neurons in the ventral tegmental area indicated that ABT-089 is a weak partial agonist that can also block the excitatory actions of (-)-nicotine.

These data demonstrate that in the present conditions ABT-089 could not be discriminated from saline in naive rats in doses up to 62 µmol/kg, and that ABT-089 induced a partial generalization in nicotine-trained rats at doses 100 times higher than (-)-nicotine. (Supported by Abbott Laboratories)

502.10

EFFECTS OF ABT-089, A NOVEL CHOLINERGIC CHANNEL MODULATOR, ON COGNITIVE PERFORMANCE IN RATS AND MONKEYS. M.W. Decker*, A.W. Bannon, P. Curzon, K.L. Gunther, J.D. Brioni, M.W. Holladay, N-H. Lin, Y. Li, J. Daanen, J.J. Buccafusco, M.A. Prendergast, W.J. Jackson, and S.P. Arneric´ Neuroscience Research, D-47W, AP-9A, Pharmaceutical Products Div., Abbott Labs, Abbott Park, IL 60064 and Dept. of Pharmacology, Medical College of Georgia, Augusta, GA 30912

ABT-089 [2-methyl-3-(2-(S)-pyrrolidinylmethoxy)pyridine dihydrochloride], a novel ligand at neuronal nicotinic acetylcholine receptors with reduced adverse effects and improved oral bioavailability relative to (-)-nicotine, was tested in a variety of cognitive tests in rats and monkeys. Acutely, ABT-089 only marginally improved the 2-platform spatial discrimination water maze performance of septal-lesioned rats. However, more robust improvement (45% fewer errors on the last training day) was observed when ABT-089 was administered continuously via osmotic pumps (min. effective dose: 1.3 µmol/kg/day, s.c.). Comparable effects are seen with (-)-nicotine, but at a higher dose (62 µmol/kg/day; Decker et al., 1995, Soc. Neurosci. Abstr. 21:159). In aged rats (20-22 mo.), continuous infusion of ABT-089 enhanced spatial learning in a standard Morris water maze, as indexed by spatial bias exhibited during a probe trial conducted after 4 days of training, but not after 7 days of training or when rats were later trained in a 2-platform spatial discrimination water maze. A small impairment was noted in young rats on the standard water maze, but not on the 2-platform task. ABT-089 did not affect performance of either the aged or young rats during inhibitory (passive) avoidance training. Continuously infused ABT-089 did not affect acoustic startle or prepulse inhibition of acoustic startle in young, aged, or septal-lesioned rats and did not affect locomotor activity in sham- or septal-lesioned rats. Acutely, ABT-089 modestly improved the delayed matching-to-sample performance of mature, adult monkeys (6-12.4 yr.) and significantly improved performance in aged monkeys (22-27.5 yr.). Improved performance in the aged monkeys was restricted to the longest delay intervals and was not accompanied by changes in response latencies. [Supported by Abbott Laboratories]

502.11

DIFFERENTIAL EFFECTS OF ABT-089 AND (-)-NICOTINE ON NICOTINIC ACETYLCHOLINE RECEPTOR DENSITY AND FUNCTION FOLLOWING SUBACUTE ADMINISTRATION.

D.J. Anderson*, D. Donnelly-Roberts, J. Campbell, J.R. Pauley[1], P. Magill[1], S.P. Arneric, and J.P. Sullivan. Neuroscience Research (D-47W), Pharmaceutical Products Division, Abbott Laboratories, 100 Abbott Park Rd, IL 60064-3500 and [1] College of Pharmacy, Univ. Kentucky, Lexington, KY, 40536.

ABT-089 [2-methyl-3-(2-(s)-pyrrolidinylmethoxy)pyridine dihydrochloride] is a cholinergic channel modulator with cognitive enhancing properties in rodents and primates; effects that are enhanced following subacute administration (see Decker et al., accompanying poster). The present study evaluated the effects of subacute (14 day) continuous administration of behaviorally effective doses of ABT-089 (1.3 µmol/kg/day) and (-)-nicotine (NIC) (62 µmol/kg/day)) on regional brain densities of the $\alpha4\beta2$ and $\alpha7$ nAChR subtypes and on nAChR-mediated functional responses. An index of the densities of $\alpha4\beta2$ receptors was measured with [^{3}H]cytisine, while [^{125}I]α-bungarotoxin (αBgT) was used for $\alpha7$ receptors. Autoradiography studies revealed that subacute administration of ABT-089 had no effect on the binding density of either [^{3}H]cytisine or [^{125}I]αBgT in any of the 37 brain regions examined. Similar results were found in radioligand binding studies to washed membranes from 5 brain regions. In contrast, subacute administration of (-)-nicotine significantly ($p < 0.05$) increased the density of [^{3}H]cytisine binding sites in all regions examined while regional differences in the ability of this agent to upregulate [^{125}I]αBgT binding sites were observed. Subacute ABT-089 had no effect on efficacy or potency of (-)-nicotine to stimulate $^{86}Rb^+$ efflux from thalamic synaptasomes in contrast to a 40% decrease in the efficacy of (-)-nicotine observed in rats following subacute (-)-nicotine treatment. Subacute ABT-089 or (-)-nicotine treatment did not alter the ability of (-)-nicotine to stimulate dopamine release from striatal slices. These data indicate that nAChR density and function is differentially modulated following continuous exposure to behaviorally effective doses of ABT-089 and (-)-nicotine. *Supported by Abbott Laboratories.*

502.12

ABT-089: A POTENT AND SELECTIVE CHOLINERGIC CHANNEL MODULATOR WITH NEUROPROTECTIVE PROPERTIES J.P. Sullivan, D. J. Anderson, C. A. Briggs, J.E. Campbell, D. Donnelly-Roberts, M. Gopalakrishnan, D. Gunn, Y. He, M.W. Holladay, D. McKenna, N. H. Lin, E. Molinari, M. Piattoni-Kaplan, I. Xue, M. Williams*, and S.P. Arneric. Neuroscience Research (D47W), Abbott Laboratories, Abbott Park, IL, 60064

Accumulating preclinical and clinical evidence data suggests that compounds that selectively activate neuronal nicotinic acetylcholine receptor (nAChR) subtypes may have therapeutic utility for the treatment of a number of neurological disorders. In the present study, the *in vitro* pharmacological properties of the novel cholinergic channel modulator ABT-089 [2-methyl-3-(2-(S)-pyrrolidinylmethoxy)pyridine), are described. In radioligand binding studies, ABT-089 was shown to display selectivity towards the high affinity (-)-cytisine binding site present on the $\alpha4\beta2$ nAChR subtype (K_i = 16 nM) relative to the [^{125}I]α-BgT binding site present on the $\alpha7$ (K_i = >10,000 nM) and $\alpha1\beta1\delta\gamma$ (K_i > 1000 nM) nAChR subtypes. In cation flux and channel current studies ABT-089 displayed a more complex profile than (-)-nicotine having agonist, partial agonist, and inhibitory activities depending on the nAChR subtype with which it interacts. ABT-089 differentially stimulated neurotransmitter release, the compound displayed a similar potency and efficacy to (-)-nicotine to facilitate ACh release but was markedly less potent and less efficacious than (-)-nicotine to stimulate dopamine release. Additionally, ABT-089 was neuroprotective against the excitotoxic insults elicted by exposure to either glutamate or Aβ1-42. The potency of ABT-089 to elicit neuroprotection was dramatically increased following subacute (7 day) treatment, a finding that parallels cognitive testing in rodents (see Decker et al., this meeting). The differential full agonist / partial agonist profile of ABT-089, as compared to (-)- nicotine and ABT-418, illustrates the complexity of nAChR activation and the potential to dissect responses at subclasses of the neuronal and peripheral receptors. [Supported by Abbott Laboratories]

502.13

PHARMACOKINETIC AND SAFETY STUDIES ON ABT-089; AN ORALLY ACTIVE CHOLINERGIC CHANNEL MODULATOR

J.E. Campbell, J.P. Sullivan, W. Arnold, Y. He, M.W. Holladay*, N.H. Lin, J. Daanen, Y. Li, K.C. Marsh, B. Peterson, H. Nellans, J. Trivedi and S.P. Arneric
Neuroscience Research (D-47W), Abbott Laboratories, Abbott Park, IL 60064-3500

ABT-089 [3-(2(*S*)-Pyrrolidinylmethoxy)-2-Methylpyridine] is a cholinergic channel modulator (ChCM) that selectively affects the function of neuronal nicotinic acetylcholine receptor (nAChR) subtypes, enhances cognitive performance and has neuroprotective activity (see accompanying posters). Unlike ABT-418 and (-)-nicotine, ABT-089 exhibits a more complex profile having agonist, partial agonist, and inhibitory activities at discrete nAChR subtypes. This report provides safety and pharmacokinetic information that clearly differentiates ABT-089 from (-)-nicotine and ABT-418. ABT-089 is orally available (33-76% in rat, dog and monkey) and studies indicate that a once-a-day formulation is feasible. In contrast, ABT-418 and (-)-nicotine have poor oral bioavailability in dog and monkey (< 5%) and moderate bioavailability in rat (17 to 29 %). In anesthetized dog, bolus administration of ABT-089 at doses up to 12,500 nmol/kg elicited no significant increases in blood pressure or heart rate. In contrast, significant increases were observed following bolus administration (500 nmol/kg) of either (-)-nicotine or ABT-418. Subacute (14 days) administration of a once a day oral formulation of ABT-089 to conscious dogs followed by infusion of increasing doses of this agent did not elicit any significant changes in diastolic blood pressure or heart rate. Maximal plasma levels in these animals were >300-fold higher than the cognitive enhancing level (i.e. 2-10 ng/ml) in rodents. ABT-089 (0.1 to 100 μM) directly applied to the smooth muscle of guinea pig ileum did not elicit contraction and did not inhibit the ability of ACh, histamine, serotonin or K^+ to produce normal responses. Thus, ABT-089 may be a safe and effective ChCM for the potential once-a-day oral treatment of neurological disorders. [Support provided by Abbott Laboratories]

502.14

SMOKER'S LEVEL OF NICOTINE ACTS VIA NICOTINIC RECEPTORS CONTAINING THE α7 SUBUNIT TO INCREASE GLUTAMATE RELEASE FROM HIPPOCAMPAL NEURONS.

K.A. Radcliffe, A. Rajan, M.M. Zarei, M. Yakehiro, J.Y. Hsu and J.A. Dani*.
Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The concentration of nicotine achieved by smokers (0.5 μM nicotine) enhances the frequency but not the amplitude of glutamatergic miniature EPSCs in cultures of hippocampal neurons. Immunocytochemistry and pharmacology indicate that presynaptic nicotinic receptors containing the α7 subunit mediate the influence of nicotine.

Rat hippocampal neurons were maintained in culture and were typically studied 10 to 30 days after plating. The neurons were whole-cell voltage clamped and mEPSCs were measured before, during, and after direct application of 0.5 μM nicotine. In another protocol, pulses of nicotine (0.5 mM) activated a rapidly desensitizing current indicative of α7-containing nicotinic receptors. In most (but not all cases), α-BGT (50 nM) or MLA (5 nM) completely inhibited the nicotinic currents, indicating α7-containing receptors. Both methods of nicotine application increased the release of glutamate as measured by an increase in the frequency (but not the amplitude) of mEPSCs. Nicotine did not have this effect in 0 external Ca. Co-localization with anti-α7 antibody and an antibody against a presynaptic marker (synaptotagmin) further supported the physiology results, suggesting that nicotine acts mainly via presynaptic nicotinic receptors containing the α7 subunit. Supported by NIH, NINDS; Smokeless Tobacco Research Council.

502.15

EFFECT OF SMOKING HISTORY ON [^{3}H]-NICOTINE BINDING IN HUMAN POSTMORTEM BRAIN.

C.R. Breese, C.E. Adams, M.J. Marks, A.C. Collins, S. Leonard. Department of Pharmacology, University of Colorado Health Sciences Center, and Veterans Administration Medical Center, Denver, CO 80262.

It is well established in animals that chronic nicotine administration evokes increases in brain nicotinic receptor numbers. There is also a genetically determined variability in nicotinic receptor numbers in rodents, differences which are thought to affect the sensitivity to nicotine, as well as the development of tolerance. Humans self-administer nicotine principally in the form of cigarettes. We have examined the level of [^{3}H]-nicotine binding in the thalamus and hippocampus of human postmortem brain tissue, from normal non-smoking subjects, and subjects with variable life-long smoking histories. A significant increase in the level of nicotinic receptors was observed in both the hippocampus and thalamus of subjects with life-long smoking histories (hippocampus: $F(36,1)=8.68$, $p<0.006$; thalamus: $F(30,1)=9.633$, $p<0.005$). There was also a modest correlation between the degree of smoking, as measured by the average packs smoked per day, and the number of nicotine binding sites found in the hippocampus and thalamus (hippocampus: $r=0.49$, $p<0.003$; thalamus: $r=0.44$, $p<0.02$). This data suggests that humans, like rodents, exhibit dose dependent increases in brain nicotinic receptor numbers following chronic nicotine exposure. [This research was supported by USPHS Grants MH44212, DA09457, and VA Medical Research Service]

502.16

THE EFFECTS OF PRENATAL NICOTINE EXPOSURE ON THE DEVELOPMENT OF BRAIN NICOTINIC RECEPTORS.

J.A. Sparks* and J.R. Pauly. PET Division, College of Pharmacy, Univ. of Kentucky, Lexington, KY 40536-0082.

In humans, prenatal exposure to cigarette smoke causes a number of developmental abnormalities. Although the causative agents have not been clearly identified, many studies have focused on nicotine. In the present study female C57Bl/6 mice received as a drinking solution either 2% saccharin or nicotine in 2% saccharin (200 μg/ml) for one month prior to mating and throughout pregnancy. This method of nicotine delivery is non-stressful and produces "smoking relevant" plasma concentrations of cotinine in the dam and the pups. Although there was no difference in maternal weight gain between the two treatment groups, pup weight was slightly reduced by nicotine exposure. [3H]-cytisine and alpha [125I]-bungarotoxin (BTX) receptor autoradiography was performed on maternal and fetal brains obtained on day 19 of gestation (GD19). The pattern of cytisine binding on GD19 is distinct from that observed in the adult CNS with the retina and the pons/medulla having the greatest number of receptors. BTX binding on GD19 more closely resembles the adult pattern as high concentrations of receptors are present in the somatosensory cortex, hippocampus, hypothalamus and dorsal tegmental nucleus. The number of CNS binding sites for each ligand was increased by prenatal nicotine exposure. The functional consequences of increased fetal receptor number remain to be elucidated. Supported by DA-08443.

502.17

AUTORADIOGRAPHIC COMPARISON OF [3H]-A 85380, [3H]-EPIBATIDINE AND [3H]-CYTISINE BINDING IN RAT AND MOUSE BRAIN.

J.R. Pauly*, D.J. Anderson AND J.P. Sullivan. College of Pharmacy, Univ. of Kentucky, Lexington, KY 40536-0082 and Abbott Labs, Neurosci. Res. D47W Abbott Park IL 60064-3500.

Recent studies have shown that [3H]-epibatidine is a useful ligand for the autoradiographic analysis of brain nicotinic receptors (nAChr's). However, it appears that [3H]-epibatidine may bind to two or more nAChr alpha subunits (α3, α4 and possibly α7). The purpose of the present study was to compare the binding of [3H]-epibatidine and [3H]-cytisine with that of [3H]-A 85380, a newly developed, high-affinity nAChr radioligand (Ki = 50pM). Adjacent sets of cryostat sections were prepared from both rat and mouse brain and autoradiography was performed as described by Perry and Kellar (1995). The binding of three different concentrations of [3H]-epibatidine and [3H]-A 85380 (80pM, 400pM or 800pM) were compared to that produced by 5nM [3H]-cytisine. Qualitatively similar patterns of binding were produced with each ligand. However significant quantitative differences between [3H]-epibatidine and [3H]-cytisine\[3H]-A 85380 binding were identified in both rat and mouse brain. Specifically, the medial habenula, the interpeduncular nucleus and the fasiculus retroflexus bound significantly more [3H]-epibatidine than [3H]-A 85380. The high affinity and subtype selectivity of [3H]-A 85380 suggest that it is an excellent ligand for measuring brain nAChr's. Supported by Abbott Labs and DA-08443.

502.18

LOBELINE POTENTLY INHIBITS [^{3}H]DOPAMINE ([^{3}H]DA) UPTAKE INTO RAT STRIATAL VESICLES AND SYNAPTOSOMES, AND PROMOTES [^{3}H]DA RELEASE FROM STRIATAL VESICLES AND SLICES.

L.H. Teng*, P.A. Crooks, P.K. Sonsalla and L.P. Dwoskin. Graduate Center for Toxicology and College of Pharmacy, University of Kentucky, Lexington, KY 40536 and Dept. Neurology, UMDNJ RW Johnson Medical School, Piscataway, NJ 08854.

Lobeline is currently being developed as a nicotine substitution therapy for tobacco smoking cessation. The present study compared the effects of lobeline and nicotine on [^{3}H]DA uptake and release in rat striatum. In contrast to nicotine, the concentration-dependent increase in [^{3}H]DA release from striatal slices evoked by lobeline was not inhibited by mecamylamine (1-100 μM), a nicotinic receptor antagonist. Also, in contrast to nicotine, lobeline-evoked [^{3}H]DA release from striatal slices was calcium independent. Thus, the effect of lobeline to evoke [^{3}H]DA release from striatal slices was via a mechanism other than stimulation of nicotinic receptors. In contrast to nicotine, lobeline potently inhibited [^{3}H]DA uptake into striatal synaptic vesicles and synaptosomes with IC50s of 0.88μM and 80μM, respectively. At concentrations < 1 mM, nicotine did not inhibit [^{3}H]DA uptake into vesicles or synaptosomes. Interestingly, lobeline evoked the release of [^{3}H]DA from preloaded synaptic vesicles. The results demonstrate that lobeline acts to release striatal DA, not by stimulation of nicotinic receptors, but by inhibition of DA uptake into presynaptic vesicles and by promotion of DA release from presynaptic vesicles. We suggest that lobeline acts in an amphetamine-like manner, in that lobeline increases cytoplasmic DA concentrations and thereby enhances DA release from the dopaminergic terminal. Supported by the Tobacco and Health Research Institute, Lexington, KY.

502.19

AMINOGLYCOSIDE ANTIBIOTICS BLOCK ACETYLCHOLINE-INDUCED CURRENTS IN α9-INJECTED XENOPUS LAEVIS OOCYTES.
A.B. Elgoyhen, C. Rothlin, M. Verbitsky, D. Vetter and S. Heinemann*. Instituto de Investigaciones Farmacológicas (CONICET), Buenos Aires, Argentina and The Salk Institute for Biological Studies, La Jolla, CA.

Ototoxicity is one of the major side effects of aminoglycoside antibiotic treatment. When these drugs are administered in high doses outer hair cells degenerate *(Schacht, Otolaryngol. Clin. North Am., 26: 845, 1993).* The injection of gentamicin to guinea pigs produces a rapid elimination of the cholinergic olivocochlear efferent system that synapses on outer hair cells. The aim of the present work was to study the effects of aminoglycosides on α9, the nAChR of outer hair cells *(Elgoyhen et al, Cell 79: 705, 1994). In vitro* transcribed α9 cRNA was injected into *Xenopus laevis* oocytes. Responses of oocytes to acetylcholine (ACh) were measured under two-electrode voltage-clamp two to seven days after injection. Application of aminoglycosides blocked ACh-evoked currents in a concentration-dependent manner. The order of potency was neomycin > gentamicin > streptomycin > amikacin, with IC_{50} of 1 μM, 6 μM, 14 μM and 102 μM respectively, measured at the EC_{50} (10 μM) for ACh. The antagonistic effect of neomycin and of gentamicin was not overcome by increasing the concentration of ACh, indicative of a non-competitive type of blockade. Our results suggest that at least part of the inner ear side effects of aminoglycosides could be due to the blockade of the cochlear nicotinic cholinergic receptor.

Supported by Fogarty International Research Collaboration, Fundación Antorchas and Strauss Foundation for Auditory Science.

502.20

NEUROSTEROIDS ACT AS ALLOSTERIC ANTAGONISTS ON BRAIN NICOTINIC RECEPTORS
A.E. Bullock*, A.L. Clark, S.R. Grady, S.F. Robinson, B.S. Slobe and A.C. Collins Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

Recent evidence demonstrates that progesterone and it's metabolites are allosteric modulators of GABAergic receptors. Brain nicotinic receptors are members of the same super gene family. With this in mind, we assessed the effects of progesterone and two of it's metabolites (5α-pregnane-3,20-dione and 5α-pregnane-3α-ol-20-one) on brain nicotinic receptor function using [^{3}H]Dopamine release from striatum and ^{86}Rubidium efflux from thalamus as measures of receptor function. Current evidence indicates that these two assays monitor function of α_3 and $\alpha_4\beta_2$ containing receptors, respectively. Each of the steroids inhibited receptor function although the potency and maximal effect of the steroids varied between the two measures. The inhibition of nicotinic receptor function was noncompetitive in nature and was slow in onset and recovery. One potential explanation for these findings is that the steroids may alter the ratio of ground state to desensitized state of the nicotinic receptor. Thus reducing the number of activatable nicotinic receptors. (Supported by DA05131 and DA00197)

502.21

Use-dependent inhibition of neuronal nicotinic ACh receptor by preadrenomeduline precursor protein (PAMP).
H. Ishibashi*, J. Nabekura , O. Murata and N. Akaike. Dept. Physiol., Kyushu Univ. Fac. Med., Fukuoka 812-82, Japan.

PAMP has been recently discovered as a powerful bioactive compound for vasodilation released from adrenal gland. This substance inhibits the release of adrenaline from chromaffin cells stimulated by nicotine, which suggests that PAMP might act as an antagonist on nicotinic acetylcholine (ACh) receptor. Therefore, to elucidate the action of PAMP on the neuronal nicotinic receptor, we employed a conventional whole-cell patch recording on acutely dissociated locus coeruleus (LC) neurons of rats, which receive massive cholinergic projection.

Nicotine as well as ACh elicited a peak followed by a gradual decrease of inward current at a holding potential of -40 mV in almost all LC neurons tested. The peak of nicotine-induced current (I_{nic}) increased in a concentration dependent manner (>10^{-5}M). Agonists and antagonists studies indicated that the nicotinic receptor in the LC are mainly composed of α4 subunits. PAMP reversibly and non-competitively inhibited the I_{nic} in a concentration-dependent manner (>10^{-9}M). In the continuous application of PAMP, this inhibition increased use-dependently. These results indicate that the PAMP exhibits the powerful antagonistic action on neuronal nicotinic ACh receptor-channel complex.

502.22

INHIBITORY EFFECTS OF LOCAL ANESTHETICS ON ALPHA-BUNGAROTOXIN-SENSITIVE NEURONAL NICOTINIC RECEPTORS IN RAT CULTURED HIPPOCAMPAL NEURONS.
W.S. Cortes[1,2,3], H. Matsubayashi[1], Y.-P. Yu[1], Y. Aracava[1,2], A.T. Eldefrawi[1]* and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, USA; [2]Lab. Mol. Pharmacol., IBCCF, Fed. Univ. Rio de Janeiro, RJ 21944, [3]Dept. Physiol. Sci., IB, UFRRJ, Rio de Janeiro, RJ 23568, Brazil.

Previous results have shown that bupivacaine and piperocaine can act as open channel blockers at muscle nicotinic acetylcholine receptors (nAChRs) (*Mol. Pharmacol.* **16:**901, 1979; **26:**304, 1984) and neuronal nAChRs (*Soc.Neurosci. Abs.* **19:**1535, 1993). In the present study we investigated the effects of bupivacaine and derivatives of piperocaine on the α-bungarotoxin-sensitive (α7 containing) receptor, the predominant neuronal nAChR in the hippocampus (*J. Pharmcol. Exp. Ther.* **265:**1455, 1994). Bupivacaine (1-100 μM) or derivatives of piperocaine (100 nM- 100 μM) were applied via a U-tube as an admixture with 100 μM or 1 mM ACh, and in the bath perfusion. Bupivacaine decreased the peak amplitude of ACh- elicited currents in a concentration-dependent manner with an IC_{50} value of 23.7 μM and Hill coefficient of 0.86. Piperocaine derivatives up to 1 μM had no effect on the ACh- evoked currents, while at 30 μM piperocaine and its quaternary derivative reduced the peak amplitude of ACh-evoked currents by 42 ± 7.4% (mean ± S.E.M.) and 30 ± 4.5% respectively, and at 100 μM piperocaine and its quaternary derivative decreased the peak amplitude of ACh-evoked currents by 75 ± 9.3% and 83 ± 3.6%. All drugs decreased the peak amplitude of the nicotinic currents in a voltage-dependent fashion. All drugs also shortened the decay phase of the currents; this effect was more clearly observed at low agonist concentration (100 μM ACh) and was not voltage dependent. The potency of either bupivacaine (previous results) or piperocaine derivatives in inhibiting the nicotinic response was much lower when these drugs were applied only via the U-tube as an admixture with the agonist. Thus, these results indicate that bupivacaine and derivatives of piperocaine may interact with the open state of the channel of α-bungarotoxin-sensitive nAChRs, in addition to interacting with a distinct site, which may be located either on the receptor or in the lipid bilayer. (*Support:* USPHS Grants NS25296 and ES05730; CNPq and UFRRJ, Brazil.)

502.23

INHIBITORY EFFECTS OF STRYCHNINE ON NICOTINIC ACETYLCHOLINE RESPONSES OF CULTURED RAT HIPPOCAMPAL NEURONS.
H. Matsubayashi*, M. Alkondon and E.X. Albuquerque. Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Strychnine is generally known to act as a specific antagonist of glycine at the glycine-gated channel with an IC_{50} value of 0.08 μM (*Br. J. Pharmacol.* **113:**165, 1994). However, our study has revealed that this drug is a potent inhibitor of neuronal nicotinic receptors (nAChRs). At least three distinct nicotinic current types, IA, II and III, have been recorded from hippocampal neurons (*J. Pharmacol. Exp. Ther.* **265:**1455, 1993), and these currents are subserved by nAChRs with different subunit compositions. In the present study, whole-cell currents of type IA and II were recorded from cultured hippocampal neurons according to the standard patch-clamp technique. The external perfusing solution contained atropine (1 μM) and tetrodotoxin (200 nM). ATP-regenerating solution was used in the patch pipette to prevent the "rundown" of type IA current. Strychnine inhibited both type IA and type II currents evoked by pulses (0.5-2 s) of ACh (0.3 mM) in a concentration-dependent manner. The IC_{50}s for strychnine in inhibiting type IA and II currents were 1.2 and 37.6 μM, respectively. Strychnine decreased the peak amplitude of type IA current in a voltage-independent manner without shortening the decay phase of these currents. The concentration-response curve for ACh (0.05-10 mM) in evoking type IA currents was shifted to the right in the presence of strychnine. These findings suggest that this toxin acts as a competitive antagonist of ACh at the α7-bearing nAChRs that subserve type IA currents. On the other hand, inhibition by strychnine of type II currents was strongly voltage dependent, and the decay phase of these currents was shortened by this toxin. These results indicate that strychnine inhibits type II nicotinic current by acting as an open-channel blocker of α4β2 nAChRs. In conclusion, strychnine is a potent antagonist of neuronal nAChRs, in addition to being a potent antagonist of glylcine at glycine-gated channels. *Support:* USPHS grant NS25296.

502.24

METHAMIDOPHOS, BUT NOT ALDICARB, IS A PURE CHOLINESTERASE INHIBITOR.
N.G. Castro*, M.D. Santos, A.L. Camara, L.E.F. Almeida, M.F.M. Braga, W.M. Cintra, Y. Aracava and E.X. Albuquerque. Lab. Mol. Pharmacol II, IBCCF, UFRJ, Rio de Janeiro, RJ 21944, Brazil & Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, USA.

Aldicarb (Ald) and methamidophos (Mphos) belong, respectively, to the carbamate and organophosphate groups of anticholinesterase (anti-ChE) agents. Here, we analyzed the anti-ChE activity of Mphos in various preparations, and the ability of both Mphos and Ald to interact with muscle nicotinic receptors (nAChRs). In homogenates of sartorius muscles of the frog *Leptodactylus ocellata*, and of diaphragm muscle, brain, and hippocampus of rats, Mphos inhibited the ChE activity with IC_{50}s of 300, 43, 50, and 30 μM, respectively. Mphos (100-300 μM) increased the amplitude and prolonged the decay-time constants of nerve-evoked and of spontaneous end-plate potentials recorded from rat diaphragm, and had no effect on end-plate potentials recorded from frog sartorius muscle. Single-channel currents evoked by ACh (0.2μM) in the presence or in the absence of one of the insecticides were recorded from isolated fibers of interosseal and lumbricoid muscles of the frog *Leptodactyllus ocellata*, using the cell-attached mode of the patch-clamp technique. Patch micropipettes were filled with ACh (0.2 μM) either alone or as an admixture with either insecticide. Ald (1-100 μM) produced a concentration-dependent increase in the number of brief closures and reduction of the duration of the openings (τ_{open}) during burst. The burst duration (τ_{burst}) was not significantly altered. At 300 μM Ald, although τ_{open} of ACh-activated channels was further reduced, both the number of openings per burst and the τ_{burst} were reduced. In addition, the mean duration of the Ald-induced channel closures increased with Ald concentration. These alterations indicated a departure from the simple sequential blockade of the channel open state by Ald. In contrast to Ald, Mphos (100 and 300 μM) had no effects on ACh-activated currents recorded from rat myoballs in culture or frog muscle fibers. Our results demonstrate that the anti-ChE Ald can interact directly with muscle nAChRs, whereas Mphos interacts only with ChE, inhibiting more potently the enzyme present in rat preparations than that present in frog preparations.

(CAPES fellowship, CNPq & FINEP grants, Mol. Pharmacol. Train. Prog.-UFRJ/UMAB).

503.1

DIVALENT CATIONS ENHANCE THE RESPONSE OF BRAIN NICOTINIC RECEPTORS TO NICOTINE. T.K. Booker*, A.C. Collins. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

Recent studies suggest that calcium (Ca) enhances the function of brain nicotinic receptors (nAChRs) expressed in chromaffin cells and Xenopus oocytes. None of these studies assessed potential effects on $\alpha_4\beta_2$ containing nAChRs. Recently, we have developed an ion (Rb^+) flux assay that monitors the function of brain (especially thalamus) nAChRs. The effects of altering Ca ion concentration on nicotine-stimulated Rb^+ ion flux from thalamus was assessed. Ca ion elicited a concentration-dependent increase in nicotine-evoked Rb^+ ion flux, with an EC_{50} of 0.42mM Ca. Maximal effects were seen at ~1mM Ca. The dose-response curves are steep, with a Hill coefficient of 1.64 ± .45, suggesting a cooperative interaction. Magnesium (Mg) and Barium (Ba) ion also enhanced agonist-stimulated Rb^+ efflux, with maximal effects similar to that of calcium. Coadministration of Ca and Mg resulted in additive enhancement of Rb^+ ion flux, suggesting these two ions produce similar effects via interactions at different sites on the receptor. None of the cations altered the potency of nicotine; only the maximal effect was changed. These effects could arise as a consequence of divalent cation-induced changes in ratio of ground-state to desensitized nAChR. Supported by DA-03194, DA-00197, DA-05681-02.

503.2

THE NICOTINIC RECEPTOR/IONIC CHANNEL COMPLEX (AChR) IN ANDROGEN-DEPENDENT SKELETAL MUSCLE CULTURES. R.L.M. Rivero,* M. Bielavsky and M.T.R.Lima-Landman. Dept. of Pharmacology, EPM/UNIFESP, São Paulo, Brazil.

The kinetics of the AChR complex is slower in myoballs from androgen dependent skeletal muscles (Perineal - P) primary cultures than in non target (Thigh - T) ones (Lima-Landman et al.; III Int. Symp. Cholinergic Synapse, P3.7, 1994). This finding could be related to the delayed differentiation of the perineal musculature (Cihak et al., J. Anat.; 106: 96, 1970). The present experiments analyzed the influence of cell differentiation and culture age on the AChR complex of these two muscles. Cells from 4 or 6 days old male rats were cultured for 7 and 15 days and used for "patch-clamp" studies on "cell-attached" configuration, 22-25° C. The single channel recordings were done in HEPES solution, pH 7.4 with TTX (300 nM) using ACh (400 nM) as agonist. The myoballs presented more than one channel conductance in P as in T cultures. The prevalent one was 34 ± 3 pS in P (n=9) and 35 ± 4 pS in T (n=6) from 4 days old rat cells cultured for 7 days (G 4/7) and 37 ± 2 pS in P (n=5) and 30 ± 3 pS in T (n=4) for cells cultured for 15 days (G 4/15). The channel conductance in the groups 6/7 and 6/15 was not statistically different from G 4/7 and G 4/15. Hyperpolarization of the cell membrane increased the frequency of channel opening and prolonged the mean channel open time. The mean channel open time (τ), at -165 mV, was 1.21 ± 0.19 ms and 7.21 ± 1.29 ms in G 4/7 and 5.51 ms and 6.36 ± 1.14 ms in G 4/15, in T and P respectively. In cells from G 6/7 τ was 5.31 ms and 5.51 ms and in G 6/15, 8.65 ± 2.86 ms and 4.24 ± 1.09 ms in P and T respectively. These results indicate that the slowed differentiation of P muscles could be responsible for the difference in τ. The androgenic influence in the AChR complex, between P and T muscles, will be confirmed by undergoing experiments treating the cultures with an anti-androgenic druchecked growing the cultures in the presence of an anti-androgenic drug. Supported by FAPESP, CNPq. Brazil.

503.3

EFFECTS OF N-(4-ARSENOSOPHENYL)-SUCCINAMIC HYDRAZIDE (ASH) ON NICOTINIC RECEPTORS R.H.Loring*, R. Moaddell, T. McHugh, X.G. Zhang, Y.Y. Wu and G.S. Jones, Jr.# Dept. Pharmaceutical Sciences, Northeastern Univ., and #Div. Pharmaceutical Sci., Mass. College of Pharmacy, Boston, MA 02115.

Adjacent cysteine residues are part of the agonist binding site of every known type of nicotinic α-subunit. We have previously demonstrated (Loring et al., *Mol. Brain Res.* **15**:113, 1992) that trivalent aromatic arsenicals, which recognize paired but not single thiols, will covalently bond to reduced nicotinic receptors and block the binding of competitive ligands, such as α-bungarotoxin (ABT). The nitrogen of our model compound, p-aminophenylarsonous acid (APA) is not reactive with most amine labeling reagents. Thus, we synthesized the novel compound, ASH, which has a reactive nitrogen. ASH has no effect on native receptors, but potently blocks ^{125}I-ABT binding (IC_{50} ~10 nM) to immunoprecipitated *Torpedo* α_1 receptors reduced with dithiothreitol (2 mM). Like APA, ASH protects against irreversible alkylation of *Torpedo* receptors by the affinity agent, bromoacetylcholine, and the antiarsenical, dimercaptopropanesulfonate (DMPS, 1 mM) restores ^{125}I-ABT binding to ASH-treated receptors. ASH also blocks ^{125}I-ABT binding to reduced chick α_7 and α_8 receptors (IC_{50}'s ~3 μM and 30 nM, respectively). ASH (10 μM) blocks the function of reduced nicotinic receptors in the chick retina, until reversed with DMPS. The amine labeling agent, N-hydroxysuccinimidyl-biotin reacts with ASH to make the biotinylated derivative, biotinyl-(N-(4-arsenosophenyl)-succinamic hydrazide) (Bio-ASH). Bio-ASH is slightly more potent than ASH, and blocks ^{125}I-ABT binding (IC_{50} ~5 nM) to reduced *Torpedo* α_1 receptors. Bio-ASH also prevents reactivation of functional nicotinic receptors in the chick retina, until reversed by DMPS. We are presently synthesizing and testing an iodinated derivative of ASH (^{125}I-Bolton-Hunter-labeled ASH), and other biotinylated ASH derivatives with longer spacer arms. Supported by NS22472 and the Smokeless Tobacco Research Council.

503.4

RJR-1647: A NOVEL NICOTINIC AGONIST WITH CNS SELECTIVITY. P.M. Lippiello*, S. Arrington, K. W. Fowler, M. E. Lovette, L. Reeves, W.S. Caldwell and M. Bencherif. Research and Development, R.J. Reynolds Tobacco Company, Winston-Salem, NC 27102.

There is a growing body of evidence from *in vitro* and *in vivo* studies indicating that nicotinic cholinergic ligands with selectivity for CNS nicotinic acetylcholine receptors (nAChR) improve cognitive and attention disorders. The recent characterization of nicotinic agonists with the ability to discriminate between the various nAChR subtypes which are targets of the endogenous neurotransmitter acetylcholine has provided the rationale for the design of compounds with an enhanced "therapeutic window". RJR-1647 exhibits a pharmacological profile suggestive of a strong selectivity for neuronal nicotinic receptors. In several aspects this analog shows a greater selectivity for neuronal nAChR than RJR-2403. The binding affinity to putative α4β2 receptors is enhanced (Ki = 9 nM vs. 26 ± 3 for RJR-2403). Its efficacy in inducing ion flux from thalamic synaptosomes is comparable to that of RJR-2403, but the potency is 2 and 3-fold greater than those for nicotine and RJR-2403, respectively (EC_{50} = 269 ± 55 nM vs. 732 ± 155 nM for RJR-2403 and 591 ± 120 nM for nicotine). There are also differences in eliciting dopamine release from striatal synaptosomes. RJR-1647 was the most efficacious of the compounds tested (Emax = 124 ± 18% compared to nicotine) with a potency 5 times greater than that for RJR-2403 (EC_{50} = 269 ± 55 nM for RJR-1647 vs. 732 ± 155 nM for RJR-2403). Similar to RJR-2403, and unlike nicotine which potently activates human muscle receptors in TE671/RD cells and autonomic ganglionic nAchR's in PC12 cells, no detectable activity was observed at these receptor subtypes with RJR-1647. Another distinguishing feature of RJR-1647 is that apparent *down*-regulation of α4β2 neuronal nAChR was observed following chronic treatment of M10 cells. The selectivity profile of RJR-1647 may provide important clues for the design of receptor selective ligands.

503.5

EXPRESSION OF THE β3 NICOTINIC RECEPTOR SUBUNIT AND OLFACTORY MARKER PROTEIN mRNAs IN PC12 CELLS. C.J.H. Keiger*, M. Bencherif, P.M. Lippiello, G.M. Hellmann and J.C. Walker. UNC Craniofacial Center, School of Dentistry, Chapel Hill, NC 27599 and R. J. Reynolds, Research & Development, Winston-Salem, NC 27102.

Recent studies have described the activation of the β3 nAChR subunit promoter as a marker of ganglion cell induction and differentiation during retinal development (Matter, *et al.*, J Neuroscience, 15, 1995). Olfactory marker protein (OMP), expressed in high levels in mature olfactory receptor neurons, is also expressed in some neuroblastoma cell lines (Servenius *et al.*, Cancer Res, 54, 1994). RT-PCR was employed to determine whether the β3 nAChR subunit and OMP genes are also expressed in PC12 cells (a model for the study of neuronal differentiation). Cells were tested after treatment with 100 ng NGF for either 2 hours or 7 days. Ion flux studies were also used to determine the function of nAChRs in these cells. Multiple nAChR subunit mRNAs (β2, β4, α3, α5, α7) were detected in both control and treated cells. β3 subunit mRNA levels were low in control cells and in those treated for only 2 hours, but increased to moderate levels following chronic NGF treatment. Low levels of OMP mRNA were observed in all three groups. As assessed by ion flux studies, functional responses indicated no changes in E_{max} and EC_{50} in response to 10 μM nicotine.

These findings may suggest a possible role of the β3 nAChR subunit and OMP genes in cell proliferation and/or differentiation of sympathetic neurons.

Supported by the RJR-UNC Collaborative Olfactory Research Program.

503.6

RJR-2429: A NICOTINIC AGONIST WITH SELECTIVITY FOR MUSCLE NICOTINIC CHOLINERGIC RECEPTORS. M. Bencherif*, P.A. Crooks[1], S. Arrington, K. W. Fowler, M.E. Lovette, L. Reeves, W.S. Caldwell, and P.M. Lippiello. Research and Development, R.J. Reynolds Tobacco Company, Winston-Salem, NC 27102, [1] University of Kentucky, Lexington, KY.

The search for nAChR subtype-selective agonists as pharmacological and/or therapeutic tools represents an important milestone to unravel the role of nicotinic acetylcholine (nAChR) receptors in health and disease. We have previously reported the development of a nicotinic agonist having selectivity for neuronal nAChR's (RJR-2403; Bencherif et al., 1995). The present report describes *in vitro* studies demonstrating that a novel heterocyclic substituted pyridine compound (RJR-2429) is a full agonist and is very potent in activating human muscle nAChR's (EC_{50}= 55 nM; by comparison, epibatidine and nicotine have EC_{50}'s of 90 nM and 80,000 nM respectively). In contrast to epibatidine, RJR-2429 exhibits a 100 to 1000-fold selectivity for activation of human muscle-type nAChR's compared to CNS subtypes. RJR-2429 binds with high affinity to the α4β2 receptor subtype (K_i = 1.5 nM). However, this binding does not result in any detectable activation, but rather in antagonism of nicotine-stimulated ion flux in thalamic synaptosomes (IC_{50} ca. 80 nM), a preparation containing putative α4β2 receptors. It is also a weak agonist at nAchR's mediating dopamine release from rat synaptosomal preparations (EC_{50} = 30,300 nM; E_{max} = 29%; epibatidine and nicotine are equipotent at this receptor subtype with EC_{50} ca. 100 nM and E_{max} = 100%). Activation of a putative α3β4-containing nAChR in the rat pheochromocytoma clonal cell line (PC12) reveals a potency intermediate between nicotine and epibatidine (EC50's of 30,000 nM for nicotine, 1,000 nM for RJR-2429 and 30 nM for epibatidine). The profile of RJR-2429 may provide important clues for the development of nAChR subtype-selective ligands as probes in the life sciences or as potential therapeutics.

503.7

NICOTINE EXCITATION OF DOPAMINE NEURONS IN THE VENTRAL TEGMENTAL AREA *IN VITRO* - INTERACTION WITH NMDA-RECEPTORS.
P. Grillner, L. Arborelius* and T.H. Svensson. Dept of Physiology and Pharmacology, Div. Pharmacology, Karolinska institute, 171 77 Stockholm, Sweden.

The mesolimbocortical dopamine (DA) system originating from the ventral tegmental area (VTA) plays an important role as part of the reward systems in the brain. Several drugs of abuse, including nicotine, stimulate this system. This study aims to: 1) test if the excitatory effect of nicotine on DA neurons in the VTA involves NMDA-receptor activation, and 2) to investigate a putative synaptic input to DA neurons mediated by postsynaptic nicotinic receptors. Intracellular recordings were made from DA neurons in VTA in the *in vitro* rat brain slice preparation of the ventral mesencephalon. To study the putative synaptic, cholinergic input, a bipolar stimulation electrode was placed close to VTA, on its caudal side, where cholinergic pathways projecting from the pedunculopontine nucleus to VTA are located. Application of nicotine receptor antagonists, mecamylamine (10 μM) and dihydro-beta-erythroidine (DHBE) (50 μM), did not significantly alter the firing rate of DA neurons, but blocks completely the excitatory effect of nicotine (10 μM) on DA neurons. Application of 2-amino-5-phosphonopentanoic acid (AP-5) (90 μM) slightly decreased the spontaneous firing activity. The increase in firing frequency induced by nicotine appeared lower in the presence of AP-5 (138.5 %), than in the absence of AP-5 (248 %), suggesting that the excitatory effect of nicotine on DA neurons may partly depend on NMDA-receptor activation. Excitatory postsynaptic potentials evoked by afferent single pulse stimulation were not affected by DHBE at a concentration sufficient to block the excitatory effect of bath application of nicotine. In conclusion, 1) the excitatory effect of nicotine on VTA DA neurons is mediated via nicotinic receptor activation, and 2) the increase in excitability of DA neurons induced by nicotine appears to require concomitant NMDA-receptor activation.

This study has been supported by The Swedish Medical Research Council (grant no 4747), the Karolinska institute, and The Council for Tobacco Research, USA, Inc..

503.8

SYSTEMIC NICOTINE INDUCED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS IS DEPENDENT ON NMDA RECEPTOR ACTIVATION IN THE VENTRAL TEGMENTAL AREA
B. Schilström, G. G. Nomikos*, P. Hertel, M. Nisell & T. H. Svensson Dep. of Physiology and Pharmacology, Div. of Pharmacology Karolinska Institutet, 171 77 Stockholm

Systemic administration of nicotine (NIC) enhances both firing rate and burst firing of dopamine (DA) neurons in the ventral tegmental area (VTA) as well as DA release in the nucleus accumbens (NAC). Nicotinic receptors in the VTA seem to be of primary importance for these actions of NIC, but recent evidence also suggest that NIC facilitates glutamatergic transmission in brain. Since both burst firing in VTA-DA neurons and DA release are regulated *inter alia* by excitatory amino acid (EAA) inputs to the VTA via NMDA receptors, the present study was undertaken to investigate the putative role of ionotropic glutamate receptors within the VTA for the DA release in the NAC, elicited by systemic NIC administration or local infusion of NIC in the VTA. For this reason the dual microdialysis approach was used, in which one probe was implanted in the VTA and another in the ipsilateral NAC. Both a subcutaneous NIC injection and local infusion of NIC into the VTA (1.0 mM) increased DA output in the NAC significantly. Also infusion of 0.3 mM of NMDA or 0.1 mM AMPA into the VTA increased accumbal DA release. This increased DA outflow was effectively attenuated by the concomitant infusion of the selective antagonists at NMDA receptors, AP-5 (0.3 mM) and non-NMDA receptors, CNQX (0.3 mM), respectively. Infusion of 0.3 or 1.0 mM of AP-5 or CNQX into the VTA did not by itself affect DA levels in the NAC. Simultaneous infusion of CNQX did not affect the DA release induced by systemic NIC. In contrast, infusion of AP-5, starting 40 min before the NIC injection, significantly attenuated the increase in accumbal DA release. Concomitant infusion of AP-5 and NIC completely prevented the increase in DA release in the NAC seen after local infusion of NIC. These results indicate that the stimulatory action of NIC on the mesolimbic DA-system is to a considerable extent dependent upon the concomitant stimulation of NMDA receptors within the VTA.

This study was supported by The Swedish Medical Council (grant no 4747), the Karolinska Institute and The Council for Tobacco Research, USA, Inc..

503.9

DEVELOPMENT AND EXPRESSION OF LIGAND-GATED ION CHANNELS IN NEURONS ACUTELY DISSOCIATED NON-ENZYMATICALLY FROM RAT HIPPOCAMPUS.
C.T.F. Barbosa[1,2], M. Alkondon[1], Y. Aracava[2], K.L. Swanson[1*] and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201, USA; [2]Lab. Mol. Pharmacol., IBCCF, UFRJ, RJ 21944, Brazil.

Ligand-gated ion channels are essential elements of neuronal function and have been studied *in vitro* using cultured neurons, brain slice preparations, and enzymatically dissociated neurons. To avoid possible deleterious effects of enzymes on channel proteins, we developed a non-enzymatic technique for isolating neurons from the CA1 area of the hippocampus of postnatal rats (2 to 26 days-old), and studied the presence of several ligand-gated ion channels by the whole-cell patch-clamp technique. Hippocampal slices of ≈500-μm thick were incubated for 3 h in Ca^{2+}-free solution bubbled continuously with a mixture of 95% O_2 and 5% CO_2. Fragments of the slices were sucked into a glass micropipette with a tip diameter of 300-400 μm and transferred to the recording chamber; the passage of the tissue through the micropipette was sufficient to disperse the neurons meanwhile conserving the long processes. Agonists were applied via a U-tube to the neurons in the whole-cell patch-clamp configuration. Application to the neurons of an admixture of NMDA (50 μM) and glycine (10 μM) elicited whole-cell currents, which increased in peak amplitude with neuronal maturation. Currents induced by quisqualic acid (20 μM) had the same peak amplitude regardless of age. Glycine (100 μM) and kainic acid (20 μM) also evoked whole-cell currents. Whereas the four agonists above induced currents in all the neurons, GABA (20 μM) only evoked currents in 80% of the neurons. ACh (1 mM) only evoked currents in 60% of the neurons, and these currents were blocked by methyllycaconitine (1 nM) and Pb^{2+} (1-10 μM), indicating the presence of α-bungarotoxin -sensitive nicotinic receptors (nAChRs) in hippocampal neurons developed *in vivo*. In addition, the amplitude of ACh-evoked currents depended on the length of the dendrites on the neurons such that it was larger in neurons with long dendrites, confirming our earlier results that many nAChRs are located on the dendrites. Thus, the new method of isolating neurons enables studies of ligand-gated channels, particularly those present on dendrites of hippocampal neurons of postnatal rats. (USPHS Grants NS25296, ES05730; CNPq, Brazil).

503.10

DIFFERENTIAL DISTRIBUTION OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBTYPES IN RAT HIPPOCAMPAL NEURONS.
E.X. Albuquerque[1,2], E.F.R. Pereira[1,2], C.R. Creveling[3*] and M. Alkondon[1]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, USA; [2]Lab. Mol. Pharmacol., IBCCF, UFRJ, RJ 21944, Brazil; [3]Technology Devel., NIDDK, NIH, Bethesda, MD 20982, USA.

Hippocampal neurons of the rat express at least 3 different subtypes of functional nAChRs, activation of which results in nicotinic currents with distinct kinetics and pharmacology (*J. Pharmacol. Exp. Ther.* **265**: 1455, 1993; **271**: 494, 1994). Because the role of each nAChR may depend on its specific location on the neurons, we investigated the distribution of two identified subtypes of nAChRs on rat hippocampal neurons in culture. For this purpose, we used an infrared video microscope to view the neuronal processes, and a nanorobot system of micromanipulators to position the recording and the agonist-delivering micropipettes at the desired locations. Whole-cell patch recording was performed at the neuronal soma, and the agonist ACh (in the presence of 1 μM atropine) was applied by pressure to identified regions of the neuron via a micropipette. Nicotinic currents sensitive to blockade by methyllycaconitine (1 nM) were elicited by application of ACh to the cell body, apical and basal dendrites of pyramidal-shaped neurons and to the cell body and both branches of bipolar-shaped neurons. The peak amplitude of the currents elicited by agonist application to the cell body was larger than that of the currents evoked by agonist application to the dendrites of pyramidal-shaped neurons. In contrast, the peak current amplitude was larger when ACh was applied to the dendrites than when it was applied to the soma of bipolar-shaped neurons. Nicotinic currents sensitive to blockade by dihydro-β-erythroidine (100 nM) were elicited by application of ACh to both cell body and dendrites, however, the amplitude of such responses elicited at the dendrite was larger than that of the responses evoked at the cell soma. The present results indicate that nAChRs are distributed differentially on the neuronal axis, and suggest that they may play a role pertaining to the dendritic compartment of the hippocampal neurons. (USPHS Grants NS25296 and ES05730).

503.11

CHARACTERIZATION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN CA1 NEURONS OF RAT HIPPOCAMPAL SLICES.
M. Alkondon[1*], E.F.R. Pereira[1,2] and E.X. Albuquerque[1,2] [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201, USA; [2]Lab. Mol. Pharmacol., IBCCF, UFRJ, RJ 21944, Brazil.

Hippocampal neurons obtained from fetal rats and cultured for 10 to 30 days express at least 3 different subtypes of functional nicotinic acetylcholine receptors (nAChRs), activation of which results in nicotinic currents with distinct kinetics and pharmacology (*J. Pharmacol. Exp. Ther.* **265**: 1455, 1993; **271**: 494, 1994). In the present study, we investigated the presence of functional nAChRs in the developing hippocampus of 10- to 17-day-old rats. Hippocampal slices ≈200-μm thick were prepared, and neurons were viewed with the assistance of infrared video microscopy. Matrix covering the neuronal soma and the dendrites were removed by applying a gentle stream of bathing solution via a micropipette. Whole-cell recordings were made from the cell soma, and different agonists were applied using a U-tube positioned on top of the cell. Application of ACh (1 mM), in the presence of 1 μM atropine, induced a whole-cell current that was accompanied by several miniature postsynaptic currents (MPSCs). Application of other agonists such as nicotine, DMPP, (+)anatoxin-a or epibatidine also evoked similar responses. Superfusion of the neurons with d-tubocurarine (10 μM) abolished the agonist responses completely and reversibly. In addition, tetrodotoxin (300 nM) also diminished to a great extent the agonist-elicited responses. The MPSCs evoked by nicotinic agonists appear to be mediated via glutamate and $GABA_A$ receptors. The present results indicate that functional nAChRs are expressed in the developing hippocampus and suggest post- and pre-synaptic roles for the nAChRs. (USPHS Grants NS25296 and ES05730).

503.12

NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS: ANALYSIS OF RECTIFYING PROPERTIES.
R. Bonfante-Cabarcas and E.X. Albuquerque*. Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, USA; Lab. Mol. Pharmcol., IBCCF, UFRJ, Rio de Janeiro RJ 21944, Brazil.

Intracellular factors that modulate inward rectification of type IA nicotinic currents in cultured hippocampal neurons (Bonfante-Cabarcas *et al.*, *JPET* **277**:432, 1996) were investigated using the whole-cell mode of the patch-clamp technique. Internal solutions with malate plus EGTA or EDTA and desired $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ were prepared using the program Calc ver. 2.2. Typically in ≈5 neurons under each condition, type IA currents were elicited by application of ACh (2 mM), and peak current amplitudes at positive holding potentials were normalized to amplitudes at -50 mV, giving SE's ≈10%. Using EGTA and 10 μM $[Ca^{2+}]_i$ without Mg^{2+}, the current-voltage relationship showed inward rectification, with normalized values of 0.32, 0.66 and 0.80 at +50, +75 and +100 mV, respectively. Using EDTA and 10 μM $[Ca^{2+}]_i$ without Mg^{2+}, the inward rectification was eliminated, normalized values being 0.95, 1.97 and 2.88 at +50, +75 and +100 mV, respectively, indicating that natural Mg^{2+} could be removed. Under this condition, the chord conductance increased when the voltage was increased from +40 to +100 mV. Using EDTA with neither Ca^{2+} nor Mg^{2+}, the peak amplitudes were significantly lower ($p<0.05$) at +50, +75 and +100 mV with values being 0.76, 1.16, and 1.44, and the chord conductance was constant from +40 to +100 mV, suggesting that internal free Ca^{2+} potentiates type IA currents at potentials above +40 mV in a voltage-dependent manner. In order to study the effect of $[Mg^{2+}]_i$, internal solutions were prepared using EDTA with 10 μM $[Ca^{2+}]_i$ and replacing Cs^+ with $TRIS^+$ and/or Mg^{2+} to maintain the osmolarity and concentration of permeant cations. Surprisingly, $TRIS^+$ (30 mM) in the absence of internal Mg^{2+} induced inward rectification, the normalized values being 0.5 and 1.47 at +50 and +100 mV, respectively. Increasing $[Mg^{2+}]_i$ (0, 0.1, 1 and 6 mM) decreased normalized peak amplitude at +50 mV with an IC_{50} = 1 mM and n_H of 0.74. Similar values were obtained at +100 mV. We suggest that Mg^{2+} or $TRIS^+$ induce inward rectification by acting by an intracellular mechanism affecting the nAChR. *Support: USPHS Grant NS25296; CONICIT-UCLA (Venezuela)*

503.13

SECOND AGONIST SITE ON THE NICOTINIC RECEPTOR (nAChR): EFFECTS OF PROTEOLYTIC ENZYMES AND NONCOMPETITIVE ANTAGONISTS. B.S. Nascimento[1,2], C.M.T. Silva[1,2], A. Maelicke[3], Y. Aracava[1,2], and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. MD Sch. Med., Baltimore, MD 21201; [2]Lab. Mol. Pharmacol., IBCCF, UFRJ, RJ 21944, Brazil; [3]Inst. Physiol. Chem. Pathobiochem., Johannes-Gutenberg Univ. Med. Sch., 55099 Mainz, Germany.

Pyrazole and 4-methylpyrazole (4-MP) act as allosteric agonists at nAChRs by binding to the so-called physostigmine site on the receptors (*J. Pharmacol. Exp. Ther.* **261**:331, 1992; *Abs. Neurosci. Soc.* **20**:1137, 1994). We have shown that in enzymatically isolated muscle fibers from the frog *L. occelata* (method described in *J.Pharmacol. Exp. Ther.* **252**:507, 1990), a high frequency of ACh (0.4 μM)-evoked single-channel currents (> 10-15 events/min) is always detected. In contrast, only in 20% of the isolated muscle fibers can 4-MP (10 μM) activate single-channel currents, and the frequency of 4-MP-induced channel activity is very low (<1 event/min). Here, we describe the optimization of the methodology to isolate muscle fibers, and we demonstrate that the region including the physostigmine-binding site can be damaged by proteolytic enzymes. During the dissociation of the muscle fibers, the concentration of enzymes and the time of enzymatic treatment were decreased by 60%, and the external Ca^{2+} concentration was reduced from 1.8 to 0.45 mM. About 1 hour before recording single-channel currents from cell-attached patches, the fibers were transferred to a solution containing 1.8 mM Ca^{2+}. Under these conditions, in the presence of α-BGT, application of 4-MP to all fibers via the recording pitpette resulted in activation of single-channel currents that were sensitive to blockade by the nAChR-specific monoclonal antibody FK1 (a ligand specific for the physostigmine site on nAChRs). The frequency of openings induced by 4-MP was extremely high (> 50 events/min) in fibers treated with low enzyme concentration; in some patches, even double and triple simultaneous openings could be induced by 4-MP. ACh-induced channel activity was also higher (4 to 5 fold, ~ 400-600 events/min) in fibers exposed to low concentrations of proteolytic enzymes. Atropine (10-50 μM, an open-channel blocker of the nAChRs) shortened the open times of ACh- and 4-MP-activated single channels, and nortriptyline, a desensitizing agent, at 10 nM eliminated most of the ACh-evoked channel activity, whereas even at 100 nM, it did not affect the 4-MP-evoked channel activity. Also, even after the nAChRs were desensitized by a high concentration of ACh (10 μM), 4-MP could activate single-channel currents. Although the kinetics of channels activated by ACh were different from those of channels activated by 4-MP, the conductance of the channels activated by either agonist was the same. Thus, our results demonstrate for the first time that the nAChR site through which 4-MP activates the nAChR channels is very sensitive to damages by proteolytic enzymes, and support the concepts that (i) the action of open-channel blockers is conserved regardless of the pathway through which the nAChR channels are activated, and (ii) desensitization is not a property of the nAChR channel, but is associated with the agonist site. *(Supp: CAPES, CNPq, Mol. Pharmacol. Train. Prog.)*

503.14

MODULATION OF NICOTINIC RECEPTOR FUNCTION BY COMPOUNDS THAT ACT THROUGH THE PHYSOSTIGMINE-BINDING SITE. E.F.R. Pereira[*1,2], A. Schrattenholz[3], A. Maelicke[3] and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, USA; [2]Lab. Mol. Pharmacol., IBCCF, UFRJ, Rio de Janeiro, RJ 21944, Brazil; [3]Inst. Physiol. Chem. Pathobiochem., Johannes-Gutenberg Univ. Med. Sch., 55099 Mainz, Germany.

Compounds such as physostigmine, galanthamine, codeine, and benzoquinonium, by binding to a novel site on nAChRs, can activate nicotinic single-channel currents in various preparations. This agonist action, while insensitive to blockade by competitive nicotinic antagonists, can be inhibited by the nAChR-specific monoclonal antibody FK1. In fact, this pharmacological profile identifies a new class of nicotinic agonists (reviewed in *Drug Dev. Today* **1**:53, 1996). The efficacy of these agonists is low, so they are unable to evoke sizable whole-cell currents. Recently, however, evidence has been provided that, by acting through this novel nAChR site (the physostigmine site), galanthamine and serotonin can potentiate nicotinic responses evoked by ACh (*Mol. Pharmacol.* **49**:1, 1996). Here, applying the whole-cell mode of the patch-clamp technique to rat myoballs in culture, we investigated the ability of decamethonium and d-tubocurarine (d-TC) to interact with the physostigmine-binding site on muscle nAChRs. Unlike the nicotinic agonist action of physostigmine and related compounds, that of decamethonium (0.1-10 μM) could be completely inhibited by α-bungarotoxin (5 μg/ml). In agreement with a previous report (*J. Neurosci.* **15**:230, 1995), we found that at low concentrations (10-40 nM) d-TC potentiates ACh (0.3 μM)-evoked whole-cell currents in rat myoballs, whereas at high concentrations (≥400 nM) it inhibits the nicotinic currents elicited by application of ACh (0.3 μM) to the cells. In our experiments, d-TC (40 nM) increased by 57.7 ± 17.8% (n = 4; mean ± S.E.M.), whereas at 1 μM it decreased by about 30% the peak amplitude of ACh (0.3 μM)-elicited currents. Also, at higher concentrations of ACh (≥ 1 μM), the potentiating effect of low concentrations of d-TC could not be observed. Initial studies show that following a 30-min perfusion of the cells with FK1-containing external solution, the potentiating effect of d-TC on the ACh-evoked currents could not be detected. Thus, it is possible that the potentiating effect of d-TC on nicotinic currents in rat myoballs is mediated via the physostigmine-binding site. Alternatively, FK1, by binding to the physostigmine site on the nAChRs, could modify allosterically the interaction of d-TC with a different nAChR site. *(USPHS Grant NS25296)*

503.15

PHARMACOLOGICAL AND BIOPHYSICAL ANALYSIS OF NICOTINIC CURRENTS IN RAT CULTURED THALAMIC NEURONS. Y. Aracava[1,2], W.S. Cortes[1,2,3], C.T.F. Barbosa[1,2], J.N. Hokoç[2*] and E.X. Albuquerque[1,2*]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201,USA; [2]Lab. Mol. Pharmacol., IBCCF, Fed. Univ. Rio de Janeiro, RJ 21944; [3]Rural Fed. Univ. Rio de Janeiro, RJ 23658, Brazil.

Studies using techniques based on radioligand binding, synaptosomal Rb^+ efflux and intracellular recordings have shown that functional nicotinic acetylcholine receptors (nAChRs) are expressed in thalamic neurons (*J. Neurosci.* **4**:2906,1984; *J. Neural Transm.* **73**:77, 1988; *J. Pharmacol. Exp. Ther.* **264**:542, 1993; *Prog. Neurobiol.* **39**:337, 1992). In this study, we characterized some functional properties of nAChRs in cultured thalamic neurons using the whole-cell configuration of the patch-clamp technique. The agonists ACh (0.001-3 mM) and (-)nicotine (0.001-0.3 μM) were applied to the neurons via a U-tube in a 0.5-2-sec pulse and the results showed that either ACh (n=8) or (-)nicotine (n=3) could elicit nicotinic currents in these neurons. All neurons tested responded to either ACh (1 or 3 mM) or (-)nicotine (100-300 μM) with currents that exhibited fast rise and decay phases. A rapid "rundown" of these currents was present and it was considerably prevented by using an ATP-regenerating internal solution. Prior exposure of these neurons to methyllycaconitine (1 nM) produced a complete and reversible blockade of these currents. In addition, the I-V relationship revealed a mild inward rectification of these nicotinic currents (n=4). In a few neurons (n=4), an increase in the frequency of miniatuare postsynaptic currents was noticed during 2-sec pulse applications of 1 mM ACh to the neurons. Thus, these results show that functional nAChRs are present in the thalamic neurons and that their pharmacological and biophysical features resemble those of α-bungarotoxin-sensitive nAChRs (α7 or α8 containing) already characterized in other brain regions. (*Support:* USPHS Grants NS25296 and ES05730; CNPq and UFRRJ, Brazil).

503.16

DEVELOPMENTAL PATTERN OF α7 NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT IN THE EMBRYONIC CHICK CEREBELLUM. W. M. Kaneko*, L. R. G. Britto, C-C. Wu, J. M. Lindstrom and H. J. Karten. Dept. of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

Previous studies have demonstrated the spatially restricted distribution of neuronal nicotinic acetylcholine receptor (nAChR) subunits in the mammalian and avian adult brain. Little, however, is known about the development of these receptor subunits. The embryonic chick provides an excellent model to study developing subunits because the developmental stages are well defined and much of the biochemistry of nAChR subunits have been conducted in the chick. Furthermore, the cerebellum, due to its well-characterized organization, provides an appropriate substrate for detailed study. The present study demonstrated changes in the pattern of expression of the neuronal nAChR subunit α7, an α-bungarotoxin sensitive subunit, using immunohistochemical techniques in the developing chick cerebellum. We found that Purkinje cell bodies were immunoreactive for α7 antibodies at embryonic day 12 (E12). This immunoreactivity increased along the dendrites until E16. On E16, the immunoreactivity diminished from the more distal portions of the dendrites and α7-immunoreactivity was expressed mainly on the cell body in a manner similar to that seen at E12. This particular developmental pattern of α7 expression is similar to the level of α-bungarotoxin (αBgt) binding sites seen during the development of the brain. The transient expression of the nAChR α7 subunit provides evidence for the involvement of this subunit in the development of αBgt-sensitive nAChRs and suggests that E12 to E16 may be a critical period for the developing neuronal cholinergic system. Supported by NIH EY06890 (HJK).

503.17

FUNCTION OF CENTRAL NEURONAL NICOTINIC RECEPTORS IN CHICK EMBRYOS EXPOSED TO NICOTINE DURING SYNAPTOGENESIS. V.A. Chiappinelli*, K.W. Wolf and J.M. Garcia-Ferrer. Dept. Pharmacol. Physiol. Science, Saint Louis Univ. Sch. Med., St. Louis MO 63104

When rat or chick embryos are exposed to nicotine during development, a significant up-regulation of ^{3}H-nicotine receptors in brain occurs (Brain Res. Bull. 23:187, 1989; Soc. Neurosci. Abstr. 21:345, 1995). Our goal in this study was to examine possible functional consequences of this increase in receptor number. We implanted L-nicotine-impregnated pellets onto the yolk sac of chick embryos at 5 days of incubation (d.i.). These sustained-release pellets provided the embryo with a continuous exposure to nicotine between 5-13 d.i., a period during which cholinergic synapses form. A dose-dependent up-regulation of ^{3}H-nicotine receptors was measured in the mesencephalon, with B_{max} increasing to 183-240% of control after exposure to 12 mg nicotine/kg/day. To assess the function of central ^{3}H-nicotine receptors in the mesencephalon, intracellular recording was done in brain slices of 14-19 d.i. chick embryos exposed to nicotine-impregnated or control pellets. The maximum depolarizing response and EC_{50} for carbachol were determined in lateral spiriform neurons (SPL) as described (Mol. Pharm. 46:993, 1994). At the later embryonic stages studied, there were no significant differences between nicotine-treated and control embryos in these functional measures. However, at 14 d.i. after exposure to 12 mg nicotine/kg/day, the apparent affinity for carbachol was reduced almost 3-fold, to 118±50 μM. No significant change in the maximum depolarization to carbachol was detected. This affinity change suggests that the structure of nicotinic receptors in the SPL was altered by nicotine. A possible explanation for the affinity change would be an altered subunit composition of the receptors. Since changes were not detected in older embryos examined 4-6 days after release of nicotine from the pellets should have ceased, the effect may be reversible after cessation of nicotine.

Supported by a grant from the Smokeless Tobacco Research Council, Inc.

503.18

NICOTINIC SINGLE-CHANNELS RECORDED FROM NEURONS IN INTACT CILIARY GANGLIA OF CHICK EMBRYO. W.R. Weaver*, J.-Z. Guo, T.L. Tredway and V.A. Chiappinelli. Dept. Pharmacol. and Physiol. Sci., Saint Louis Univ. Sch. of Med., St. Louis MO 63104

There is evidence for multiple nicotinic channel conductances in acutely dissociated neurons of chick embryo ciliary ganglia (Margiotta et al., Dev. Bio. 135:326-339, 1989). To avoid altering the receptor proteins during dissociation, we developed an enzyme treatment method that kept the ganglia intact while still allowing formation of tight seals. Ciliary ganglia isolated from 15-16 day old chick embryos were superfused with a mixture of collagenase (200 U/ml) and thermolysin (10 U/ml) at 32 °C for a 30 min period. This process was monitored under Nomarski optics at 400X magnification to avoid over-treatment. Preparations were then superfused with normal chick hepes tyrode at room temperature. Pipettes with or without acetylcholine (ACh) were used to obtain cell-attached single channel recordings. Lucifer yellow (0.5 mM) was used to verify the recording site. Forty seven of 76 recordings with ACh (3-300 μM) showed inward currents at holding potentials of -40 to -120 mV. Current-voltage analysis showed single channel slope conductances from 27.7 to 54.7 pS at ACh concentrations 3 to 30 μM (30 μM, 37.9 ± 3.2 pS (n=5)). While at 100 to 300 μM ACh, the slope conductance range decreased to 15.8 to 24.8 pS (300 μM, 21.3 ± 1.7 pS (n=4)). The conductances of these two groups (30 and 300 μM) were significantly different ($p<0.005$). All neurons showed similar resting membrane potentials upon switching to whole cell configuration. These results suggest at least two nicotinic channels that show different sensitivities to ACh exist in 15-16 day old chick embryo ciliary ganglia.

Supported by NIH Grants NS17574 and NS33135 to V.A.C.

503.19

DIRECT MEASUREMENT OF FUNCTIONAL NICOTINIC RECEPTORS ON PRESYNAPTIC TERMINALS USING WHOLE-CELL PATCH-CLAMP. J.S. Coggan* and D.K. Berg. Dept. of Biol., UC San Diego, La Jolla, CA 92093.

Nicotinic acetylcholine receptors (AChRs) are widely expressed in the central and peripheral nervous systems but their functions are largely unknown. A popular hypothesis is that the receptors are often presynaptic in location and modulate transmitter release. The large calyces formed on chick ciliary ganglion neurons by preganglionic terminals permit a direct test of the hypothesis in situ.

Whole-cell patch-clamp recordings were made from calyces in embryonic ganglia while perfusing with 20-100 μM nicotine. The agonist induced a small inward current often followed by a train of action potentials (APs) that overrode the voltage clamp (the dimensions of the calyx prevent complete space clamp). Recordings from ganglionic neurons demonstrated that full excitatory postsynaptic currents (epscs) were triggered by the nicotine, presumably in response to presynaptic APs, and that increases in the frequency of spontaneous miniature epscs sometimes occurred as well. Tetrodotoxin (1 μM) blocked the nicotine-induced APs in the calyces but not the inward currents. α-Bungarotoxin at 60 nM completely blocked the presynaptic responses to nicotine. The results demonstrate that AChRs sensitive to α-bungarotoxin can increase the excitability of the presynaptic terminal and enhance transmitter release. **(NIH RO1 NS12601 & NS35469, and NIH F32 NS09589)**

503.20

ASSEMBLY AND FATE OF NICOTINIC RECEPTORS CONTAINING THE α7 GENE PRODUCT IN A STABLY TRANSFECTED CELL LINE. P.D. Kassner and D.K. Berg*. Dept. of Biol., UC San Diego, La Jolla, CA 92093.

One of the most abundant nicotinic acetylcholine receptors (AChRs) expressed in the central and peripheral nervous systems is a species that binds α-bungarotoxin (αBgt), fluxes calcium, and contains the α7 gene product. We have stably transfected the quail fibroblast cell line QT6 with wildtype and epitope-tagged chick α7 gene constructs to examine the assembly and fate of α7-containing AChRs.

The stable transfectants express the α7 gene product and are nearly a quarter as efficient as primary neurons at assembling the protein into a species that binds αBgt. Essentially all of the toxin binding species is found on the cell surface and, when solubilized, sediments at 10S as does the native species from neurons. Moreover, competition binding studies demonstrate that both the wildtype and epitope-tagged transfected species that bind toxin are indistinguishable pharmacologically from native receptors. The stability of the toxin binding species measured under conditions that blocked appearance of new receptors yields a metabolic half-life of about 10 hours. In contrast, α7 protein incapable of binding αBgt is degraded within minutes. Transfected QT6 cells should permit analysis of mechanisms controlling the assembly and stability of neuronal AChRs destined for the cell surface. **(NIH RO1 NS12601 & NS35469, and NIH F32 NS09830)**

503.21

DIVERSITY OF Ca^{2+} RESPONSES TO NICOTINIC RECEPTOR ACTIVATION IN BOVINE ADRENAL CHROMAFFIN CELLS

S. Donoghue & R. J. Bookman* Dept. of Mol. and Cell. Pharmacology, University of Miami School of Medicine, Miami, FL, 33101

Neuronal nicotinic receptors (nAChRs) possess an intrinsic Ca^{2+} permeability. In adrenal chromaffin cells, influx of Ca^{2+} (and other cations) after stimulation of nAChR depolarizes the membrane potential thereby activating voltage-gated Ca^{2+} channels. The resulting increase in $[Ca^{2+}]_i$ may also release Ca^{2+} from internal stores. We have developed a pharmacological approach (i.e., *killer soup* = thapsigargin + agatoxin + conotoxin + DHP) to isolate the contribution of Ca^{2+} influx through nAChR to the overall $[Ca^{2+}]_i$ signal. Changes in $[Ca^{2+}]_i$ were measured by ratio-imaging video microscopy using fura-2. The agonist-stimulated increases in $[Ca^{2+}]_i$ in the absence of *killer soup* can be classified into two types. Type A responses are characterized by an initial peak and a quick recovery to baseline, even in the presence of the agonist, DMPP. The type B responses typically have smaller peak values but show a maintained elevation of Ca^{2+} during a 5 min agonist application, suggesting a lack of desensitization. Surprisingly, the same types of responses were recorded in experiments with *killer soup* present. The relative peak amplitudes and recovery rates were similar. While we cannot rule out a change in receptor function, these results suggest that the Ca^{2+} influx through nAChRs is the main determinant of the whole cell $[Ca^{2+}]_i$ response in adrenal chromaffin cells. If correct, this finding then raises the question of whether the observation of secretion under voltage clamp with capacitance measurements or amperometry will reveal a similar diversity of secretory responses to nAChR activation.

(Supported in part by AHA/FL)

503.22

SUBTYPE-SPECIFIC MODULATION OF NEURONAL nAChRs BY PGE2

C. Du & L. W. Role*. Dept. Anat. & Cell Biol., Columbia Univ., P & S 722 W 168th St., New York, NY 10032.

Our previous studies characterized multiple subtypes of neuronal nAChR channels expressed by individual chick sympathetic neurons. Four of these subtypes are distinguished from one another by biophysical and pharmacological profile (i.e. conductance, kinetics, opening probability (Po), susceptibility to neuronal-bungarotoxin, and rate of desensitization). Each subtype has been seen in isolation without transitions to other states. In view of the differential the levels of expression, clustering and segregation of these nAChR subtypes during development, we have now addressed whether nAChRs might be differentially modulated. We focused initial studies on PGE2 because prior experiments indicate nAChRs are modulated by arachidonic acid (AA; Berg and colleagues: J. Neurosci.15:3679) and specific AA derivatives including prostaglandins (PG)E_2 (Tan, Siegelbaum & Role, submitted).

Single channel currents gated by ACh (2-20 μM) were recorded in cell-attached patches. Baseline records of 5-10 min. were followed by superfusion with PGE2 (10 μM;) for 2 min.; changes in nAChR channel activity were examined over the next 5-10 min.. Under control conditions, activity was relatively stable for up to 30 min. (n=4 patches) and most patches contained 3-5 nAChR subtypes, with γ=15-65 pS. Examination of each channel subtype in the presence of PGE2 reveals that the Po of 15pS nAChRs was reduced by 60%, due to decreases in opening frequency (*f*) (0.8 Hz vs. 0.6 Hz) and τ_o (1.0 ms vs. 0.5 ms). In contrast, Po of 25pS nAChRs was increased >3 x, due to increased open duration (from 1.1 ms to 5.7 ms); *f* was only slightly increased. Larger conductance nAChRs appear to be inhibited by PGE2 although the low *f* and τ_o, preclude accurate quantitation. These results indicate differential modulation of specific nAChRs by AA derivatives that may permit selectively activation of one nAChR with concurrent suppression of others.

(Supported by NS29832.)

503.23

IN VITRO ACTIVATION OF ALPHA-4 BETA-2 nAChR BY RJR-2403 SUGGESTS DIFFERENTIAL DESENSITIZATION RELATIVE TO NICOTINE.

J. Watterson[1], B. Moulton[1]*, P. Lippiello[2], M. Bencherif[2] and R. L. Papke[1]. [1]Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL 32610 and [2]Research & Development, R. J. Reynolds Tobacco Co., Winston-Salem, NC 27102

Because of the growing need for therapies for neurodegenerative diseases such as Alzheimer's and Parkinson's, recent endeavors have involved a search for compounds which have distinct activation and desensitization profiles for specific neuronal nicotinic acetylcholine receptor subtypes, particularly the predominant brain α4β2 subtype. Nicotine is well known to be a potent activator of these receptors, but concentrations of nicotine required to produce significant activation also lead to inhibition and/or desensitization. In whole cell voltage clamp studies of Xenopus oocytes co-expressing the α4 and β2 neuronal receptor subunits, the CNS-selective nicotinic agonist RJR-2403, (E)-N-methyl-4-(3-pyridinyl)-3-butene-1-amine (Bencherif et al. 1995; Lippiello et al., 1996), had a concentration-response relationship of comparable potency to nicotine but with greater peak currents relative to nicotine. Based on the measurement of peak currents, the efficacy of RJR-2403 was comparable to acetylcholine and 30% greater than nicotine. The application of 100 μM RJR-2403 produced a maximal response. Moreover, RJR-2403 did not cause the apparent inactivation which consistently occurs subsequent to activation by nicotine. There was effectively complete recovery of control response amplitude following a 5 min washout of the compound at concentrations up to and including 100 μM. These preliminary findings with RJR-2403 hold promise for future therapeutic development of compounds which are equal or better activators than nicotine, but do not cause the significant inhibition characteristic of nicotine. Further experiments will determine the effects of this new compound on other receptor subtypes, using the oocyte expression system.

Bencherif et al., Abst.Soc.Neurosci. 21: 605 (1995) Lippiello et al., in Alzheimer's Disease, Therapeutic Strategies, Birkhauser Boston (In Press)

503.24

THE α5 ACETYLCHOLINE RECEPTOR (ACHR) SUBUNIT ALTERS THE SINGLE CHANNEL PROPERTIES OF HUMAN α3 ACHRS EXPRESSED IN *XENOPUS* OOCYTES. M.E. Nelson* and J.M. Lindstrom. Department of Neuroscience, University of Pennsylvania Medical School, Philadelphia, PA 19104.

The human neuroblastoma cells IMR-32 and SH-SY5Y express α3, α5, α7, β2, and β4 AChR subunits with the IMR-32 cells having considerably more functional α3 AChRs than SH-SY5Y. There are at least two AChRs expressed in these "ganglionic-like" cells, one that is αBGT-sensitive (α7 AChRs) and one that is not (α3 AChRs). Several lines of evidence have suggested that a major ganglionic α3 AChR subtype also contains the β4 and α5 subunits. The aim of this work was to characterize cloned human α3 AChRs expressed in *Xenopus* oocytes at the single-channel level and to compare their properties to native neuroblastoma AChRs. The chord conductance for IMR-32 ACh-activated channels was 38 pS and the channel open times were 0.6 and 6.3 ms. The channel conductance for heterologously expressed α3β4 AChRs was 28 pS and the open times were 0.5 and 1.9 ms, while the conductance of α3β2 AChRs was 25 pS and the open times were 0.7 and 2.6 ms. When the α5 subunit was coexpressed with α3 and β4, the channel conductance was slightly increased (30 pS) and the channel open times became longer. Additionally, the channel bursts were longer when compared to α3β4 or α3β2 AChRs. The channels resulting from coexpressing α3, α5, and β4 subunits more closely resembled the channels expressed by IMR-32 cells than did α3β4 or α3β2 AChRs. These results suggest that the α5 subunit contributes to the functional properties of AChRs expressed by IMR-32 cells.

(Supported by grants to J.L. from NINDS, STRC, CTR, and MDA)

503.25

A PATCH-CLAMP STUDY OF THE HUMAN α4β2 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR. B. Buisson*, M. Gopalakrishnan, S.P. Arneric, J.P. Sullivan and D. Bertrand. University of Geneva, 1211 Geneva, Switzerland; Abbott Laboratories, Abbott Park, Illinois 60064-3500, USA.

HEK 293 cells stably transfected with human α4 and β2 cDNAs were characterized with patch-clamp and fast drug application techniques. The rank order of potency of four nAChR ligands to activate human α4β2 receptors is: (-)-nicotine > ACh > (-)-cytisine > ABT-418. At saturating concentrations, the efficacy of these ligands is: ABT-418 >> (-)-nicotine > ACh >> (-)-cytisine > GTS-21. Coapplication of ACh with dihydro-beta-erythroidine (DHβE) and methyllycaconitine (MLA) reversibly reduces the current with respective IC_{50}'s of 80 nM and 1.5 μM. The current-voltage relationship of human α4β2 displays a strong rectification at positive potentials. Experiments of ionic substitutions demonstrate that human α4β2 nAChRs are permeable to sodium and potassium ions. In the "outside-out" configuration, ACh evokes unitary currents (main conductance 46 pS) characterized by a very fast run-down. In contrast to previously characterized nAChRs the human α4β2 subtype is inhibited by increasing the extracellular $[Ca^{2+}]$.

Work supported by the Swiss National Foundation and OFES.

503.26

CHARACTERIZATION OF F11 CELLS, A DORSAL ROOT GANGLION CELL LINE: EVIDENCE FOR FUNCTIONAL NEURONAL NICOTINIC RECEPTORS - A. Manelli, D. Donnelly-Roberts, S.P. Arneric*, and P.S. Puttfarcken, Neuroscience Research, D-47W, Abbott Laboratories, 100 Abbott Park, IL 60064-3500

Previous studies have identified nicotinic acetylcholine receptors (nAChRs) in nociceptive pathways. (±)-Epibatidine, the potent neuronal nAChR agonist, has been reported to possess more potent analgesic properties (Sullivan et al, JPET:271 1994), relative to morphine. However to date, the potential mechanism underlying the effect of nAChRs on pain transmission remain unclear. To begin to understand the role of nAChRs in the modulation of pain, we investigated the ability of nicotinic agonists to activate nAChR channels in F11 cells. The F11 cell line is a dorsal root ganglion (DRG) hybrid cell line previously reported to possess properties of authentic, peptidergic type-C DRG neurons (Francel et al., J. Neurochem.:48 1987). In support, immunohistochemical studies indicated that the F11 cells possess very similar characteristics to primary cultures of rat DRG cells. Both models expressed neuronal markers and a subpopulation of substance P- and calcitonin gene related peptide-containing (CGRP) neurons. (-) Nicotine and (±)-epibatidine evoked a concentration-dependent stimulation of $^{86}Rb^+$ efflux with EC_{50} of 34.1 μM and 0.32 μM, respectively. These agonists exhibited maximal efficacy of 100% at 100 μM for (-)-nicotine and 102% at 1 μM for (±)- epibatidine. Both events can be specifically blocked with mecamylamine. Additionally, preliminary studies indicate that (±)-epibatidine evoked CGRP release from these cells. This is the first evidence of nAChRs present in this cell line and that these recpetors regulate the release of CGRP. These results suggests that F11 cells may be a suitable model to investigate the role of nicotinic receptors in nociception. *This work was supported by Abbott Laboratories.*

ACETYLCHOLINE RECEPTORS: NICOTINIC—BINDING

504.1

ASSOCIATION BETWEEN STRAIN-SPECIFIC α5 AND α7 NICOTINIC RECEPTOR SUBUNIT LOCI AND SENSITIVITY TO THE HIGH DOSE EFFECTS OF NICOTINE. J.A. Stitzel*, J.M. Blanchette, and A.C. Collins. Institute for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309.

Previous studies have demonstrated that mice with higher levels of hippocampal α-Bungarotoxin (αBTX) binding sites are more susceptible to the convulsant effects of high doses of nicotine. We have identified strain-specific rflps for the α7 nicotinic receptor subunit gene which codes for the αBTX binding receptor subunit in brain. Strain-specific rflps also have been identified for a nicotonic receptor subunit, α5, that some reports suggest can bind αBTX and/or is a subunit that contributes to a brain αBTX-binding receptor. To determine whether the strain-specific variants of α5 and/or α7 co-segregate with individual differences in sensitivity to high doses of nicotine, F2 mice derived from a C3H (a strain sensitive to the high dose effects of nicotine) by DBA (a strain resistant to the high dose effects of nicotine) cross were tested for high dose sensitivity to nicotine and genotyped for the α5 and α7 rflps. Animals were scored for overall sensitivity to high doses of nicotine on a scale of one (least affected) to five (death) and for seizure frequency. Animals homozygous for the C3H variant of either α5 or α7 had significantly higher sensitivity scores (3.22 ± 0.18 and 2.88 ± 0.17, respectively) and seized with a higher frequency (43.5% and 32.4%) than those animals homozygous for the DBA variants of α5 and α7 (2.02 ± 0.18 and 2.04 ± 0.2, respectively, for the sensitivity score and 6.9% and 4.0%, for seizure frequency). Results from heterozygotes (α5: score = 2.16 ± 0.15, 9.4% seized; α7: score = 2.38 ± 0.16, 18.2% seized) suggest that the DBA variant of α5 has a dominant effect towards nicotine resistance while the α7 variants appear to act additively. Animals homozygous for the C3H variants of both subunit genes exhibited a sensitivity score of 3.6 ± 0.16 with a 60% seizure frequency while animals homozygous for the DBA variants of both subunits had a score of 1.83 ± 0.4 with no animals seizing. Thus, strain specific variants of the α5 and α7 loci appear to contribute, in a combinatorial fashion, to genetic differences in sensitivity to nicotine. Supported by DA-03194, DA-10156, and DA-00197.

504.2

ALPHA7 IS THE ONLY SUBUNIT DETECTED BY WESTERN BLOT IN PURIFIED RAT BRAIN MEMBRANE α-BTX BINDING SITES D. N. Chen* and J. W. Patrick. Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030

The rat alpha7 subunit of the neuronal nicotinic receptor forms homo-oligomeric nicotine gated ion channels which are blocked by α-bungarotoxin (α-BTX) when expressed in *Xenopus* oocytes. However, while alpha7 subunit is found in purified α-BTX binding molecules from rat brain or cultured PC12 cells, other proteins are co-purified with the alpha7 subunit. Purified chick brain α-BTX binding sites are heterogeneous, and the chick alpha7 subunit associates with the chick alpha8 subunit to form hetero-oligomers. In this study, our goal was to understand whether native rat α-BTX binding molecules are homomers of alpha7 subunits, or heteromers containing other subunits besides alpha7.

We used affinity purified polyclonal antibodies in western blot analysis of affinity purified α-BTX binding sites. We report here that our experimental data strongly suggests that the α-BTX binding receptor in rat brain membrane is either a homo-oligomer of alpha7 subunits or contains some subunit(s) yet to be cloned that do not cross react with our most pan-specific antibodies.

This work was partly supported by the grants from NINDS and NINA of the National Institutes of Health.

504.3

ROLES OF N-LINKED GLYCOSYLATION IN THE FORMATION AND FUNCTION OF α7 NICOTINIC RECEPTOR H. Dang*, D. N. Chen and J. W. Patrick Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030

The α7 subunit of the nicotinic acetylcholine receptor forms homomeric ion channels in *Xenopus* oocytes which are blocked by the snake toxin, α-bungarotoxin. The N-terminal extracellular domain of α7 has three consensus sites (NXT/S) for N-glycosylation ($N_{77}DS$, $N_{90}MS$, and $N_{133}AS$). In this study, we show that α7 expressed in transfected COS cells and in rat brain membrane are glycoproteins. We also demonstrate by site-directed mutagenesis and COS cell transfection that all three sites are utilized for glycosylation.

To elucidate the roles of the glycosylation sites in the formation and function of the α7 receptor, effects of replacing either the asparagines or serines with alanines on the receptors expressed in oocytes were measured by two-electrode voltage clamp. The number of α-BTX binding sites or the amount of receptor protein on the surface were determined by either the ^{125}I-α-BTX binding assay or an antibody binding assay. Both the N46A and S48A mutants showed increased whole cell currents and surface expression of receptor proteins compared to WT indicating that glycosylation at N46 limits the rate of surface expression of the α7 receptor. However, both asparagine and serine mutations at the N90 and N133 sites greatly reduced the whole cell currents but not the surface protein expression suggesting that the carbohydrate moieties attached at these sites may be involved in the function of ligand binding domain.

This work was in part supported by grants from NINDS and NINA of NIH.

504.4

COMPARISON OF L-NICOTINE-STIMULATED [^{3}H]DOPAMINE RELEASE FROM FOUR MOUSE BRAIN REGIONS. S.R. GRADY*, A.L. CLARK, and A.C. COLLINS. Inst. for Behav. Genetics, U. of Colo., Boulder, CO 80309.

Several brain regions known to release dopamine were compared in order to further characterize the actions of L-nicotine (NIC). Striatum (ST), olfactory tubercle (OT), frontal cortex (FC), and nucleus accumbens (NA) were dissected from C57/6JIBG ♀ mouse brain slices (1mm thick) made using a mouse brain block. A P2 synaptosome preparation was made from each region and [^{3}H]dopamine release was assayed as previously described (Grady et al. J. Neurochem. 59:848-856, 1992). Release was stimulated by NIC from all four regions. This release was Ca^{++}-dependent and inhibited by mecamylamine and dihydro-ß-erythroidine. Dose response for a 1 min exposure to NIC was determined for each region. EC_{50} values (μM), calculated by curve fits assuming $n_H = 1$, were: ST 0.35 ± 0.04; OT 0.15 ± 0.04; FC 0.17 ± 0.04; NA 0.13 ± 0.03. Maximum release values (stimulated release/baseline), using the indicated amounts of tissue from one mouse, were: ST(1/8) 6.45 ± 0.11; OT(1/4) 7.38 ± 0.19; FC(1/4) 2.91 ± 0.06; NA(1/4) 4.28 ± 0.17. In addition, a 10 min exposure to NIC revealed biphasic desensitization as previously shown for striatum (Grady et al. J. Neurochem. 62:1390-1398, 1994). The regions, however, appeared to differ in the ratio of persistent response to total response with NA having the lowest ratio and ST the highest. ST was significantly different from the other regions ($P < 0.05$).

Supported by DA03194 and DA00197.

504.5

HETEROGENEITY OF EPIBATIDINE BINDING SITES IN MOUSE BRAIN. M.J. Marks*, B. Coll, K.W. Smith and A.C. Collins. University of Colorado, Boulder, CO.

Epibatidine, an alkaloid isolated from the skin of the Ecuadorean poison dart frog, *Epipedobates tricolor*, is a potent nicotinic agonist in vivo and in vitro. [^{3}H]Epibatidine binds to brain with high affinity and apparently labels several distinct nicotinic receptor subtypes. [^{3}H]Epibatidine binding to mouse brain was measured using regionally dissected tissue and by quantitative autoradiography. The binding of [^{3}H]epibatidine (400 pM) to membranes prepared from olfactory bulbs was inhibited by cytisine, nicotine, acetylcholine, and methylcarbachol. The inhibition profile obtained for each agonist was biphasic with approximately 45% of the binding inhibited at relatively low agonist concentrations. Since the IC_{50} values estimated for cytisine (4.1 nM and 460 nM) differed more than those of the other agonists, cytisine was used to inhibit binding in ten additional regions. The IC_{50} values for high and low affinity cytisine inhibition did not differ among the regions, but total [^{3}H]epibatidine binding and the percentage inhibited at low cytisine varied among the regions. These results suggest a differential distribution of nicotinic receptors in mouse brain. Quantitative autoradiographic analysis of [^{3}H]epibatidine (350 pM) binding for samples incubated with 0, 5, 50, 150, 500 and 10000 nM cytisine revealed a distinct and restricted pattern for the distribution of sites with low affinity for cytisine: accessory olfactory bulb, medial habenula, interpeduncular nucleus, and fasciculus retroflexus showed high binding, while medial and lateral geniculate nuclei, and inferior and superior colliculi had somewhat less, but still substantial, binding. These sites were virtually undetectable in other brain areas. [^{3}H]Epibatidine binding sites with high affinity for cytisine had identical distribution to those measured with [^{3}H]nicotine. These results confirm that epibatidine binds to at least two nicotinic receptor subtypes, one of which is identical to the high affinity nicotine binding site (presumably α4/ß2) and another relatively rare site. Supported by DA03194, DA10156 and DA00197.

504.6

3H-EPIBATIDINE BINDING: CHARACTERIZATION IN RAT AND HUMAN BRAIN. M.W. Arriola, D.G. Costello, P.W. Sweetnam, and T.F. Seeger*. Pfizer Inc., Central Research Division, Groton, CT 06340.

Epibatidine (EPI), a potent analgesic, appears to mediate its central and peripheral actions through nicotinic receptors. Using [3H]EPI homogenate binding and autoradiographic techniques, we report on the further characterization of EPI binding sites in the rat and human CNS. Competition studies revealed the rank order of potency (EPI>cytisine>nicotine>lobeline>carbachol) at the [3H]EPI site (final conc. 250 pM) was consistent with previous reports. Further studies revealed binding in both species was saturable, reversible, and of high affinity. Two pharmacologically distinct binding sites were discriminated by Scatchard analysis. Apparent affinity was 30 and 350 pM in rat cortex, while in human parietal cortex the corresponding affinities were <10 and 100 pM. In contrast to previous reports, the detection of two sites was dependent on the presence of ions, particularly calcium, in the assay buffer. Analysis of 3H[EPI] autoradiography in the rat brain confirmed it to be reversible, and to consist of more than one binding component, delineated by displacement with nicotine. The majority of [3H]EPI binding areas in rat brain were displaced by nicotine with an apparent IC50 of 25-50 nM. This component was widely distributed throughout the rat brain, with highest densities found in thalamic nuclei. The second component was insensitive to nicotine at up to 1 uM. This site was present in very high density in medial habenula, fasciculus retroflexus, and interpeduncular nucleus. Epibatidine may serve as an important tool for the molecular characterization of nicotinic receptors other than the α2ß4 subtype which predominates in the rat CNS. (Funded by Pfizer Inc.)

504.7

N-OCTYLNICOTINIUM IODIDE (NONI), A NOVEL NICOTINIC RECEPTOR ANTAGONIST: COMPETITIVE DISPLACEMENT OF [^{3}H]NICOTINE BINDING FROM RAT STRIATAL MEMBRANES AND BRAIN REGION-SPECIFIC DISPLACEMENT OF [^{3}H]CYTISINE BINDING. L.H. Wilkins*, P.A. Crooks, J.R. Pauly and L.P. Dwoskin. College of Pharmacy, University of Kentucky, Lexington, KY 40536.

A series of N-substituted nicotine analogues was synthesized and evaluated for inhibition of nicotine-evoked [^{3}H]dopamine release from rat striatal slices and for displacement of [^{3}H]nicotine binding from rat striatal membranes (Crooks et al., Drug Dev. Res. 36:91, 1995). NONI was found to be the most potent (IC50 = 1 μM) and efficacious compound, completely inhibiting nicotine's effect in the release assay. Although NONI completely displaced [^{3}H]nicotine binding, it was the least potent (IC50 = 50 μM) analogue examined in the binding assay. In the present study, we assessed if NONI was a competitive or noncompetitive antagonist as determined by the pattern of [^{3}H]nicotine displacement from striatal membranes. A parallel rightward shift in the displacement curves was observed with increasing concentration of NONI. Scatchard analysis revealed an increased Kd value (from 1.7 nM at 0 NONI to 30 nM at 22 μM NONI) with no change in Bmax (68.7 fmol/mg protein) with increasing concentration of NONI, suggesting a competitive interaction of NONI with the [^{3}H]nicotine binding site. Similar results were obtained when NONI was used to displace [^{3}H]cytisine (5 nM) binding from rat brain sections. Low concentrations of NONI (0.5 μM) did not significantly displace [^{3}H]cytisine binding, but higher concentrations (10 μM) reduced binding by 20-40%, depending on the brain region analyzed. Thus, NONI is an excellent lead compound for the development of novel, subtype-selective nicotinic antagonists. Supported by the Tobacco and Health Research Institute, Lexington, KY.

504.8

[^{3}H]-CYTISINE BINDING TO α4β2 NEURONAL NICOTINIC RECEPTORS EXPRESSED IN XENOPUS OOCYTES. M.J. Parker, C.W. Luetje* Department of Molecular and Cellular Pharmacology, University of Miami, Miami FL, 33101.

The high affinity cytisine binding site in rat brain (Kd=0.96 nM, Pabreza et al., 1990, Molec. Pharmacol. 39:9-12) is thought to be composed of the α4 and β2 neuronal nAChR subunits (Flores et al., 1991, Molec. Pharmacol. 41:31-37). In order to determine the structural basis for high affinity agonist binding to neuronal nAChRs, we have begun using radioligand binding techniques to study neuronal nAChRs exogenously expressed in *Xenopus* oocytes. Equilibrium binding assays were performed with [^{3}H]cytisine in a crude membrane homogenate. To increase receptor expression, the α4 and β2 subunits were engineered into the high expression vector, pGEMHE. Expression of α4β2, as measured by two-electrode voltage clamp and [^{3}H]cytisine binding, was increased by approximately 3-fold when the RNA was transcribed from pGEMHE constructs, rather than from pSP64/65 constructs. The Kd for [^{3}H]cytisine binding to α4β2 expressed in oocytes was 0.88 nM. This is similar to the Kd for [^{3}H]cytisine binding to the rat brain site reported by Pabreza et al., further supporting the conclusion that the high affinity cytisine binding site in rat brain is α4β2. Using this radioligand binding assay, we plan to characterize the affinity of the α4β2 receptor for other agonists and to determine the affinity of other neuronal nAChR subunit combinations for agonists. Ultimately we will use chimeric and mutant subunits to identify amino acid residues responsible for high affinity agonist binding.

[Support: NIH DA-08102 and AHA-FL to CWL]

504.9

[^{125}I]IPH, AN EPIBATIDINE ANALOG BINDS WITH HIGH AFFINITY TO NEURONAL NICOTINIC RECEPTORS. M.I. Dávila-García*, J.L. Musachio[1], D.C. Perry[2], Y. Xiao, A. Horti[3], E.D. London[1,3], R.F. Dannals[1] and K.J. Kellar. Department of Pharmacology, Georgetown University School of Medicine, Washington, DC 20007. [1]The Johns Hopkins University Medical Institutions, Baltimore, MD 21287. [2]George Washington University School of Medicine, Washington , DC 20037, [3]Division of Intramural Research, National Institute on Drug Abuse, Baltimore, MD 21224.

[^{125}I]IPH ((±)-exo-2-(2-[^{125}iodo]-5-pyridyl)-7-azabicyclo[2.2.1] heptane), an epibatidine analog was prepared with specific activities from 188 to 1920 Ci/mmol, via a Cu-assisted exchange reaction from bromide to [^{125}I]iodide. [^{125}I]IPH has high affinity for neuronal nicotinic acetylcholine receptors in membranes from rat brain, rat adrenal gland and stably transfected cell lines expressing the α2/ß4 subunit combination. In all cases, nonspecific binding was low. In adult rat forebrain, this radiolabeled ligand has a similar K_D value (≈50pM) to that of its analog [^{3}H]epibatidine ([^{3}H]EB), and nicotinic drugs compete with a similar rank order of potency for the binding sites labeled by the two radioligands: (±)EB >> cytisine > nicotine > dihydro-ß-erythroidine (DHßE) > carbachol > curare. In competition studies, most of the drugs tested show Hill coefficients close to 1 (>0.8), with the exception of DHßE (0.6) and curare (0.7). [^{125}I]IPH also labels nicotinic receptors in autoradiographic studies in rat brain. The fact that [^{125}I]IPH labeled to a high specific radioactivity binds with high affinity to neuronal nicotinic acetylcholine receptors makes it a useful compound to study the pharmacology and regulation of these receptors, particularly in tissues with low receptor density.

This work was supported by NIH grants AG09973 and DA06486 to KJK.

504.10

CHARACTERISATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR OF *MANDUCA SEXTA* : A NICOTINE INSENSITIVE, TOBACCO FEEDING INSECT. H. M. Eastham*, R. J. Lind, A. J. Wolstenholme, S. E. Reynolds & S. Wonnacott. Sch. of Biol. & Biochem., Univ. Bath, Bath, BA2 7AY, U.K.

Nicotine is a potent insecticide, causing a depolarising blockade of the nicotinic acetylcholine receptor (nAChR). However, the larvae of the tobacco hornworm, *Manduca sexta*, feed on tobacco yet suffer no detrimental effects from the ingestion of nicotine. Trimmer & Weeks (J. exp. Biol. 144:303-337, 1989) postulated that this reduced nicotine sensitivity could reflect in part an altered nAChR, making it a natural, nicotine-insensitive, mutant receptor.

Binding studies on brain membranes from adult *Manduca* show that [^{125}I]α-bungarotoxin (α-bgt) labels a specific site with Kd=7.2±2.4nM and Bmax= 186±14.5fmol/mg. Displacement studies show that (-)nicotine (IC_{50}=2.3±1.0μM), (±)epibatidine (1.0±0.23μM) and imidacloprid (1.8±0.3μM) bind with similar affinities, while ACh (59±14.3μM, with neostigmine) and another tobacco alkaloid, anabasine (37.9±5.8μM) have lower binding affinities. Honeybee, a nicotine-sensitive insect, gives IC_{50} values for α-bgt, imidacloprid or nicotine (Tomizawa *et al.*, J. Pesticide Sci. 20:57-64, 1995) comparable to those of *Manduca* determined here. These results suggest that the nicotine insensitivity of *Manduca* is not reflected in a lowered nicotine binding affinity at this α-bgt-labelled nAChR.

To see if an α-bgt-insensitive nAChR is present in *Manduca* brain we performed binding assays using [^{3}H]epibatidine. At 1nM and 10nM concentrations no specific [^{3}H]epibatidine binding displaceable by excess nicotine or α-bgt was demonstrated. Using a variety of PCR techniques we have obtained the complete coding sequence of a nicotinic α subunit for *Manduca*. This shows no obvious changes in candidate residues that determine nicotine affinity, consistent with the binding data.

Supported by EC, BBSRC and Zeneca Agrochemicals.

504.11

A NEURONAL CELL LINE EXPRESSING A UNIQUE, CNS NICOTINIC ACETYLCHOLINE RECEPTOR SUBTYPE. L. Lucero* and R.J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Our previous work has shown that clonal cell lines of different derivation are excellent models for studies of fundamental properties of diverse, naturally-expressed nicotinic acetylcholine receptor (nAChR) subtypes. The CATH.a cell line (Central, Adrenergic, Tyrosine Hydroxylase-positive cell line "a"), which was isolated by Suri et al. (J. Neurosci. 13:1280, 1993) and a generous gift of Dona Chikaraishi, was derived from a tumor occurring at the ventral aspect of the midbrain/rostral brainstem boundary in a transgenic mouse carrying the SV40 large T antigen oncogene under transcriptional control of rat tyrosine hydroxylase 5'-flanking sequences. In support of our reasoning that CATH.a cells might represent a long-sought neuronal cell line that expresses some form of CNS nAChR, we find that they express specific, high-affinity binding sites for [^{3}H]epibatidine (EBDN; 6 pM K_D). There are no specific ^{125}I-labeled α-bungarotoxin binding sites, and EBDN binding sites are found in both intracellular and cell surface pools. Numbers of membrane-bound EBDN binding sites increase ~150% on chronic exposure of CATH.a cells to nicotine. However, EBDN binding competition studies indicate CATH.a cells have lower affinity for agonists such as cytisine and nicotine than do rat or mouse brain α4β2-nAChR, but higher affinity for antagonists such as alcuronium and d-tubocurarine. These relationships are reversed for comparisons of CATH.a cell nAChR and IMR-32 cell α3β4-nAChR. Northern analyses indicate expression as mRNA of nAChR α4, β2, and other subunits. Collectively, these studies suggest that CATH.a cells express a unique nAChR subtype that contains α4, β2, plus other nAChR subunits. We hypothesize that the CATH.a cell nAChR subtype is involved in the control of catecholamine release from mesolimbic dopaminergic cells and/or adrenergic cells of the locus coeruleus. Funded by NIDA and ADCRC.

504.12

IMAGING NICOTINIC ACETYLCHOLINE RECEPTORS IN BABOON BRAIN BY PET AND SPECT. V.L. Villemagne[1], U. Scheffel*[1], A. Horti[2], J.L. Musachio[1], H.T. Ravert[1], P. Finley[1], M. Stathis[1], E.D. London[1,2], and R.F. Dannals[1]. [1]The Johns Hopkins Medical Institutions, and [2]Intramural Research Program, NIDA, Baltimore, Maryland 21205.

Nicotinic acetylcholine receptors (nAChRs) have been implicated in a variety of central processes, such as learning & memory, and modulation of appetite. These receptors also mediate the reinforcing properties of nicotine in tobacco products and are decreased in the brains of individuals who have died from dementia of the Alzheimer type. In an effort to prepare PET/SPECT radiotracers for in vivo studies of nAchR, we have prepared radiohalogenated [F-18, I-123] analogs of epibatidine, a high affinity potent nAchR agonist. The distribution and kinetics of [F-18]-FPH and [I-123)-IPH were evaluated using PET and SPECT, respectively. After intravenous injection of 5 mCi of high specific activity [F-18]-FPH or [I-123]-IPH into a 20 kg anesthetized baboon (Papio Anubis), sequential tomographic data were acquired over a period of 150 min. Brain activity peaked for both compounds within 60 min post injection, and the binding showed reversible characteristics. Elimination was more rapid from the cerebellum (clearance t1/2 ~ 3 h), and than from the thalamus (t1/2 ~ 16 h). Radioactivity concentrations at ~ 100 min post injection were highest in the thalamus; intermediate in the neocortex; and lowest in the cerebellum. Subcutaneous injection of 1 mg/kg cytisine 45 min after injection of [F-18]-FPH reduced brain activity at 130 min by 67, 64, 56, and 52% of control values in the thalamus, hypothalamus, hippocampus, and cerebellum, respectively. High correlation with known densities of nAChR measured in vitro in rat brain was found for [F-18]-FPH (r = 0.90) and [I-123]-IPH (r = 0.87). These results demonstrate the feasibility of imaging nAChR in vivo and suggest that both [F-18]-FPH and [I-123]-IPH can be used to study nAChR in the human brain.

504.13

CELLULAR LOCALIZATION OF α-BUNGAROTOXIN BINDING AND α7 mRNA IN THE HIPPOCAMPUS RELATED TO AUDITORY GATING IN THE AWAKE, BEHAVING RAT. Y.D. Rollins, C. Breese, C. Adams, C. Drebing*, G.M. Rose, and S. Leonard. Depts. Of Pharmacology and Psychiatry, UCHSC, and Medical Research, VAMC, Denver, CO 80262

Previous studies have shown that both α-bungarotoxin (α-BTX) and methyllycanconitine (MLA), specific antagonists at the α7 containing nicotinic neuronal receptor, are able to partially disrupt gating of auditory evoked potentials in hippocampus of the awake, behaving rat (Rollins et al., 1993). The α7 antagonists at lower concentrations decrease the inhibition of the test response normally seen in this paradigm while at higher concentrations they decrease the initial or condition response. To explain these biphasic effects, autoradiography with [^{125}I]-α-BTX was analyzed at the light microscopic level in the rat hippocampus to determine the cellular localization of the receptor. Two types of receptor binding were found: 1) High levels of α-BTX binding were seen on cell bodies and dendrites of many cells of differing morphology in the polymorphic region of the fascia dentata and on scattered interneurons located in all layers of the hippocampus and, 2) Dense, diffuse binding was identified in the CA3c subregion of the hippocampus that was not associated with cell bodies and appeared to be presynaptic from lesioning studies. In contrast, *in-situ* hybridization revealed that α7 mRNA was located primarily in hippocampal pyramidal cells and in scattered interneurons, indicating the binding found in CA3c may derive from pyramidal cells. The diverse localization of the receptor may provide a mechanism for both stimulatory and inhibitory modulation by the α7 neuronal nicotinic receptor in the auditory gating paradigm. [This research was supported by USPHS Grants MH44212 and the VA Medical Research Service]

504.14

BINDING OF ^{125}I-α-BUNGAROTOXIN AND EXPRESSION OF mRNA FOR THE α7 NICOTINIC, CHOLINERGIC RECEPTOR IN NEURAL CREST-DERIVED TISSUES OF THE RAT. S. Leonard*, C.R. Breese, J. Logel, Y. Rollins and C. E. Adams. Department of Psychiatry, University of Colorado Health Sciences Center., Denver, CO 80262.

Cells within the chick cervical ganglia, a derivative of the neural crest, have been reported to express mRNA for the α7 nicotinic, cholinergic receptor. This finding raised the possibility that expression of the α7 nicotinic receptor might be a common characteristic of cells of neural crest origin. The hypothesis was examined in the rat in a variety of organs containing neural crest-derived cells using ^{125}I-α-bungarotoxin receptor autoradiography and *in situ* hybridization for α7 mRNA. Cells within the thymus, adrenal gland, trigeminal ganglia, dorsal root ganglia, superior cervical ganglia, lung, heart and skin were found to both bind ^{125}I-α-bungarotoxin and express α7 mRNA. Other tissues, including the testis, pancreas, ovary, intestine, olfactory bulb and retina, exhibited α-bungarotoxin binding with little to no expression of α7 mRNA. These results suggest that expression of the neuronal nicotinic receptor α7 is not limited to the CNS but is also present in peripheral tissues of neural crest origin. [This research supported by USPHS Grants MH44212, DA09457 and VA Medical Research Services]

504.15

NICOTINIC ACETYLCHOLINE RECEPTOR mRNAs DURING DEVELOPMENT OF THE SCN, OTHER BRAIN REGIONS, AND FOLLOWING NICOTINE EXPOSURE *IN VIVO* AND *IN VITRO*. B.F. O'Hara*, V.H. Cao, S.W. Wiler, H.C. Heller, J.D. Miller# and T.S. Kilduff. Ctr. for Sleep & Circadian Neurobiology, Depts. of Psych. and Biological Sciences, Stanford Univ., Stanford, CA 94305. # Dept. of Pharm., Texas Tech Univ. HSC., Lubbock, TX 79430.

We have previously found substantial changes in levels of nicotinic acetylcholine receptor (nAChR) subunit mRNAs during development that may underlie the sensitivity of the fetal suprachiasmatic nuclei (SCN) to nicotine (Dev. Brain Res. 84(1995)46-54). Specifically, α7 mRNA increases in the SCN from fetus to adult while α4 mRNA decreases as determined by Northern analysis. β2 levels remain relatively constant. We have now examined several other subunits and find that α2 and α3 follow similar patterns to α4 in the SCN and other brain regions examined. α5, β3 and β4 mRNAs were barely detectable in our brain samples. We have examined both rat and mouse tissue and generally find similar mRNA sizes, however, some mRNAs do vary between the two species and some vary between brain regions and throughout development. More recently we have been examining changes in nAChR gene expression following nicotine exposure in the fetus, adult and hypothalamic cell cultures. Other groups have found that nicotine exposure can increase nAChR protein levels without any change in mRNA. However, in hypothalamic cell cultures (from E18 rat fetuses) exposed to 100μM nicotine for one hour, we find a several fold increase in α7 mRNA levels. We find a more modest increase in α7 mRNA in the hypothalamus and hippocampus following a 1mg/kg s.c. dose of nicotine. In both *in vivo* and *in vitro* conditions, the tissue was frozen after one hour of nicotine exposure. Additional timepoints with both acute and chronic nicotine are now being examined. The *in vitro* studies utilize cells grown for 3 or 10 days in culture. The 10 day old culture exhibits the highest α7 mRNA levels suggesting possible glial expression. In contrast, β2 and other subunit mRNAs are nearly absent in these cultures. (Supported by NIH grants DA00187 and HD29732).

504.16

INDIVIDUAL RAT INTRINSIC CARDIAC NEURONS DISPLAY VARIABLE NICOTINIC PHARMACOLOGIES AND NEURONAL nAChR β SUBUNIT mRNA EXPRESSION PATTERNS K. Poth*, R.J. Bookman, C.W. Luetje, Department of Molecular and Cellular Pharmacology, University of Miami, Miami FL, 33101.

Neurons can assemble nAChRs from a potential pool of at least 11 different subunits. In *Xenopus* oocytes, each functional subunit combination has distinct pharmacological properties. Sensitivity to the agonist cytisine is largely dependent on the identity of the β subunit (Luetje & Patrick, 1991, J. Neurosci. 11: 837-845). Single cell RT-PCR demonstrates that individual neurons cultured from rat intracardiac ganglia express different arrays of nAChR subunit mRNAs (Poth, et.al., 1995, Soc. Neurosci. Abstr. 21: 1335). To determine the effect of differential subunit expression on the pharmacological properties of nAChRs expressed by these neurons, we are assessing agonist pharmacology by measuring changes in $[Ca^{2+}]_i$ with fura-2 fluorescence imaging, and probing subunit mRNA expression with single cell RT-PCR. We find that both whole cell agonist pharmacology and β subunit expression are variable. The ratio of peak Ca^{2+} responses to cytisine and ACh ranged from 0.23 to 1.85. Single cell RT-PCR showed that while some neurons express either β2 or β4 mRNA, many neurons express both β subunit mRNAs with the ratio of β2 to β4 mRNA varying from neuron to neuron (in one experiment the β2:β4 ratio ranged from 0.6 to 4.6). This suggests that the pharmacological differences from neuron to neuron are due to differences in the relative expression of neuronal nAChR subunits. We are currently examining this possibility directly by performing pharmacological measurements and RT-PCR in the same neurons. [Support: NIH DA-08102 and AHA-FL]

504.17

SEVERAL SUBUNITS OF THE NICOTINIC RECEPTOR GENE FAMILY ARE EXPRESSED IN THE RAT PERIPHERAL VESTIBULAR SYSTEM. H. Hiel, H.K. Happe*, A.B. Elgoyen, B.J. Morley. Boys Town Nat'l Research Hospital, 555 North 30th Street, Omaha, NE 68131.

A major efferent neurotransmitter in the peripheral vestibular organ is probably acetylcholine (ACh), but the cholinergic receptor(s) have not been identified. Pharmacological data suggest that at least one receptor is a nicotinic cholinergic receptor (nAChR) (Guth et al., Hear. Res. 75, 225-232, 1994). We studied the mRNA expression of the α3-α7, and β2-β4 subunits in the peripheral vestibular system by *in situ* hybridization with ^{35}S-labeled riboprobes. Alpha 4, α5, α6, α7, β2 and β3 nAChR subunits, but not α3 or β4 are expressed in the primary afferent vestibular ganglion (Scarpa's ganglion).

The vestibular sensory epithelia were devoid of detectable hybridization signal for the α3-α7 and β2-β4, but vestibular sensory hair cells express the α9 subunit, the subunit recently reported in the cochlear outer hair cell by Elgoyhen *et al.* (Cell 79, 705-715, 1994). This observation is consistent with a recent report Guth *et al.* (Hear. Res. 75, 225-232, 1994) in which the pharmacological profile for the vestibular efferent receptor was reported to be similar to the nAChR on the cochlear outer hair cell.

Supported by NIH/NIDCD grant DC002115-12 to BJM.

504.18

IDENTIFICATION OF THE SUBUNITS OF THE NICOTINIC CHOLINERGIC RECEPTORS IN THE RAT COCHLEA USING RT-PCR AND *IN SITU* HYBRIDIZATION. B.J. Morley*, H.-S. Li, H. Hiel, D.G. Drescher, A.B. Elgoyen, Boys Town Nat'l Research Hospital, 555 North 30th Street, Omaha, NE 68131.

There are two neuronal tissues in the mammalian cochlea that are post-synaptic to cholinergic efferent fibers: the outer hair cells (OHCs) and the dendrites of the afferent fibers of the Type I spiral ganglion cells. The unusual nicotinic-like pharmacology of cochlear cholinergic responses and the unique embryonic development of cochlear tissues suggest that the nicotinic cholinergic receptor (nAChR) may be different than those described previously at synapses in the mammalian brain, autonomic ganglia, or skeletal muscle. In this study, we determined the mRNA expression of the α2-7, α9, and β2-4 subunits of the nicotinic acetylcholine receptor (nAChR) family in the rat cochlea. In micro-dissected tissue from the organ of Corti, spiral ganglion, and stria vascularis, we found mRNA expression of the α7 and α9 subunits in the organ of Corti and α5-7, and β2 and β3 in the spiral ganglion using RT-PCR. Using *in situ* hybridization with ^{35}S-riboprobes, α9 was localized in hair cells and α6, α7 and β2 were localized in the Type I cells of the spiral ganglion. No evidence of nAChR subunit mRNA expression was found in supporting cells, but β2 was expressed in Type II spiral ganglion cells, which are neither cholinergic nor cholinoceptive.

Supported by NIH/NIDCD grant DC002115-12 to BJM.

504.19

BINDING TO NICOTINIC RECEPTORS LABELED BY [^{3}H]EPIBATIDINE AND [^{3}H]CYTISINE IS INCREASED IN BRAINS OF SMOKERS. D.C. Perry[1], M.I. Dávila-García[2], C.A. Stockmeier[3]*, G.E. Dilley[3], L. A Shapiro[3] & K.J. Kellar[2] Depts. of Pharmacology, [1]George Washington University Medical Center, Washington, DC 20037, [2]Georgetown University Sch. Of Med., Washington, DC 20007 and [3]Dept. of Psychiatry, Case Western Reserve University Sch. of Med., Cleveland, OH 44106.

To assess the effects of smoking on nicotinic receptors in human brain, we used homogenate binding assays and quantitative receptor autoradiography to compare the density of nicotinic receptors in prefrontal cortex (area 10), anterior temporal cortex (area 38) and hippocampus. Brains were collected from 8 smokers (>20 cigarettes /day) and 8 nonsmokers matched by age (54±4y v. 54±6y) and post mortem interval (21±2h v. 16±3 h). Receptors in homogenates were labeled using [^{3}H]epibatidine ([^{3}H]EB) or [^{3}H]cytisine ([^{3}H]Cyt) at saturating concentrations to reflect total receptor density (1 nM [^{3}H]EB or 7 nM [^{3}H]Cyt). Nonspecific binding was determined with 300 μM nicotine tartrate. In homogenates from prefrontal cortices of smokers, the density of nicotinic receptors labeled by either ligand increased by ~300% compared to nonsmokers ($p<0.0001$). In anterior temporal cortex from smokers, nicotinic receptor density measured by either ligand increased by ~250% compared to nonsmokers ($p<0.0001$). For autoradiographic analysis, 20 μm frozen sections were mounted on slides and incubated with 1 nM [^{3}H]EB or 4.5 nM [^{3}H]Cyt and after rinsing and drying apposed to tritium sensitive film for approximately 3 months. Preliminary analysis of autoradiographic images confirms the large increases seen in both cortical regions of smokers. Increases were seen across the laminae, but a particularly dense band of binding was apparent in the deepest layers of the frontal cortex (layer VI), and in two deep bands in temporal cortex (layers IV and VI). Data from hippocampal autoradiography will also be presented. Supported by DA06486 & MH45488.

504.20

THE REDUCED EXPRESSION AND FUNCTIONAL LOSS OF CENTRAL NICOTINIC RECEPTORS IN A STRAIN OF GENETICALLY HYPERTENSIVE RATS (SHR) M. Gattu*, A.V.Terry, Jr., J.R. Pauly and J.J. Buccafusco, Alzheimer's Research Center, and Dept. Pharmacol. & Tox., Medical College of Georgia, and the VA Med. Ctr., Augusta, GA, 30912 and the School of Pharmacy, University of Georgia, Athens, GA.

Studies from this and other laboratories have shown that the expression of brain nicotinic receptors is dramatically reduced in SHR compared with age-matched normotensive rats. However, it has not yet been determined whether this decrease in expression is reflected in a decrease in nicotinic receptor-mediated function, or whether the hypertensive state causes the decreased expression. We estimated the number of nicotinic receptor binding sites in the brains of 12 week old SHR its normotensive counterpart the WKY strain by using quantitative autoradiographic techniques. Coronal sections were exposed to saturating concentrations of [^{3}H]-cytisine (α_4 subunit-containing sites) and [^{125}I]-α-bungarotoxin (α_7 sites). In general, [^{3}H]-cytisine binding was reduced (by up to 25%) in the brains of SHR *vs.* WKY. Significant reductions were observed in about 50% of the regions analyzed. SHR exhibited only regional reductions in [^{125}I]-α-bungarotoxin binding sites. These were relegated to the frontal cortex and anterior olfactory nucleus (interstrain differences 29 and 32%). Similarly, the magnitude of nicotine-stimulated $^{86}Rb^+$ efflux from synaptosomes derived from the frontal cortex and striatum of SHR was significantly lower than that from normotensive rats. Since central nicotinic receptors have also been shown to play are role in learning and memory, we employed a navigational memory task (NMT) and a passive avoidance (PA) task to elucidate any strain differences in memory-related task performance. SHR exhibited significantly impaired performance in learning and re-learning phases of NMT and PA tasks compared to age-matched WKY rats. Thus, the decreased expression of nicotinic receptors in SHR may result in functional impairment both neurochemically and behaviorally, and may be related to the cognitive impairment known to occur in untreated human essential hypertension. Supported by the VA Medical Research Service.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY VI

505.1

NEUROTOXIC ACTIONS OF N-METHYL-D-ASPARTATE (NMDA)/ GLUTAMATE RECEPTOR ANTAGONISTS IN THE RAT ENTORHINAL CORTEX. G. Wong, J. Väisänen, A-M Lindén, E. Castrén*. A.I. Virtanen Institute, University of Kuopio, PL 1627, Kuopio, Finland 70211

NMDA/glutamate receptors are formed from a family of ligand-gated ion channels which together mediate many of the neurotoxic effects produced by chemical agents, hypoxia-ischemia, and mechanical injury. As a consequence, antagonists to the channel site have been developed as a strategy to attenuate neuronal injury produced through activation of these receptors. These agents include the channel blockers MK-801, ketamine, and phencyclidine. Nonetheless, these substances have been observed to produce paradoxical neurotoxicity in cingulate, retrosplenial and medial entorhinal cortices as determined by formation of intracellular vacuoles, and induction of mRNAs for immediate early genes, heat shock proteins, and brain-derived neurotrophic factor. The aim of the present study was to determine the underlying mechanisms involved in the neurotoxic actions of MK-801 in the rat entorhinal cortex. In these studies, receptor autoradiography using [^{3}H]MK-801 and modulators of the complex (e.g. glutamate, glycine, polyamines) revealed heterogeneity of NMDA receptors in the entorhinal cortex, which was dependent on the layer observed, and is most likely the result of differential NMDAR1 and NMDAR2A,B subunit expression. Furthermore, *in situ* hybridization studies were used to correlate the specific cortical layers where paradoxical neurotoxic effects were observed (as measured by c-FOS expression) and the expression of various receptor subtypes. Taken together, these results suggests a role for NMDA/glutamate receptor subtypes in mediating neuronal injury.

*This investigation was supported by The Academy of Finland and the Sigrid Juselius Foundation.

505.2

CGS19755 SENSITIVE ELECTRICAL COMPONENT THAT CONTRIBUTES TO SYNAPTICALLY MEDIATED EXCITOTXICITY. J.R. McLeod, M. Shen, S.A. Thayer* Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

Reducing the extracellular Mg^{2+} concentration ($[Mg^{2+}]_o$) bathing cultured rat hippocampal neurons to 0.1 mM elicited an aberrant pattern of excitatory synaptic activity. 0.1 mM $[Mg^{2+}]_o$ elicited repetitive bursts of action potentials associated with large increases in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$ spikes) as indicated by combined-whole-cell current clamp and indo-1-based microfluorimetry. Digital imaging experiments showed that the $[Ca^{2+}]_i$ spikes were synchronized, suggesting that these cultures would be suitable for relating synaptic activity to viability. 24 hr exposure to 0.1 mM $[Mg^{2+}]_o$ resulted in the death of 18 ± 4 % of the neurons. The NMDA receptor antagonist CGS19755 (10 μM) protected the network from 0.1 mM $[Mg^{2+}]_o$ - induced cell death (3 ± 6 % cell death). CGS 19755 blocked the slow depolarization upon which the action potential burst lay, blocked a slow inward current of comparable duration (n=10) and reduced $[Ca^{2+}]_i$ spike amplitude by 76 ± 10 % and frequency by 68 ± 13 %. These data suggest that a specific component of the aberrant electrical activity elicited by 0.1 mM $[Mg^{2+}]_o$ preferentially contributes to cell death rather than the integrated somatic response represented by the somatic $[Ca^{2+}]_i$ spike.

Supported by the NIH and the NSF.

505.3

INTRACEREBRAL NMDA INJECTION STIMULATES PRODUCTION OF INTERLEUKIN-1β (IL-1β) IN NEONATAL RAT BRAIN. P. Hagan*, S. Poole, A.F. Bristow, F. Tilders and F.S. Silverstein. Depts. of Pediatrics and Neurology, Univ. Michigan, Ann Arbor, MI, USA; NIBSC, Potters Bar, Herts. UK and Dept. of Pharmacology, Free University of Amsterdam, Netherlands.

Two lines of evidence suggest that IL-1β is a mediator of excitotoxic injury in the developing brain. Intra-cerebral NMDA injection in 7 day old (P7) rats stimulates IL-1β mRNA expression acutely (Stroke 26:1995); adenovirus-mediated over-expression of IL-1 receptor antagonist (which blocks the biological actions of IL-1β) in the brain, confers resistance to NMDA-induced injury (Hagan *et al*, Neuroscience, in press). The goal of this study was to evaluate the duration, magnitude, and distribution of IL-1β production induced by intra-striatal injection of NMDA (10 nmol) in P7 rats. We used a rat-specific IL-1β ELISA to quantify tissue homogenate IL-1β content and immunocytochemistry (ICC) using a monoclonal anti-rat IL-1β antibody to visualize IL-1β distribution in the brain. At 2h post-lesioning, IL-1β increased 3-fold ($p<0.001$, t-test) over basal levels; concentrations peaked at 6h. Treatment with MK-801(1mg/kg), a non-competitive NMDA antagonist, blocked NMDA-induced IL-1β production. ICC demonstrated that at 6h post-injury IL-1β was concentrated in neurons and ependyma of the lesioned hemisphere. These data provide direct evidence that excitotoxic injury stimulates IL-1β production *in vivo*.

Supported by a grant from the Hearst Foundation (to FSS).

505.4

EXCITOTOXIC NEURONAL DAMAGE IN ISOLATED RETINAE INVOLVES *DE NOVO* NEUROSTEROID SYNTHESIS. P. Guarneri[a*], C. Cascio[b], D. Russo[c], G. De Leo[d], F. Piccoli[b] and R. Guarneri[a]. [a]IBS and [c]ISMEDA, CNR; [b]Inst. Neuropsych. and [d]Inst. of Biology, University of Palermo, Italy.

Isolated retinae have the ability to synthesize pregnenolone (P5) and other neurosteroids. The cytochrome P450scc enzyme responsible of P5 formation is mainly located in retinal ganglion cells where a tonic GABAergic control regulates their steroidogenic activity.

It has been observed that following metabolic stress (ischemia, hypoglycemia, hypoxia), the activation of NMDA receptors leading to retinal degeneration is associated with GABA release from amacrine cells. Moreover, neurosteroids have been shown to modulate NMDA-gated Ca^{2+} currents. In the present study, we examined whether neurosteroid synthesis may be involved in NMDA-mediated neurotoxic events. When isolated retinae were exposed to 1-100 μM NMDA, P5 and P5 sulfate synthesis was increased. The histological evaluation of the retinal damage revealed that the maximum stimulatory effect on neurosteroid synthesis (50μM NMDA for 30 min) corresponded to an edematous swelling and nuclear pyknosis of neuronal cells in the inner nuclear layer and few cell bodies in the ganglion cell layer. 10 μM MK 801 or 200 μM CPP reverted NMDA effects on the activated neurosteroid synthesis and retinal cytology. Similarly, a reduction of these effects was reported after the addition of a $GABA_A$ receptor antagonist, bicuculline (1-100 μM), or 4,4`-diisothiocyano-2,2`-stilbenedisulfonate (400 μM), an anion channel blocker. When isolated retinae were incubated with P5 sulfate (10 μM), cytopathological reactions were observed which closely resemble the excitotoxic damage following NMDA exposure. The results suggest that (i) NMDA receptor stimulation increases neurosteroid synthesis, (ii) this effect is mediated by $GABA_A$ receptors, and (iii) neurosteroids newly formed (i.e. P5 sulfate) are implicated in retinal neurotoxicity.

505.5

MECLOFENAMATE BLOCKS NMDA RECEPTOR-MEDIATED NEUROTOXICITY IN CORTICAL CELL CULTURES. S.J. Hewett*[1], L.L. Dugan[2], S.P. Yu[2], L.M.T Canzoniero[2], S.L. Sensi[2] and D.W. Choi[2] [1]Dept of Pharmacol, Univ. CT Health Center, Farmington, CT 06030; [2]Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Previous studies have suggested that free radicals may be important downstream mediators of excitotoxic neuronal death. One plausible source of free radical generation in neurons exposed to excitotoxins is lipid degradation initiated by Ca^{2+}- activation of phospholipase A_2, followed by cyclooxygenase and lipoxygenase pathway metabolism. We set out to determine if inhibition of cyclooxygenase/ lipoxygenase pathways would attenuate excitotoxic death in murine cortical cell cultures containing both neurons and astrocytes.

We turned to the dual cyclooxygenase/lipoxygenase pathway inhibitor, meclofenamate sodium, used clinically as a non-steroidal anti-inflammatory drug. Neuronal death induced by brief intense exposure to NMDA (200 μM for 5 min) was concentration-dependently decreased by addition of 30-300 μM meclofenamate. Electrophysiological and $^{45}Ca^{2+}$ flux study indicated that meclofenamate was not an NMDA antagonist. Furthermore, KCl-induced increases in intracellular free Ca^{2+} were unaffected by meclofenamate (300 μM), arguing that meclofenamate protection was not due to block of voltage-gated calcium channels. Several other inhibitors of cyclooxygenase or lipoxygenase pathways, did not match the strong protective effect of meclofenamate against rapidly-triggered NMDA receptor-mediated neurotoxicity. These results raise the possibility that meclofenamate might be therapeutically useful in neurological diseases associated with excessive NMDA receptor activation.

Supported by NIH NINDS grants NS 32636 (LLD) and NS 30337 (DWC).

505.6

NITROUS OXIDE, UNLIKE OTHER ANESTHETIC AGENTS, FAILS TO BLOCK MK-801 NEUROTOXICITY V. Jevtovic- Todorovic*, J. Labruyere and J.W. Olney. Depts of Anesthesiology and Psychiatry, Washington Univ Med School, St. Louis, MO.

MK-801 and other antagonists of NMDA glutamate receptors protect neurons against excitotoxic degeneration in conditions such as stroke or head trauma, but these agents also can cause neurotoxic side effects (vacuole formation) in posterior cingulate/retrosplenial (PC/RS) neurons of rat brain. Loss of GABAergic inhibition is a critical feature of the mechanism by which NMDA antagonists cause neurotoxic side effects. Therefore, various agents that facilitate $GABA_A$ neurotransmission, including benzodiazepines, barbiturates and anesthetics such as halothane, isoflurane and propofol, protect against these side effects. While it is desirable to prevent these effects, it is also useful to have anesthetic agents that do not prevent them. For example, in PET or MRI studies investigating the effects of NMDA antagonists on neurons in the brain of non-human primates, it is necessary to anesthetize the subjects with an agent that does not interfere with the $GABA_A$-linked mechanism of action of the NMDA antagonist. Nitrous oxide, an anesthetic that acts by an unknown mechanism, is not thought to facilitate $GABA_A$ transmission. In the present study we administered nitrous oxide to rats at several concentrations, the highest being 70%, at which concentration the rats were sedated and motionless with reduced response to paw pinch. This level of anesthesia was maintained for 4 hours following administration of MK-801 at a dose (0.5 mg/kg sc) that reliably produces a vacuole reaction in PC/RS neurons. Control rats received this dose of MK-801 without nitrous oxide. Four hours after MK-801 treatment, histological examination of the brains revealed that the control and experimental rats (n = 8 per group) sustained the same degree of injury to PC/RS neurons [217 ± 29 (SD) and 211.6 ± 21 (SD) vacuolated neurons per section in experimentals and controls respectively]. Thus, nitrous oxide is an anesthetic that does not interfere with the $GABA_A$ receptor-linked mechanism by which NMDA antagonists injure cerebrocortical neurons.

Supported by AG 11355, DA 05072 and RSA MH 38894 (JWO).

505.7

EXCITOTOXICITY INDUCED BY EXPRESSION OF NMDA RECEPTORS USING VACCINIA VIRUS IN HUMAN KIDNEY CELLS . J. Renart* M. Díaz-Guerra, M. García-Gallo, C. Moratilla, and M.M. Behrens. Instituto de Investigaciones Biomédicas-CSIC and Departamento de Bioquímica-UAM. 28029-Madrid. Spain.

In order to develop an *in vitro* excitotoxicity assay that could allow us to generate mutant cell lines resistant to glutamate, we are developing systems to overproduce or express the NMDA receptor subunits R1 and R2A in a regulated fashion.

By combining transient transfection with constructs containing different NMDA receptor subunits under T7 viral promoters and infection with recombinant vaccinia viruses expressing the T7 RNA polymerase we obtain high levels of expression of the NMDA receptor. In these conditions, NMDA-dependent cell death (estimated by cotransfection of a luciferase reporter gene) is only induced if both NR1 and NR2A subunits are cotransfected. Moreover, treatment of the cells with receptor antagonists (APV, MK 801) protects cells from excitotoxicity.

As a complementary approach, we are generating stable cell lines that express NMDA receptor subunits using a tetracycline-dependent system that allows regulation of the amount of protein produced.

Supported by grants PB93-0143 from CICYT-Spain and AE00287/95 from Comunidad Autónoma de Madrid-Spain

505.8

NMDA-INDUCED EXCITOTOXICITY IN CULTURED HIPPOCAMPAL SLICES. Prabu V. Ayyagari* and Fulton T. Crews. Center for Alcohol Studies and Department of Pharmacology, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC 27599-7178.

We describe a novel method to investigate NMDA-induced excitotoxicity in the hippocampus. Organotypic hippocampal slices were prepared from 7 day-old rat pups, placed on a membrane at the interface of air and medium and cultured for 7 days. Slices (350 μm) showed intact cytoarchitecture. Hippocampal slices were stained with Trypan Blue, a vital dye that is excluded by living and intact cells. Slices were periodically placed in phosphate buffered saline (PBS) containing 10% Trypan Blue at 37°C for 10 min. Slices were washed 5X in PBS and Trypan Blue staining was quantified using a light box, CCD camera and imaging software. The use of Trypan Blue staining as a measure of cell death was validated by parallel studies that estimated the enzyme Lactate dehydrogenase (LDH) in the medium, and by Hematoxylin Eosin (H&E) staining of the slices. To study excitotoxicity, slices were exposed to 100 μM NMDA at 37°C for 30 min. followed by a PBS wash and periodic staining and imaging. NMDA induced significant ($p < 0.01$) cell death in the slices as measured by Trypan Blue staining or LDH release, when compared to control slices exposed to PBS. NMDA-induced cell death was first observed at 4 hours post-treatment and progressed up to 24 hr post-treatment. NMDA-induced excitotoxicity was blocked by a 15 min. pre-treatment with the NMDA antagonist, MK-801. Organotypic brain slices obtained by this simple culture technique, coupled with the use of Trypan Blue staining as a measure of cell death, show great potential in toxicity studies and drug screening protocols. (Supported by NIAAA-AA06069).

505.9

TRANSIENT EXPOSURE TO N-METHYL-D-ASPARTATE (NMDA) INDUCES DELAYED NEURONAL DEATH THROUGH APOPTOSIS IN EXPLANT CULTURE OF RAT HIPPOCAMPUS. K. Lee, R. A. Wilson, J. L. Ross, C. Shin*, Departments of Neurology, Pharmacology and Molecular Neuroscience, Mayo Foundation, Rochester, MN 55905

Excitotoxicity is thought to be a critical mechanism in neuronal injury from a variety of insults including ischemia, trauma and epilepsy. The mode of neuronal death subsequent to the overstimulation of glutamate receptors may take the form of necrosis or a programmed cell death such as apoptosis. We hypothesized that the severity of excitotoxic insult may determine the type of neuronal death, and that delayed neuronal damage is through apoptosis.

Hippocampal explant cultures prepared from 6-10 day old rats were exposed to low (50 μM) and high (1 mM) NMDA for 30 minutes at 10 DIV. The cultures were then processed at 3, 18, 24, 48 and 72 hours for (1) gel electrophoresis of DNA; (2) TdT dUTP nick end labeling(TUNEL) staining; and (3) acridine orange/ethidium bromide staining.

In cultures exposed to 50 μM NMDA, DNA fragmentation was evident by TUNEL staining and DNA gel electrophoresis at 24, 48 and 72 hours, but not at 3 or 18 hours. Confocal microscopy with acridine orange/ethidium bromide staining showed nuclear chromatin condensation consistent with apoptosis at the corresponding time points. On the other hand, cultures exposed to 1 mM NMDA showed the pattern of necrosis within hours after the exposure with no evidence of DNA fragmentation.

The results demonstrate that moderate excitotoxic neuronal damage cause delayed neuronal injury through apoptotic process. More severe insult causes immediate necrosis. These findings may eventually lead to a therapeutic window to minimize neuronal damage in acute CNS insults.

[Supported by funding from Mayo Foundation.]

505.10

HYPOXIA INDUCES TYROSINE DEPHOSPHORYLATION OF NMDA RECEPTOR SUBUNITS AND SUPPRESSION OF *SRC*-FAMILY KINASE ACTIVITY. Y.T. Wang*, Jodi L. Braunton, Victor Wong and Wei Wang. Dept. Pathol., Hosp. Sick Children, Univ. Toronto, Toronto, Canada M5G 1X8.

Overactivation of the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor has been implicated in brain hypoxia-induced neuronal injury. Because NMDA receptors are phosphorylated and regulated by protein tyrosine kinases (PTKs) and PTK activity can be altered during hypoxia, in the present study we investigated whether the level of tyrosine-specific phosphorylation of NMDA receptor subunits is altered by hypoxic stimulation. Acute hypoxia was simulated *in vitro* by incubating rat hippocampal slices in glucose-free artificial cerebrospinal fluid saturated with 95% N_2 and 5% CO_2. Protein tyrosine phosphorylation was analyzed by immunoblotting of slice homogenates. Hypoxic challenge induced a marked decrease in the level of tyrosine phosphorylation of several proteins including a sharp band migrating at 180 kDa. The effect was partially blocked by dephostatin, a membrane-permeable inhibitor of protein tyrosine phosphatases (PTPs), but was not affected by the presence of glutamate receptor antagonists, nor by removing extracellular Ca^{2+}. Immunoprecipitation and Western blot confirmed that the 180 kDa tyrosine phosphorylated protein is the 2A/2B subunit(s) of the NMDA receptor, and that upon hypoxic challenge its level of phosphorylation was decreased. Enzyme assays showed that hypoxia resulted in a selective reduction in *src*-related PTK activity without affecting PTP activity. Thus the present work demonstrates that 2A/2B subunit(s) of NMDA receptors is tyrosine-dephosphorylated as a result of decreased *src*-family PTK activity under hypoxic conditions and such a change in receptor phosphorylation states may be important for altering NMDA receptor function during brain hypoxic insults. (Supported by Canadian MRC and Fealdman Fund.)

505.11

NMDA RECEPTOR ANTAGONISTS REVEAL DIFFERENCES IN NMDA- AND GLUTAMATE-INDUCED CALCIUM FLUXES AND NEUROTOXICITY IN VARIOUS BRAIN NEURONS. A. Wägner*, G. Cebers and A. Kalda, Dept of Clinical Neuroscience, Karolinska Institute, Karolinska Hospital, S-17176 Stockholm, Sweden.

Transient periods of ischemia induce excessive release of the excitatory neurotransmitter glutamate followed by neuronal cell death both in in vitro cell cultures and in vivo in the living brain. Accumulated evidence suggests that the release of glutamate triggers a marked inflow of Ca^{2+} into the postsynaptic neurons, an event, which is considered to be involved in glutamate-produced neurotoxicity. In this study we compared the effects of various NMDA receptor antagonists, CGS19755 (Selfotel), CGP39551, and CGP37489 on NMDA/glutamate-induced functional Ca^{2+} responses ($^{45}Ca^{2+}$ and $[Ca^{2+}]_i$) and neurotoxicity (LDH activity) in primary cultures of rat cerebellar and cortical brain neurons. Our data indicate that although all the NMDA receptor antagonists were potent inhibitors of NMDA/glutamate-produced Ca^{2+} responses and neurotoxicity, there were marked differences in their potency between the two types of brain neurons. The observed interneuronal differences in the neuroprotective properties of these agents may be relevant for the observed variations of these drugs in the treatment of ischemia-induced brain lesions in the rat and for the clinical usefulness of these agents in the management of brain damage induced by ischemia in stroke patients.

505.12

ETHAMBUTOL MEDIATED OPTIC NEUROPATHY IS MEDIATED THROUGH PERTURBATION OF MITOCHONDRIAL CALCIUM E. B. Dreyer*, C. Vorwerk, and J. E. Heng, Massachusetts Eye & Ear Infirmary & Harvard Medical School, Boston, MA, 02114

Ethambutol is a mainstay in the management of tuberculosis and other mycobacterial infections. Although its ability to cause optic neuropathy and visual loss is well known, a mechanism has not heretofore been established. We have established that ethambutol is directly toxic to retinal ganglion cells *in vitro*, and that this toxicity is mediated through the NMDA receptor. Furthermore, this toxicity appears to involve a perturbation of the mitochondria's ability to buffer cellular calcium levels. Other investigators have demonstrated that the ability of mitochondria to buffer calcium is critical for the cell to resist an excitotoxic challenge. Dissociated neonatal rat retinal ganglion cells, previously labeled with the mitochondrial sensitive dye rhodamine-2, were incubated with ethambutol. Sister cultures were also labeled with the calcium sensitive dye calcium green. Ethambutol caused an approximate doubling in calcium-dependent rhodamine-2 fluorescence over a period of 3-4 minutes. Ethambutol did, however, cause a decrease in calcium green fluorescence, suggesting a decline in cytosolic calcium. Both changes could be blocked with either rotenone or FCCP. These results indicate that ethambutol toxicity is most likely mediated through a perturbation in mitochondrial calcium homeostasis. Supported by the Lions, NIH, Potts & Glaucoma Fnds.

505.13

GLUTAMATE-INDUCED DEPOLARIZATION OF THE MITOCHONDRIAL MEMBRANE IN CULTURED CENTRAL NEURONS. DEPENDENCE ON Na^+. O.V.Vergun, T.P.Storozhevikh, N.P.Vinskaya, V.G.Pinelis and B.I.Khodorov*, Inst. of Gen. Pathology, Moscow 125315, Russia, Inst. of Pediatrics, Moscow117963, Russia.

Changes in mitochondrial membrane potential were monitored in individual cerebellar granule cells loaded with rhodamine 123 (Rh123); an increase in its fluorescence was considered as a result of mitochondrial depolarization (MD). A prolonged 10-15-min treatment of the culture by 100 μM glutamate (GLU) (0 Mg^{2+}, 10 μM glycine) induced in most of cells relatively small MD: the Rh 123 fluorescence increased in these cells on the average by about 20% from control.This GLU-induced MD could be greatly (on the average by about 100%) increased by replacement of Na^+ in the GLU solution by N-methyl-D-glucamine (NMDG). Returning of the cell to the Na^+-containing GLU solution led to a partial repolarization of the mitochodrial membrane. Replacement of Na^+ by Li^+ did not enhance the GLU-induced MD. The latter both in Na^+- or NMDG- containing solutions was effectively prevented by removal of external Ca^{2+}. Control experiments with fura-2-loaded cells showed that a prolonged 100 μM GLU application induced about a 10-fold increase in $[Ca^{2+}]_i$ which did not undergo appreciable changes after replacement of Na^+ with NMDG. Therefore the observed effects of external Na^+ removal on the GLU-induced MD were interpreted as a result of $[Na^+]_i$ lowering which is known to inhibit the mitochondrial Na^+/Ca^{2+} exchange system. At high $[Ca^{2+}]_i$ this inhibition should promote the mitochondrial Ca^+ overload culminating in the permeability transition pore opening and mitochondrial depolarization.This process is apparently promoted by an additional cytoplasmic acidification induced by replacement of Na^+ with NMDG in the GLU-containing medium. (Supported by RFFI and ISF).

505.14

MITOCHONDRIAL REGULATION OF NMDA RECEPTOR-MEDIATED CALCIUM ENTRY AND EXCITOTOXICITY. S.L. Budd* and D.G. Nicholls. Neurosciences Institute, Department of Pharmacology, University of Dundee, Dundee, Scotland, U.K., DD1 9SY.

Neuronal death is caused by Ca^{2+} influx after intense NMDA receptor activation, but the role of Ca^{2+} in the initial intracellular events following an excitotoxic insult remain unclear. This series of experiments sought to clarify the role of mitochondria during NMDA receptor activation in cerebellar granule cells cultured from rats.

Acute exposure of cells to 100μM NMDA in the absence of Mg^{2+} causes calcium loading of mitochondria. Mitochondrially accumulated calcium can then be released following exposure to protonophore. Furthermore, fluorescent monitoring of the mitochondrial membrane potential with rhodamine-123 shows a partial depolarisation of the mitochondria after addition of NMDA.

Exposure of cells to glutamate for 1h results in cytoplasmic Ca^{2+} deregulation and collapse of the cellular ATP/ADP ratio; extensive cell death is seen after just 5h. The mitochondrial respiratory chain inhibitor rotenone (5μM), potentiates Ca^{2+} deregulation by forcing the mitochondrial ATP synthase to reverse in order to support the mitochondrial membrane potential. Cells maintained by glycolytic ATP in the presence of the mitochondrial ATP synthase inhibitor oligomycin also show a cytoplasmic Ca^{2+} deregulation and considerable cell death in response to glutamate indicating that prevention of ATP synthase reversal alone is not neuroprotective. In contrast collapse of the mitochondrial membrane potential and hence mitochondrial Ca^{2+} transport by rotenone plus oligomycin confers complete protection against glutamate excitotoxicity. Thus excitotoxicity is associated with mitochondrial Ca^{2+} accumulation. Cells with depolarised mitochondria maintain a low cytoplasmic free Ca^{2+} concentration in the presence of glutamate and accumulate little $^{45}Ca^{2+}$; this is consistent with a Ca^{2+}-mediated feedback inhibition of the NMDA receptor which can be overridden by mitochondrial Ca^{2+} uptake. (SLB is the recipient of a Medical Research Council studentship.)

505.15

PHARMACOLOGY OF THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE (mPTP) IN CULTURED ASTROCYTES AND NON-SYNAPTOSOMAL MITOCHONDRIA. J.M. Dubinsky and B.S. Kristal, Physiology Departments University of Minnesota and University of Texas Health Science Center.

The mPTP, associated with hypoxic injury in liver and heart, was studied in 2 preparations. In non-synaptosomal mitochondria isolated from adult rat CNS, decreases in the A_{450} reflect mitochondrial swelling. In digitonin permeabilized, cultured astrocytes mitochondria were observed microscopically at 530nm with the dye jc-1. Ca-mediated mPTP induction was stimulated by phosphate or phenylarsine oxide but not by tert-butylhydroperoxide. Cyclosporin A (CsA), an inhibitor of liver mPTP, slowed PTP activation in isolated CNS mitochondria. Mitochondrial swelling in astrocytes was only partially prevented by high μM doses of CsA. In both systems, PTP opening slowed substantially in the presence of ADP or ATP. In combination with 1μM CsA, ADP more fully inhibited PTP induction. Ruthenium red (RuR, 10μM), an inhibitor of mitochondrial Ca^{2+} uptake, prevented Ca^{2+} induced astrocyte PTP opening. The RuR block was potentiated by addition of 10μM of the Na/Ca exchange inhibitor CGP37157. RuR and CGP37157 were ineffective in 4BrA23187 treated astrocytes where Ca^{2+} entry was unregulated. Monobromobimane, a thiol crosslinker, prevented mPTP opening in response to oxidants. Supported by the MN Medical Foundation and the MN American Heart Association.

505.16

ALS-LIKE DEGENERATION OF MOTONEURONS INDUCED BY SYSTEMIC ADMINISTRATION OF L-GLUTAMATE IN THE RAT. M. Ishida*, Y. Uyama, T. Saitoh, H. Suzuki and H. Shinozaki. Dept. Pharmacol., The Tokyo Met. Inst. Med. Sci., Tokyo 113, Japan

L-Glutamate has been implicated in the pathogenesis of neuronal damage in the mammalian central nervous system. Systemic administration of high doses of L-glutamate to neonatal rats or mice induces obesity with selective neuron damage in the arcuate nucleus of the hypothalamus and the retina. Recently we found that repeated subcutaneous injection of L-glutamate (4 mg/g/day) in the first 5 - 6 days of life caused irreversible paraplegia in the rat, accompanied by severe muscular atrophy and urinary incontinence. The first signs of parapregia usually begin within 5 days of the first administration of L-glutamate, although, for unknown reason, the incidence of paraplegia was very low (less than 4%) and was not constant. Paraplegic rats did not respond to nociceptive stimuli to lower parts of the body, such as the hindlimbs, the back and the tail. Spinal reflexes, recorded extracellularly from the L3 - L5 ventral roots of 7 day-old paraplegic rats, and induced by electrical stimulation of the dorsal root of the same segment, were considerably decreased, but were still detectable. The cytoarchitecture of the lower spinal cord of the paraplegic rat was much disordered. In the paraplegic rat, ventral roots in lower parts of the spinal cord showed marked atrophy, and the cells of the ventral horn apparently decreased in number. Microscopic observation revealed that the decrease in number of motoneurons first became noticeable about 5 weeks after birth, and developed until 9 - 15 weeks, when most of the motoneurons had disappeared. However, dorsal roots and dorsal ganglion cells in paraplegic rats were intact. The paraplegic rat should provide a useful animal model for elucidating the mechanism underlying certain neurological diseases, in particular, amyotrophic lateral sclerosis (ALS).

505.17

MECHANISMS OF 3-NITROPROPIONIC ACID TOXICITY IN PRIMARY CULTURES OF STRIATAL NEURONS. J.G. Greene*, S-S. Sheu, R.A. Gross, and J.T. Greenamyre University of Rochester Medical Center, Rochester, NY 14642, and Emory University, Atlanta, GA, 30322.

We have examined the effects of the succinate dehydrogenase (SDH) inhibitor 3-nitropropionic acid (3NP) on neuronal survival, ATP content, and N-methyl-D-aspartate (NMDA)-induced cytosolic calcium concentration ($[Ca^{2+}]_c$) increases in cultured rat striatal neurons. 3NP caused concentration-dependent ATP depletion and neuronal death following 24 hour incubation. The NMDA antagonist MK-801 prevented the neurotoxicity, but not the ATP depletion, caused by 3NP. Pretreatment of cultures with 3NP (3 mM) for 3 hours did not cause toxicity, but reduced ATP content by 35%. This pretreatment paradigm synergistically enhanced NMDA toxicity, and this enhancement remained pronounced in Mg^{2+}-free solutions. Pretreatment with 3NP did not affect baseline $[Ca^{2+}]_c$ as determined by fura-2 microfluorimetry. NMDA (30 μM) caused a relatively rapid rise in $[Ca^{2+}]_c$, the magnitude of which was not affected by 3NP. However, after a 1 hour treatment, $[Ca^{2+}]_c$ was dramatically higher in 3NP-treated neurons. This increased calcium load was washed out slowly and only partially, although calcium in control neurons washed out rapidly and almost completely. These results suggest that in striatal cell culture, the enhancement of NMDA toxicity caused by SDH inhibition may be caused predominantly by disruption of neuronal calcium regulation. *(Supported in part by an Individual MD/PhD NRSA (JGG), a Mallinckrodt Scholar Award (JTG), PHS grant AG11755, and the Lucille P. Markey Foundation.)*

505.18

THE NEUROPROTECTIVE EFFECTS OF NMDA ANTAGONISTS IN THE *SPASTIC* HAN WISTAR MUTANT. K.L. Brunson*, A. Khanna, A. Martinez, and R.W. Cohen. Department of Biology, California State University, Northridge, CA. 91330.

We are studying *spastic* Han-Wistar (HW) mutant rats which develop neurodegeneration in two brain regions (hippocampus and cerebellum). Previous data suggest that the neuronal degeneration is the result of an alteration in glutamate receptor expression. This work is the first of a two-part study of the glutamate receptors in the HW mutant. Here, we analyze the neuroprotective effects of two NMDA antagonists, MK-801 and Ketamine. Mutants were injected (in a two week interval) with either Ketamine (10 mg/Kg) twice a week or MK-801 (1 mg/Kg) once a week starting at 30 days. Both MK-801 injected mutants (n=12) and Ketamine injected mutants (n=6) exhibited longer life spans (27% increase and 10% increase, respectively) compared to saline injected mutants (controls; n=27). Motor skill deterioration was monitored in an open-field test for two minute sessions. After 50 days of age the MK-801 injected mutants displayed 20% greater motor skill activity in the open field test than the controls. Ketamine mutants showed a 39% increase. The neuroprotective effects in the cerebellum were observed with cresyl violet staining of the Purkinje cells. In MK-801 injected mutants there was a 38% increase in Purkinje cells compared to controls. Staining in the hippocampus showed a slight increase in CA3 pyramidal cells in the MK-801 injected mutants as compared to controls. An increase in spatial learning ability of MK-801 mutants was assessed in a water maze. MK-801 injected mutants (n=3) made 13.3% less errors finding the platform than the controls (n=2), corresponding to the observed pyramidal cell survivorship. The data show that mutants derive protective effects from NMDA antagonists, suggesting a role of glutamate-induced excitotoxicity in neuronal degeneration of our model. This work was supported by CSUN Foundation grants to KLB and RWC.

505.19

THE NEUROPROTECTIVE EFFECTS OF NON-NMDA ANTAGONISTS IN THE *SPASTIC* HAN WISTAR MUTANT. A.A. Nisim*, M.T. Martin, M.A. Abdullah and R.W. Cohen. Department of Biology, California State University, Northridge, CA. 91330.

Associated excitotoxicity due to altered glutamate receptor expression has been attributed to neuronal degeneration in many human disorders. The *spastic* Han Wistar (HW) rat, a possible model of glutamate excitotoxicity, exhibits hyperactivity and fore limb tremor as early as 25 days postnatally. Previous studies with this mutant have shown a progressive loss in Purkinje cells in the cerebellum which is manifested by progressive hind limb paresis and ataxia, culminating in death at approximately 65 days of age. In this study, possible neuroprotection derived from the administration of the AMPA/Kainate receptor antagonist GYKI 52466 hydrochloride (GYKI) in *spastic* HW rats was examined. Starting at 30-32 days of age, the mutants were injected with GYKI (15 mg/Kg) twice a week for three weeks. GYKI-injected mutants (n=6) exhibited increased life span (15%) compared to saline-injected mutants (controls; n=7). GYKI-treated animals showed prolonged weight gain (7% heavier after 45 days of age; 11% heavier after 60 days of age) compared to controls. Motor skill deterioration was monitored by an open-field test which lasted for two minutes. At 50 days, GYKI-treated mutants displayed 24% greater motor skill activity compared to controls. GYKI-treated mutants displayed an improved limb strength as indicated by prolonged periods of hanging on an inverted screen compared to controls at 40 days (23% increase) and 50 days (52% increase). These findings suggest that non-NMDA receptors play a significant role in the observed neurodegeneration in this rat. Supported by a CSUN Research and Sponsored Projects Grant.

506.1

CHANGES IN PHF- AND GFAP- LIKE IMMUNOHISTOCHEMISTRY FOLLOWING SYSTEMIC KAINIC ACID INJECTIONS IN RATS. JB Pan *, AL Nikkel, L Bednarz and KE Asin. Neuroscience Discovery, Abbott Laboratories, D-47U, AP9A, Abbott Park, IL 60064.

We have examined limbic and cortical pathologic changes in rats systemically injected with kainic acid (KA) by using anti-glial fibrillary acidic protein (GFAP) and anti-paired helical filament (PHF) antibodies.

Systemic injections of kainic acid (10 mg/kg, sc) induced wet dog shakes, tremors and seizures. Rats were sacrificed by perfusion with buffered 4% formaldehyde three, ten, or thirty days post-injection. The brains were sectioned from anterior to posterior at 35 um increments and collected serially. Standard immunocytochemical techniques were used to assess GFAP or PHF expression in cortical and limbic regions.

In rats that survived three days, there was severe tissue damage in the amygdaloid complex. Gross cellular damage that was evident at three days was partly replaced by astroglial cells in the rats sacrificed at ten days. As a consequence of the KA-induced prolonged neuronal depolarization, swelling and neuronal death, resting astrocytes became reactive. Astrocytes within the entorhinal cortex were most obviously involved in these changes. The astrocytes still showed enlarged cell bodies and more thickened processes in rats at ten days post-injection. Interestingly, anti-PHF labeled, degenerating neurites evident in consecutive brain sections from ten-day rats, were no longer evident in sections from thirty-day rats. At thirty days, GFAP+ astrocytes were observed in the neurodepleted area

The systemic administration of kainic acid induces not only neuronal damage, as evidenced by increased PHF-like immunoreactivity, but also leads to astrocytic activation. These phenomena may provide a useful animal model for drug discovery efforts in the search for neuroprotective agents.

[Supported by Abbott Labs.]

506.2

GLUTAMATE REGULATES PHOSPHORYLATION OF NEUROFILAMENT HEAVY SUBUNITS BY AMPA/KAINATE RECEPTORS IN RAT SPINAL CORD CULTURES. N. Vartiainen*, K. Kaasinen and J. Koistinaho. A.I. Virtanen Institute, University of Kuopio, P.O.B. 1627, FIN-70211 Kuopio, Finland.

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease of motor neurons characterized by a slow loss of upper and lower motor neurons. ALS is largely a sporadic disease, with only 10% of cases being of the familiar form. Increasing evidence suggests that excitotoxicity, autoantibodies to L-type voltage-gated calcium channels (VGCC) and abnormal neurofilament (NF) function contribute to motor neuron loss in the sporadic ALS (sALS). Here we provide immunohistochemical evidence linking the function of kainate (KA) receptors and VGCC to hypophosphorylation of NF heavy subunits in spinal cord (SC) neurons.

SC neurons from 14-day old rat embryos were cultured for 10-14 days and processed for immunocytochemistry or Western blotting using monoclonal antibodies recognizing either phosphorylated (pNF-H), nonphosphorylated (nNF-H) or both forms of NF heavy subunits. A 24-h exposure to 2-20 uM KA induced dose-dependent increase in the number of pNF-H neurons; this induction was detectable even following a 3-day exposure to KA. Glutamate (20 uM) dramatically induced NF-H phosphorylation, whereas trans-(1S,3R)-ACPD (20-100 uM) a metabotropic receptor agonist, and NMDA (10 uM) were without clear effects. The KA-induced NF-H dephosphorylation was blocked by CNQX (100 uM), a KA/AMPA-receptor antagonist, and partially prevented by nifedipine, an L-VGCC blocker. The glutamate-induced NF-H phosphorylation was reversed when glutamate (20 uM) was administered in the presence of MK-801 (10 uM), an NMDA-receptor antagonist.

The results indicate that chronic exposure to excitotoxins leads to NF-H dephosphorylation in SD neurons specifically via KA receptors. The alteration in the NF-H phosphorylation involves active L-VGCC. Dephosphorylation enhances the susceptibility of NFs to degradation representing an early change in neuronal injury.

506.3

KAINIC ACID INDUCED BEHAVIORAL SYNDROME: CHALLENGE WITH NICOTINIC RECEPTOR LIGANDS. R.D. Shytle*, C.V. Borlongan, and P.R. Sanberg. Div. of Neurological Surgery, Dept. of Surgery, Neurology, Psychiatry and Pharmacology, Univ. of South Florida Coll. of Med., Tampa, FL 33612-4799.

Systemic administration of kainic acid (KA) in rats results in a behavioral syndrome characterized by wet dog shakes (WDS), seizures and convulsions. While a number of drugs have been found to either potentiate or prevent this KA-induced syndrome, little is known about the possible influence of cholinergic nicotinic receptor ligands. In the present study, each rat was placed in an open field and pretreated with either saline, nicotine base (0.25-0.5 mg/kg), mecamylamine (0.3-3.0 mg/kg), or lobeline (1.0-10.0 mg/kg) s.c. followed 15 min. later by 12.0 mg/kg KA s.c. Experimenters blind to the pretreatment conditions counted the number of WDS and scored the occurrence of seizures occurring between 45-180 min. post-KA injection. In contrast to our previous findings under home cage testing conditions, rats pretreated with nicotine exhibited no differences in WDS or seizures relative to rats pretreated with saline. Moreover, mecamylamine, at doses thought to be selective in blocking nicotinic receptors, had no effect on KA-induced WDS or seizures. However, lobeline dose-dependently abolished both KA-induced WDS and seizures. These findings indicate that the testing environment influences the behavioral effects of KA and suggests that lobeline may have neuroprotective properties in animal models of excitotoxicity. (Supported, in part, by grants provided by NINDS, TSA, and the USF President's Council).

506.4

AMPA INDUCES TWO TYPES OF TOXICITY OF CEREBELLAR PURKINJE CELLS (PCs) IN THE SLICE PREPARATION. Jean C. Strahlendorf and Howard K. Strahlendorf*♣. Departments of Physiology & Pharmacology♣. Texas Tech University Health Sciences Center, Lubbock, TX 79430.

PCs are selectively vulnerable to AMPA-induced damage, evincing a type of delayed neurotoxicity referred to as dark cell degeneration (DCD) that morphologically resembles apoptosis. The appearance of DNA laddering produced by nucleosomal breakdown is considered a biochemical hallmark of apoptosis. In cerebellar slices, AMPA (30 μM, 30 min exposure followed by 90 min recovery) induced an early (within 15 min) appearance of DNA laddering as assessed by gel electrophoresis. In addition, aurintricarboxylic acid, which decreases Ca^{2+}-dependent endonuclease activity, and cycloheximide, a translational inhibitor, were effective in attenuating AMPA-induced DCD of PCs, further suggesting that DCD may involve a type of programmed cell death. Studies are currently utilizing Hoechst 33258, a fluorescent dye that reveals nuclear condensation/aggregation within apoptotic cells and in situ DNA end nick labeling to detect DNA damage in PC nuclei. AMPA also produces edematous damage (ED) in approximately one third of PCs. Cyclothiazide and diazoxide, two antagonists of AMPA receptor desensitization, reversed DCD and unveiled a fulminating ED. Studies have addressed the ionic nature and pharmacological sensitivity of AMPA-induced ED. These experiments indicate that AMPA can elicit more than one type of degeneration in the same neuronal population and a primary determining factor for the type of toxicity expressed may be AMPA receptor desensitization. Supported by National Ataxia and Whitehall Foundations.

506.5

DOWNREGULATION OF GluR2 mRNA IN GERBIL HIPPOCAMPAL CA1 NEURONS IS ASSOCIATED WITH AN INCREASE OF AMPA RECEPTOR MEDIATED CYTOSOLIC Ca^{2+} AT A LATE POST-ISCHEMIC STAGE. J. A. Gorter*, J.J. Petrozzino#, E. Aronica, D. Rosenbaum, M.V.L. Bennett, J. A. Connor#, R.S. Zukin. Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461. #Roche Institute of Molecular Biology, Roche Research Center, 340 Kingsland Street, Nutley, NJ, 07110.

A transient ischemic episode can result in an extensive delayed death of hippocampal pyramidal CA1 neurons. We tested whether Ca^{2+} permeable (GluR2 subunit lacking) AMPA receptors could be a contributing factor to neuronal cell death in the 2-vessel occlusion model of global forebrain ischemia in the gerbil. *In situ hybridization* revealed that GluR2 mRNA is specifically downregulated in the CA1 region but with a slower time course than previously described after 10 minute four-vessel occlusion in the rat (Pellegrini-Giampetro et al., 1992). Intracellular calcium levels ($[Ca^{2+}]_i$) in individual CA1 pyramidal cells in dorsal hippocampal slices were measured using *optical imaging* techniques with fura-2 as Ca^{2+} indicator. With AP-5 (50 μM), MK-801 (20 μM), TTX (3 μM) and Cd^{2+} (100 μM) added in the bathing solution (solution A), a CA1 pyramidal neuron displayed low resting $[Ca^{2+}]_i$ in control slices and in 48 and 72 hrs postischemic slices (50-100 nM). Slices were changed to solution A + AMPA (30 μM) and cyclothiazide (10 μM, to prevent desensitization of AMPA receptors). In control and 48 hrs post-ischemic CA1 neurons, AMPA receptor stimulation did not cause a noticeable Ca^{2+} change. In 72 hrs post-ischemic neurons however, a significant increase was observed. The $[Ca^{2+}]_i$ increased to a mean somatic value of 257±66 nM. These findings suggest that Ca^{2+} permeable AMPA receptors play a role in the mechanisms of delayed neuronal cell death following global ischemia.

(J.A.G. was supported by a HFSPO grant LT522/94).

506.6

AMPA AND KAINATE RECEPTOR-MEDIATED APOPTOSIS AND NECROSIS IN CULTURED MURINE CORTICAL NEURONS. J. A. Larm, N. S. Cheung and P. M. Beart*. Dept. of Pharmacology, Monash Univ., Clayton, Vic 3168, Australia.

Glutamate receptor-mediated excitotoxicity occurs by over-activation of ionotropic glutamate receptors, particularly those of the NMDA subtype. This study investigated the neurotoxicity of AMPA and kainate (KA) in cultured cortical neurones, and the types of cell death involved. Primary cerebral cortical cultures were prepared from 15d fetal mice in chemically defined, serum-free medium. Immunocytochemistry using neuron-specific enolase and glial fibrillary acidic protein indicated that 95% of cells were neurons. At 6d cultures were exposed to AMPA or KA for 15 min to 24h in antioxidant-free maintenance medium. After a further 1-8h, cellular viability was examined by the MTT procedure, and DNA fragmentation was examined by both the TUNEL technique and agarose gel electrophoresis. AMPA and KA produced concentration-dependent excitotoxicity (EC_{50} values; 3 & 65 μM) with morphological features of necrosis, including neuronal swelling at 1h, and DNA fragmentation was not observed (8h). LY293558 attenuated the excitotoxicity produced by AMPA, while CNQX attenuated the actions of both agonists. AMPA and KA also produced cell death in cells exposed to fetal calf serum (10%, day zero, 24h) and cycloheximide-sensitive DNA fragmentation, but not neuronal swelling was observed. Excitotoxicity induced by non-NMDA receptors involves necrosis and apoptosis dependent upon experimental milieu. Supported by the NHMRC (Australia).

506.7

QUISQUALATE SENSITIZATION IN ANOXIC HIPPOCAMPAL SLICES. L.A. Chase* and J.F. Koerner. Department of Biochemistry, Medical School, University of Minnesota, Minneapolis, MN 55455.

Quisqualate sensitization describes a phenomenon in which rat hippocampal CA1 neurons become sensitized to the depolarizing effects of phosphonate analogs of glutamate only after the application of the non-NMDA agonist, quisqualic acid. We found that the potency of L-2-amino-6-phosphonohexanoic acid (L-AP6) for depression of the antidromic population spike is enhanced 6-fold in anoxic quisqualate-sensitized hippocampal slices incubated in the presence of 95% N_2/5% CO_2 (IC_{50} = 4 μM) in comparison with those which were oxygenated (IC_{50} = 24 μM). The rate of quisqualate uptake into hippocampal slices, measured by HPLC, does not change in anoxia compared with oxygenated conditions, suggesting that the enhanced potency of L-AP6 observed in anoxia is not a result of increased intracellular quisqualate concentration. Theophylline (200 μM) did not prevent the increase in L-AP6 potency observed in anoxic, quisqualate-sensitized slices suggesting that neither A1 nor A2 adenosine receptor activation caused this effect. Additionally, increasing the extracellular [K^+] had no effect on L-AP6 potency in oxygenated hippocampal slices. In contrast, the IC_{50} of L-AP6 in quisqualate-sensitized slices does appear to be related to the Nernst potential for Ca^{2+} (manipulated by changing the extracellular [Ca^{2+}]). We suggest that an increase in intracellular [Ca^{2+}] during anoxia may mediate the observed shift in L-AP6 potency in quisqualate-sensitized slices. Finally, in contrast to oxygenated slices, a 10 min exposure to 2 mM glutamate in anoxic slices partially reverses quisqualate sensitization. We hypothesize that glutamate is a more efficient competitive agonist at quisqualate uptake and release site(s) in anoxia due to the decreased rate of sodium dependent glutamate uptake under these conditions.

(Supported by NIH NS17944; LAC supported by a Shevlin Fellowship).

506.8

Zn^{2+} PERMEATION THROUGH Ca^{2+} PERMEABLE AMPA/KAINATE CHANNELS AND SELECTIVE NEURODEGENERATION. H.Z. Yin*, D.H. Ha and J.H. Weiss. Depts. of Neurology, and Anatomy and Neurobiology, U.C. Irvine, Irvine CA 92717.

Neurons possessing Ca^{2+} -permeable AMPA/kainate channels can be identified by a histochemical stain based on kainate stimulated Co^{2+} uptake (Co^{2+}(+) cells), and are unusually vulnerable to AMPA/kainate receptor-mediated injury. Among neurons which we have found to be highly kainate sensitive and to be Co^{2+}(+) are cortical somatostatin (SS), parvalbumin (PV) and SMI-32 (a neurofilament marker that labels subsets of pyramidal neurons) immunoreactive neurons, and basal forebrain cholinergic neurons (BFCN). The divalent cation, Zn^{2+}, is synaptically released at many excitatory synapses. In prior studies using fluorescent imaging of intracellular Zn^{2+} combined with Co^{2+} staining, we found that Zn^{2+} appears able to permeate Ca^{2+} permeable AMPA/kainate channels with relative ease.

We have now examined toxic effects of brief kainate and Zn^{2+} exposures on cortical SS, PV and SMI-32 neurons, and on BFCNs. Brief (5-15 min) exposures of neuronal cultures to 100 μM concentrations of either kainate or Zn^{2+} trigger little neuronal injury. However, with Zn^{2+} / kainate co-exposures, relatively little overall injury but substantial damage to cortical SS, PV and SMI-32 neurons, and to BFCNs is seen the next day.

Further studies aim to correlate Zn^{2+} and Ca^{2+} permeation through AMPA/kainate channels with AMPA/kainate subunit expression in hippocampal neurons. Many large neurons in hippocampal cultures, often with pyramidal morphology, are Co^{2+}(+), with prominent dendritic Co^{2+} staining. Labeling with antibodies to AMPA subunits reveals most of these neurons to lack staining with antibody to GluR2/3, and usually to be GluR4- or GluR1-positive. Fluorescent imaging reveals kainate to preferentially trigger Ca^{2+} or Zn^{2+} accumulation in these neurons. Entry of Ca^{2+} or Zn^{2+} through Ca^{2+} permeable AMPA/kainate channels of some central neurons may serve signalling functions and may contribute to degeneration under certain conditions. *Supported by NIH grant NS 30884 and by the Pew scholars program in the biomedical sciences.*

506.9

DIFFERENTIAL NEURAL VULNERABILITY TO AMPA/KAINATE RECEPTOR-MEDIATED INJURY: ROLES OF Ca^{2+} AND FREE RADICAL PRODUCTION. S.G. Carriedo*, H.Z.Yin, and J.H. Weiss. Depts. of Neurology and Psychobiology, U.C. Irvine, Irvine CA 92717.

Neurons possessing Ca^{2+} -permeable AMPA/kainate channels can be identified by a histochemical stain based on kainate stimulated Co^{2+} uptake (Co^{2+}(+) cells), and are unusually vulnerable to AMPA/kainate toxicity, probably reflecting rapid Ca^{2+} entry through these channels. We have previously found GABAergic neurons in cortical cultures (identified by GAD staining; "GAD(+) neurons") and putative motor neurons in spinal cultures (identified by staining with the neurofilament antibody SMI-32; "large SMI-32(+) neurons") to be Co^{2+}(+), and to show greater vulnerability than Co^{2+}(-) cells to AMPA or kainate injury. However, preliminary direct comparison of the vulnerability of these neurons to slow (24 h) AMPA/kainate toxicity suggests that the motor neurons are more vulnerable (10 μM kainate or 2.5 μM AMPA kill about 50% of large SMI-32(+) neurons but < 20% of GAD(+) neurons), supporting the possibility that factors downstream from Ca^{2+} entry are contributory. Tyrosine hydroxylase staining labels dopaminergic neurons in midbrain cultures (TH(+) neurons). In preliminary studies we find TH(+) neurons are Co^{2+}(-), yet are more vulnerable than most other Co^{2+}(-) neurons to slow AMPA/kainate injury, again suggesting a critical role of factors downstream from agonist triggered ion entry.

Toxic effects of free radicals may contribute to degeneration of motor neurons (in ALS) and dopaminergic neurons (in Parkinson's disease). In preliminary studies using the superoxide sensitive fluorescent dye, hydroethidine, we find kainate to preferentially trigger fluorescence increases in large SMI-32(+) neurons. Ongoing studies aiming to elucidate "downstream" mechanisms that may contribute to the high vulnerability of large SMI-32(+) neurons and TH(+) neurons to AMPA/kainate injury are using fluorescent imaging to see if these cells are relatively deficient their ability to maintain intracellular Ca^{2+} homeostasis, or show unusually high agonist triggered free radical production. *Supported by NIH grant NS 30884 (JHW) and by an American Psychological Association fellowship (SGC).*

506.10

CYCLOTHIAZIDE-INDUCED HIPPOCAMPAL INJURY IN IMMATURE RAT BRAIN. W. H. Trescher*. Kennedy Krieger Research Institute, Johns Hopkins Medical Institutions, Department of Neurology, Baltimore, MD 21205.

Cyclothiazide, a benzothiadiazide diuretic, selectively inhibits α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor desensitization. To assess *in vivo* cyclothiazide neurotoxicity, postnatal day (PND) 7 rats received an intrahippocampal injection of one of three doses of cyclothiazide (25 nmol - 250 nmol/0.5 μl). In a second experiment, single doses of chlorothiazide (250 nmol) and hydrochlorothiazide (250 nmol) were injected in the hippocampus. Injury was assessed on PND 14 by comparing regional cross-sectional areas of the dorsal hippocampi on coronal Nissl stained sections. In a third experiment, *in vitro* receptor autoradiography was used to evaluate the site of cyclothiazide activity. Cyclothiazide produced dose dependent hippocampal injury with 53.0 ± 4.3% damage after injection of 250 nmol/0.5 μl ($p < 0.001$). Injury produced by chlorothiazide and hydrochlorothiazide was not different than control. With receptor autoradiography, a single concentration of cyclothiazide (100 μM) reduced [^{3}H]AMPA binding in the dentate granule cell molecular layer, hilus, and CA3 and CA1 regions by 38% to 48% compared to control ($p < 0.001$). In contrast, cyclothiazide had no effect on [^{3}H]kainate binding. In summary, cyclothiazide alone produced hippocampal injury in the immature rat brain, likely through selective action at the AMPA receptor. The findings support the hypothesis that receptor desensitization may be a protective mechanism against overactivation of glutamate receptors. Supported by NIH NS01482

506.11

CORTICAL CELL CULTURES FROM A TRANSGENIC MOUSE MODEL OF FALS ARE MORE VULNERABLE TO AMPA AND KAINATE TOXICITY

F. Facchinetti[1,2], M.E. Gurney[4], T.M. Dawson[1,3] and V.L. Dawson [1,2,3].Depts. Of Neurology[1], Physiology[2] and Neuroscience[3]. Johns Hopkins School Of Medicine, Baltimore, MD 21287, Upjohn Lab[4]., Kalamazoo, MI 49001

Transgenic mice (line GH1) expressing a human SOD1 containing a gly^{93} -ala mutation develop a motoneuron disease similar to familial amyotrophic lateral sclerosis (FALS). To investigate a possible interaction between excitotoxicity and the FALS-SOD1 mutation we compared dose-dependent toxicity of glutamate receptor agonists in cortical cell culture derived from GH1 mice vs non-transgenic mice. The human mutated SOD-1 is expressed in primary neuronal cultures as assessed by western blot analysis. A more pronounced toxic effect of either kainate (12.5 μM) and AMPA (5 μM) is observed in cultures from GH1 mice when compared with controls. Dose-dependent toxicity to either glutamate and NMDA did not significantly differ in GH1 cultures from controls at the 3 concentrations tested (20, 100, 500 μM). Nitric oxide can react with superoxide to form the highly toxic peroxynitrite. Of the two NO donors, SNP and NOR-3, we observed a small but significant increase of SNP (10, 50 μM) toxicity in cultures from GH1 mice while NOR-3 toxicity is unchanged. Our results suggest a selective vulnerability to non-NMDA agonists of cortical cell cultures expressing a FALS-linked form of human SOD1.

Funded by Muscular Dystrophy Association

506.12

MECHANISMS OF KAINATE-INDUCED EXCITOTOXICITY IN RETINA: UNIQUE ROLE OF CHLORIDE. Q. Chen*, J. Olney, P. Lukasiewicz[1] and C. Romano[1], Dep't. Psychiatry and [1]Dep't. Ophthalmology, Washington Univ. Sch. Med. St. Louis, MO 63110.

Kainate is a powerful excitotoxin in isolated chick embryo retina. We demonstrated last year that this is due to non-desensitizing activation of AMPA-preferring glutamate receptors. Activation of AMPA receptors leads to a Na^+ or Na^+/Ca^{++} influx through cation channels, and the subsequent membrane depolarization may also lead to a Cl^- influx through anion channels. In the present study, the role of these ion influxes in excitotoxicity was investigated. Isolated chick retina (embryo 14-15 days) was exposed to kainate for 30 min, and cell damage was assessed 24 hours later by LDH assay. In retina exposed to kainate at 32 μM, Na^+ omission (Na^+ replaced by a non-permeable cation, choline) during the kainate exposure provided full protection, whereas Ca^{++} omission (Ca^{++} replaced by Mg^{++}, plus 100 μM EGTA) potentiated the neurotoxicity. However, neither Na^+ omission alone nor the combined Na^+/Ca^{++} omission reduced the more severe neurotoxicity by kainate at 320 μM. In contrast, kainate-induced neurotoxicity at both concentrations was completely prevented by replacing 75% of Cl^- with a non-permeable anion, methylsulfate. Consistent with these findings, whole cell voltage clamp studies in ganglion neurons of salamander retina demonstrated that Cl^- constituted a significant component of the whole cell currents induced by kainate, and that replacing extracellular Cl^- with methylsulfate modified the currents, presumably by changing the Cl^- equilibrium potential. Together, these results suggest that excessive Cl^- influx plays a pivotal role in excitotoxicity in the retina.

Supported by EY08089, EY08922, EY09370, EY02687, DA07261 and an unrestricted grant from Research to Prevent Blindness, Inc.

507.1

NR2B SPECIFIC INTERACTIONS BETWEEN HALOPERIDOL AND THE NMDA RECEPTOR Lynch, D.R. , Reddy, U.*, Huang, H., and Gallagher, M.J., Department of Pharmacology and Pediatrics, University of Pennsylvania, Children's Seashore House, Philadelphia, PA 19104-4318

Many compounds which interact with the *n*-methyl-D-aspartate (NMDA) receptor and modulate its function in a subunit specific manner. The dopaminergic agent haloperidol has been previously shown to exert an 8-10 fold preference for NR2B over NR2A containing NMDA receptors, showing the same subunit dependence observed for the antagonist ifenprodil. We have mapped a determinant of high affinity haloperidol inhibition to the region near amino acid 198 of the NR2B subunit using chimeric NR2A/ NR2B receptors. A conserved glutamate residue (E201) in this region was mutated to either asparagine (the residue found in NR2A) or arginine, causing a >10 fold loss in the ability of haloperidol to displace ^{125}I-MK 801 from these receptors. Mutation to aspartate or alanine caused negligible change in apparent affinity. E201 does not govern the interactions of ifenprodil, since all of the mutants at E201 exhibited wild-type affinity for ifenprodil. Haloperidol interaction is independent from Arg-337, previously shown to cause a 400-fold loss in apparent affinity for ifenprodil. Since haloperidol has been suggested to interact with the strychnine-insensitive glycine site, we also tested the ability of 7-chlorokynurenic acid (glycine site antagonist) to inhibit NMDA-induced currents in oocytes expressing NR1A/ E201N mutant receptors, and found no change in the apparent affinity for glycine for these receptors, suggesting that E201 affects haloperidol, but not glycine or ifenprodil interaction with NMDA receptors. (This work was supported by the Grants F32-DA05675, DA07130, and NS01789 from the National Institutes of Health).

507.2

PROTECTIVE EFFECTS OF ELIPRODIL IN RETINAL AND NEURONAL CELLS AND TISSUES. I.-H. Pang*, E.M. Wexler, R.J. Walters, M. Reyes, A. Reyes, D.L. Shade, S. Nawy, P.K. Stanton, M.A. Kapin. Alcon Laboratories, Fort Worth, TX 76134 and Departments of Neuroscience, Neurology & Ophthalmology and Visual Sciences, Albert Einstein Coll. Med., Bronx, NY 10461.

Eliprodil, an NMDA-receptor spermine-site antagonist, was tested for its neuroprotective actions in retinal and neuronal cells and tissues. Eliprodil prevented death of cultured rat retinal ganglion cells induced by 100 μM glutamate with an EC_{50} of 1 nM. At 100 nM of eliprodil, 100% of cells survived the glutamate insult. Eliprodil also protected against glutamate-induced toxicity in differentiated SHSY5Y (human neuroblastoma) cells (EC_{50} = 2 nM). In both cultured ganglion cells and retinal slices, eliprodil suppressed NMDA (200 μM)-activated currents recorded under patch-clamp.

In rat hippocampal slices *in vitro*, eliprodil markedly reduced pharmacologically isolated NMDA EPSPs, and protected Schaffer collateral-CA1 synaptic transmission from hypoxia-induced irreversible damage, measured as enhanced recovery of EPSPs following hypoxia (EC_{50} = 500 nM bath applied). In long-term organotypic slice cultures, 5 μM eliprodil prevented ischemia (hypoxia with hypoglycemia)-induced delayed neuronal death in field CA3, but was much less effective in protecting CA1 pyramidal neurons from cell loss measured with propidium iodide. Thus, eliprodil can be protective against cytotoxic insults by excitatory amino acids or hypoxia in both retinal and neuronal tissues and cells. (Supported by Alcon Laboratories, Inc.)

507.3

PREVENTION OF NMDA-INDUCED BRAIN INJURY IN NEONATAL RATS BY SUBTYPE SELECTIVE NMDA (NR2B) ANTAGONISTS. M.G. Vartanian*, P.A. Boxer Neuroscience Therapeutics, Parke-Davis Pharmaceutical Research, Div Warner-Lambert Co., Ann Arbor, MI 48105.

The non-competitive NMDA antagonists, eliprodil and ifenprodil, are selective antagonists of the NR1A/2B subtype of N-methyl-D-aspartate (NMDA) receptors. Haloperidol and trifluperidol are less potent but share this NMDA subtype selectivity, although they are more potent as dopamine D_2 antagonists. Since perinatal rats have a much higher density of NR1A/NR2B (vs NR1A/2A) receptors, we have investigated the effects of ifenprodil, eliprodil, haloperidol, and trifluperidol in a model of NMDA-induced brain injury. In addition, remoxipride, a D_2 antagonist with no affinity for NR1A/NR2B receptors was tested to control for non-specific dopaminergic effects. Unilateral striatal microinjections of NMDA (15 nmol/0.5 µl) were performed at postnatal day (PND) 7. Brain injury was calculated on PND 12 by comparing the wet weights of the cerebral hemisphere ipsilateral and contralateral to the injection site. The greatest neuroprotection was seen with haloperidol and eliprodil, which each significantly prevented NMDA-induced injury (67.2 ± 8.6% and 56.0 ± 9.5%, respectively; $p<0.002$) after dosing 3 x 1 mg/kg at hour intervals starting 15 minutes post injection. Ifenprodil and trifluperidol (3 x 1 mg/kg) provided less but still significant protection (35.3 ± 11.6% and 24.6 ± 7.3%, respectively; $p<0.03$, and $p<0.002$). Remoxipride (2 x 30 mg/kg; dosed 15 min and 2.25 h) was not neuroprotective. These results demonstrate that NR1A/NR2B selective agents are potently neuroprotective in this model of NMDA-mediated brain injury in perinatal rats, although there is not a direct correlation between in vivo potency and affinity at the receptor. [Supported by Warner-Lambert Co.]

507.4

PROTECTIVE EFFECTS OF THE POLYAMINE ANTAGONIST, ELIPRODIL HYDROCHLORIDE IN RETINA SUBJECTED TO AN EXCITOTOXIC- OR ISCHEMIC-INSULT. M.A. Kapin*, R. Doshi, L. Chapman, L. Watson, S. Porter, B. Scatton, and M.L. Chandler. Alcon Laboratories, Inc., Fort Worth, TX 76134 & Synthélabo Recherche, Bagneux, France.

Ischemia and excitotoxicity have been implicated in a myriad of retinal and optic nerve head disorders, including glaucoma. In animal models of ocular ischemia or excitotoxicity, N-methyl-D-aspartate (NMDA) antagonists have been shown to ameliorate retinal injury. Presented here is the neuroprotective profile of the polyamine antagonist, eliprodil hydrochloride in retina subjected to ischemia or NMDA administration. Total retinal ischemia, as measured by a cessation of ERG activity, was induced in the anesthetized rabbit by elevating IOP at 140 mmHg for 65 minutes. Following ischemia, restoration of ERG was assessed at 24 and 48 hours in animals (n = 4-6/group) treated i.v. and i.p. with vehicle or eliprodil (1.0-10.0 mg/kg). In vehicle treated animals, A and B waves were suppressed by 60-70% at 24 hours. At 10 mg/kg, eliprodil significantly ameliorated the A and B wave depression throughout the 48 hour experiment. To evaluate retinal excitotoxicity, intravitreal NMDA (5 µl, 20 nmoles) was injected in rats which resulted by 7 days in a dose-related decrease in cells of the ganglion cell layer (GCL) and choline acetyltransferase (ChAT) activity. In animals treated with NMDA but not eliprodil, the loss of cells was 24% and 30%, compared to the non-injected contralateral eyes or vehicle injected eyes in different animals, respectively. At 10 mg/kg, i.p., eliprodil completely prevented the loss of ChAT as well as cells in the GCL. From these results we conclude that eliprodil is neuroprotective of retina subjected to an ischemic or excitotoxic-challenge. (Supported by Alcon Laboratories, Inc.)

507.5

INHIBITION OF NMDA-INDUCED BEHAVIORAL RESPONSES BY CP-96,345 IN THE MOUSE. R. A. Velazquez*, K. F. Kitto and A. A. Larson. Dept. of Vet. Pathobiology, University of Minnesota, St. Paul, MN 55108.

The non-peptide compound CP-96,345 has been found to inhibit substance P (SP) binding in brain membranes. Several behavioral, physiological and electrophysiological experiments have shown the selectivity of this compound in inhibiting the neurokinin 1 (NK-1) receptor compared to NK-2 and NK-3. Also, CP-96,345 inhibits in a non-stereospecific manner Ca^{++} channels which can account for the non-specific effects seen after the administration of this drug. Intrathecal injections of SP or *N*-methyl-D-aspartate (NMDA) produce a well characterized caudally-directed biting and scratching (CBS) response that desensitizes following multiple injections. In contrast, repeated injections of kainic acid (KA) increase the number of CBS behaviors. In this study, we examined the effect of 30-min pretreatment with 0.002 to 2.0 nmoles of CP-96,345 on four consecutive intrathecal injections of 22.5 pmoles SP, 25 pmoles KA and 0.3 nmoles NMDA. The number of CBS behaviors were counted over a 2-min interval after each injection. At these dose ranges, CP-96,345 did not inhibit the behaviors elicited by the first injection of SP or KA but it inhibited SP desensitization and KA sensitization to repeated injections in a dose-related fashion. In contrast, at doses as low as 0.02 nmoles, CP-96,345 inhibited NMDA-induced behaviors and blocked NMDA-induced desensitization. The inhibition of NMDA behaviors by CP-96,345 at a dose that failed to inhibit the CBS effect of SP suggest that a non-selective inhibition of Ca^{++} channels may be responsible for these results or that the CBS effect of i.t. injected SP is not mediated by NK-1 sites. [This study was supported by U. S. Public Service Grant DA04090 to AAL.]

507.6

SUBTYPE-SELECTIVE ANTAGONISM OF NMDA RECEPTORS BY NYLIDRIN. E. R. Whittemore*, V. I. Ilyin, and R. M. Woodward. CoCensys, Inc., 213 Technology Drive., Irvine, California 92718.

The structurally-related di-substituted piperidines ifenprodil, eliprodil and haloperidol have been shown to exhibit non-competitive subtype-selective antagonism of the NMDA 1a / 2B receptor combination, as measured in expression systems and in cultured neurons. To further investigate the structural requirements for selective blockade of NR 1a / 2B receptors, we assayed NMDA antagonist activity of the β-adrenergic agonist nylidrin, a compound structurally related to ifenprodil in which the piperidine ring has been opened to generate a secondary amine. In *Xenopus* oocytes expressing cloned NMDA receptors, nylidrin was a potent antagonist of 1a / 2B subunit combinations (IC_{50} ~ 0.2 µM) while it was >100-fold weaker at 1a / 2A (IC_{50} ~ 27 µM) and 1a / 2C (IC_{50} ~ 33 µM). Antagonism of 1a / 2B receptors was independent of glycine concentration and associated with a slight increase in apparent affinity for glutamate. Antagonism at the 1a / 2B subunit was clearly insurmountable with respect to both agonists. Blockade of the 1a / 2B subunit by nylidrin did not exhibit voltage dependence, while blockade of 1a / 2A and 1a / 2C subunits was strongly voltage-dependent. Nylidrin also inhibited NMDA responses in primary rat cortical neurons cultured for 7-10 days *in vitro*, which have been shown to express primarily the 1a / 2B subunit combination. Potency of inhibition in neurons (IC_{50} ~ 0.23 µM) was similar to that observed for 1a / 2B receptors expressed in oocytes. Our results suggest that nylidrin is a potent and selective antagonist of the NMDA 1a / 2B subunit combination, and that it may interact with the same allosteric site as previously described for the piperidine compounds mentioned above. Further, nylidrin may be a valuable starting point for directed synthesis in this area. *(Supported by CoCensys, Inc.)*

507.7

THE EFFECTS OF NEUROACTIVE STEROIDS ON RECOMBINANT NMDA RECEPTORS. N. Yaghoubi*, A. A. Malayev, T. T. Gibbs, and D. H. Farb, Laboratory of Molecular Neurobiology, Department of Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Recent studies have shown that neuroactive steroids can modulate NMDA receptor responses in cultured neurons. Molecular studies have revealed an important diversity in the NMDA receptor family, which is now known to consist of NR1 and NR2 subunit classes. As a first step towards the characterization of steroid modulation of recombinant NMDA receptors, we have examined the effects of different steroids on NMDA receptors expressed in *Xenopus* oocytes.

Similiar to what has been observed with neuronal NMDA responses, pregnenolone sulfate (PS) was found to potentiate currents through recombinant $NR1_{100}$:NR2A receptors in oocytes at all NMDA concentrations tested. An EC_{50} value of 74 μM was found for PS potentiation of responses induced by 100 μM NMDA. Pregnanolone sulfate (5β3αS) and epipregnanolone sulfate (5β3βS) were found to decrease the E_{max} of the NMDA dose-response curve, consistent with a mechanism of non-competitive inhibition. NMDA responses in the presence of 200 μM PS were inhibited by 5β3βS to the same extent as in the absence of PS (EC_{50} values of 124 μM and 150 μM, respectively), suggesting the existence of independent modulatory sites for inhibitory and potentiating steroids. Preliminary studies have shown that homomeric $NR1_{100}$ receptors were also modulated by steroids in a manner similiar to modulation of $NR1_{100}$:NR2A receptors. These results show that expression of recombinant NMDA receptor subunits is sufficient to confer steroid sensitivity. Supported by NIMH MH49469.

507.8

Distinct Sites for Positive and Negative Modulation of NMDA Receptors by Sulfated Steroids. M. Park-Chung*, F-S. Wu, R.H. Purdy, T.T. Gibbs and D.H. Farb. Laboratory of Molecular Neurobiology, Department of Pharmacology, Boston University School of Medicine, Boston, MA 02118

We have shown previously that the naturally occurring sulfated steroid pregnenolone sulfate (PS) potentiates N-methyl-D-aspartate (NMDA) response at a site distinct from the glycine recognition site. Here we use voltage-clamped chick spinal cord neurons to study further interactions between sulfated steroids and the NMDA receptor. Drug solutions were applied to single neurons by pressure ejection from 7-barrel pipets. Epipregnanolone sulfate (3β-hydroxy-5β-pregnan-20-one sulfate; 5β3βS), which differs from PS only in the presence of a double bond between C-5 and C-6, stereospecifically inhibits the NMDA response. The inhibition of the NMDA response by 5β3βS is a voltage- and agonist-independent, suggesting that 5β3βS does not act as an open channel blocker. Potentiation by PS and inhibition by 5β3βS is also observed in *Xenopus* oocytes expressing only NMDAR1 and NMDAR2A subunits. These results argue against the hypothesis that PS and 5β3βS act upon different receptor populations of NMDA receptors. Both PS and 5β3βS act independently of other NMDA receptor modulators such as spermine, arachidonic acid, or redox agents. Surprisingly, 5β3βS and PS is also act independently of each other, suggesting that there are at least two independent steroid modulatory sites that are distinct from previously identified modulatory sites of the NMDA receptor. We suggest that sulfated steroids have the capacity to exert powerful inhibitory and excitatory modulatory effects on nervous system activity through the modulation of NMDA receptors. Supported by NIMH MH49469.

507.9

[^{3}H]AMPA BINDING AUTORADIOGRAPHY: CYCLOTHIAZIDE BUT NOT BDP-37 HAS LOWER POTENCY IN BRAIN AREAS THAT ARE RICH IN FLOP SUBUNITS. M.S. Mutneja, M. Kessler* and G. Lynch. CNLM, Univ. of California Irvine, CA 92717.

Since AMPA receptors vary across brain regions in regard to subunit composition, drugs that act on these receptors can be expected to have regionally selective effects if they have a preference for certain subunits. The latter question is usually explored using homomeric AMPA receptors expressed in non-neuronal cells. Studies of this kind have indicated in physiological (Partin *et al.*, 1994) and binding experiments (Hennegriff *et al.* this meeting) that cyclothiazide (CTZ) has about ten times greater affinity for flip isoforms of AMPA receptor subunits. Conversely, BDP-37, a benzoylpiperidine type drug, had a slight preference for flop subunits (Hennegriff *et al*). The present study examined whether native AMPA receptors in the brain show drug preferences that conform with *in situ* hybridization results according to which area CA3 and outer cortex appear to have comparatively higher densities of flip isoforms (Sommer *et al.*, 1990). Drug potencies were assessed by measuring the effect on [^{3}H]AMPA binding to rat brain sections. CTZ inhibited binding in hippocampal area CA3 with an EC_{50} of 24 μM but had much lower affinity in area CA1 (EC_{50}: 66 μM). A significant difference was also observed in the cortex with CTZ being more potent in the outer (19 μM) vs. inner layers (41 μM). BDP-37 exhibited less obvious regional differences but was, if anything, slightly more potent in CA1 and in *inner* cortical layers. These results show that drug preferences of brain AMPA receptors correspond to those extrapolated from homomeric receptors expressed in non-neuronal cells and demonstrate the usefulness of the latter in investigating drug effects. The data further indicate that benzoyl-piperidine and cyclothiazide type drugs differ in the brain regions they preferentially target; centrally active variants of CTZ might thus produce behavioral effects that differ from those reported for the benzoylpiperidine drugs. (Grant 95-I-0304 from AFOSR and CP-19982 from Cortex Pharmaceuticals).

507.10

SCN^- BLOCK OF AMPA RECEPTORS: INTERACTION WITH THE CYCLOTHIAZIDE BINDING SITE. S.D. Donevan*[1], N.G. Carlson[2] and M.A. Rogawski[3]. [1]Dept. of Neurology, [2]Dept. of Neurobiology & Anatomy, University of Utah, Salt Lake City, UT 84112 and [3]Neuronal Excitability Section, NINDS, NIH, Bethesda, MD 20892.

Previous studies have demonstrated that the chaotropic anion, SCN^-, non-competitively blocks AMPA-evoked responses (Bowie & Smart, 1993). We sought to evaluate the hypothesis that SCN^- blocks AMPA receptors by facilitating desensitization at the site where cyclothiazide reduces desensitization. Whole cell and patch recordings were carried out from cultured hippocampal neurons. Native AMPA receptors in cultured hippocampal neurons were blocked in a concentration-dependent fashion by SCN^- (IC_{50}, 1.1 mM). In outside-out patches from these neurons, SCN^- enhanced the rate of AMPA receptor desensitization with little effect on the peak AMPA current. Several lines of evidence were consistent with the view that SCN^- and cyclothiazide interact at a single binding site. First, cyclothiazide produced a rightward shift of the SCN^- concentration-response relationship. Second, SCN^- appeared to exhibit a similar subunit selectivity to cyclothiazide as the magnitude of SCN^- block was correlated with the degree of cyclothiazide potentiation, which varied over a wide range from cell to cell. Finally, the rate of onset of SCN^- block was slowed 100-fold in the presence of cyclothiazide and was similar to the rate of recovery from cyclothiazide potentiation. Taken together, these results support the view that SCN^- acts as an inverse agonist at the site where cyclothiazide potentiates AMPA receptors responses and reduces desensitization.

507.11

SUBTYPE-SPECIFIC MODULATION OF STABLY EXPRESSED RECOMBINANT HUMAN N-METHYL-D-ASPARTATE RECEPTORS BY ZINC IONS. S. Grimwood, P.H. Hutson*, A.J. Macaulay and T. Priestley. Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR.

Zinc ions (Zn^{2+}) are known to antagonise native NMDA receptor function in a complex manner which involves both a weak voltage-sensitive component and a voltage-insensitive component. We have investigated the effect of Zn^{2+} on cells stably expressing human NR1a/NR2A and NR1a/NR2B receptors using both $^{45}Ca^{2+}$-influx and whole-cell voltage-clamp techniques. In the presence of 100 μM L-glutamate and glycine, Zn^{2+} inhibited $^{45}Ca^{2+}$-influx into cells expressing NR1a/NR2B receptors with an IC_{50} of 4.66 (4.38, 4.96) μM [geometric mean (-SEM, +SEM) n=4]. Inhibition of $^{45}Ca^{2+}$-influx into NR1a/NR2A expressing cells by Zn^{2+} was concentration-independent over the range 0.3 - 100 μM [54.2 ± 9.4 % inhibition @ 100 μM (mean ± SEM, n=4)]. Electrophysiologically, Zn^{2+} inhibited both NR1a/NR2A and NR1a/NR2B receptor-mediated currents in a monophasic manner with significantly different IC_{50} values of 100 (83.9, 120) μM (n=5) and 6.70 (6.11, 7.35) μM (n=4), respectively ($p<0.0001$, unpaired t test).

The electrophysiological results show that Zn^{2+} has ~15-fold higher affinity for NR1a/NR2B receptors compared to NR1a/NR2A receptors. However, the manner in which Zn^{2+} ions modulate NR1a/NR2A receptor function, as measured by $^{45}Ca^{2+}$-influx is not straightforward. The effect of a number of other ligands proposed to inhibit NMDA receptor function through an interaction with the zinc site have also been investigated for subtype-selectivity using the procedures described above.

MSD Ltd.

507.12

FUNCTIONAL EXPRESSION OF A RAT BRAIN cDNA WHICH ENCODES NAALADase, A GLUTAMATE-GENERATING NEUROPEPTIDASE. R.E. Carter* and J.T. Coyle. Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital and Consolidated Department of Psychiatry, Harvard Medical School, Boston, MA.

There is a growing body of evidence that the N-acetylated alpha-linked acidic dipeptidase (NAALADase) and its neuropeptide substrate N-acetylaspartylglutamate (NAAG) participate in nervous system signaling via glutamate receptors. In order to define the functional relationships of this peptidase and its substrate to glutamatergic communication further, we have undertaken the isolation of NAALADase cDNA clones. We have recently reported the characterization of one NAALADase species, the investigational prostate cancer marker PSM, whose corresponding cDNA was cloned from a prostate cancer cell line (Carter et al. [1996], Proc. Natl. Acad. Sci. U.S.A. 93, 749-753). We have subsequently characterized a NAALADase mRNA expressed in brain by using a combination of immunoscreening and RT-PCR techniques to construct a full-length rat brain cDNA clone. The rat brain clone shows a high degree of sequence similarity to the PSM cDNA, and the two predicted proteins also demonstrate many common structural features. Importantly, transfection of the rat brain cDNA into a NAALADase-negative mammalian cell line (PC3) confers NAAG-hydrolyzing activity which is sensitive to the NAALADase inhibitors quisqualic acid and β-NAAG. Thus, we have extended the molecular characterization of NAALADase species to the nervous system and can now begin to address its expression, and ultimately its function, therein.

This work was supported by NIH grant MH-572901 and a NRSD Senior Investigator Award to J.T.C.

507.13

RELEASE OF GLUTAMATE FROM PRIMARY SENSORY NEURONS IN CULTURE CAN BE MODULATED BY GROWTH FACTORS

M. Rydh-Rinder [1,2], N. Kerekes [2], J. Näsström [1]* and T. Hökfelt [2]. [1] Astra Pain Control AB, Novum Unit 141 57 Huddinge and [2] Dept Neuroscience, 171 77 Karolinska Institute, Sweden.

Glutamate (GLU) has been shown to be a sensory neurotransmitter in the spinal cord with a substantial role in nociception. We have studied the effect of different growth factors on potassium (K^+)-, bradykinin (BK)- or capsaicin (CAPS)-induced release of GLU from primary sensory neurons in culture. METHOD: Dorsal root ganglions (DRG) were dissected from adult male rats, dissociated and plated on 8-chamber slides. The cells received control medium or medium containing either NGF (100ng/ml), BDNF (10ng/ml) or βFGF (10 ng/ml) (3 wells per group), during three days. After a thorough rinse in PBS the cells were equilibrated in ACSF during 5 minutes prior to stimulation and were then subsequently exposed to ACSF containing K^+ (60mM), BK (1μM) or CAPS (10μM). Controls received pure ACSF. Samples were drawn after 15, 30, 60, 120 and 300s. GLU levels were determined using HPLC. Additional experiments were performed using glial cultures derived from peripheral nerve. RESULTS: In cultures treated without growth factors a 6-fold increase was seen after BK stimulation compaired to basal release. All growth factors reduced the BK evoked release, βFGF being the most potent showed almost a total depletion of the response. Capsaicin only induced a minor increase in 0-treated cultures while K^+-stimulation only revealed a small increase of GLU levels in the βFGF group. Glial cultures did not exhibit any increased GLU release upon BK stimulation. CONCLUSION: The release of GLU may be regulated by growth factors, whether this is a direct effect or mediated by induction of other regulatory mechanisms remains to be clarified.

EXCITATORY AMINO ACIDS: PHARMACOLOGY—SYNAPTIC RECEPTORS

508.1

FUNCTIONAL AND PHARMACOLOGICAL PROPERTIES OF AMPA RECEPTORS IN TYPE I AND TYPE II CULTURED RAT HIPPOCAMPAL NEURONS.

M.A. Rogawski*[1] and S.D. Donevan[2], [1]Neuronal Excitability Section, NINDS, NIH, Bethesda, MD 20892 and [2]Dept of Neurology, University of Utah, Salt Lake City, UT 84112.

The majority of cultured rat hippocampal neurons (Type I) show Ca^{2+}-impermeable, outwardly rectifying AMPA receptor responses. However, recent studies (Iino *et al.*, 1990) have identified a small population of hippocampal neurons (Type II) that have reduced expression of the GluR2 AMPA receptor subunit and exhibit inwardly rectifying, Ca^{2+}-permeable AMPA responses. We have used whole cell and outside-out patch recording techniques to compare the functional and pharmacological properties of AMPA receptors of Type I and Type II neurons. The degree of rectification of kainate-evoked AMPA receptor responses was well correlated with the extent of Ca^{2+} permeability as assessed by reversal potential measurements in a high Ca^{2+}/0 Na^+ extracellular buffer. The time course of desensitization of AMPA responses in outside-out patches from Type II neurons was almost two-fold faster (τ_{des}~5 ms) than in patches from Type I neurons and the extent of desensitization was greater in Type II neurons, suggesting that Type II neurons contain predominately *flop* subunits. Accordingly, cyclothiazide produced only a two-fold potentiation of kainate currents in Type II compared with the 8-fold potentiation in Type I neurons. The arthropod toxin philanthotoxin produced a potent use- and voltage-dependent block of Ca^{2+}-permeable AMPA receptors of Type II neurons, but only minimally affected AMPA receptors in Type I neurons. In contrast, the use-dependent blocker pentobarbital and the competitive antagonist NBQX exhibited moderate selectivity for AMPA receptors in Type I neurons. The allosteric antagonist GYKI 52466 was equieffective at AMPA receptors of both cell types These studies demonstrate that native Ca^{2+}-permeable AMPA receptors differ functionally and pharmacologically from the more common Ca^{2+}-impermeable type. It may be possible to exploit these differences in the development of novel therapeutic agents that selectively target Ca^{2+}-permeable AMPA receptors.

508.2

PDC-STIMULATED STRIATAL GLUTAMATE RELEASE IS ATTENUATED BY REDUCING EXTRACELLULAR CALCIUM AND BLOCKING GLUTAMATE RECEPTORS.

S.M. Rawls* and J.F. McGinty. Dept. Anatomy & Cell Biology, East Carolina Univ. School of Medicine, Greenville, NC 27858-4354.

L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC) reverses plasma membrane glutamate transporters and elevates extracellular glutamate levels in vivo. We hypothesized that PDC-stimulated glutamate release is partially mediated by an increase in transynaptic activity. Therefore, the possible involvement of calcium-dependent glutamate release and glutamate receptor stimulation was investigated via reverse dialysis of (1) reduced calcium ACSF, (2) CPP, an NMDA receptor antagonist, or (3) LY293558, a kainate/AMPA antagonist via reverse microdialysis. The reduced calcium did not alter basal glutamate levels; however, a significant attenuation of 1mM PDC-evoked glutamate release was observed. In separate experiments, 100 uM CPP or 100 uM LY293558 administration significantly attenuated PDC-evoked glutamate release without altering basal levels. These data suggest that glutamate transporter reversal by PDC evokes glutamate release, resulting in postsynaptic glutamate receptor stimulation and elevated striatal output. This would indirectly lead to increased thalamostriatal and cortiostriatal activity which would contribute to the PDC-stimulated increase in extracellular glutamate levels. Reduction of extracellular calcium would directly decrease action potential-dependent glutamate release. In addition, glutamate receptor blockade would reduce medium spiny neuronal firing and indirectly attenuate thalamostriatal and corticostriatal activity, resulting in an overall depression of PDC-stimulated glutamate levels. Supported by DA 09256.

508.3

GLUTAMATE RECEPTOR FUNCTION DETERMINED BY STIMULATION OF RUBIDIUM FROM MOUSE HIPPOCAMPAL SYNAPTOSOMES.

Jeanne M. Wehner* and Ying Lu. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

The ability of glutamate receptor agonists to activate ion channels resulting in Na^+ and K^+ fluxes has been used to develop a functional assay of mouse hippocampal synaptosomes. The efflux of $^{86}Rb^+$ from synaptosomes was monitored by a B-RAM radioactivity HPLC detector using continuous superfusion methods. Ion flux was evoked in a concentration dependent manner by glutamate receptor agonists, glutamate, kainate, AMPA, and quisqualate upon a 2-sec stimulation. The EC_{50} values for these agonists were in the range of low uM, similar to those obtained in other functional assays. Pharmacological antagonisms were demonstrated by showing that CNQX shifted the kainate concentration-response curve to the right in a parallel manner. Cyclothiazide, an AMPA receptor modulator, augmented the ion flux mediated by AMPA and quisqualate. Ethanol (25-200 mM) inhibited kainate-mediated $^{86}Rb^+$ flux from hippocampal synaptosomes. These results indicate that a glutamate-receptor-mediated ion flux can be measured in hippocampal tissues and that the ion flux should serve as a useful assay to examine the effects of ethanol and other drugs on glutamate receptor function in the central nervous system. Supported by AA-03527, AA-00141, and MH-48663.

508.4

NMDA-LIKE GLUTATHIONE-STIMULATED INCREASES IN INTRACELLULAR CALCIUM, ([Ca]*i*) IN THREE BRAIN REGIONS: INHIBITION BY ETHANOL.

W. R. Wilson* and S. W. Leslie. Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas, Austin, TX 78712.

In the continuing effort to understand ethanol's interaction with NMDA receptors we have examined further the NMDA-like stimulated increases in [Ca]*i* produced by both reduced (GSH) and oxidized (GSSG) glutathione in cerebellar, cortical and hippocampal brain regions. Acutely dissociated rat pup neurons were loaded with fura-2 to monitor GSH/GSSG-stimulated increases in [Ca]*i*; ethanol inhibition of the response was also examined. Both GSH and GSSG produced concentration dependent, saturable increases in [Ca]*i*. The response in cerebellum was significantly less than the response in cortex and hippocampus. In addition, GSSG was more efficacious than GSH, producing a maximal response that was almost 2-fold greater than GSH in all brain regions. Furthermore, the GSSG maximal response was comparable to the maximal response produced by NMDA (100μM). Ethanol inhibition of GSH/GSSG-stimulated increases in [Ca]*i* was more potent in cortex and hippocampus than in cerebellum. The results demonstrate brain region differences in GSH/GSSG-stimulated increases in [Ca]*i*, that GSSG is more efficacious than GSH and that ethanol is not as effective an inhibitor of GSH/GSSG-stimulated changes in [Ca]*i* as it is of NMDA-induced changes in [Ca]*i*.

Supported by NIAAA grants RO1 AA09337 and T32 AA07471.

508.5

INTERACTIONS OF HALOTHANE AND CLONIDINE ON THE HIPPOCAMPAL POPULATION SPIKE AND ALTERATIONS BY PICROTOXIN

M. D. Sokoll * T. Gerhold and L.R. Davies. Department of Anesthesia Laboratories, University of Iowa College of Medicine, Iowa City, IA 52242.

The mechanisms of the mode of action of inhalation anesthetics remains to be elucidated. Clonidine, an a 2 adrenergic agonist has long been used as an adjunct in veterinary anesthetics and is reported to decrease the minimum alveolar concentration of the anesthetic. Dexmedatomidine, a similar acting agent is now being studied in man. We examined the effects of clonidine on the amplitude, latency and slope of onset of the hippocampal slice CA1 population spike (PS). Clonidine was studied at concentrations of 10,30 and 100μM with and without the prior application of 1% halothane. Clonidine was also applied after picrotoxin (30μM).

Clonidine alone produced a dose related decrease in the population spike amplitude. When applied after halothane there was a further decrease in amplitude. The prior application of picrotoxin produced a partial inhibition of the clonidine induced PS amplitude decrease. Clonidine augments the effect of halothane. This may be partially related to its effect on inhibitory systems.

Supported by the Department of Anesthesia Trust Fund and NIH grant RO1 GM38541

508.6

NMDA-ANTAGONISTS MODULATE LOCAL INHIBITORY CIRCUITS IN A DOSE-DEPENDENT MANNER.

H.C.R. Grunze[1,2], D.G. Rainnie[1], M. Berger[2], R.W. McCarley[1], Brian F. O'Donnell[1*], and R.W. Greene[1]. [1]Harvard Medical School & VAMC Brockton, Brockton MA 02401. [2]Psychiatrische Universitätsklinik, 79104 Freiburg, Germany

We have previously reported the occurrence of an NMDA-dependent modulation of local inhibitory circuits in area CAI of the rat hippocampus (Grunze et al., 1996), whereby NMDA antagonists could suppress evoked IPSP's, as well as their LTP. Here, we further characterize the dose-dependency of the IPSP amplitude suppression in response to bath application of the specific NMDA antagonists APV, PCP, and NAAG. The IPSP amplitude, evoked in response to alvear stimulation, was measured from whole cell patch clamp recordings obtained from CAI pyramidal neurons manually held at - 60 mV. Results are given as the percentage decrease of the baseline IPSP amplitude (± SE) in the presence of increasing concentrations of antagonist.

For APV: 0.4μM- 12±13%; 1μM- 8±16%; 1.5μM- 12±9%; 3μM- 15±3%; 5 μM- 15±4%; 10μM- 36±13%; 25μM- 31±9%; 50μM- 47±9%. For PCP: 10μM- 24±21%; 25μM- 9±16%; 50μM- 46±6%; 100μM- 48±19%. PCP also has antagonist activity at the sigma opioid receptor, hence, we examined the response to PCP in the presence of the nonspecific opiate antagonist, naloxone. In the presence of naloxone (10μM), 50μM PCP reduced the IPSP amplitude by -40% which is close to that observed in control ACSF. In 4/8 neurons tested, the non-NMDA antagonist, DNQX (5μM), abolished the IPSP completely, and in the other 4 neurons by 80±22% when compared to baseline values. The endogenous mixed NMDA antagonist NAAG was also capable of reducing the IPSP amplitude by -11±9% at 50μM and -43±15% at 100μM, respectively. Furthermore, in 2/4 neurons tested at 50μM PCP, and in all 4/4 neurons tested at 100μM PCP, a pronounced and reversible increase, up to 50 ms, was observed in the action potential duration evoked in response to depolarizing current injections. Taken together, these results emphasize the role of NMDA receptor activation in generating local inhibitory IPSP's at physiological resting membrane potentials.

Supported by the Department of Veterans' Affairs

508.7

STEREOSELECTIVITY OF 2.3-BENZODIAZEPINES ON AMPA RECEPTORS AND SYNAPTIC EVENTS ON NEONATAL RAT SPINAL NEURONES.

D. Lodge, N.A. Moore* and M.L. Woolley, Lilly Research Centre, Windlesham, Surrey. GU20 6PH, U.K.

Glutamate receptors mediate fast and slow synaptic excitation in the CNS. The role of AMPA receptors in these two phases of synaptic transmission and the stereoselectivity of 2,3-benzodiazepines were examined by testing the (+) and (-) isomers of LY300168 (GYKI 53655) on dorsal root to ventral root reflexes (DR-VRR) in hemisected spinal cords of 3-6 day old rats.

The fast paucisynaptic and slow polysynaptic phases of the DR-VRR were blocked by (-)-LY300168 with EC50s of approximately 2 and 10 uM respectively. The (+) isomer, up to 20uM, did not reduce either fast or slow phases. The NMDA antagonist, CPP 100uM, reduced the fast and slow phases by about 5 and 30% respectively.

In nominally magnesium-free medium, (-)-LY300168 10uM and CPP 100uM each reduced the fast phase of the DR-VRR by less than 20%, and each reduced the slow phase by 0 and 65% respectively. The two antagonists applied together, however, produced a near complete abolition of both phases of the DR-VRR.

Responses to exogenous application of AMPA 3uM, but not those of NMDA 30uM, in control medium were reduced by (-)-LY300168 with an IC50 of about 1uM whereas CPP, IC50 versus NMDA of about 0.3uM, had the reverse selectivity and (+)-LY300168 was inactive.

The present results demonstrate the stereoselectivity of 2,3-benzodiazepines as AMPA antagonists and the co-activation of AMPA and NMDA receptors in both fast and slow phases of the DR-VRR in neonatal rat spinal cords.

508.8

NPS 846·HCl, A NOVEL OPEN-CHANNEL BLOCKER OF THE NMDA RECEPTOR: *IN VITRO* CHARACTERIZATION.

L.D. Artman[1], B.C. VanWagenen[1], M.F. Balandrin[1], E.G. DelMar[1], L.G. Hammerland[1]*, S.D. Donevan[2], M.A. Rogawski[3], K. Williams[4], and A.L. Mueller[1]. [1]NPS Pharmaceuticals, Inc. 420 Chipeta Way, Salt Lake City, UT 84108, [2]Dept. of Neurology, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132, [3]Epilepsy Res. Branch, NIH, Bethesda, MD 20892, and [4]Dept. of Pharmacology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

NMDA receptor (NMDAR) antagonist activity was determined *in vitro* in a fura2-based assay of cytosolic Ca^{2+} in rat cerebellar granule cells (RCGCs) and a [^{3}H]MK-801 binding assay. 3,3-diphenylpropylamine (DPPA), obtained from a commercial source, possessed modest potency in the RCGC assay (IC_{50} = 435 nM). Initial medicinal chemistry efforts on this lead compound led to the preparation of NPS 846·HCl (3,3-bis(3-fluorophenyl)-1-propylamine HCl; FW 283) with a 6-fold increase in potency (IC_{50} = 72 nM). Each compound was less potent in the [^{3}H]MK-801 binding assay (IC_{50}s = 2.1 μM and 285 nM, respectively). MK-801 was equipotent in both assays (IC_{50}s = 5 nM). DPPA and NPS 846·HCl were noncompetitive with respect to either NMDA or glycine in RCGCs. DPPA and NPS 846·HCl blocked I_K in N1E-115 cells and L-type Ca^{2+} channels in RCGCs at concentrations ranging from 3-10 μM. Patch clamp studies in hippocampal pyramidal cells demonstrated that DPPA and NPS 846·HCl were selective for NMDARs over non-NMDARs, and that their actions were use- and voltage-dependent, and occluded by Mg^{2+}, indicative of open-channel blockade. The NMDAR subtype selectivity of NPS 846·HCl was examined using a *Xenopus* oocyte expression system (NR2A, NR2B, NR2C and NR2D subunits coexpressed with NR1A). NPS 846·HCl (1 μM) inhibited all subtypes equally; MK-801 (30 nM) preferentially inhibited NR2A and NR2B. NPS 846·HCl possesses neuroprotectant and anticonvulsant activities (Y. Chien *et al.*, this meeting) and an *in vivo* profile distinct from that of most open-channel blockers (A.L. Mueller *et al.*, this meeting).

Source of support: commercial.

508.9

NPS 846·HCl, A NOVEL OPEN-CHANNEL BLOCKER OF THE NMDA RECEPTOR: *IN VIVO* CHARACTERIZATION.

A.L. Mueller[1*], L.D. Artman[1], H. Li[2], R.L. Balster[2], D. Taylor[3] and T.N. Parks[3]. [1]NPS Pharmaceuticals, Inc. 420 Chipeta Way, Salt Lake City, UT 84108, [2]Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298, [3]Dept. of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

NPS 846·HCl, a small synthetic molecule (FW 283), is a novel open-channel blocker of NMDA receptors (L.D. Artman *et al.*, this meeting). NPS 846·HCl possesses neuroprotectant and anticonvulsant activities in the dose range of 1-3 mg/kg i.p. (Y. Chien *et al.*, this meeting). In the present study, no behavioral symptoms were observed when NPS 846·HCl was administered to freely moving rats at doses up to 10 mg/kg i.p. The administration of NPS 846·HCl at doses of 30 mg/kg i.p. and above elicited a characteristic behavioral syndrome consisting of hyperexcitability, increased reactivity to touch, increased muscle tone, and tremors. MK-801-like behaviors (head weaving, backwards shuffling) were rarely observed. NPS 846·HCl, at doses up to 10 mg/kg i.p., did not substitute for PCP in rats trained to discriminate PCP (2 mg/kg) from saline. The 10 mg/kg i.p. dose of NPS 846·HCl produced a marked decrease in overall response rates, indicating that this dose was behaviorally active. MK-801, used as a positive control, fully substituted for PCP at doses of 0.075 and 0.15 mg/kg i.p. The potential for NPS 846·HCl to induce neuronal vacuolization was examined as well. NPS 846·HCl produced no vacuoles in neurons of the retrosplenial and cingulate cortices of rats (0 out of 2 rats treated with 10 mg/kg i.p. and 0 out of 3 rats treated with 30 mg/kg i.p.). MK-801 (5 mg/kg i.p.) produced vacuoles in 6 out of 7 rats. The absence of vacuolization is consistent with the unique behavioral profile of NPS 846·HCl. Taken together, these results demonstrate that NPS 846·HCl, a novel NMDA receptor antagonist, does not elicit the *in vivo* profile characteristic of most open-channel NMDA blockers.

Source of support: commercial.

508.10

NEUROPROTECTANT AND ANTICONVULSANT PROPERTIES OF NPS 846·HCl, A NOVEL NMDA RECEPTOR ANTAGONIST.

Y. Chien[1], J.L. Raszkiewicz[1*], S. Draper[1], H.S. White[2], and A.L. Mueller[1]. [1]NPS Pharmaceuticals, Inc. 420 Chipeta Way, Salt Lake City, UT 84108 and [2]Dept. of Pharmacology, University of Utah School of Medicine, Salt Lake City, UT 84132.

NPS 846·HCl, a small synthetic molecule (FW 283), is a novel open-channel blocker of NMDA receptors (L.D. Artman *et al.*, this meeting). To test its potential neuroprotective effects *in vivo*, NPS 846·HCl was administered (1 mg/kg i.p.) at different times after ischemic insult in male adult Wistar rats subjected to a 2-hr intraluminal occlusion of the middle cerebral artery (the suture model). In all cases, a second dose was administered 5-6 hrs after the first dose. Total, cortical, and striatal infarct volumes were measured by 2,3,5-triphenyltetrazolium staining 48 hrs after suture occlusion. Compared to saline-treated controls, NPS 846·HCl reduced the total infarct volume (corrected for edema) by 71% ($p < 0.05$), 64% ($p < 0.05$) and 46% ($p < 0.05$) when given immediately, 1 hr, or 2 hr after the onset of cerebral ischemia, respectively. The neuroprotective effects of NPS 846·HCl were observed both in cortex and striatum. No apparent behavioral side effects were noted in this study (see also A.L. Mueller *et al.*, this meeting). The anticonvulsant activity of NPS 846·HCl was determined in the Frings audiogenic mouse model of reflex epilepsy. NPS 846·HCl produced a dose-dependent reduction in the seizure score with an ED_{50} of 2.1 mg/kg i.p. NPS 846·HCl was active in this model following oral administration as well, with an ED_{50} of 9.7 mg/kg p.o. Motor toxicity was determined by rotorod; TD_{50} (toxic dose) values were 19.9 mg/kg i.p. and 21.8 mg/kg p.o. These results demonstrate the neuroprotective efficacy of NPS 846·HCl in this rat model of temporary focal ischemia, and suggest that this novel NMDA receptor antagonist possesses a therapeutic window of opportunity of at least 2 hrs. The compound also possesses anticonvulsant activity with a favorable therapeutic index in the Frings audiogenic model of reflex epilepsy.

Source of support: commercial.

508.11

KINETICS OF SPONTANEOUS MINIATURE EXCITATORY POSTSYNAPTIC CURRENTS IN PRESENCE AND IN ABSENCE OF CYCLOTHIAZIDE. B. Atassi[1], J.-M. Godfraind*[,3] and M.I. Glavinovic[1,2] Departments of Anaesthesia Research[1] and Physiology[2], McGill University, Montreal, P.Q. H3G 1Y6 Canada, and Departments of Physiology[3] and Pharmacology[3], Universite Catholique de Louvain, Brussels, Belgium.

It is still controversial whether desensitization of AMPA receptors plays any role in shaping the time course of the fast component of spontaneous excitatory unitary postsynaptic currents (mEPSCs). Similar duration of the time constants of desensitization in outside-out patches and of mEPSCs in whole cell recordings argues for such a role, but implies that transmitter concentration remains high and essentially constant throughout the synaptic cleft (Edmonds et al., Ann. Rev. Physiol. 57, 495, 1995). Prolongation of mEPSCs by compounds that suppress desensitization may be partly due to slower deactivation. We examined in rat hippocampal pyramidal cells (CA1 region; 12 cells) how the relationship between the amplitude and the kinetics of mEPSCs changes as desensitization is suppressed by cyclothiazide (0.1 mM). Both rise (τ_r) and decay (τ_d) times of mEPSCs become much more amplitude dependent in cyclothiazide (slopes increased from 0.12 to 0.67 ms/pA and from 0.40 to 2.09 ms/pA for τ_rs and τ_ds respectively; V_H = -50 mV and T = 32 °C) with membrane depolarization (from -100 to -50 mV; slopes increased from 0.01 to 0.12 ms/pA and from 0.04 to 0.40 ms/pA for τ_rs and τ_ds respectively; T = 32 °C), and with lower temperatures (from 32 to 22 °C; slopes increased from 0.12 to 0.42 ms/pA and from 0.40 to 1.30 ms/pA for τ_rs and τ_ds respectively; V_H = -50 mV). No upward shift of the kinetics vs amplitude relationship was observed in cyclothiazide arguing that it does not affect channel deactivation. These observations support the proposition that desensitization plays an important role in shaping the time course of mEPSCs.

Supported by the Medical Research Council of Canada.

508.12

IN VIVO EVALUATION OF AMPA ANTAGONISTS IN THE CA1 REGION OF HIPPOCAMPUS IN RATS. C. Mathiesen, M. Lauritzen, J. Hounsgaard and L.H. Jensen *. NeuroSearch, Smedeland 26B, DK-2600 Glostrup, Denmark.

Single unit recording have been used to evaluated the in vivo antagonism of neuronal firing evoked by microiontophoretic ejection of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) or the N-methyl-D-aspartate (NMDA) in the CA1 region of the hippocampus in rats. The antagonists were administrated by intravenous injection (i.v.) in the femoral vein. NBQX (30-60 µmol/kg) inhibited both AMPA and NMDA evoked firing at the highest dose (60 µmol/kg i.v.) whereas 45 µmol/kg given i.v. only inhibited AMPA responses. GYKI 52466 (30 and 61 µmol/kg) selectively inhibited AMPA evoked firing, whereas lower dose (15 µmol/kg i.v.) potentiated NMDA evoked firing in 3 out of 5 experiments. PNQX was tested at doses ranging from 9.0-36 µmol/kg) but were only significantly inhibited AMPA evoked firing at the highest doses uses (36 µmol/kg i.v.) It also have at minor inhibitory effect on NMDA evoked firing. The novel compound NS377 (7-Ethyl-5-phenyl-1,6,7,8-tetrahydro-1,7-diaza-as-indacene-2,3-dione) significantly inhibited AMPA and NMDA evoked firing at doses 2-4 times lower (12-24 µmol/kg i.v.) than the other compounds tested. The antagonists NS377 and NBQX did not only inhibited AMPA but also NMDA evoked firing, which could be related to lack of selectivity at high doses or indirect effect (due to a lack of depolarisation induced relief of $Mg2^+$ block of the NMDA channel when the fast depolarisation is removed by AMPA channel inhibition). The non-competitive antagonist GYKI 52466 selectively block the AMPA evoked activity. PNQX only inhibited the AMPA evoked activity statistically significant which could indicate some degree of in vivo selectivity, but there were only minor differences between the degree of AMPA and NMDA inhibition.

The result indicates, that the AMPA antagonists (like NBQX and NS377) inhibited NMDA responses indirectly which may be advantageous in ischemic therapy. The non-competitive AMPA antagonist GYKI 52466 shown in vivo selectivity, which could be related to a higher degree of receptor selectivity or some unknown mechanism.

508.13

DICARBOXYPHENYLGLYCINES AS SELECTIVE ANTAGONISTS OF AMPA-INDUCED DEPOLARIZATIONS IN NEONATAL RAT MOTONEURONES. D. E. Jane, N. K. Thomas, M. Gardener and J. C. Watkins*. Pharmacology Dept, University of Bristol, Bristol, BS8 1TD, U.K.

Ionotropic glutamate receptors have been categorized in three main groups according to the selective agonists that activate them, the N-methyl-D-aspartate (NMDA), (S)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA) and kainate receptors. It is known that both AMPA and kainate induce depolarizations in neonatal rat spinal motoneurones. However selective antagonists capable of discriminating between the effects of these two antagonists are not widely available. Although Pook *et. al.* (*Br. J. Pharmacol. 1993, 108: 179*) have reported that 6-nitro-7-sulphamobenzo(f)quinoxaline-2,3-dione (NBQX) has a 12-fold selectivity as an antagonist of AMPA- vs kainate-induced spinal motoneuronal depolarization, additional, and preferably more selective antagonists would be useful particularly in view of the diversity of sub-types of AMPA and kainate receptors now known to exist. As part of a search for such compounds we report the actions of (RS)-3,4-dicarboxyphenylglycine (3,4-DCPG) and (RS)-3,5-dicarboxyphenylglycine (3,5-DCPG) on AMPA- and kainate-induced motoneuronal depolarizations in the neonatal rat spinal cord preparation. Both 3,4-DCPG and 3,5-DCPG antagonized AMPA-induced depolarizations (apparent K_D = 137 ± 20 µM (n=3) and 167 ± 13 µM (n=5) respectively). However, 3,5-DCPG (1 mM) weakly potentiated responses to kainate while 3,4-DCPG (1 mM) displayed very weak antagonism of these responses. It is likely that an increase in potency and AMPA receptor selectivity will be observed once the individual enantiomers have been synthesized and tested. This work is in progress, together with an investigation of the actions of the substances on metabotropic glutamate receptors.

This work was funded by the Medical Research Council (U.K.).

508.14

NMDA AND AMPA RECEPTORS MEDIATE A POPULATION EPSP IN NEONATAL SPINAL CORD. D. L. Tauck* and J. J. Kendig. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

Glutamate is the neurotransmitter at the synapse between sensory and motor neurons that drives the monosynaptic reflex in the spinal cord. Stimulating and recording from non-homologous pairs of dorsal and ventral roots, respectively, allows one to record population EPSPs uncontaminated by superimposed population spikes. Using spinal cords isolated from 3 to 7 day old rats, we show that this glutamatergic population EPSP (pEPSP) is mediated by NMDA and AMPA receptors. A competitive glutamate receptor antagonist with high selectivity for the kainate receptor, NS-102 had no effect on the pEPSP. The selective NMDA antagonists AP5 and CPP both reversibly inhibit a slow to develop and long-lasting component of the pEPSP. Both CNQX, an AMPA/kainate antagonist, and NS-257, a selective AMPA antagonist, completely and reversibly blocked that part of the pEPSP not affected by NMDA antagonists. When applied alone, both CNQX and NS-257 depress the fast component of the pEPSP as well as the slower, long-lasting component of the pEPSP - leaving a small, slow response that is blocked by NMDA antagonists. Supported by NIH grants GM47818 and NS13108 to JJK.

508.15

ETHANOL DEPRESSES GLUTAMATE AMPA AND NMDA RECEPTOR-MEDIATED TRANSMISSION IN NEONATAL RAT SPINAL CORD. J. J. Kendig*, E. G. Fong and S. M. E. Wong. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

Ethanol, an intoxicant, is also a general anesthetic agent, preventing movement in response to a noxious stimulus, a spinal effect. The effects of ethanol were examined in isolated 1-7 day old rat spinal cord on the monosynaptic reflex (MSR) (AMPA+compound action potential); slow ventral root potential (sVRP)(NMDA+metabotropic); dorsal root potential, (DRP)($GABA_A$ via glutamate-excited interneurons); and population EPSP, (pEPSP)(AMPA+NMDA, see abstract by D.L. Tauck and J.J. Kendig). At a general anesthetic concentration (130 mM) ethanol significantly ($P<0.05$) depressed MSR amplitude (86.0%±6.15 of control, mean±SE), sVRP area (30%±1.51), DRP area (59.0%±3.47) and pEPSP area (62.4%±5.15). Effects were selective for NMDA versus metabotropic receptor-mediated sVRP components ($P<0.01$) and for NMDA versus AMPA-mediated pEPSP components ($P<0.05$). In the integrated spinal cord ethanol (1) depresses both AMPA and NMDA neurotransmission, NMDA more than AMPA and (2) ethanol depressant effects on glutamate excitatory transmission override $GABA_A$ enhancement when the $GABA_A$-mediated DRP is evoked via glutamate-activated interneurons. Supported by NIH grants GM47818 and NS13108 to JJK.

508.16

ANTAGONISM OF NMDA-EVOKED CURRENTS IN RAT CORTICAL CULTURES BY ARL15896AR. G.A.R. Mealing, [1]T. Lanthorn*, M.A. Black, N.B. Laferriere, D.L. Small, & P. Morley. National Research Council, Ottawa, Canada & [1]Astra Arcus USA, Rochester, NY, USA

The low-affinity NMDA antagonist ARL15896 protects primary cultures of rat cortical neurons from NMDA- or glutamate-mediated toxicity and, like memantine, rapidly blocks NMDA-triggered [Ca^{2+}], responses. This whole-cell voltage-clamp study examines the antagonism of NMDA-evoked currents by ARL15896 in this same culture model. ARL15896 caused a rapid and reversible inhibition of NMDA-evoked currents from neurons held at -60 mV, with an IC_{50} of 8 ± 1 µM. The EC_{50} for NMDA was not significantly affected by 10 µM ARL15896 ($p>0.05$), consistent with a non-competitive mechanism of block. ARL15896 antagonism is use-dependent, since application of ARL15896 60 s prior to NMDA exposure did not attenuate the initial NMDA-evoked current although the block developed rapidly thereafter. Once bound, ARL15896 remained trapped upon removal of NMDA until subsequent NMDA re-exposure, whereupon currents rapidly recovered. The forward and reverse binding-rate constants were estimated to be 2.406 x 10^4 M^{-1} s^{-1} and 0.722 s^{-1}, respectively. Antagonism was strongly voltage-dependent, as the K_D s at 0 and -60 mV were 60 µM and 11 µM, respectively. Additionally, there was a small component (≈25%) of ARL15896 antagonism that was voltage-insensitive. This component of the block does not act at the glutamate site, since it was not influenced by NMDA concentration.

In contrast to some uncompetitive NMDA antagonists, such as MK-801, ARL15896 exhibits rapid kinetics. This property could mediate the high margin of safety seen in preclinical studies, while maintaining the efficacy associated with use-dependent NMDA antagonists. ARL 15896 is in clinical trials for stroke.

This work was supported by the Canada/Astra Fight Stroke Program

509.1

SUBUNIT SELECTIVITY OF BENZODIAZEPINE LIGANDS WITH DIFFERENT PHARMACOLOGICAL PROFILES. M.R. Witt*, J. Drejer, S.E. Westh-Hansen and M. Nielsen. Research Institute of Biological Psychiatry, St. Hans Hospital, Roskilde, DK-4000 Roskilde, Denmark.

Classical benzodiazepines, which are widely used as anxiolytics, hypnotics and antiepileptics, mediate their activity via an interaction with the GABA$_A$ receptor complex. During recent years a series of benzodiazepine ligands which do not exert the full spectrum of classical benzodizepine activity has been identified. Compounds such as NNC13-8199, RP60503 and U78875 have in animal models been characterized as anxiolytics with minor or no sedative activity. In order to elucidate the molecular mechanism of the pharmacological interaction of these compounds with the GABA$_A$ receptor complex, a series of experiments were performed on recombinant GABA$_A$ receptors. Cloned human GABA$_A$ receptor subunits in the combinations $\alpha_x\beta_3\gamma_y$ (x=1,2,3 and 5; y=1,2 and 3) were transiently expressed in Sf9 cells and ^{3}H-Ro15-1788 binding was performed on membrane preparations from the cells in the absence and presence of GABA (100 μM). The classical benzodiazepine diazepam showed as expected positive GABA ratios predictive of agonistic activity on all receptor subunit combinations. On the other hand NNC13-8199 was found to be a weak partial agonist in a few subunit combinations (GABA ratio ~ 1.3) and an antagonist in other combinations (GABA ratio ~ 1). RP60503 appeared to be a pure antagonist at all combinations tested. U78875 showed partial agonism in some combinations and inverse agonism in others. The data suggest that the lack of sedative activity of the three anxiolytic compounds tested might be explained not only by partial agonism at the benzodiazepine binding sites but also by subunit selectivity.

509.2

MOLECULAR DETERMINANTS OF BENZODIAZEPINE-MEDIATED POTENTIATION OF THE RAT $\alpha1\beta2\gamma2$ GABA$_A$ RECEPTOR. J Amin†, AR Brooks-Kayal*‡, and DS Weiss§. †Dept. of Pharm.&Therap., USF Col. of Med., Tampa, FL 33612; ‡Depts. of Peds & Neurol., Univ of Penn. & Children's Hosp, Phila., PA 19104; §Neurobiol. Res. Ctr., Univ. of Alabama, Birmingham, AL 35294.

Benzodiazepine (BZD) mediates its actions in the CNS, at least in part, through a potentation of the GABA$_A$ receptor, although the molecular components underlying these actions are not fully understood. Two separate, but homologous, domains each comprised of a tyrosine and threonine on the extracellular region of the β subunit, have previously been shown to play an important role in GABA-mediated activation. Here, we examined the role of the corresponding tyrosines of the α and γ subunits on diazepam-mediated potentiation and [3H]Ro 15-1788 binding. Mutant $\alpha1$ and $\gamma2$ subunits were coexpressed with their wild type counterparts in Xenopus oocytes or HEK293 cells. Mutation of either of the two conserved tyrosines (to serine) in the α subunit dramatically impaired diazepam-mediated potentiation of GABA-activated chloride currents in oocytes and abolished the binding of [3H]Ro15-1788 to the transfected HEK293 cells. In contrast, the γ subunit mutants had a minimal effect on the EC50 for diazepam (2-fold increase) and did not significantly alter the binding of [3H]Ro15-1788. These data suggest that these two tyrosines of the α subunit may contribute to the BZD binding site. Furthermore, the correspondance of these residues to those in the β subunit critical for GABA-mediated activation suggests that the BZD binding site may have evolved from the GABA binding site. (Supported by NIH AA09212 & NS35291)

509.3

IDENTIFICATION OF GABA AND DIAZEPAM-RESPONSIVE REGIONS OF THE GABA$_A$ RECEPTOR USING $\gamma2/\alpha1$ SUBUNIT CHIMERAS. A. J. Boileau, N. V. Cozzi, P. Chen and C. Czajkowski*. Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Although GABA$_A$ receptor α subunits are important for binding GABA and benzodiazepines (BZDs), the presence of a γ subunit is required for high affinity BZD binding (Pritchet *et al.*, Nature 338, 1989). In order to determine which portions of the γ subunit confer BZD binding, we generated chimeric protein combinations of rat $\gamma2$ and $\alpha1$ subunits (Liu, *et al.*, Science 266, 1994) and expressed them with wild type $\beta2$ and/or $\alpha1$ subunits. We have generated over two dozen chimeric subunits with crossovers in the 5' extracellular region. Restriction enzyme mapping and DNA sequencing revealed seven distinct $\gamma2/\alpha1$ chimeras which encode complete ORF's. Of those expressed with wild type $\alpha1$ and $\beta2$ subunits in HEK 293 cells, two exhibited significant levels of [3H]flunitrazepam binding, and when co-expressed with wild type $\alpha1$ and $\beta2$ cRNA in *Xenopus* oocytes displayed diazepam potentiation of GABA-mediated chloride current. By comparing where the cross-over occurred in chimeras that were responsive to BZDs with those that were not, we have identified a 45 amino acid residue region in the 5' extracellular portion of the $\gamma2$ subunit that is necessary for BZD binding and BZD potentiation of the GABA-mediated chloride current. Using the same chimeras we have also identified a 42 amino acid region of $\alpha1$ which confers GABA-responsiveness when expressed with $\beta2$ subunits alone. This work was supported in part by funds from U.W.-Madison and a Basil O'Connor Starter Scholar Research Award from the March of Dimes to C.C.

509.4

[^{3}H]FLUNITRAZEPAM AND [^{3}H]RO15-4513 PHOTOAFFINITY LABEL DIFFERENT DOMAINS OF HUMAN RECOMBINANT GABA$_A$ RECEPTORS. M. Davies[a]*, I.L. Martin[a], A.N. Bateson[a,b], K.L. Hadingham[c], P.J. Whiting[c] and S.M.J. Dunn[a,b] [a]Department of Pharmacology and [b]Division of Neuroscience, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2H7. [c]Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Harlow, Essex, CM20 2QR, U.K.

We have used permanently transfected cell lines that express the human $\alpha1\beta1\gamma2$, $\alpha1\beta3\gamma2$, and $\alpha6\beta3\gamma2$ GABA$_A$ receptor subtypes to map the recognition sites for [^{3}H]flunitrazepam and [^{3}H]Ro15-4513. To identify specific areas of the receptor labelled by these compounds, we used a combination of photoaffinity labelling and chemical cleavage by hydroxylamine. Hydroxylamine cleaves at asparagine-glycine amide bonds, which in the receptor subtypes utilized in this study occur only in the α subunits. The photolabelled fragments were then resolved using SDS-PAGE. Using this approach, we determined that $\alpha1$-containing receptors incorporated [^{3}H]flunitrazepam within residues 1-103, while labelling by [^{3}H]Ro15-4513 occurred between residue 104 and the C-terminal of the α subunit. When membranes prepared from the $\alpha6\beta3\gamma2$-expressing cell line were photolabelled with [^{3}H]Ro15-4513, two major protein species were labelled, the sizes of which were consistent with the $\alpha6$ and $\gamma2$ subunits. Cleavage of these photolabelled receptors resulted in a gel profile consistent with incorporation of photolabel into residues lying between 103 and the C-terminal. These results show that the sites photolabelled by the agonist [^{3}H]flunitrazepam and the partial inverse agonist [^{3}H]Ro15-4513 occur within distinct domains of the human GABA$_A$ receptor. Supported in part by MRC Canada.

509.5

INCREASED SENSITIVITY TO BENZODIAZEPINES IN AGED BASAL FOREBRAIN NEURONS. **S.C. Hall* and W.H. Griffith. Dept. Medical Pharmacol. & Toxicol., College of Medicine, Texas A&M University, College Station, TX 77843-1114.**

The pharmacology of the γ-aminobutyric acid$_A$/benzodiazepine (GABA$_A$/BDZ) receptor complex in young and aged rats was explored using whole-cell patch-clamp recording techniques on acutely dissociated neurons from the medial septum/diagonal band. Previous findings have shown an increase in sensitivity to the GABA$_A$ enhancing BDZ drug, midazolam (MD), in aged rats (Griffith and Murchison, J. Neurosci., 15: 2407-2416, 1995). The purpose of this study was to further characterize pharmacologically the increased sensitivity of aged animals to the BDZs. We studied the differences in GABA$_A$ receptor pharmacology between young (1-3 months) and aged (24-26 months) Fischer 344 rats by comparing concentration-response curves of GABA (0.3-100 μM) and MD (0.03-3 μM). GABA-induced currents were enhanced by MD in both age groups at low GABA concentrations ($\leq$3 μM; young, n=22; aged, n=8). In contrast, during 10 μM GABA, 1 μM MD decreased currents in young (n=12), but continued to increase currents in aged cells (n=4). Flumazenil (1-3 μM) blocked both effects of MD in both age groups (10/12 neurons). Our data suggest MD may change the efficacy and affinity of the GABA$_A$/BDZ receptor in aged rats. (Supported by NIH grant AG07805).

509.6

ANXIOLYTIC PROFILE OF FULL AND PARTIAL AGONIST GABA$_A$ RECEPTOR MODULATORS IN SQUIRREL MONKEYS, RATS AND MICE. B. Dubinsky*, A.H. Vaidya, S. DeLuca, C.B. Davis, D.I. Rosenthal, C.T. Cheo-Isaacs, J.J. Crooke, and R.P. Shank. The R.W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477.

Allosteric modulators of GABA$_A$ receptors can induce a wide variety of CNS-related effects. An aim of drug discovery researchers who target GABA$_A$ receptors is to identify compounds that selectively induce only the effects that are therapeutically useful, e.g. anxiolytic activity. Hypothetically, the ideal compound may exert a low level of modulatory activity on all of the various types of GABA$_A$ receptors (a partial agonist), or selectively modulate only GABA$_A$ receptors associated with a specific CNS function (a selective agonist or a mixed agonist/antagonist). We examined the validity of these concepts by comparing the in vitro and in vivo activity of some full agonist modulators [alprazolam, chlordiazepoxide, clonazepam, diazepam and lorazepam] to that of partial agonist modulators [abecarnil, bretazenil, imidazenil, panadiplon and Ro 19-8022]. Our results in vitro indicate that the so-called full agonists as well as partial agonists have an ability to differentially modulate different types of GABA$_A$ receptors. This differentiation is due to differences in affinities and intrinsic activities. In vivo, the partial agonists usually exhibited lower maximum responses than the full agonists in anxiolytic tests (e.g., Vogel conflict and elevated plus maze in rats and food-reward/tail shock conflict in monkeys) and side-effect tests (e.g., motor impairment, ethanol sleep potentiation, reduced spontaneous motor activity and muscle relaxation). Furthermore, the partial agonists often exhibited a separation between anxiolytic activity and side-effects, but the magnitude of separation differed markedly between the species studied. Also, dose-response curves for partial agonists were often biphasic, i.e. effects observed at low doses disappeared at higher doses. Our results indicate that the simplistic terminology "full agonist", "partial agonist" or even "selective agonist" does not adequately define the activity of most GABA$_A$ receptor modulators acting at the benzodiazepine site.

509.7

PAIRED-PULSE DEPRESSION OF SLOW IPSPs SHOWS NO INVOLVEMENT OF GABA$_B$ AUTORECEPTORS IN REDUCTION OF GABA INHIBITION IN THE HIPPOCAMPAL CA1 REGION AFTER CHRONIC FLURAZEPAM TREATMENT. Xu Zeng* and E.I. Tietz, Dept. of Pharmacology, Medical College of Ohio, Toledo, OH 43699

The findings of intracellular recordings in CA1 neurons from 1 week flurazepam (FZP)-treated rats showed a reduction in monosynaptic GABA$_A$ and GABA$_B$-mediated IPSP amplitudes. Whole-cell patch clamp recordings from CA1 neurons showed a decreased frequency of spontaneous, but not miniature IPSCs, with this FZP treatment. Thus, in addition to postsynaptic alterations, presynaptic changes may contribute to reduced GABA inhibition in FZP tolerant rats. Since postsynaptic GABA$_A$, but not GABA$_B$-mediated responses were reduced by FZP treatment, the role of presynaptic GABA$_B$ autoreceptors in mediating GABA release was assessed by paired-pulse depression of slow IPSPs in *in vitro* hippocampal slices (500 μm) from FZP-tolerant rats (100 mg/kg × 3 days; 150 mg/kg × 4 days in 0.02% saccharin) sacrificed 2 days after treatment. CA1 neurons were recorded with glass micropipettes using intracellular techniques (3M KAc, 60-80 MΩ, I_H = -60 mV). Monosynaptic IPSPs were activated in stratum radiatum/lancanosum <0.5 mm from the recording electrode in the presence of APV (50 μM) and DNQX (10 μM). Fast IPSPs were blocked with picrotoxin (50 μM) and slow IPSPs of equivalent amplitude (Control: 4.67 ± 0.05 mV, FZP-Treated: 4.53 ± 0.06 mV, p>.05) were activated at interpulse intervals from 250-1250 ms. There were no significant differences (p>.05) in paired-pulse depression between control (n=6) and FZP-treated neurons (n=6). Reduction of interneuron firing rate, rather than of GABA$_B$ autoreceptor function, may be related to decreased presynaptic GABA-mediated inhibition in the hippocampus associated with benzodiazepine anticonvulsant tolerance. Supported by NIDA grant R01-DA04075 and RSDA K02-DA00180 to E.I.T.

509.8

DIFFERENTIAL CHANGES IN GABA$_A$ RECEPTOR (GABAR) α_2, β_1, β_3 AND γ_2 SUBUNIT PROTEINS IN FLURAZEPAM TOLERANT RATS. S. Chen, X. Zeng, W. Sieghart† and E.I. Tietz*, Dept. of Pharmacology, Medical College of Ohio, Toledo, OH 43699 and Dept. Biochemical Psychiatry†, Univ. Clinic of Psychiatry, A-1090 Vienna, Austria.

Prolonged administration of benzodiazepines (BZs) to animals results in the development of functional tolerance *in vivo* and *in vitro*. One week flurazepam (FZP) treatment decreased GABAR-mediated early IPSP and spontaneous IPSC amplitude in CA1 pyramidal neurons. GABA/BZ receptors were also functionally uncoupled, locally in CA1 subregions. Using *in situ* hybridization methods, decreased α_1 and β_3, but not α_5 or γ_2 subunit mRNA were detected in FZP-treated rat brains. We previously demonstrated the sensitivity and reliability of computer-assisted image analysis for quantitation of immunostaining density (Huang et al., 1996). Using this method, we analyzed levels of GABAR α_2, β_1, β_3 and γ_2 subunit proteins in rat brain sections (10 μm). Rats were sacrificed immediately after 1 week FZP treatment (100 mg/kg X 3 days; 150 mg/kg X 4 days in 0.02% saccharin water). Control rats received saccharin water for the same time period. Specific GABAR α_2, β_1, β_3 and γ_2 subunit antibodies (W. Sieghart) were used as 1° antibodies and biotinylated anti-rabbit IgG F(ab)$_2$ fragments as 2° antibodies. Immunostaining was visualized with DAB and staining intensity was measured over images acquired with NIH Image software. As previously found for the α_1 subunit protein, β_3 subunit protein significantly decreased in FZP-treated rat brains in parallel with β_3 subunit mRNA in the CA1 s. oriens (-18.9%), s. pyramidale (-20%) and s. radiatum (-25%); molecular (-16%) and granular layer (-18%) of dentate gyrus; and in CA3 and cortex. Though γ_2 subunit mRNA had not significantly decreased in the dentate molecular layer (-12%), a significant decrease in γ_2 subunit protein (-7%) was detected. No changes were found in α_2 or β_1 subunit antibody staining in any brain region despite a significant decrease in α_2 mRNA in caudate (-32%). mRNA/protein mismatches may relate to differences in the timing of transcription/translation. Regional decreases in GABAR subunit antibody staining may be one postsynaptic mechanism associated with BZ tolerance. R01-DA-04075 and RSDA K02-DA00180 to EIT.

509.9

Diazepam Increases the Maximal Response to Kojic Amine: Evidence for a Two-State Model of GABA$_A$ Receptor Modulation. Y. T. Lee, T. T. Gibbs* and D. H. Farb, Laboratory of Molecular Neurobiology, Department of Pharmacology, Boston University School of Medicine, Boston, MA 02118.

The A-type γ-aminobutyric acid receptor (GABA$_A$R) is a heteropentameric chloride ion channel. The GABA$_A$R is activated by full agonists such as GABA and muscimol, and agonist function is subject to positive and negative modulation by benzodiazepines, barbiturates, neurosteroids, and heavy metal divalent cations. The design of new therapeutic agents would be aided by a general model for GABA$_A$R modulation. As a first step, we have proposed a simple two-state model for GABA$_A$R modulation. This model predicts that, while the efficacy of a full agonist should not be affected by a positive modulator, the efficacy of a partial agonist can be positively modulated. To test this model, we examined the effect of diazepam on the response to kojic amine, which we have shown to be a partial agonist at the GABA$_A$R, using whole cell voltage-clamp recordings of primary chick spinal cord neurons in culture. In the presence of the positive modulator diazepam, the maximum response to kojic amine was increased while the maximum response to GABA remained the same. The results obtained are consistent with the two-state model for GABA$_A$R modulation. Supported by NIMH MH49469.

509.10

CHARACTERIZATION OF THE BINDING OF ME 3127, A NEW BENZODIAZEPINE AGONIST, TO NATIVE AND RECOMBINANT GABA$_A$ RECEPTORS. T.H. Johansen[1,2]*, M. Nielsen[2], T. Hiranuma[3], S.E. Westh-Hansen[2], F. Wätjen[1], and E.Ø. Nielsen[1], [1]NeuroSearch A/S, DK-2600. Glostrup, Denmark. [2]Research Institute of Biological Psychiatry, St.Hans Hospital, DK-4000 Roskilde, Denmark, [3]Meji Seika Kaisha, Ltd., Pharmaceutical Research Center, Yokohama 222, Japan.

ME 3127 is a new benzodiazepine agonist which induces a robust anticonflict effect in rats. In this study we present a characterization of the binding profile of ME 3127. At native receptors in cortex homogenate ME 3127 displaced [^{3}H]flunitrazepam binding competetively with a K_i = 2.9 ± 0.5 nM (n = 11). The affinity of ME 3127 was increased 1.6 fold by addition of 30 μM GABA compared to a 1.5 fold increase for flunitrazepam. Saturation of the benzodiazepine binding site by ME 3127 increased non-equilibrium [^{35}S]TBPS binding to the same extent as diazepam did, 1.29 ± 0.03 fold and 1.31 ± 0.03 fold (n = 4), respectively. This indicates that ME 3127 is a benzodiazepine agonist with full intrinsic activity.

The affinity of ME 3127 for recombinant human GABA$_A$/benzodiazepine receptor complexes was studied at $\alpha_x\beta_x\gamma_{2S}$ receptors expressed in baculovirus-transfected Sf-9 insect cells. ME 3127 displaced [^{3}H]Ro 15-1788 with some subtype selectivity being approximately 5 times more potent at receptors containing the α_1 or the α_5 subunit compared to receptors containing the α_2 or the α_3 subunit. The ratio between IC_{50} values for ME 3127 in the absence and presence of GABA (GABA ratio) depended on the subunit combination. At $\alpha_3\beta_2\gamma_2$ and $\alpha_3\beta_3\gamma_2$ receptors the GABA ratios were 2.0 ± 0.4 and 2.1 ± 0.3 (n = 3), respectively, whereas ME 3127 had a GABA ratio of 0.9 ± 0.2 at $\alpha_3\beta_1\gamma_2$ (n =3) indicating that ME 3127 acts as an antagonist at this subunit combination.

The binding [^{3}H]ME 3127 to rat brain homogenate, brain slices and recombinant GABA$_A$ receptors is presently being characterized.

509.11

A REGIONAL DIFFERENCE IN THE EFFECTS OF MIDAZOLAM ON SYNAPTIC TRANSMISSIONS IN RAT HIPPOCAMPAL NEURONS. Y. Sato, Y. Fujito*, and M. Aoki, Dept. of Physiology, School of Medicine, Sapporo Medical University, Sapporo 060, Japan.

Midazolam, an imidazobenzodiazepine derivative, is widely used as an intravenous anesthetic. Its effects are considered to be mediated mainly through the GABA$_A$ receptor, enhancing the inhibitory action of GABA. In the present study, we compared the effects of midazolam on synaptic transmissions in CA1 pyramidal cells (PCs) and on dentate gyrus granule cells (GCs) in rat hippocampal slices using intracellular recordings. Midazolam (75 μM) markedly depressed orthodromic evoked spikes in PCs, compared with those in GCs. A GABA$_A$ receptor antagonist bicuculline (10 μM) almost completely antagonized the depressant effects of midazolam on spike generation in PCs, while bicuculline little antagonized midazolam effects in GCs. Midazolam produced depolarizing or hyperpolarizing effects on resting membrane potentials (RMP) with an input resistance decrease in PCs, whereas it produced slight depolarizing effect on RMP without affecting an input resistance in GCs. Midazolam significantly increased the amplitudes of monosynaptic inhibitory postsynaptic potentials in PCs evoked by stimulation near the recording electrode in the presence of glutamate receptor antagonists, but slightly decreased those in GCs. These results clearly demonstrated that midazolam has differential depressant effects on synaptic transmissions in hippocampal neurons. The mechanism of this difference could be due to the different densities and types of GABA$_A$/benzodiazepine receptors between PCs and GCs.

This work was partly supported by a grant from Foundation of Sapporo Medical University.

509.12

NALOXONE ANTAGONIZES GABA$_A$/BENZODIAZEPINE RECEPTOR FUNCTION IN RAT CORTICOHIPPOCAMPAL SYNAPTONEUROSOMES. A.I. Svensson*, J.A. Engel and B. Söderpalm, Inst. of Physiol. and Pharmacol., Dept. of Pharmacol., Göteborg Univ., Medicinaregatan 7, S-413 90 Göteborg, Sweden.

Low serotonin (5-HT) neurotransmission has been linked to anticonflict effects and impulsive/aggressive behaviors in rats, and to impulse control deficiency, aggression, suicide etc. in humans. Impulsive behavior after extensive 5-HT depletion in rats may be reversed with negative modulators of brain GABA$_A$/benzodiazepine receptors. In an accompanying abstract we report that naloxone reduces 5-HT depletion induced anti-conflict and aggressive behaviors, and that amobarbital in turn reverses the naloxone effect in the conflict model used. Also Ro 15-4513, a partial inverse benzodiazepine receptor agonist, reduces 5-HT depletion induced aggression. Naloxone thus produces behavioral effects similar to those produced by negative modulators of GABA$_A$/benzodiazepine receptors in 5-HT depleted animals, and GABA$_A$ antagonistic properties of naloxone have been proposed. Hence, weak GABA$_A$/benzodiazepine receptor antagonistic effects of naloxone may explain our behavioral data. Here we have studied the effects of naloxone on rat GABA$_A$/benzodiazepine receptor function *in vitro*.

Naloxone (0.1 - 1000 μM) in a concentration-dependent manner reduced the GABA-induced (10 μM) $^{36}Cl^-$-uptake in corticohippocampal synaptoneurosomes, with a 60% reduction at naloxone 1000 μM. This concentration of naloxone did not by itself affect $^{36}Cl^-$-uptake. Furthermore, the concentration-response curve for GABA-induced $^{36}Cl^-$-uptake (GABA 3, 10, 30, 100 μM) was shifted to the right by naloxone 1000 μM. The naloxone-induced (1000 μM) reduction of GABA-mediated (10 μM) $^{36}Cl^-$-uptake was concentration-dependently reversed by amobarbital (10 - 1000 μM) but not by flumazenil (10 - 1000 μM).

These results indicate that naloxone is a weak negative modulator of GABA$_A$/-benzodiazepine receptor function and suggest, together with our behavioral data, that this property may be involved in its anti-impulsive/anti-aggressive effects.

Supported by the Swedish MRC (grants no 11583 and 4247).

509.13

THE EFFECT OF DIAZEPAM UPON THE PHYSIOLOGICAL CHARACTERISTICS OF NEURONES IN THE IMHV OF THE DOMESTIC CHICK P.M.Bradley, B.D.Burns, B.A.Clark and A.C.Webb.(SPON:BRAIN RESEARCH ASSOCIATION) Department of Neurobiology, Medical School, Newcastle upon Tyne,United Kingdom. NE2 4HH.

The avian brain contains an area known as the intermediate and medial hyperstriatum ventrale (IMHV) which is essential for early learning. Local electrical stimulation of the IMHV in an *in vitro* brain slice preparation elicits a characteristic extracellular field response. This response is under a considerable degree of inhibition mediated by the $GABA_A$ receptor system.The benzodiazepine receptor is thought to play a role in the modulation of GABAergic inhibition and the IMHV has been shown to contain a high density of these receptors compared to other forebrain regions.

We therefore examined the effect of the benzodiazepine receptor agonist diazepam (12μ M) upon the electrophysiological properties of the IMHV in slices taken from 3-4 day old birds (N =10). Analysis of the data using the paired t-test showed no significant difference in responses before and after perfusion with the drug. However, comparision of the measurements made under diazepam with those made under the control periods showed that the drug produced a significant ($p = 0.0025$) increase in the mean peak amplitude (14% $\pm$ 2.8) of the post-synaptic component of the field response. It has previously been shown that the peak amplitude is dependent upon glutamate receptors of the non-NMDA sensitive subtype. The duration of the response, which is dependent upon NMDA receptors showed no significant change.

Intracellular recordings from single cells (N = 7) showed that diazepam did not alter the membrane resistance, threshold to internal stimulation, resting membrane potential or duration of response to an external stimulus of the cells. However, 4 of the 7 cells displayed irregularly occuring spontaneous bursts of EPSP's and diazepam appeared to reduce the amplitude, duration and frequency of the bursts in these cells.

This work was supported by the BBSRC.

509.14

PHYSIOLOGICAL, BEHAVIORAL AND SUBJECTIVE EFFECTS OF FLUMAZENIL-PRECIPITATED WITHDRAWAL IN BENZODIAZEPINE-DEPENDENT METHADONE CLIENTS, J. M. Frey*, C. R. Rush and R. R. Griffiths. Johns Hopkins University School of Medicine, Baltimore, MD 21224.

The effects of i.v. administered flumazenil (FLU, Romazicon™; 0-4 mg/70 kg) were studied in six methadone clients with a recent history of chronic benzodiazepine use (at least 450 mg diazepam equiv./mo.). Subjects were converted to p.o. lorazepam administration (5-12 mg/day) during a 7 day stabilization period prior to testing. During the stabilization period, subjects were evaluated for benzodiazepine tolerance development and monitored for sedative intoxication using a variety of psychomotor/behavioral performance, observer and subject-rated measures. All six subjects were determined to be benzodiazepine dependent in a semi-structured clinical interview diagnostic (SCID) for the DSM IIIR. After at least two i.v. practice sessions, FLU was injected (42ml/5 min) in ascending doses in three days of dose run-up testing; dosing run-ups were followed in four subjects by a randomized crossover study up to the highest dose of FLU tolerated during the run-up period. Precipitated withdrawal symptoms reliably occurred within 1-2 min and peaked within 5 min after initiating FLU injections. Composite scores from a 22-item "Subject-rated Withdrawal Symptoms Questionnaire" and the Spielberger Y-1 (State) Anxiety Index indicated that the effects of FLU were both dose and time-dependent from 0.25-4.0 mg/70 kg. Precipitated withdrawal was further characterized by anecdotal self-reports of confusion/difficulty concentrating, anxiety, irritability, dysphoria, dizziness, stomach cramping/pain and numbness in the extremities. This study demonstrates that rapid infusion of FLU in benzodiazepine-dependent volunteers can precipitate symptomology commonly associated with benzodiazepine withdrawal.

Supported by NIDA grant DA-03889.

GABA$_A$ RECEPTORS: ETHANOL

510.1

POSTNATAL ETHANOL EXPOSURE INHIBITS MATURATION OF $GABA_A$ RECEPTORS IN DEVELOPING MEDIAL SEPTUM/ DIAGONAL BAND (MS/DB) NEURONS. S.-H. Hsiao[1]*, J.R. West[2], J.C. Mahoney[2] & G.D. Frye[1]. Dept. Med. Pharmacol. & Toxicol.[1], Dept. Med. Anat.[2], Texas A&M Univ. Coll. Med., College Station, TX 77843-1114.

The $GABA_A$ receptor is a ligand-gated Cl^- channel playing a prominent inhibitory role. Postnatally, expression of $GABA_A$ receptor subunits undergoes major changes and may represent a target for ethanol as part of the fetal alcohol syndrome (West et al., *Metab. Brain Dis.* 9:291, 1994). The present study examined whole cell $GABA_A$ currents in MS/DB neurons, acutely dissociated from Sprague-Dawley rats (days PN 12-16). During days PN 4-9, animals were artificially reared (pup-in-the-cup) and exposed twice daily to ethanol (4.5 g/kg/day) or milk alone. GABA (0.3-300 μM) responses were concentration and ethanol treatment dependent. Maximum GABA response for neurons in the ethanol exposed group (209.08 $\pm$ 15.2 pA/pF, n = 36) was smaller than that of cells from artificially reared controls (305.0 $\pm$ 15.18 pA/pF, n=6; $p < 0.05$). The EC_{50} values were also smaller after ethanol (4.85 $\pm$ 0.44 μM vs 7.68 $\pm$ 0.93 μM; $p < 0.05$), but Hill coefficients were unchanged. Meanwhile, there were no significant differences between artificially vs normally reared controls, respectively (maximum response: 305.0 $\pm$ 47.93 vs 348.75 $\pm$ 71.9 pA/pF; $p>0.05$. EC_{50}: 7.68 $\pm$ 0.93 vs 7.66 $\pm$ 1.50 μM; $p>0.05$). These data suggest that the chronic postnatal ethanol exposure significantly changes development of $GABA_A$ receptors in MS/DB neurons. Supported by AA06322 (GDF) and AA05523 (JRW).

510.2

CHRONIC ETHANOL DOES NOT UNCOUPLE ALLOSTERIC MODULATION OF $GABA_A$ RECEPTORS BY MIDAZOLAM OR LORECLEZOLE IN MEDIAL SEPTUM / DIAGONAL BAND (MS/DB) NEURONS. K.A. Wallace*, A.S. Fincher, G.D. Frye. Dept. of Med. Pharmacol. & Toxicol., Texas A&M Univ., College Station, TX 77843-1114.

$GABA_A$ receptor activity is acutely increased by a wide range of positive allosteric modulators acting at distinct sites on the chloride channel complex. However, chronic treatment with modulators such as ethanol may lead to uncoupling of other allosteric interactions with the $GABA_A$ receptor. In the present study, Spraque-Dawley rats were made physically dependent on ethanol via a liquid diet. The ability of midazolam (1 μM) or loreclezole (10 μM) to potentiate $GABA_A$ mediated chloride whole cell currents was measured in acutely dissociated MS/DB neurons. In neurons from diet-fed controls, currents activated by 3 μM GABA were significantly potentiated by midazolam and loreclezole (GABA alone: 73.9 $\pm$ 7.3 pA/pF, plus midazolam: 119.5, $\pm$ 16.7 pA/pF; $p < 0.01$ - - GABA alone: 79.8 $\pm$ 12.4 pA/pF, plus loreclezole: 153.2 $\pm$ 28.6 pA/pF; $p < 0.01$). Chronic ethanol treatment did not change the level of midazolam or loreclezole induced potentiation (GABA alone: 86.7 $\pm$ 10.0 pA/pF, plus midazolam: 161.3 $\pm$ 34.3 pA/pF; - - GABA alone: 71.3 $\pm$ 6.3 pA/pF, plus loreclezole: 118.2 $\pm$ 12.0; $p > 0.05$ for both drugs relative to control). Near maximum currents GABA (30 μM) were not potentiated by either drug regardless of pretreatment. Thus, loreclezole and midazolam potentiation at the concentrations tested is limited to $GABA_A$ responses induced with lower levels of GABA and this enhancement does not show uncoupling after chronic ethanol treatment. Supported in part by AA06232 (GDF).

510.3

ETHANOL POTENTIATES AND INHIBITS $GABA_A$ CURRENTS IN ADULT HIPPOCAMPAL AND CEREBRAL CORTICAL NEURONS. G.D. Frye* and A.S. Fincher. Dept. of Med. Pharmacol. & Toxicol., Texas A&M Univ., Col. of Med., College Station, TX 77843-1114.

$GABA_A$ currents in neurons isolated from medial septum/n. diagonal band of adult rats exhibit a range of sensitivities to ethanol ranging from enhancement to inhibition (Frye et al., Brain Res. 635: 283-292, 1994). In this study, acutely isolated adult neurons from Sprague-Dawely rat hippocampus (hippo) or cerebral cortex (cortex) exhibited concentration dependent currents in response to GABA (0.3-300 μM) with EC_{50}s of 13.9 and 19.8 μM, respectively. Coapplication of ethanol (10, 30 or 100 mM) and 3 μM GABA did not enhance mean currents. However, many cells showed either potentiation (ie., $\geq$ 110% of control) or inhibition (ie., $\leq$ 90% of control) of GABA currents by at least one concentration of ethanol. Ethanol potentiated GABA currents in one subset of neurons (cortex = 3 of 14; hippo = 4 of 14), but inhibited in another subset (cort = 8 of 14; hippo = 6 of 14). Currents in one cortical neuron were both potentiated and inhibited by ethanol. Thus ethanol sensitivity of $GABA_A$ receptors on adult hippocampal and cerebral cortical neurons varies widely from cell to cell ranging from enhancement to inhibition. Supported in part by AA06322 (GDF).

510.4

ETHANOL ENHANCES $GABA_A$ IPSC RESPONSES IN ETHANOL SENSITIVE BUT NOT ETHANOL INSENSITIVE MOUSE HIPPOCAMPAL NEURONS. W.R. Proctor* and T.V. Dunwiddie. Dept. of Pharmacology, University of Colorado Health Sciences Center, and Veterans Admin. Medical Res. Service, Denver, CO.

The mechanisms of ethanol enhancement of GABAergic neurotransmission are still poorly understood. Although the hippocampus has been reported to be relatively insensitive to acute ethanol treatment, recent observations have shown significant potentiation of selectively activated $GABA_A$-mediated IPSCs in CA1 pyramidal cells by ethanol. In the present study, we compared the ethanol sensitivity of IPSCs in stable, inbred long sleep (ILS) and inbred short sleep (ISS) mice, which are differentially sensitive to the behavioral effects of ethanol. $GABA_A$-mediated IPSCs, elicited by stimulation in the somal layer instead of in the stratum radiatum, were recorded under voltage clamp conditions with K^+-gluconate whole cell electrodes. The IPSCs were pharmacologically isolated by blocking EPSCs with APV (40 μM) and DNQX (10 μM). The resulting IPSCs could be blocked by application of 30 μM bicuculline. Ethanol had no significant effect on IPSCs in ISS mice, but at concentrations of 80 and 160 mM, the IPSCs from ILS neurons were significantly potentiated (128 $\pm$ 8.6% of control, n = 10). Lower concentrations of ethanol had no effect. These results suggest that the ethanol sensitivity of $GABA_A$ receptors may contribute to the behavioral differences between ILS and ISS mice.

Supported by AA 03527 and the Veterans Administration Medical Research Service.

510.5

GABA$_A$ RECEPTOR GENE EXPRESSION IN CNS OF RATS "BEHAVIOURALLY DEPENDENT" TO ETHANOL. G. Pinna*, M. Eravci, S. Kley, J. Wolffgramm and A. Baumgartner. Departments of Nuclear Medicine and Neuropsychopharmacology, Klinikum Benjamin Franklin, Free University of Berlin, 12200 Berlin, Germany.

The sensitivity of GABA$_A$ receptors to ethanol (ETH) may be related to the GABA$_A$ receptor subunit formulation. For this reason, we examined the receptor gene expression of several GABA$_A$ receptor subtypes (α_1; α_2; α_3; β_1; β_2; γ_2) in various brain areas of rats chronically exposed to ETH. An animal model was developed to render rats "behaviourally dependent" to ETH. Rats were administered ETH following two different designs: in group A, animals had the free choice between water and two different concentrations of ETH (5 and 20% v/v), in a three bottle system, during 9 months. The group B received a 5% ETH solution as sole drinking fluid during 9 months. After 3 months period (mo. 10-12) of "abstinence" the animals were re-exposed to a choice between water and ethanol for two months (mo. 13-14). In this phase, rats could choose between water and, again, two concentrations of ETH. In month 14, all the alcohol solutions were adultereted with 0.1 g/l quinine hydrochloride. Group A, but not group B, full filled the criteria for "behavioural dependence" which was defined as a "loss of control" over drug ingestion, maintained even after 3 months' "forced abstinence" and characterised by an unusually high ETH consumption, even when ETH was adulterated with a highly aversive substance such as quinine hydrochloride. This result indicates that the possibility of free choice paradigm for alcohol or water is important for the induction of "behavioural dependence". Forced ETH application may affect many biochemical parameters, but will not result in "behavioural dependence". After the "retest" rats were withdrawn from ETH for one month before sacrification. Quantification of mRNA steady-state levels was performed by Multiple Oligonucleotide Solution Hybridization (MOSH) and densitometric evaluation of the autoradiographs. In cerebral cortex, in group A the mRNA of all GABA$_A$ receptor subtypes investigated was decreased, no changes were detected in B. On the other hand, in hippocampus no changes were detected in both groups. These results support the hypothesis that ETH comsumption modulates the expression of GABA$_A$ receptors in a regional-specific way. Moreover, in cerebral cortex the dynamics of GABA$_A$ receptor subunit gene expression was modified specifically in "behaviourally dependent" rats. We conclude that the alteration of GABA$_A$ receptor gene expression may play a critical role in the "behavioural dependence" to ethanol. Supported by grant Ba 932/6-1 of the Deutsche Forschungsgemeinschaft.

510.6

FUROSEMIDE AND DMCM ACTIONS ON CEREBELLAR GABA$_A$ RECEPTORS OF ALCOHOL-SENSITIVE ANT RATS. R. Mäkelä[1,2], H. Lüddens[2] and E. R. Korpi[*,2,3]. [1]Tampere Brain Research Center, University of Tampere Medical School, [2]Department of Alcohol Research, KTL, Helsinki, [3]Department of Pharmacology and Clinical Pharmacology, University of Turku, Finland and [4]Department of Psychiatry, University of Mainz, Germany.

We have shown that furosemide, a loop diuretic, specifically reverses the inhibition by GABA of [^{35}S]TBPS binding through a novel binding site in the GABA$_A$ receptors with an $\alpha6\beta2/3\gamma2$ subunit composition, but not in an $\alpha1\beta1\gamma2$ receptor subtype (Korpi *et al.*, Mol. Pharmacol. 47:283-289; 1995). We now present that the ability of furosemide to increase [^{35}S]TBPS binding and antagonize GABA-induced inhibition of [^{35}S]TBPS binding in cerebellar membranes of alcohol-sensitive (ANT) rat line is reduced as compared to those of alcohol-insensitive (AT) rat line. This reduced antagonism ability was not shared by other GABA$_A$ receptor antagonists, e.g., SR 95531. The single amino acid mutation in the benzodiazepine (BZ) binding site of ANT rats (Korpi *et al.*, Nature 361:356-358; 1993) rendering cerebellar BZ-insensitive sites sensitive to BZ agonists did not explain the blunted furosemide antagonism, as confirmed with recombinant receptor studies. DMCM, a β-carboline, acts at low concentrations as an inverse agonist on BZ binding site and at high concentrations, it potentiates GABA$_A$ receptor function through a loreclezole binding site in β2 and β3 subunits (Stevenson *et al.*, Mol. Pharmacol. 48:965-969; 1995). Using quantitative autoradiography with [^{35}S]TBPS, we demonstrate similar biphasic effect of DMCM on [^{35}S]TBPS binding in the presence of GABA in cerebellar granule cell layer of AT and ANT rats. These experiments suggest that the cerebellar GABA$_A$ receptors of the ANT rats are altered also otherwise than in their BZ-site mutation, either by endogenous compounds, other mutations, or altered subunit composition.

(The study was partly supported by the Finnish Alcohol Research Foundation)

510.7

PRESENCE OF mRNA FOR SPECIFIC GABA$_A$ RECEPTOR SUBUNITS CORRELATES WITH RESPONSES TO ZOLPIDEM AND ETHANOL. H. E. Criswell* T. J. McCown, A. G. S. Oxford, L. Morrow, and George R. Breese. University North Carolina, Chapel Hill, NC 27599.

When ethanol is locally applied to neurons, *in vivo*, it enhances the action of iontophoretically applied GABA for some neurons but not for others. Responsiveness of a neuron to the type I benzodiazepine agonist zolpidem predicts whether that neuron will respond to ethanol. (J.P.E.T. 273:526-536,1995). Because studies in recombinant systems have shown that the α1 subunit for the GABA$_A$ receptor in combination with a β and a γ2 subunit must be present for low concentrations of zolpidem to enhance the action of GABA, we used whole-cell patch clamp recording followed by RT-PCR of cytoplasm from that cell to relate the presence of mRNA for specific subunits to the action of zolpidem and ethanol. Concentration-response curves for zolpidem showed the presence of a high affinity receptor (10-30 nM), a low affinity receptor (100-300 nM) or both, on individual cells. Those cells that responded to low concentrations of zolpidem universally expressed mRNA for the α1 subunit, while cells which required the higher concentrations contained the α2 and/or the α3 subunit mRNA. Neurons that showed a response to the low concentration of zolpidem with an increased response to the higher concentrations invariably expressed mRNAs for the α1, as well as the α2 and/or α3 subunits. While fewer neurons showed an enhancement of the effect of GABA by ethanol, the α1 subunit was always present when ethanol was effective. Supported by AA-09122.

GABA$_A$ RECEPTORS: ANESTHETICS

511.1

A NOVEL CLASS OF GABA-A RECEPTOR SUBUNIT CONFERS INSENSITIVITY TO ANESTHETIC AGENTS

E.F. Kirkness*, P.A. Davies, M.C. Hanna and T.G. Hales. The Institute for Genomic Research, 932 Clopper Road, Gaithersburg, MD 20878 and Dept. Anesthesiology, UCLA, Los Angeles, CA 90025.

A novel class of GABA-A receptor subunit (epsilon; ε) has been cloned from human and rat tissues. The human ε1 subunit is 506 amino acids in length, and exhibits 20-40% identity with related receptor subunit classes (α, β, γ, δ, ρ, π). The human ε1 subunit gene encodes an mRNA of ~3.5 kb that is expressed in discrete brain regions and some peripheral tissues. The human ε1 subunit gene has been localized on the X-chromosome.

When expressed transiently in HEK293 cells, the ε1 subunit does not appear to form homomeric GABA-A receptors. Combinations of α/ε, or β/ε subunits also fail to express GABA-gated chloride channels. However, combinations of α, β and ε subunits can assemble to form GABA-A receptors that exhibit unique biophysical and pharmacological properties. GABA-evoked currents that are mediated by α/β receptors exhibit outward rectification, whereas activation of α/β/ε receptors yields currents that display a relatively linear relationship to voltage. Also, in contrast to α/β receptors, which are modulated by low concentrations of anesthetic agents (e.g. pregnanolone, pentobarbital, propofol), the α/β/ε combinations are relatively insensitive. However, in common with α/β receptors, the α/β/ε combination can be activated directly by high concentrations of these agents. Expression of the ε1 subunit may explain the differential sensitivity of GABA-A receptors to anesthetic agents that has been detected in brain tissues. In addition, the ε1 subunit will be useful for dissecting the dual actions of anesthetic agents at GABA-A receptors.

Funding sources: Grants from TIGR and NIH.

511.2

ISOFLURANE MODULATION OF ALTERED RHO 1 GABA RECEPTOR SUBUNITS. Eric P. Greenblatt*. Dept. of Anesthesia, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Inhibitory neurotransmitter receptors for GABA and glycine may be important targets in the mechanism of action of volatile anesthetics. The anesthetic isoflurane has been shown to enhance chloride ion currents through these receptors. In contrast, the rho 1 subtype of GABA receptor is insensitive to isoflurane. In an effort to identify a site of isoflurane action on such proteins, mutations were introduced in human rho 1 subunits to induce amino acid substitutions corresponding to residues shared by the structurally related, isoflurane-sensitive GABA$_A$ α_1 and glycine α_2 receptor subunits. Amino acid residues in two putative transmembrane domains were targeted, given the expectation that volatile anesthetics should interact at hydrophobic portions of the protein. Mutants designated rho1m1-2 and rho1m2-1 were generated, coding for amino acid substitutions M297I and S325T, respectively. Effects of isoflurane on GABA-gated chloride currents were examined using two electrode voltage clamp in *Xenopus* oocytes injected with mutant or wild type rho subunit cRNAs . Currents measured in oocytes expressing wild-type rho subunits exhibited characteristic GABA pharmacology and were unaffected by coadministration of isoflurane. In oocytes expressing each mutant, isoflurane inhibited GABA-evoked currents. Although isoflurane-sensitive GABA and glycine receptors typically show enhancement of agonist-gated currents, these results may reflect the possibility that neither single amino acid substitution was sufficient to generate the structural determinant(s) which may define a volatile anesthetic binding site. However, each substitution may have resulted in alterations in protein structure that permitted a functionally significant molecular interaction with isoflurane, and may be relevant to the interaction of isoflurane with its target site on native receptors in the central nervous system.

Supported by a Foundation for Anesthesia Education and Research Young Investigator Award.

511.3

UPREGULATION OF GABA$_A$ RECEPTOR α3 and β3 SUBUNIT mRNA LEVELS BY CHRONIC PENTOBARBITAL TREATMENT IN IMMORTALIZED GT1-7 NEURONS. L.-H. Lin* and W.-T. Jang Department of Pharmacology, Chang Gung College of Medicine and Technology,Taiwan.

The present study investigated how GABA$_A$ receptor expression was regulated by drugs exerting actions on this receptor complex. Immortalized GT1-7 cell line containing single cell population was chosen as the experimental preparation. The cells were treated for 48 h with various GABA$_A$ receptor agonists (i.e. GABA, pentobarbital, 3α-OH-DHP, propofol and ethanol), antagonists (i.e. bicuculline, picrotoxin and SR 95531) and inverse agonist (i.e. Ro15-4513). We found that pentobarbital not only increased GABA$_A$ receptor α3 and β3 subunit mRNA levels, but also reduced the mRNA level for the house keeping gene GAPDH. The elevated α3 and β3 mRNA levels decremented, within 24-48 h after discontinuation of the drug treatment, to control levels. The other agents showed little effects. To investigate whether pentobarbital modified gene expression via GABA$_A$receptor activation or other mechanisms, we treated GT1-7 neurons with picrotoxin for 1 h, followed by a co-treatment of pentobarbital/ picrotoxin for another 48 h. We found that picrotoxin abolished the pentobarbital effects on α3 and β3 subunits, but changed little on the pentobarbital effect on GAPDH. It is thus likely that pentobarbital upregulates α3 and β3 subunit mRNA levels by increasing the chloride channel activity via GABA$_A$ receptor activation. Pentobarbital downregulates GAPDH mRNA level via some biochemical targets other than GABA$_A$ receptor. (Supported by grants from Chang Gung Memorial Hospital and National Science Council, Taiwan)

511.4

PENTOBARBITAL OPENING OF A HOMOMERIC CHLORIDE ION CHANNEL REQUIRES A SPECIFIC ASPARAGINE RESIDUE, I. N. Cestari* and J. Yang. Dept. Anesth. Pain Mgmt and Neurosci. Grad. Prog., UTSW Med. Sch., Dallas, TX.

The murine GABA$_A$ receptor β3 subunit when expressed by itself forms homomeric ion channels opened by pentobarbital (PNB). The β1 subunit with 82% amino acid identity with the β3 subunit does not form channels responsive to PNB. Here we report the results of our chimera and point-mutation studies designed to identify the molecular-structural basis for this profound pharmacological difference between the receptors formed by highly homologous murine β1 and β3 subunits.

Six chimeras with different portions of the wild-type β1 sequence replaced by the β3 sequence were constructed and their responsiveness to PNB determined. Only chimeras with a critical segment of the β3 subunit amino acids 250 - 341 (numbering based on the mature β3 polypeptide) formed PNB responsive receptors. In this critical region of 92 amino acids, there are 17 amino acid difference between the two parent subunits. Site-directed mutagenesis of one specific Asn residue in the M2 - M3 linker region of the wild-type β3 subunit with a Ser present at the homologous site on the wild-type β1 subunit abolished the response to PNB. The converse mutation of the serine residue in the wild-type β1 to an Asn residue formed PNB-responsive receptors with a dose-response indistinguishable from the wild-type β3 subunit receptor. Substitution of this residue with Gly, Ala, Glu, or Thr gave non-responsive receptors indicating a critical role of Asn not replaced by other amino acids. The dependence of the peak current response on the ratio of wild-type β3 to β3(N-to-S) mutant cRNA suggests that all five subunits in a pentameric assembly must be wild-type for successful PNB gating of the ion channel. Our results suggest that PNB opening of the ion channel requires a concordant interaction with all five subunits analogous to the neurotransmitter gating mechanism.
(Supported by R01-GM52325 and CNPq of Brazil)

511.5

THE GABA$_A$ RECEPTOR SUBUNIT GAMMA 2 LONG/SHORT RATIO IS ALTERED BY PENTOBARBITAL USING A MECHANISM DISTINCT FROM RECEPTOR OCCUPATION. R.F. Tyndale*, S.V. Bhave, E. Hoffmann, P.L. Hoffman, B. Tabakoff, A.J. Tobin and R.W. Olsen Addiction Research Foundation and Dept of Pharmacology, Univ of Toronto, Toronto; M5S 1A8; Dept of Pharmacol, Univ. of Colorado, Denver; Depts of Biol and Pharmacol, UCLA, Los Angeles.

Pentobarbital (PB) treatment of cerebellar granule cells in culture caused a change in the γ-aminobutyric acid (GABA)$_A$ receptor subunit mRNA γ2Long/Short ratio which was dose and time dependant (Society for NSc. abstract, 1995). The γ2L subunit mRNA contains an extra 24 base-pair exon coding for 8 amino acids which contain a protein kinase C phosphorylation consensus site. The 8 amino acid insertion occurs in the cytoplasmic domain between the putative third and fourth trans-membrane regions and can be phosphorylated (demonstrated *in vitro*). In the present study, in order to identify whether PB acts directly at the GABA$_A$ receptor to cause the shift in the γ2L/γ2S ratio, other GABA agonists and the channel blocker picrotoxin (PX) were tested. Cerebellar granule cells were cultured from postnatal day 7 rats for 4 days before 3 day drug treatments. In a single blind study, RT-PCR was used to detect the GABA$_A$ receptor subunit mRNAs, using non-neuronal enolase mRNA as an intemal standard. PB decreased the γ2L/γ2S ratio up to 65 percent, while ethanol (100 mM) and 3-α, 5-α dihydroprogesterone (allopreg-nanolone;1 or 2 μM) did not. Both isomers of hexobarbital (500 μM) caused a small change in the γ2L/γ2S ratio. Picrotoxin (10 μM) for 1 or 3 days had no effect alone on γ2L/γ2S ratio and was unable to block the effect of PB. These data suggest that barbiturates alter the γ2L/γ2S RNA ratio by a mechanism which does not involve direct occupation of the GABA$_A$ receptor itself. Supported by NIAAA, NINDS, MRC of Canada and the Addiction Research Foundation.

511.6

IN VIVO IMAGING OF VOLATILE ANESTHETIC ACTION IN HUMANS: EVALUATION OF ^{11}C-FLUMAZENIL (FMZ) PET UNDER ANESTHESIA F. Gyulai, M. Mintun*, L. Firestone, J. Price, P. Winter
Depts. Anesthesiology/CCM, Radiology, and PET Facility, U. Pittsburgh, PA 15261

One of the proposed sites of volatile general anesthetic (GA) action, the γ-aminobutyric acid receptor type A (GABA$_A$-R), can now be studied *in vivo* by positron emission tomography (PET) using ^{11}C-FMZ. However, GA effects on regional cerebral blood flow (rCBF) could confound anesthetic-related changes in ^{11}C-FMZ binding, by altering ligand delivery. To assess this, following IRB approval (#950389), ^{11}C-FMZ binding and rCBF was measured in 7 healthy volunteers before and during 1.2 vol% isoflurane anesthesia. The effect of a wide range (25-200%) of rCBF values on ^{11}C-FMZ binding was also studied by computer simulation. The distribution volume (DV) of ^{11}C-FMZ, a reflection of both receptor-specific and nonreceptor binding, was estimated using a 2-compartment model (J. Cereb. Blood Flow Metab. 11:735, 1991) to assess how both isoflurane-induced, and simulated rCBF changes affected this parameter. Absolute rCBF was measured using ^{15}O-water. Regions of interest included areas of high (frontal, occipital cortex), intermediate (thalamus, cerebellum), and low (pons) GABA$_A$-R density. Comparisons were made by a paired *t*-test at $p < 0.05$. Least squares regression was used to correlate GA-related DV changes to rCBF alterations and literature values of GABA$_A$-R density.

GA *increased* DV in each examined brain region ($p < 0.05$), and DV change correlated highly with GABA$_A$-R density ($r = 0.86$, $p < 0.001$). rCBF *increased* by ~ 30% in 3, and *decreased* by ~ 30% in 4 subjects. Measured rCBF changes did not correlate with DV alterations ($p > 0.05$). Finally, large simulated rCBF alterations were associated with trivial (± 2%) changes in ^{11}C-FMZ DV. These results indicate that GA effects on ^{11}C-FMZ binding, measured by PET, occur independently from rCBF alterations in humans.
Supported by FAER (F.G.); IARS (L.F.); GCRC/RR00056.

511.7

BARBITURATE ENHANCEMENT OF SINGLE AXON INHIBITORY POSTSYNAPTIC POTENTIALS IN HIPPOCAMPUS. H.M. Pawelzik*, J. Deuchars, J. Hahn and Alex M. Thomson. Dept. Physiology, Royal Free Hospital Sch. Med., London NW3 2PF, UK.

To determine the potency with which pentobarbitone facilitates the inhibition elicited by single identified interneurones, dual intracellular recordings with biocytin-filling were performed in 450μm thick transverse slices of adult rat hippocampus. Presynaptic interneurones were identified by their electrophysiological properties and ability to elicit inhibitory postsynaptic potentials (IPSPs) in a simultaneously recorded pyramid. Single axon IPSPs were 0.5 to 1.6mV in amplitude, had 10-90% rise times of 4.8 to 14.8ms and widths at half amplitude of 35 to 59ms under control conditions (n = 4). These interneurones displayed firing patterns distinguishable from pyramidal cells, but none recorded to date displayed the classical fast spiking pattern often considered typical of inhibitory interneurones. On light microscopic analysis the presynaptic interneurone somata were in or close to stratum pyramidale (SP) in CA1 (n=3) and CA3 (n=1), their dendrites were aspiny and beaded and 3 of these axons ramified in SP. Sodium pentobarbitone at concentrations up to the anaesthetic dose (250μM) greatly enhanced both the amplitude (by 72 ± 63% SD) and duration of these IPSPs (rise time by 116 ± 93% and half width by 92 ± 19%). This work was supported by the MRC.

511.8

DIFFERENTIAL SUBUNIT INFLUENCE FOR THE ACTIONS OF GENERAL ANESTHETICS ALPHAXALONE AND ETOMIDATE ON GABA$_A$ RECEPTORS. E. Sanna*, A. Murgia, F. Amato, T. Deserra, M. Usala, P.J. Whiting and G. Biggio. Dept. of Experimental Biology, Univ. of Cagliari, Italy, and Merck, Sharp & Dohme Research Labs, Harlow, Essex, U.K.

Recent studies have suggested that multiple actions of several general anesthetics on GABA$_A$ receptors are subunit-dependent. In this study, we have investigated the role of different β-subunits in the actions of the anesthetics alphaxalone (AX) and etomidate (ET) on GABA$_A$ receptors expressed in *Xenopus* oocytes. Mixtures of cDNAs encoding human α1β1γ2s, α1β2γ2s or α1β3γ2s subunits of the GABA$_A$ receptor were injected into oocytes and modulatory effects of GABA (EC_{20}) responses and direct "agonist" action of AX and ET were measured using standard two-electrode voltage-clamp recording. AX (10^{-7}-10^{-4}M) and ET (10^{-7}-10^{-4}M) enhanced currents induced by GABA in all three receptors. For AX, higher efficacy (220-280%) was detected with receptors containing β1 or β3 compared to those with the β2 subunit (150%). β2- or β3-containing receptors showed higher sensitivity (250-300%) to potentiation by ET compared to those with the β1 subunit (140%). When we studied the direct effects of AX and ET, we found that the rank order of receptor sensitivity in relation to the β subunit was β1>β2≥β3 and β3≥β2>β1 for AX and ET, respectively. In addition, β1 homomeric receptors were sensitive to direct activation by ET but, as already shown (Sanna et al., 1995), not by AX. Conversely, α1γ2s receptors were strongly activated by AX but were only poorly sensitive to ET. These results further demonstrate that β subunits influence the action of general anesthetics on GABA$_A$ receptors, and indicate that the effects of AX and ET differentially vary according to receptor composition. The data support the idea that AX and ET have different sites of action at the level of the GABA$_A$ receptor complex.

511.9

PROPOFOL INDUCES TYROSINE-PHOSPHORYLATION OF THE GABA$_A$-RECEPTOR β-SUBUNIT. K. Björnström[1], Å. Schippert[2], A. Sjölander[2] and C. Eintrei*[1]. Departments of Anaesthesiology[1] and Cell Biology[2], Linköping University, 581 85 Linköping, Sweden.

Little is known about the action of anaesthetic drugs on the central nervous system. The major theories have involved changes of the cell membrane and its associated proteins. Recently, increasing evidence have been raised that the GABA-receptor-complex might be a target for anaesthetic drugs. The GABA-complex is the major inhibiting system in the brain, and it fits well with the desired action of anaesthetics, i e to reduce nervous excitability. We have previously shown that propofol in neurons induced an immediate intracellular calcium signal.

We used primary cell cultures from new-born rats, cultivated for 10 days in cell culture flasks. The cells were stimulated with 0, 3, 30 μg/ml propofol, by the commercial available solution Diprivan®, for 0, 60 or 120 sec respectively. We used electrophoresis combined with Western blot technics to detect tyrosine-phosphorylation and the GABA$_A$β-subunit.

The stimulation by propofol gave an increase in tyrosine-phosphorylation. One of these bands was also detected by an specific antibody against the GABA$_A$β-subunit. The time maximum of tyrosine-phosphorylation was 120 sec.

We conclude that the anaesthetic drug propofol probably cause its anaesthetic effect by changing the GABA$_A$-receptor by tyrosine-phosphorylation.

Sponsored by Östergötlands County Council.

511.10

PROPOFOL MODULATION OF GABA$_A$ RECEPTOR ACTIVATION AND DESENSITIZATION IS MEDIATED BY DISTINCT MECHANISMS. LC McAdam[1,2], JF MacDonald[2], BA Orser[1,2]*. [1]Departments of Anaesthesia & [2]Physiology, Sunnybrook Health Science Centre & University of Toronto, Ontario, Canada, M5S 1A8

Propofol (PRO, 2,6 di-isopropylphenol) may act at distinct sites on the GABA$_A$ receptor to 1) potentiate GABA-evoked responses, 2) directly activate the receptor in the absence of GABA, and 3) decrease desensitization (Orser *et al.*, J Neurosci 14:7747, 1994). In contrast, the sedative-hypnotic benzodozepine, midazolam (MDZ) does not directly activate the GABA$_A$ receptor but potentiates GABA-evoked responses by binding to a flumazenil-sensitive site. The purpose of this study was to investigate the interactions between PRO and MDZ at the GABA$_A$ receptor.

Whole cell currents were recorded from embryonic cultured hippocampal neurons voltage clamped at -60mV. Currents activated by PRO in the absence of GABA were enhanced by MDZ (0.5 μM). The EC_{50}'s and Hill coefficients for the currents activated by PRO or PRO + MDZ were 22.3 ± 4.3 μM and 15.9 ± 3.1 μM, and 1.26 ± 0.16 and 1.58 ± 0.29, respectively (mean ± SEM, n=4). Saturating concentrations of GABA (600 μM) activated inward currents which peaked (I_p) then declined to an apparent steady-state (I_{ss}). The extent of desensitization, estimated from the I_{ss}/I_p ratio, was 0.156 ± 0.026. In the presence of PRO (10 μM) or PRO (10 μM) + MDZ (0.5 μM), the I_{ss}/I_p ratios were 0.198 ± 0.022 and 0.193 ± 0.022, respectively (n=7). These data indicate that MDZ increased the apparent affinity of the GABA$_A$ receptor for PRO but did not effect PRO-induced modulation of desensitization. This suggests that there may be distinct mechanisms through which PRO modulates activation and desensitization. Supported by the International Anesthesia Research Society Frontiers in Anesthesia Award (B.A.O) and a Medical Research Council grant (J.F.M.)

511.11

EFFECTS OF RUN-DOWN AND INTRACELLULAR CALCIUM BUFFERING ON PROPOFOL'S MODULATION OF GABA$_A$ RECEPTOR DESENSITIZATION. S. Roder[1]*, J. F. MacDonald[1] and B. A. Orser[1,2]. [1]Departments of Physiology & [2]Anaesthesia, University of Toronto & Sunnybrook HSC, Toronto, Ontario, Canada, M5S 1A8.

The desensitization of GABA$_A$ receptors observed with saturated GABA concentrations is decreased by anaesthetics such as propofol (PRO). Intracellular Ca^{2+} buffering reduces the time dependent run-down of GABA$_A$ mediated whole cell currents, and may also effect GABA$_A$ receptor desensitization. However, the effects of run-down and intracellular Ca^{2+} buffering on PRO modulation of GABA$_A$ receptor desensitization are not known. Therefore, the whole cell patch clamp recording technique was used to investigate these effects on the modulation by PRO of GABA$_A$ receptor desensitization. Whole cell currents were recorded from cultured embryonic hippocampal neurons using intracellular solutions containing either the low affinity Ca^{2+} chelator, EGTA (11mM), and $CaCl_2$ (1mM) or the high affinity Ca^{2+} chelator, BAPTA (11mM), and no added $CaCl_2$. Using a rapid perfusion system, GABA (600μM) in the absence and presence of PRO (10μM) was applied at 10 min intervals to neurons held at -60 mV. The extent of receptor desensitization was determined from the ratio of steady state to peak current (I_{ss}/I_p). In the presence of either EGTA or BAPTA, I_{ss}/I_p of the GABA induced currents decreased over a 30 minute period by 46±7% (n=6) and 26±11% (n=7), respectively. However, PRO increased I_{ss}/I_p by approximately 30% at all time intervals tested and did not depend on either intracellular Ca^{2+} buffer. These data suggest that neither the time dependent run-down of GABA induced whole cell currents nor differences in intracellular Ca^{2+} buffering altered PRO modulation of GABA$_A$ receptor desensitization. (Supported by the IARS Frontiers in Anesthesia Award to B.A.O. and an MRC grant to J. F. M.)

511.12

SUBUNIT-DEPENDENT MODULATION OF RECOMBINANT GABA$_A$ RECEPTORS BY GENERAL ANESTHETICS. S.M. O'Shea[1]*, M.D. Krasowski[1], C.E. Rick[1], P.J. Whiting[2], K.L. Hadingham[2], C. Czajkowski[3], N.L. Harrison[1]. [1]U. Chicago, Chicago, IL; [2]Merck, Sharpe, & Dohme, U.K; [3]U. Wisc, Madison, WI.

We have investigated the role of the α subunit in the modulation of GABA$_A$ receptors by the general anesthetics propofol (PRO), methohexital (MTX), and trichloroethanol (TCEt; the active metabolite of chloral hydrate). Fibroblast cell lines which stably express either $\alpha_1\beta_3\gamma_2$ or $\alpha_6\beta_3\gamma_2$ GABA$_A$ receptors were studied by both electrophysiological and radioligand assay techniques. Using the whole-cell patch clamp technique, PRO, MTX, and TCEt applied at clinical concentrations significantly potentiated submaximal GABA chloride currents in cells expressing either subunit combination. PRO and TCEt potentiated responses in $\alpha_1\beta_3\gamma_2$ receptors to a far greater degree than those in $\alpha_6\beta_3\gamma_2$ receptors. This differential effect was reflected in the maximal efficacy of modulation by PRO and TCEt, but not in their apparent affinities. This difference is not related to levels of expression, since MTX potentiation of chloride currents did not vary significantly between $\alpha_1\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ receptors. Similarly, enhancement of ^{3}H-muscimol binding by PRO was significantly greater in $\alpha_1\beta_3\gamma_2$ than $\alpha_6\beta_3\gamma_2$ receptors; whereas MTX enhances ^{3}H-muscimol binding significantly more in $\alpha_6\beta_3\gamma_2$ than $\alpha_1\beta_3\gamma_2$ receptors. These results taken altogether demonstrate that the difference in efficacy of PRO and TCEt between $\alpha_1\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ receptors is dependent on the specific anesthetic-receptor interaction and is not a general feature of these two subunit combinations. The relative anesthetic potentcies of PRO and MTX correlate better with modulation of GABA responses in $\alpha_1\beta_3\gamma_2$ than $\alpha_6\beta_3\gamma_2$ receptors, suggesting that the $\alpha_6\beta_3\gamma_2$ receptor subtype may not be a relevant general anesthetic target.

Supported by: GM 45129, GM 00623 (NLH) and DA 07255 (SMO).

511.13

GABA PHARMACOLOGY OF CEREBELLAR GRANULE CELLS IN MICE DEVOID OF THE ALPHA 6 SUBUNIT OF THE GABA$_A$ RECEPTOR. C.E. Rick*, G.E. Homanics[1], L.L. Firestone[1] & N.L. Harrison, Dept. Anesthesia & Critical Care, Univ. Chicago, Chicago, IL 60637; [1]Dept. Anesthesia & Critical Care, Univ. Pittsburgh, Pittsburgh, PA 15261.

The presence of the alpha 6 (α6) subunit confers benzodiazepine insensitivity on recombinant GABA$_A$ receptors[1]. In the CNS, GABA$_A$ receptors containing the α6 subunit are found almost exclusively in cerebellar granule neurons. The subunit composition of cerebellar granule neurons is age dependent; early receptors are benzodiazepine-sensitive whereas late ("mature") receptors show the benzodiazepine insensitive pharmacology thought to be associated with the α6 isoform[2].

Mice lacking a functional α6 subunit (-/-) were created by embryonic stem cell gene targeting, and their cerebellar granule neurons were compared to wild type mice (+/+). Cells from the cerebellum of 6-7 day old pups were cultured in a high K^+ medium in order to promote differentiation of cerebellar granule cells. Whole cell patch clamp recordings were made from early (culture day 6-10) and late (culture day 21+) cultures.

All experiments from early cultures, from both +/+ and -/- mice, had GABA EC_{50} 8-20μM; currents in response to submaximal concentrations of GABA were potentiated by the benzodiazepine, midazolam (500nM). Preliminary data suggest that mature cultures from -/- mice maintain their benzodiazepine sensitivity, with no shift in apparent affinity for GABA.

Supported by: NIH grants GM52035, AA10422

1.Wieland *et al.*, J. Biol. Chem. (1992) 257 1426-9.
2. Mathews *et al.*, Neuron, (1994) 13 149-58.

511.14

PRODUCTION AND CHARACTERIZATION OF MICE DEVOID OF THE BETA 3 SUBTYPE OF THE GABA$_A$ RECEPTOR. GE Homanics*, C Ferguson, JJ Quinlan, KD Snyder, ZP Mi[1], and LL Firestone. Dept. Anes./CCM, [1]Dept. Neurobiology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

The beta 3 (β3) subtype of the GABA$_A$ receptor has been implicated in craniofacial development (PNAS 91:2815, 1994). To test this hypothesis, we used gene targeting in embryonic stem cells to create mice that lack a functional β3 gene. Beta 3 null mice (-/-) were created by replacing exons 1-3 and ~1.8kb of promoter with a neomycin cassette. Hybridization of neonatal whole brain mRNA with an exon 1-3 specific probe and with an exon 9 specific probe showed that the ~6.0kb β3 mRNA in wild type (+/+) mice was completely absent in -/- mice, confirming successful creation of a null allele. Approximately 60% of -/- pups are born with cleft palate and die within 24h of birth; ~35% of -/- pups with apparently normal palate also die within 24h of birth due to unknown causes; ~5% of -/- mice survive to adulthood. These survivors display a nervous phenotype, but have grossly normal brain histology, normal gait, and no ataxia. The affinity of GABA$_A$ receptors in neonatal whole brain homogenates for ^{3}H-Ro15-4513 and ^{3}H-muscimol was similar in +/+ and -/- mice. However, -/- brains reveal a marked reduction in total GABA$_A$ receptor density (from 1.8±0.4 to 0.7±0.1 pmol/mg for Ro15-4513, p=0.003; and from 1.9±0.2 to 1.0±0.2 pmol/mg for muscimol, p<0.002). Both -/- males and females are fertile but females fail to care for offspring. In sum, these results indicate that β3 is intimately involved in normal palate formation, and production of the normal complement of brain GABA$_A$ receptors.

Support: NIH grants GM52035, AA10422, and Dept. Anes., Univ. of Pittsburgh.

511.15

PRODUCTION AND CHARACTERIZATION OF MICE DEVOID OF THE ALPHA 6 SUBTYPE OF THE GABA$_A$ RECEPTOR. LL Firestone*, JJ Quinlan, E Korpi[1], JL Daggett, C Ferguson, ZP Mi[2], XH Wang[3], DR Grayson[3], GE Homanics. Dept. Anes./CCM, [2]Dept. Neurobiol., Univ. of Pittsburgh, Pittsburgh, PA 15261; [1]Biomedical Research Center, Alko Ltd., Helsinki, Finland; [3]Med. Col. of Penna, Pittsburgh, PA 15212.

The alpha 6 (α6) subtype of the GABA$_A$ receptor has been implicated in the intoxicating effects of ethanol. To test this hypothesis, we used gene targeting in embryonic stem cells to create mice that lack a functional α6 gene. Alpha 6 null mice (-/-) were created by inserting a neomycin cassette into exon 8. Null mice are viable, fertile, and have grossly normal brain morphology. RT-PCR and Northern blot analyses were used to demonstrate that the targeting event disrupted production of α6 mRNA. Autoradiography of histological sections of adult brains demonstrated that the diazepam-insensitive binding of ^{3}H-Ro15-4513 to the cerebellar granule cell layer of wild type (+/+) mice was completely absent in -/- mice. Together, these analyses establish that the gene targeting event results in a nonfunctional α6 allele. Semi-quantitative RT-PCR showed no significant difference in expression of other α subtypes in cortex and cerebella of -/- mice as compared to +/+ mice. Sleep time response (mean$\pm$SEM; n=14-15/group) of +/+ and -/- mice to ip injection of ethanol (3.5g/kg BW) following pretreatment with vehicle (V) or Ro15-4513 (Ro; 10mg/kg BW) were: 49.3$\pm$5.3 for V,+/+; 41.7$\pm$5.9 for V,-/-; 27.8$\pm$3.5 for Ro,+/+; and 28.6$\pm$4.4 for Ro,-/-. The effect of genotype was nonsignificant; pretreatment with Ro decreased sleep time ($p<0.001$; ANOVA). Thus, the α6 subtype of the GABA$_A$ receptor is not required for normal brain development, normal viability and fertility, nor does it appear to be a critical or unique component of the neuronal pathway mediating the hypnotic effect of ethanol and its antagonism by Ro in mice, as reflected by this sleep time assay.
Support: NIH grants GM52035, AA10422, and Dept. Anes., Univ. of Pittsburgh.

511.16

BARBITURATE SENSITIVITY OF THE HUMAN GABA$_A$ RECEPTOR: STUDIES USING CHIMERIC GABA/GLYCINE RECEPTORS.
V.V. Koltchine*, Q. Ye, S.E. Finn and N.L. Harrison. Anesthesia and Pharmacology, The University of Chicago, Chicago IL 60637.

The mammalian GABA$_A$ receptor (GABA$_A$-R) can be modulated by a range of general anesthetics including barbiturates (BARB). Although the native GABA$_A$-R typically consists of a heteropentameric complex of α, β, and γ subunits, the γ subunit is not required for BARB modulation. The glycine receptor (Gly-R) α subunit forms a functional homomeric receptor which is insensitive to barbiturates. We have used this pharmacological selectivity to investigate the specific domains of the GABA$_A$ receptor that confer sensitivity to BARB, by studying the chimeric GABA/Gly-R receptor subunits described in the adjoining poster by Ye et al. Chimeras X2(β1-gly), X4(α2/Gly), S1(Gly/β1) and S3 (Gly/α2) did not form functional receptors when expressed alone in 293 cells, but did form functional receptors when co-expressed with the complementary wild-type GABA$_A$-R subunit, as follows: X2+α2, X4+β1, S1+α2, S3+β1. All of these receptors showed full barbiturate sensitivity, showing both strong potentiation by MTX of the response to GABA and direct gating by MTX. Chimeras X1(Gly-β1), S2 (β1-Gly) and S4 (α2/Gly) formed functional homomeric receptors gated by glycine. Like the wild-type Gly-R α2 subunit, these three chimeras were completely insensitive to 500μM MTX. These results demonstrate that neither the large C-terminal domain 3' from site X (including the cytoplasmic loop and TM4), nor the extreme N-terminal domain 5' to site S, in either α2 or β1 subunit, contributes significantly to the modulation of the GABA$_A$-R by MTX. BARB sensitivity must therefore be conferred by the remaining ~300 amino acid residues of either (or both) the GABA$_A$-R α or β subunits.
Supported by GM 45129, GM 00623 (NLH) and DA 07255 (VVK).

511.17

CONSTRUCTION AND EXPRESSION OF CHIMERIC GABA$_A$/GLYCINE RECEPTORS.
Q. Ye, V.V. Koltchine, S.E. Finn and N.L. Harrison*, Anesthesia and Pharmacology, The University of Chicago, Chicago, IL 60637

GABA$_A$ receptors (GABA-R) and glycine receptors (Gly-R) are close relatives in the 'gene superfamily' of ligand-gated ion channels, but they show distinctly different pharmacology. In order to resolve the structural elements involved in various pharmacological modulations, two series of chimeras between the human GABA-R and human Gly-R were constructed and expressed. The first series, the "S" series, was constructed by introducing an XmaI site into a conserved region in the N-termini of the GABA-R (α2 and β1 subunits) and Gly-R (α2 subunit) cDNAs, enabling an exchange between the GABA-R and Gly-R cDNA fragments. The resulting S1 and S3 chimeras were mostly GABA-R α2 or β1 with the N-terminal ~30 amino acids replaced by the corresponding region of the Gly-R, while S2 and S4 were mostly Gly-R α2 with the same N-terminal region replaced by the GABA-R α2 or β1 counterparts. S2 and S4 both formed glycine-gated homomeric channels in HEK 293 cells, with EC_{50} values of 503 and 16.6 uM (EC_{50}= 50.2 uM in w.t Gly-R α2). Chimeras S1 and S3 failed to form functional channels by themselves. However, when co-expressed with their complementary GABA-R subunits, S1/GABA α2 and S3/GABA β1 both formed GABA-gated heteromeric channels, with EC_{50} values of 17.2 and 22.5 uM (EC_{50}=8.4 uM in w.t. GABA-R α2/β1). The second series of chimeras, the "X" series, was constructed by introducing an XbaI site into the C-terminal end of the third transmembrane domain (TM3), allowing an exchange of the large cytoplasmic loop (C-loop) and TM4 between the GABA-R and Gly-R. The constructed X1 chimera has the N-terminus and TM1-3 from Gly-R α2 joined by the C-loop and TM4 from GABA-R β1, resulting in a homomeric glycine-gated channel (EC_{50}=364 uM). The chimeras X2 and X4 have the N-termini and TM1-3 from GABA-R α2 or β1 joined by the C-loop and TM4 from Gly-R. X2/GABA-R α2 and X4/GABA-R β1 both formed heteromeric GABA-gated channels (EC_{50}=51.8 and 8.2 uM). Further pharmacological analyses of these and other chimeras are presented in the adjoining posters by Koltchine et al. and Mihic et al. Supported by GM45129, GM00623 (NLH) and DA07255 (VVK).

511.18

A SEARCH FOR SITES OF ACTION OF VOLATILE ANAESTHETICS ON GABA AND GLYCINE RECEPTORS. S.J. Mihic*, M.J. Wick, N.L. Harrison, Q. Ye, S.E. Finn and R.A. Harris. Dept. Pharmacology, U. Colorado Hlth. Sci. Ctr., Denver, CO 80262 and Dept. Anesthesia, U. Chicago, Chicago, IL 60637.

Current evidence suggests that the *in vivo* effects of volatile anaesthetics result from their interactions with membrane-bound ligand-gated ion channels, such as the GABA and glycine receptors, which are the primary mediators of inhibitory neurotransmission in the CNS. The function of GABA$_A$ and glycine receptors is markedly enhanced by pharmacologically-relevant concentrations of volatile anaesthetics. However, GABA receptors formed of ρ subunits instead show a weak inhibition. Because homomeric glycine α receptors show significant amino acid sequence homology with homomeric GABA ρ receptors, we are attempting to identify the amino acids responsible for the positive effects of volatile anaesthetics on glycine receptors, by creating receptor chimeras. The X junction site (see Ye *et al.*) in the C-terminal end of the third transmembrane (TM) domain allowed for the exchange of the TM3 - TM4 intracellular loop plus TM4 between the receptors. The X-5 chimeric receptor contained glycine α2 sequence from the N-terminal end to the X junction site, and GABA ρ1 sequence from the X junction site to the C-terminal end. The X-6 chimera was the converse of X-5 at the same junction site. X-5 was gated by glycine but not GABA and, like glycine α receptors, showed potentiation by enflurane. X-6 was gated by GABA but not glycine and, like GABA ρ1 receptors, showed weak inhibition by enflurane. These data exclude the TM3 - TM4 intracellular loop as well as TM4 as contributing to the effects of volatile anaesthetics on these receptors. Supported by AA06399, GM47818 (RAH) and GM45129, GM00623 (NLH).

511.19

A SEARCH FOR SITES OF ACTION OF ALCOHOLS ON GABA AND GLYCINE RECEPTORS. M.J. Wick, N.L. Harrison, S.J. Mihic, Q. Ye, S.E. Finn, S. Bhave* and R.A. Harris. Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262 and Dept. Anesthesia, Univ. Chicago, Chicago, IL 60637.

Alcohols produce sedation, loss of righting reflex and, at high enough concentrations, anaesthesia. These effects may be produced by their positive modulation of GABA$_A$ and glycine receptor function. In contrast, alcohols potently inhibit the function of homomeric GABA ρ1 receptors (Mihic and Harris, *JPET* 277:411). Using the chimeric receptor approach discussed in the adjoining posters by Ye *et al.* and Mihic *et al.*, we are attempting to identify which amino acids are responsible for the positive effects of alcohols on glycine receptors. The X junction site (see Ye *et al.*) in the C-terminal end of the third transmembrane (TM) domain allowed for the exchange of the TM3 - TM4 intracellular loop plus TM4 between the receptors. The X-5 chimeric receptor contained glycine α2 sequence from the N-terminal end to the X junction site, and GABA ρ1 sequence from the X junction site to the C-terminal end. The X-6 chimera was the converse of X-5 at the same junction site. X-5 receptors, like glycine α receptors, showed potentiation by ethanol. However, the function of X-6 receptors, like GABA ρ1 receptors, showed marked inhibition by ethanol. These data exclude the TM3 - TM4 intracellular loop as well as TM4 as contributing to the effects of ethanol on these receptors. Thus far, we have not been able to differentiate the sites of action of alcohols and volatile anaesthetics at the glycine receptor. Supported by AA06399, GM47818 (RAH) and GM45129, GM00623 (NLH).

512.1

GAS CHROMATOGRAPHY-MASS FRAGMENTOGRAPHY WITH NEGATIVE ION CHEMICAL IONIZATION TO MEASURE NEUROSTEROIDS IN BRAIN AND CSF. D.P. Uzunov*, E. Costa and A. Guidotti. The Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612.

Neurosteroids [allopregnanolone (ALLO), progesterone (PROG), 5α-dihydroprogesterone (5α-DHP), and dehydroepiandrosterone (DHEA)] are pharmacologically potent modulators of neuronal activity either by regulating transmitter action on ligand-gated channels ($GABA_A$) or by controlling transcriptional regulation of specific genes. It is not clear, however, whether significant amounts of pharmacologically active neurosteroids are synthesized *de novo* in brain tissue. We report the development of a specific and sensitive method combining high performance liquid chromatography (HPLC) and gas chromatography-mass fragmentography (GC-MF) with negative ion chemical ionization (NICI) to provide quantification of these neurosteroids in small brain parts (1-10 mg tissue), the CSF (0.1 mL), or plasma (0.01 mL); the procedure allows identification of the chemical structure of the product being measured. We found in normal male human subjects plasma levels of ALLO of 0.59 ± 0.017 pmol/mL and of 5α-DHP 0.65 ± 0.060 pmol/mL; the content of ALLO in the CSF is considerably lower (0.15 ± 0.029 pmol/mL). By administering appropriate blockers of 5α-reductase (SKF-105111) and of 3α-hydroxysteroid oxidoreductase (3α-HSOR) (indomethacin, fluoxetine) to adrenalectomized/castrated (ADX/CX) rats, we have demonstrated that 5α-DHP and ALLO are synthesized in the brain by the coordinated action of 5α-reductase and 3α-HSOR, with the highest efficacy in the olfactory bulb. The brain concentration of ALLO does not depend on that in peripheral organs because: *a)* peripheral tissues of ADX/CX rats do not synthesize ALLO and *b)* in ADX/CX rats ALLO is virtually absent in plasma but is only marginally reduced in the brain. Moreover, ALLO is found in significant concentration (approximately 1 nM) in rat brain microdialysates. Taken together these data strongly suggest that the rat brain can synthesize, store, and release ALLO into the extracellular fluid in concentrations that may be sufficient to positively modulate the intensity of GABA-gated Cl⁻ currents. MH 49486-04.

512.2

PROGESTERONE WITHDRAWAL PRODUCES BENZODIAZEPINE INSENSITIVITY: A BEHAVIORAL STUDY. M.H. Moran, M. Goldberg, S.J. Wieland*, D. Bitran[†] and S. S. Smith. Dept. of Neurobiology and Anatomy, MCP and Hahnemann University, Philadelphia, PA; [†]Dept. of Psychology, College of the Holy Cross, Worcester, MA.

Previous studies from this laboratory have demonstrated that withdrawal (wd) from three cycles of progesterone (P) administration prevents the normally potentiating effect of the benzodiazepine lorazepam (LZM) on GABA-evoked Cl- current (Costa et al, 1995). This effect was shown to be due to the GABA-active metabolite 3 α-OH-5-pregnan-20- one (allopregnanolone). The purpose of the present study was to examine the withdrawal effects of P on the sedative potency of LZM using a variety of hormone administration paradigms as models for PMS/post-partum conditions. Towards this end, rats were treated with P, by i.p injection (500 µg, 1 or 2xdaily) or via implant (10mm/100 gm body weight). Implants of crystalline P were constructed of silastic tubing (0.132" i.d., 0.183" o.d.) and incubated overnight in 1% gelatin-saline prior to subcutaneous implantation. Three wd cycles of P administration (5 days P, 2 days wd/week) were effective in reducing the sedative potency of LZM (0.50 mg/kg, i.p.) as assessed by position slip during a treadmill locomotion paradigm (i.e., "riding" the treadmill). Position slip frequency was significantly increased following LZM administration to control rats ($P<0.05$) but was depressed in response to LZM following P wd (7.6 ± 1 vs. 49.6 ± 9.8, $P<0.05$). A shorter paradigm (2 days P, 1 day wd for three cycles) was effective in depressing position slip frequency in response to LZM using P implants (4.5 ± 1.2 vs. 9.6 ± 1.9. $P<0.05$), or P injections (2xdaily, 5.7 ± 3.1 vs. 26 ± 6.0, $P<0.05$). A 3 week exposure to P implants was ineffective in altering the sedative potency of LZM. These results suggest that wd after ierntermittent exposure to P markedly reduces the sedative potency of the BDZ LZM. This effect may reflect cross-tolerance of the two GABA-active compounds, LZM and allopregnanolone, as has been reported behaviorally. Current studies are underway to investigate the effects of pseudopregnancy-induced P fluctuations on the sedative potency of LZM. (Supported by DA 09618 to SSS)

512.3

TOWARD AN ANIMAL MODEL OF OVARIAN HORMONE-RELATED MOOD DISORDERS. Daniel Bitran*, Phyllis Renda, Steven M. Solano, & Sheryl S. Smith[†]. Dept. of Psychology, College of the Holy Cross, Worcester MA 01610; & [†]Dept. of Anatomy, Hahnemann Univ., Philadelphia PA, 19102.

Progesterone (P) is thought to play a role in the etiology of premenstrual syndrome and postpartum depression. Both disorders are characterized by anxiety and an abrupt decrease in P levels (i.e., P withdrawal). Whereas acute P injection elicits anxiolytic effects via the formation of the $GABA_A$ receptor-active neurosteroid, allopregnanolone (Bitran et al., 1993; 1995), P withdrawal has been observed to produce anxiogenic effects (Gallo & Smith, 1993; Costa et al., 1995). The animal model described herein used pseudopregnancy (PsdP) to test the effect of withdrawal from physiological elevation of P. Prepubertal female rats (21 days of age) were injected with 50 IU pregnant mare serum gonadotropin. Fifty six hours later (day 0 of PsdP), females received 25 IU of human chorionic gonadotropin. Control rats received SC injections of saline vehicle. On days 4, 10, 14, and 18 of PsdP, separate groups of animals (n=6-7 per group) were tested for 5 minutes in a novel open field, followed by a 10 minute test in the elevated plus-maze. Immediately after the behavioral tests, animals were killed, and samples of blood, cortex, hippocampus, cerebellum, diencephalon, uterus, ovary, kidney, and adrenal glands were taken. Blood serum P levels were markedly elevated in PsdP rats on days 4, 10, and 14, with peak levels on day 10. An age-dependent increase in open-field exploration was found in both control and PsdP animals. PsdP produced a delay in adult-like levels of exploratory activity, as evidenced by a decrease in exploration observed on day 10 of PsdP. Anxiolytic effects were observed in the plus-maze during the the first 5 min test period on day 4 of PsdP. The lack of anxiolytic effect on PsdP days 10-18 could be a result of tolerance to the chronically-elevated levels of P. Current experiments are testing the tolerance hypothesis as well as the effects of P withdrawal using ovariectomy of PsdP animals on day 10, the day of peak P levels. This research was funded by the UPSH Grant MH 50450 to DB.

512.4

PROGESTINS CAN HAVE MEMBRANE-MEDIATED EFFECTS IN RAT MIDBRAIN FOR SEXUAL RECEPTIVITY. C. A. Frye* and S.G. Gardiner. Neuroscience Laboratories, Depts. of Psychology and Zoology, Connecticut College, New London, CT 06320.

Does progesterone (P) have membrane-mediated actions in the rat ventral tegmental area (VTA) to facilitate sexual receptivity? Ovariectomized (ovx) rats (n=10) with cannula over the ventromedial hypothalamus (VMH) and VTA were primed with 2µg, 17β-estradiol (E). After a pre-test for receptivity, animals received implants of P, P conjugated to BSA (P:BSA), or cholesterol control (CHOL) to the VMH, and were retested. Two hours later, animals were again pre-tested for receptivity, and then P, P:BSA, the P metabolite, 5α-pregnan-3α-ol-20-one (3α,5α-THP), or CHOL implants were applied to the VTA. Subjects were re-tested 0, 30, 90, and 150 minutes later. Animals that received P in the VMH and had P, P:BSA, or 3α,5α-THP applied to the VTA, exhibited facilitated receptivity immediately, 30, 90, and 150 minutes post-VTA implantation compared to all other combination implants. Whether P's effects are due to actions at the $GABA_A$-benzodiazepine receptor complex (GBR) was investigated. Ovx (n=10), E-treated animals received GBR antagonist, bicuculline, or agonist, muscimol, infusions into the VTA and were tested for sexual receptivity. Animals receiving bicuculline to the VTA and 300 µg P showed significant reductions in lordosis 0, 30, and 60 minutes after infusion. Animals receiving muscimol and 0, 50, or 100 µg P showed significant increases in lordosis quotients immediately and 30 minutes after infusion. These data suggest that P can act at neuronal membranes within the VTA to modulate lordosis behavior and that these effects are likely due to actions at GBRs.
Supported by CAREER grant 9514463 from the National Science Foundation.

512.5

Co 5-2791: A REMARKABLY POTENT NEUROACTIVE STEROID $GABA_A$ RECEPTOR POTENTIATOR. J.E. Hawkinson*, M. Acosta-Burruel, K.C. Yang, C.S. Konkoy, E.R. Whittemore, R.M. Woodward, R.B. Carter and R.B. Upasani. CoCensys, Inc., 213 Technology Dr., Irvine, CA 92618.

The progesterone metabolite 3α-hydroxy-5α-pregnane-20-one (3α,5α-P) potentiates $GABA_A$ receptor function acting through a unique site on the receptor complex. Despite extensive synthetic work to optimize several key features of the neurosteroids, increasing their potency has proven to be an elusive goal. Here we report that appropriate substitution in the *para* position of 3β-phenylethynyl-substituted neuroactive steroids greatly increases potency. In general, hydrogen bond accepting groups are preferred, with *p*-acetyl conferring optimal potency. 3β-(4′-Acetyl)-phenylethynyl-3α-hydroxy-5α-pregnane-20-one (Co 5-2791) is ~11-fold more potent than 3α,5α-P as an inhibitor of [^{35}S]TBPS binding in rat brain membranes (IC_{50} 4.7 and 51 nM, resp.). In *Xenopus* oocytes expressing α1β2γ2L receptors, Co 5-2791 is ~200-fold more potent than 3α,5α-P in potentiating 5 % GABA responses (EC_{50} 0.78 and 160 nM, resp.). *In vivo*, i.p. Co 5-2791 is ~ 5-fold more potent than 3α,5α-P in protecting mice from PTZ-induced seizures (ED_{50} 0.6 and 2.8 mg/kg, resp.). In contrast to 3α,5α-P, Co 5-2791 apparently has good oral bioavailability (p.o. ED_{50} 0.7 mg/kg). The high potency of Co 5-2791 indicates that the neuroactive steroid binding site contains a large pocket accessible from the 3β-position and suggests that hydrogen bond accepting groups on extended 3β substituents can interact with residues of the pocket, resulting in a marked increase in potency.

512.6

Subunit-selective actions of 5α-pregnan-3α-ol-20-one and 5α-pregnan-3α,20α-diol on mammalian recombinant $GABA_A$ receptors. D. Belelli,* C. Hill-Venning, S. E. Shepherd, J. A. Peters and J. J. Lambert. Neurosciences Institute, Department of Pharmacology, Dundee University. Dundee, DD1 9SY Scotland, U.K.

The activity of the neurosteroids, 5α-pregnan-3α-ol-20-one (5α3α-P) and 5α-pregnan-3α,20α-diol (5α,20α-diol), to modulate GABA-evoked currents was investigated in *Xenopus laevis* oocytes expressing human recombinant $GABA_A$ receptors of $\alpha_x\beta_1\gamma_{2L}$ composition (where x=1,2,3,6 and y=1,2) and rat forebrain mRNA. 5α3α-P produced a concentration-dependent potentiation of the inward current evoked by the bath-application of GABA at EC_{10} at all human receptor subunit combinations tested and at the heterologously expressed rat $GABA_A$ receptors. The potency of the steroid varied little across human α_1-, α_2-, α_3- and $\alpha_6\beta_1\gamma_{2L}$, $\alpha_1\beta_2\gamma_{2L}$ and rat $GABA_A$ receptors, By contrast, the magnitude of the steroid maximal effect was approximately doubled (132 ± 6% of the GABA maximum in the presence of 3µM 5α3α-P) in the $\alpha_6\beta_1\gamma_{2L}$ receptors when compared to the other human or the rat recombinant $GABA_A$ receptors. 5α3α-P enhanced the currents evoked by a maximally effective concentration of GABA (i.e. 3mM) to 302 ± 43% of control in the $\alpha_6\beta_1\gamma_{2L}$ receptor subunit combination whereas it elicited little or no potentiation of the GABA maximal response in the α_1-, α_2-, or $\alpha_3\beta_1\gamma_{2L}$ recombinant receptors. Notably, 5α20α-diol elicited a reduced potentiation of the GABA-evoked current when compared to 5α3α-P. Furthermore, in contrast to 5α3α-P, the magnitude of the pregnanediol effect did not vary across the α_1-, α_2-, α_3- and $\alpha_6\beta_1\gamma_{2L}$ $GABA_A$ receptor subtypes, while the potency was only marginally affected by the type of α subunit.

These findings suggest that the α subtype is an important molecular determinant of the neurosteroid action at the $GABA_A$ receptor. Furthermore, comparison of the α_6 and $\alpha_{1\text{-}3}$ amino acid sequences may be instructive for the identification of the molecular locus of the neurosteroid binding site on the receptor protein.
The work was supported by the MRC.

512.7

NEUROSTEROID MODULATION OF GABA$_A$ RECEPTOR DEPENDS ON δ SUBUNIT. WJ.Zhu*, JF.Wang, KE.Krueger+ and S.Vicini
Depts. of Physiol. & Cell Biol+, Georgetown Univ Med Cntr Washington DC 20007

Neurosteroid modulation of GABA$_A$ receptors has been observed with all subunit combinations investigated, however, heterooligomeric GABA$_A$ receptors containing δ subunits were not previously studied. We describe the effect of δ subunit expression on THDOC (3α, 21dihydroxy-5α-pregnan-20-one)-induced potentiation of GABA-gated currents in HEK293 transfected cell and in cerebellar granule cell in vitro. THDOC (10-100 nM) significantly potentiated GABA-gated currents in HEK293 cells transfected with combinations of α1, α6, β3, and γ2 subunit cDNAs while the cotransfection of δ subunit cDNA inhibited this potentiation. In contrast, the direct Cl^- channels activation by THDOC at higher concentration (1-10 μM) was not significantly dependent on δ subunit cotransfection. These results suggest that the δ subunit expression inhibits GABA$_A$ receptor modulation but not the direct activation by neurosteroids implying the existence of distinct domains on the receptor protein for these actions. The δ subunit is predominantly expressed in the granule neurons of cerebellum and hippocampus in a development-dependent manner. THDOC potentiation of the GABA-gated currents was greater in 4 days than in 14 days in vitro cerebellar granule cells, indicating a development-dependent change in neurosteroid modulation. Single cell RT-PCR analysis of the mRNAs expressed in cultured cerebellar granule cells, shows that an increased number of granule cells at 14 DIV express δ subunit mRNAs as compared to 4 DIV granule cells. The presence of δ subunit mRNAs correlated well with the lack of sensitivity to THDOC. These results suggests that developmental expression of GABA$_A$ receptor δ subunits may play an important role in determining the neurosteroid-induced modification of fast inhibitory synaptic function. Supported by NINDS grants R01 NS32759 and K04 NS01680 to S.V.

512.8

STEROID MODULATION OF GABA$_A$ RECEPTORS IN HYDRA VULGARIS. A.Concas*, P. Pierobon#, M.C. Mostallino, G. Marino#, R. Minei# and G. Biggio,
Dept. of Experimental Biology, Chair of Pharmacology, University of Cagliari. 09123 Cagliari, Italy, #Cybernetics Institute C.N.R., 80072 Arco Felice, Naples, Italy.

GABA$_A$ receptors are present in membrane preparations from Hydra vulgaris, one of the primitive living forms possessing a nervous system (Life Sciences 56:1485, 1995). These receptors are sensitive to muscimol and benzodiazepines and seem to be involved in the regulation of the feeding response. We have investigated the effect of neurosteroids on GABA$_A$ receptors in membrane preparations from Hydra and their role in the living animal. Allopregnanolone (AP) and tetrahydrodeoxycorticosterone (THDOC) increased [^{3}H]GABA binding with nanomolar potency (EC_{50} = 142 ± 21 nM AP and 623 ± 43 nM THDOC) and great efficacy (maximal effect AP: 78 ± 6.5% at 3 μM; and THDOC: 62 ± 5.2% at 10 μM). The effects of steroids were mimicked by other positive allosteric modulators of GABA$_A$ receptors. In fact, diazepam (100 μM), abecarnil (30 μM), pentobarbital (300 μM) and alphaxalone (10 μM) enhanced by 20, 23, 78 and 30% [^{3}H]GABA binding to Hydra membranes, respectively. To clarify the functional role of GABA$_A$ receptors we studied the modulatory effect elicited by the above drugs on the feeding response. AP and THDOC (1-10 μM) prolonged, in a dose dependent manner, the times of mouth opening induced by 10 μM glutathione; the maximal effect was elicited by 10 μM AP (+32 ± 2.5%) and 10 μM THDOC (+27 ± 1.3%). Similarly, alphaxalone (10 μM) increased (+34 ± 7.5%) the action of glutathione, while pentobarbital did not modify significantly this response. The effects elicited by steroids were suppressed by administration of TBPS (1 μM), a specific Cl^- channel blocker. These results suggest that modulation of GABA$_A$ receptors by steroids appeared very early in evolution.

512.9

CHRONIC NEUROSTEROID TREATMENT DECREASES THE GABA RECEPTOR mRNA LEVELS R. Yu* and M.K. Ticku. Dept of Pharmacology, Univ. of TX Hlth. Sci. Ctr., San Antonio, TX 78284-7764.

The GABA$_A$ receptors are an important site of action and modulation for a variety of centrally acting drugs including neurosteroids. We have previously reported that chronic 5α-pregnane-3ol-20-one(5α3α) treatment while not altering the benzodiazepine binding, downregulated GABA and TBPS binding, and produced heterologous uncoupling, and decreased efficacy at the GABA$_A$ receptor complex in cortical neurons (Mol. Pharmacol. 45:618, 1994; J. Pharmacol. Exp. Ther. 275:784, 1995). In order to understand the underlying mechanisms of the phenomenon, we investigated the effect of chronic 5α3α (1μM, 5 days) treatment on the GABA$_A$ receptor subunit mRNA levels, using RNase protection assay. We found that 5α3α treatment decreased the α and β subunit mRNA levels, while not altering the γ2s subunit mRNA levels in cortical neurons. The decrease in the α3 and α2 subunit mRNA levels may provide an explanation for the heterologous uncoupling phenomenon and decreased efficacy observed in our earlier studies. The decrease in the β2 and β3 subunit mRNA levels may explain the downregulation of GABA and TBPS binding, and decreased GABA-induced Cl-influx. In summary, chronic 5α3α treatment produced downregulation of the GABA$_A$ receptor α and β subunit mRNA levels, and these changes may be associated with the downregulation, heterologous uncoupling, and decreased efficacy of GABA$_A$ receptor complex in the mammalian cortical neurons.

512.10

THE AMYGDALA MEDIATES THE ANXIOLYTIC-LIKE ACTIVITY OF THE NEUROSTEROID ALLOPREGNANOLONE. K. T. Britton*, Y. Akwa, R. H. Purdy and G. F. Koob. Dept. of Psychiatry, V.A. Medical Center and the UCSD School of Medicine, San Diego, CA 92161 and Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The amygdala is a limbic structure that has long been implicated in the neurobiology of fear and anxiety. Recent studies have shown that amygdaloid nuclei can differentially mediate some of the anxiolytic effects of benzodiazepines in different animal models of "anxiety". We have previously demonstrated that systemic injections of the neurosteroid, allopregnanolone (3α-hydroxy-5α-pregnan-20-one) can have an "anxiolytic-like" effect in the conflict paradigm, and this effect seems to be independent of the benzodiazepine site of action on GABA/benzodiazepine receptor-chloride ionophore receptors (Soc Neurosci. Abs. n° 207.13, 1995). In the present experiment, allopregnanolone (0-15 μg) was infused bilaterally into the central amygdaloid nuclei of Wistar male rats which were then tested in a Geller-Seifter conflict test modified for incremental shock. Allopregnanolone produced a substantial increase in punished responding compared to the vehicle-infused controls. At the highest dose (15 μg) the unpunished responding was significantly decreased, indicative of mild sedation. These results suggest that the central nucleus of the amygdala may play a role in the anti-conflict activity of allopregnanolone in the rat. This work was supported by V.A. Merit Review (K.B) and grant AA06420 (G.K).

512.11

ANTAGONISM OF EPIPREGNANOLONE ON A COMMON NEUROSTEROID MODULATORY SITE OF THE GABA$_A$ RECEPTOR COMPLEX IN CNS. S. Fiszer de Plazas* and L. Pignataro. Instituto de Biologia Celular. Facultad de Medicina. Paraguay 2155, 1121, Buenos Aires, Argentina.

The GABA$_A$ receptor complex is modulated by several drugs including neuroactive steroids. Althoug the 3β hydroxylated isomer of pregnanolone (5β-pregnan-3β-ol-20-one) , epipregnanolone (EPI), was found inactive in modulating [^{3}H]flunitrazepam ([^{3}H]FNZ) binding, high concentrations of this isomer proved to antagonize competitively the [^{3}H]FNZ binding potentiation induced by neuroactive steroids.

The aim of the present work was to study the selective nature of the steroid recognition sites present in the GABA$_A$ receptor complex of chick optic lobe. Accordingly, synaptic membranes were isolated from adult chicks and [^{3}H]FNZ binding was assayed. Results indicated that 3α-hydroxy-5α-dihydroprogesterone (3α-OH-DHP) as well as alphaxalone (AFX) modulated [^{3}H]FNZ binding in a concentration-dependent manner with half maximal concentrations (EC_{50}) of 1.28±0.32 and 6.56±0.86 μM and, maximal enhancements (E_{max}) of 97.65±7.29 and 62.98±3.73 %, respectively. The addition of 16 μM EPI to either 3α-OH-DHP or AFX resulted in a decrease in EC_{50} values to 0.54±0.09 and 1.04±0.30 μM, respectively, with no significant change in E_{max} values. Moreover, when a maximal concentration (16μM) of AFX was added to 3α-OH-DHP concentrations ranging from 16 nM to 16 μM, additivity was only observed for low concentrations of the latter. When high concentrations were reached, both steroids failed to enhance [^{3}H]FNZ binding above E_{max} for 3α-OH-DHP. These results indicate that both neurosteroids act through a common site and that EPI antagonizes their interaction with this site to a different degree, suggesting marked stereospecificity for such neurosteroids.

Supported by grants from Universidad de Buenos Aires and CONICET

512.12

ALLOSTERIC MODULATION OF GABA$_A$-MEDIATED CURRENTS IN NEURONS OF THE PREOPTIC AREA FROM MALE AND FEMALE RATS. S. T. Smith*[1], and L. P. Henderson[1,2], Depts. of [1]Physiol. and [2]Biochem, Dartmouth Medical School, Hanover, NH 03755.

The preoptic area (POA) is known to play a key role in neuroendocrine function and the expression of sexual behaviors in rats. Within the POA, transmission mediated by GABA$_A$ receptors is known to have significant effects on the expression of sexual behaviors in both males and females. GABA$_A$ receptors are hetero-oligomeric proteins for which five different subunit families (α1-6, β1-4, γ1-3, δ, ρ1-2) have been identified. Transient expression of GABA$_A$ receptor cDNAs in heterologous systems has shown that a γ subunit is required to confer high-affinity binding for central benzodiazepine allosteric modulators, and that γ subunit subtypes influence the pharmacology of expressed receptors. High levels of γ1 subunit mRNA have been shown via *in situ* hybridization to be restricted to the POA, medial amygdaloid nucleus, and the bed nucleus of the stria terminalis (Ymer et al, 1990, Wisden et al, 1992) suggesting that the role of this subunit in determining the biophysical and pharmacological properties of native receptors may be best determined in neurons from the POA. To this end, we have characterized currents elicited by rapid perfusion with GABA of POA neurons acutely isolated from rats of both sexes. Sensitivity of GABA$_A$-mediated currents to allosteric modulators, such as zolpidem and the β-carbolines has been assessed. Results obtained from acutely isolated neurons will be compared to analysis of spontaneous synaptic currents in intact slice preparations. Supported by NSF IBN-9319523, NIH NS28668 and an NRSA fellowship (MH11391) to STS.

Ymer et al. (1990). The EMBO J. 9(10); 3261
Wisden et al. (1992). J. Neurosci 12(3): 1040

512.13

HORMONE-DEPENDENT AND SEX-SPECIFIC DIFFERENCES IN EXPRESSION OF GABA$_A$ RECEPTOR γ SUBUNIT MRNAS IN THE VENTROMEDIAL NUCLEUS AND PREOPTIC AREA OF THE RAT. L. P. Henderson*[1,2], P.K. Chang[2], M. K. Hristova[2], J.M. Pace[3], S. Robinson[3], A.S. Clark[3] Depts. of [1]Physiol. and [2]Biochem., Dartmouth Medical School, Dept. of [3]Psychology, Dartmouth College, Hanover, NH 03755.

Gonadal steroids act during a neonatal critical period to induce permanent sexual dimorphisms in the ventromedial nucleus (VMN) and medial preoptic area (POA) of the rat. GABA$_A$-mediated synaptic transmission within these regions is known to play a key role in the expression of adult sexual behavior, to be sexually dimorphic, and subject to steroid regulation. It is likely that structural differences in receptor subtype contribute to the functional role of GABA$_A$ receptors in mediating sexual behaviors. To test this hypothesis, we have analyzed differential expression of the individual γ subunit mRNAs of the GABA$_A$ receptor in the VMN and the POA of rats of different sexes and ages, and in rats subjected to manipulations in the hormone environment. We find that there is high level of expression of the γ1 subunit mRNA in the POA, consistent with previous reports (Ymer et al, 1990; Wisden et al, 1992, Herbison and Fénelon, 1995) and that sex- and hormone-dependent differences in the levels of γ1 mRNA are evident. In addition, we have examined the expression of the short and long forms of the γ2 subunit mRNA in the VMN since sex-specific differences in the levels of these subunits (which differ in the presence of a PKC target consensus sequence) may contribute to sex-specific differences in current decay kinetics that we have observed previously. Supported by NSF IBN-9319523 (LPH/ASC).

Ymer et al. (1990). The EMBO J. 9(10); 3261
Wisden et al. (1992). J. Neurosci 12(3): 1040
Herbison and Fénelon (1995) J. Neurosci. 15(3): 2328.

512.14

THE SEXUAL DIMORPHISM IN GABA$_A$ RECEPTOR mRNA DISTRIBUTION AND EFFECTS OF TESTOSTERONE RECEPTOR ANTAGONISTS ON THEIR EXPRESSION IN THE EMBRYONIC RAT CNS. L. Zhang*, W. Ma, A.N. Feldman, G. Xing, F. Lahjouji, J.L. Barker, D.R. Rubinow BPB., NIMH and Lab. Neurophysiology, NINDS, NIH, Bethesda, MD 20892

We used in situ hybridization techniques to study GABA$_A$ receptor subunit (α1, β2, γ2, α2, α3 ,α5) mRNA expressions in normal controls and rats treated with testosterone receptor antagonists, flutamide (0.5 mg/kg/day) and cyproterone (250 ug/kg/day), from embryonic(E) day 14 to 21. We found that GABA$_A$ receptor subunit expression was sexually dimorphic in certain brain regions at the transcription level. For example, higher level of α5 transcripts were found at E21 in the male hypothalamus(220% of female), while higher levels of α2 mRNA were discovered in the female thalamus(130% of male). Testosterone antagonists differentially altered the expression of GABA$_A$ receptor mRNA in male and female CNS tissues. For example, β2 transcript abundance in cortical and thalamic regions was upregulated by antagonists in males but was unchanged in females. In contrast, female but not male thalamic α2 mRNAs were downregulated by the testosterone antagonists. The results indicate that testosterone levels may have detectable if not important effects on the expression of different GABA$_A$ subunit mRNAs in different embryonic CNS regions. Such effects on mRNA expression are regional, subunit selective, and age dependent. Although it is unclear how the testosterone receptor antagonists alter GABA$_A$ receptor mRNA expression, effects at both genomic and non-genomic sites may contribute. Testosterone changes in GABA$_A$ receptor subunit expression could play an early and possibly critical role in masculinizing the CNS.

512.15

SEX DIFFERENCES IN THE ANESTHETIC ACTION OF ALFAXALONE ARE CORRELATED WITH DIFFERENTIAL ELIMINATION M. E. Brewster*, W. R. Anderson, A. Webb and E. Pop, Pharmos Corp., Rehovot, ISRAEL and Alachua, FL, USA and the College of Veterinary Medicine, University of Florida, Gainesville, FL USA

Alfaxalone is a steroid anesthetic chemically related to progesterone. The poor water solubility of this agent prompted us to reformulate the steroid in a 2-hydroxypropyl-β-cyclodextrin vehicle. The anesthetic potency of alfaxalone in male and female Sprague-Dawley rats was assessed using sleeping time as an endpoint. Consistent with earlier reports, a sex difference after i.v. treatment was not observed below 100 μmol/kg (33 mg/kg). On the other hand, significant sex-related potency differences were observed at higher doses such that female animals slept 2.6-times longer at 60 mg/kg and approximately 3-times longer at 72 mg/kg than the respective male group. Sex differences were also observed after i.p. administration. The disposition of alfaxalone in male and female rats after a 50 mg/kg dose of alfaxalone was assessed using a novel HPLC method in which alfaxalone was derivatized (precolumn) using dinitrophenylhydrazine. Results of pharmacokinetic analysis of the plasma data indicated that alfaxalone was more rapidly eliminated from male as compared to female animals. In addition, areas under the concentration curve (AUC) were lower for males than females. For example, plasma AUC's were 906 and 1812 mg*min/L for males and females, respectively. Other tissue compartments manifested similar sex differences. These data are in contrast to those of Fink (Nature 298:270-273 (1982)) who reported that alfaxalone was eliminated with equal efficacy from males and females although the dose used in this study was 12 mg/kg. Since there is a sex difference in activity (after i.v. administration) only above doses of 30 mg/kg, it is possible that pharmacokinetic differences may also only be manifested at higher dose levels.

512.16

EFFECTS OF THE NEUROACTIVE STEROID ALLOPREGNANALONE ON THE SEXUAL EHAVIOR AND ULTRASONIC VOCALIZATIONS OF MALE RATS. B. Zimmerberg* and E.W. Fish, Williams College, Williamstown, MA 01267

Male sexual behavior is mediated, in part, by GABA-ergic activity. This experiment investigated the effects of i.p. injections of the neuroactive steroid allopregnanalone (AP), a positive modulator of the GABAa receptor, on the sexual motivation and sexual performance of male rats. Sexually experienced adult male rats in control, vehicle (20% beta-cyclodextrin), 1.25 mg/kg, 2.5 mg/kg, and 5.0 mg/kg AP conditions were tested in a T-maze to determine preference for an estrous vs. non-estrous female. The first two AP doses reduced estrous preference. Compared to males in control and 2.5 mg/kg conditions, fewer males in the 1.25 mg/kg, 5.0 mg/kg, and vehicle conditions achieved ejaculation during mating tests with an estrous female. Ultrasonic vocalizations in the presence of estrous bedding, the post-ejaculatory song, and post-ejaculatory activity were not affected by AP. There was a dose dependent reduction of grooming behavior by AP in the T-maze. These results suggest that neuroactive steroids may modulate both sexual motivation and performance in male rats, in addition to an anxiolytic effect as measured by grooming behavior.

GABA$_B$ AND GABA$_C$ RECEPTORS

513.1

POSITIVE COUPLING OF GABA$_B$ RECEPTORS TO ADENYLYL CYCLASE IN RAT OLFACTORY BULB. M.C. Olianas* and P. Onali, Dept. Neurosciences, Univ. Cagliari, Cagliari, Italy.

Activation of GABA$_B$ receptors has been reported to either inhibit or stimulate cyclic AMP formation in the brain. While the inhibitory effect results from a negative coupling to adenylyl cyclase through Gi/Go proteins, the stimulation of cyclic AMP formation has been detected only in intact cell preparations and has been considered to be a secondary event mediated by intracellular messengers. In this study we show that in membranes of rat olfactory bulb, the GABA$_B$ receptor agonist (−) baclofen produced a concentration–dependent increase of adenylyl cyclase activity (EC_{50} 30 μM). The (−) baclofen stimulation was higher in the granular and external plexiform layers than in the glomerular and olfactory nerve layers. The stimulatory effect of (−) baclofen was mimicked by 3–aminopropylphosphinic acid and GABA, but not by (+) baclofen, and was counteracted by CGP 35348 and CGP 55845A, two selective GABA$_B$ receptor antagonists. The (−) baclofen stimulation did not require Ca^{2+}, was abolished by pertussis toxin and inhibited by the addition of the α subunit of transducin, used as a scavenger of Gβγ. The data demonstrate that in the olfactory bulb GABA$_B$ receptors can directly stimulate adenylyl cyclase activity, likely by promoting the release of βγ subunits of Gi/Go and the activation of the Ca^{2+}–independent forms of the enzyme.

513.2

GAMMA HYDROXYBUTYRIC ACID INHIBITS EXCITABILITY OF DOPAMINE NEURONS THROUGH GABA$_B$ RECEPTORS IN THE RAT MIDBRAIN SLICE T.E. Madden and S.W. Johnson* Dept. of Physiol. & Pharmacol., Oregon Health Sciences University, Portland, OR 97201

Gamma hydroxybutyric acid (GHB), a precursor and metabolite of GABA, concentrated in the hypothalamus and basal ganglia has abuse potential. Intracellular electrophysiological recordings were made to characterize the effect of GHB on dopamine neurons in the ventral tegmental area and substantia nigra of the rat midbrain slice. 0.3 mM GHB slowed and 1mM blocked firing of dopamine neurons. Application of GHB (0.1-10 mM) resulted in concentration-dependent hyperpolarization and reduction in input resistance (EC_{50} = 0.60 mM) with the maximum mean effects reached at 1.25 mM (56% reduction of input resistance [74 MΩ] and 16 mV hyperpolarization). Changes in voltage and resistance were blocked by CGP 54626 (300 uM), but not by bicuculline (30 uM) or strychnine (10 uM). Single electrode voltage clamp recordings demonstrated that increasing concentrations of extracellular K^+ significantly shifted the reversal potential to more depolarized potentials. These data support the hypothesis that GHB hyperpolarizes dopamine neurons by GABA$_B$ receptor-mediated opening of K^+ channels.

Supported by MH 40416 and NIDA 5T32DA07262.

513.3

EFFECT OF BACLOFEN ON NMDA MEDIATED SYNAPTIC RESPONSES IN RAT MIDBRAIN DOPAMINE NEURON IN VITRO. Y. N. Wu, W. E. Cameron*, and S. W. Johnson. Department of Physiology and Pharmacology, Oregon Health Sciences Univ., Portland, OR 97201.

Effects of baclofen on exitatory postsynaptic potentials (EPSPs) were studied on dopamine neurons in ventral tegmental area and substantia nigra zona compacta of rat brain slices using the patch clamp whole cell recording technique. Baclofen was more potent for reducing the amplitude of the EPSP mediated by NMDA receptors (EC_{50} = 0.27 μM) compared with inhibition of the EPSP mediated by AMPA receptors (EC_{50} = 1.05 μM). But $GABA_A$ receptor agonist isoguvacine reduced the amplitude of NMDA (51 ± 3%, n = 7) and AMPA (55 ± 5%, n = 10) EPSPs to the same extent. The increase in membrane conductance produced by baclofen (E_{max} = 2.5nS) was less than that for isoguvacine (E_{max} = 12nS). Membrane hyperpolarization was positively related to baclofen inhibition of the NMDA mediated EPSP. The data suggest that $GABA_B$ receptor activation may play an important role in regulating NMDA activated channels in dopamine neurons. (Supported by PHS grant MH40416.)

513.4

MUSCIMOL & BACLOFEN MICROINJECTED INTO THE ARCUATE NUCLEUS AFFECT VENTILATION DIFFERENTLY IN MALE RATS. E.H. Schlenker* and X-M Lin. Dept. of Physiology and Pharmacology, University of South Dakota School of Medicine, Vermillion, SD 57069.

The arcuate nucleus (AN) of the hypothalamus contains high levels of gamma-aminobutyric acid (GABA) which may act on either GABA-A and/or GABA-B receptors to modulate autonomic function. The purpose of the present study was to determine the effects of microinjecting (200nl) muscimol (M), a GABA-A receptor agonist, in doses of 0, 1 and 5 nmol or baclofen (B), a GABA-B receptor agonist in doses of 0, 5 and 10 nmol into the AN of conscious male rats on their ventilation (VE), O_2 consumption, and ventilatory responses to hypoxia (HX) and to hypercapnia (HC). M significantly decreased VE ($F=11.42$, $P<0.001$) especially at the 5 nmol dose as a result of a decreased tidal volume (Vt). In contrast, B significantly stimulated VE ($F=8.69$,$P<0.001$) at the 10 nmol dose due to an increase in Vt. Neither agonist affected O_2 consumption, HX, nor HC. Thus, in the arcuate nucleus of male rats GABA may affect VE by acting on both receptor subtypes, and the effect is not associated with changes in O_2 consumption. NIH Grant # HD30390

513.5

MULTIPLE POSTSYNAPTIC $GABA_B$ ACTIONS IN RAT HIPPOCAMPUS: EVIDENCE FOR SYNAPTIC AND EXTRA-SYNAPTIC RECEPTORS. T.M. Pham* and J.-C. Lacaille. Center for Research in Neurological Sciences and Department of Physiology, University of Montréal, Montréal. Qc, Canada H3C 3J7.

Postsynaptic $GABA_B$ receptors in the hippocampus are coupled to heterogeneous K^+ conductances differentially sensitive to Ba^{2+}. Our aim was to determine with intracellular recordings in rat hippocampal slices, if synaptically-released GABA also activated heterogeneous K^+ conductances via $GABA_B$ receptors. In the presence of $GABA_A$, NMDA and non-NMDA antagonists, $GABA_B$ IPSPs were induced either by direct electrical stimulation of interneurons or by bath application of 100 μM 4-AP. Monosynaptic IPSPs evoked by stimulation of different layers, oriens (OR; n=13), lacunosum-moleculare (LM; n=13), and radiatum (RAD; n=14), showed no significant differences in amplitude (~-5 mV), peak latency (165ms), recovery time (410ms), or E_{rev} (-92mV). Bath application of the $GABA_B$ antagonist CGP55845A (5μM) suppressed all 3 types of monosynaptic $GABA_B$ IPSPs (amplitude ~-5% of control; n=2 OR, 4 LM, 5 RAD). Using recording solution containing GTPγS (25mM), monosynaptic IPSPs were significantly reduced 60 minutes after impalement (amplitude ~4% of control, n=3 OR, 3 LM, 3 RAD). Bath application of 1mM Ba^{2+} completely suppressed all 3 types of monosynaptic IPSPs (n=12). Spontaneous IPSPs induced by 4-AP had similar properties (-8.2mV amplitude, 230ms rise time, 1.9s recovery time, and -104mV E_{rev}). 4-AP induced IPSPs were totally blocked by bath application of CGP55845A (n=2) and reduced to 6.2±3.4% control (n=5) during recordings with GTPγS-containing solutions. 4-AP induced IPSPs were also completely suppressed by Ba^{2+} (n=10). These results indicate that GABA, released synaptically by stimulation of interneurons or by 4-AP application, may activate, via $GABA_B$ receptors and G-proteins, only Ba^{2+}-sensitive K^+ conductances. Other postsynaptic $GABA_B$ actions involving Ba^{2+}-insensitive K^+ conductances may thus be activated only via extrasynaptic receptors.

[Supported by the MRC, FRSQ, FCAR and Savoy Foundation]

513.6

MUTATIONS IN GABA RECEPTOR'S M2 DOMAIN OPEN THE CHANNEL GATE AND PRODUCE DUAL AGONIST EFFECTS. Zhuo-Hua Pan*, Dongxian Zhang, Xishan Zhang, and Stuart A. Lipton. Department of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

The chloride mediated $GABA_C$ (or ρ) receptors belong to the ligand-gated ion channel superfamily. We used rat homomeric GABA ρ1 receptors, site-directed mutagenesis, and the *Xenopus* oocyte expression system to explore the role of amino acid residues in the M2 domain on the receptor-channel function. Point mutations were made by replacing the threonine at position 314 with an alanine [ρ1(T314A)] or several other amino acids. Oocytes injected with ρ1(T314A) mutant cRNA displayed spontaneous inward current when clamped at -70 mV. The current, which reversed at the Cl^- reversal potential, was blocked by the channel blocker picrotoxinin but not by the competitive $GABA_C$ receptor antagonist 3-APMPA, indicating that receptor-channels were constitutively open in the absence of agonist. Moreover, low concentrations of GABA (nM) suppressed the spontaneous current (IC_{50} = 0.11 μM and n_H = 0.8), while higher concentrations (>0.1 μM) elicited additional inward current. Both of these GABA-evoked effects were blocked by 3-APMPA. Substituting alanine for leucine at position 317 (but not other nearby sites) produced similar effects. Our findings provide electrophysiological evidence that amino acid residues at positions 314 and 317 of the GABA ρ1 receptor constitute the gate of the channel. The dual but opposing actions of GABA provide direct physiological evidence for multiple conformational transitions during receptor activation, possibly reflecting binding of multiple agonist molecules.

Supported by an AmFAR Award and NIH grant R01 EY05477.

513.7

IMMUNOCYTOCHEMICAL LOCALIZATION OF $GABA_C$ RECEPTORS IN THE MAMMALIAN CNS. R. Enz, J.H. Brandstätter, P. Koulen, H. Wässle* and J. Bormann Max-Planck-Institut für Hirnforschung, D-60528 Frankfurt, Germany.

The bicuculline-insensitive $GABA_C$ receptors are thought to be formed by the ρ subunits in the vertebrate retina. RT-PCR and *in situ* hybridization studies have shown the expression of ρ1 and ρ2 subunits in the retina and brain of the rat (Enz et al., 1995, *Eur. J. Neurosci.* 7: 1495-1501). We have generated polyclonal antibodies against the N-terminus of the rat ρ1 subunit in order to investigate the distribution of the ρ subunits in the mammalian CNS. The obtained antiserum recognized ρ1/2/3 subunits but not the $GABA_A$-receptor subunits α1–3, β1–3, γ2, δ or the glycine-receptor subunits α1 and β on Western blots or transfected HEK-293 cells. On retina sections immunostaining was present in the outer and more prominently in the inner plexiform layers of different species. Labelling was punctate, indicating synaptic localization. Pre-incubation of the antibodies with the antigen resulted in no staining. Double-labeling experiments with antibodies against the ρ1 subunit and with antibodies staining either rod and cone bipolar cells or amacrine cells showed expression of ρ subunits at dendrites and axon terminals of rod and cone bipolar cells. Prominent staining of neurons with the ρ1 antiserum was observed also in rat spinal cord. These findings suggest an important physiological role of $GABA_C$ receptors in mediating inhibitory synaptic interaction in the mammalian CNS.

Supported by the Deutsche Forschungsgemeinschaft (SFB 269)

513.8

MOLECULAR CHARACTERIZATION OF A NOVEL GABA RECEPTOR TRANSIENTLY EXPRESSED IN THE HIPPOCAMPUS OF NEONATAL RATS. M. Mladinic, E. Cherubini*and A. Bradbury. Biophys. Lab, Int Sch. Adv. Studies (SISSA), 34013 Trieste, Italy.

We have recently described in the hippocampus of neonatal rats a novel GABA response which is bicuculline and baclofen insensitive and is mediated through Cl^- permeable receptor channels. This response which is transiently expressed during development closely resembles that one present in the retina and assumed to be mediated by $GABA_C$ receptors. It is conceivable that this new receptor channel is an heterooligomeric protein assembled from the rho1 or rho2 subunits. In the attempt to clone the novel GABA receptor gene(s) expressed in the neonatal hippocampus during the critical period of postnatal development, two different approaches have been used: RACE (Rapid Amplification of cDNA ends) and screening of a neonatal hippocampus cDNA library. In both approaches we have used the probe previously employed in *in situ* hybridization experiments (rho1+rho2 probe: nucleotide sequence of the rho1 gene 1130-1174 and nucleotide sequence of the rho2 gene 1042-1086) which gave a high signal in neonatal but not in adult rat brain. The RACE protocol generates cDNA by using PCR to amplify copies of the region between a single point in the transcript and 3' and 5' end. The 450bp fragment was obtained as a 3' RACE product using rho1+rho2 and TnTAG primers. The same fragment was obtained by PCR performed on the neonatal hippocampus cDNA library. The fragment was cloned into the Bluescript plasmid and sequenced. The sequence obtained is novel and not present in the database. It is most similar to the expressed sequence tag HFBEL69 from the human fetal brain. We are using this fragment as well as rho1+rho2 oligonucleotide as the probes to screen the neonatal hippocampus cDNA library to obtain the whole length of the gene of interest.

513.9

MUTATION OF A CONSERVED LEUCINE IN THE M2 REGION OF HOMOMERIC ρ1 GABA RECEPTORS. Y. Chang*, DS Weiss. Neurobiology Research Center and Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294-2001.

γ-aminobutyric acid (GABA) binds to its receptor and opens an integral, chloride-selective pore. Previous studies demonstrated that mutations of a conserved leucine residue of the putative pore-lining region in the nicotinic receptor alters the channel's gating characteristics. In this study, we assessed the role of the conserved leucine residue in the M2 region of the ρ1 GABA receptor expressed in *Xenopus laevis* oocytes. The conserved leucine (L301) was mutated either to another hydrophobic amino acid: glycine (L301G), alanine (L301A), phenylalanine (L301F), isoleucine (L301I), valine (L301V) or to a hydrophilic amino acid: tyrosine (L301Y), serine (L301S), threonine (L301T). All hydrophilic substitutions, as well as L301G and L301A, created spontaneous-opening channels, evident as an unusually high inward current in the absence of GABA. The holding current dropped in response to low concentration of GABA (0.01-0.3μM), but at higher GABA concentrations (1-1000μM) the response was biphasic. The results are consistent with the idea that the residue at this position needs both proper hydrophobicity and size for the channel to gate normally. (Grant # NS35291.)

513.10

FUNCTIONAL EXPRESSION OF RAT GABA RECEPTOR ρ3 SUBUNIT. Ryuzo Shingai*, Kazuya Yanagi, Teruyuki Fukushima, Kazumi Sakata and Tarou Ogurusu. Department of Information Science, Faculty of Engineering, Iwate University, Morioka 020 Japan

Cloned cDNA encoding GABA ρ3 subunit was isolated from cDNA library of rat retina. The amino acid sequence of this clone has 63 and 61% homology to human ρ1 and ρ2 subunit respectively. The expression of mRNA for ρ3 subunit in the retina was shown by Northern analysis. The cRNA made from ρ3 cDNA was injected into *Xenopus* oocytes and sensitivity to drugs was investigated under the two electrodes voltage clamp condition. Bicuculline did not block the GABA current, while 10 μM picrotoxin eliminated inward currents induced by 10 μM GABA. This receptor was almost insensitive to diazepam, pentobarbital, and neurosteroids tetrahydrodeoxycorticosterone (THDOC) and 3α-hydroxy,5α-pregnan-20-one (3α-OH-DHP). Thus ρ3 subunit can form a homo-oligomeric ionotropic receptor, and has pharmacological properties common to the ρ-type GABA receptor subunits, and therefore could participate in functions of GABAc receptor in the retina.

513.11

PROTON SENSITIVITY OF THE RAT ρ_1 GABA RECEPTOR

M. Pasternack[1]*, C. Rivera[1], K. Wegelius[1], M. Reeben[2], and K. Kaila[1]
Dept. of Biosciences, Div. of Animal Physiology, P.O.Box 17, and [2]Institute of Biotechnology P.O.Box 56, FIN-00014 University of Helsinki, Finland.

Normal and pathophysiological activity induce marked pH changes in nervous tissue. These pH changes have a profound effect on the general excitability of the brain. A sensitivity of $GABA_A$ and NMDA receptors to external proton ions has been postulated to underlie the pH-dependence of neuronal activity.

We have cloned the ρ_1 GABA receptor cDNA from rat retina and transiently expressed the receptor in a HEK 293 cell line. We studied the sensitivity of this receptor to external protons using the whole cell patch-clamp technique. We found the ρ1 receptors to be markedly sensitive to external pH. An increase in external pH (pH_o) from 7.4 to 8.4 increased the GABA-induced conductance by about 70%, whereas a decrease in pH_o from 7.4 to 6.4 decreased the conductance to about 50%. GABA concentration-response experiments revealed that external protons did not affect the EC_{50} of GABA, but rather that protons inhibited the conductance in a manner independent of the GABA concentration.

We have previously shown that $GABA_A$ receptors in pyramidal neurons of rat hippocampus are modulated in a dual manner by external protons. At higher GABA concentrations the conductance was upmodulated by protons, whereas at lower GABA concentrations downmodulation was observed. This suggests the presence of more than one $GABA_A$ receptor population in the hippocampal pyramidal neurons. We have examined the distribution of ρ_1 and ρ_2 transcripts in the rat brain using *in situ* hybridization and RT-PCR techniques and found that both ρ_1 and ρ_2 are expressed in the rat hippocampus, albeit at a relatively low level. As the ρ_1 receptors studied here have a relatively high affinity for GABA (EC_{50} = 1.5 μM) they may well be responsible for the downmodulatory effect of protons seen in the hippocampal neurons at low GABA concentrations.

Supported by the Academy of Finland.

GABA: GAD, GAT AND GABA STUDIES

514.1

Regulation of the mouse glutamate decarboxylase gene expression.

Y. Yanagawa*[1], T. Kobayashi[1,2], T. Kamei[1], K. Ishii[1], M. Nishijima[2], A. Takaku[2], H. Asada[3], K. Obata[3], Y. Sasahara[1], T. Kobayashi[1], and S. Tamura[1]
[1] Inst. Development, Aging and Cancer, Tohoku Univ., Sendai, 980, Japan; [2] Toyama Med. and Pharmaceu. Univ., Toyama, 930, Japan; [3] Natl. Inst. for Physiol. Sci., Okazaki 444, Japan

Mouse glutamate decarboxylase (mGAD65 and mGAD67) is the enzyme responsible for synthesizing GABA. We used P19 cell line to examine the developmentally regulated expression of mGADs, and we cloned the 5' flanking region of mGAD67 gene to investigate the regulatory mechanisms controlling transcription of this gene.

(1) P19 mouse embryonal carcinoma cells can be induced to differentiate along the neuroectodermal lineage. Western blot analysis showed that mGAD65 and mGAD67 were not expressed during 4 days after retinoic acd (RA) treatment, but appeared 6 days after RA treatment when neurite outgrowth was observed. The addition of a protein phosphatase (PP) inhibitor, okadaic acid, into the culture medium had an inhibitory effect on the expression of mGAD65 and mGAD67, but that of cyclosporin A did not. These results suggested that GAD expression coincidented with neural differentiation and that PP1 or PP2A, but not PP2B, was associated with the regulation of GAD expression.

(2) 5' -RACE method showed mGAD67 gene transcripts have two different 5' untranslated regions. Analysis of the genomic clones encompassing the 5' -exons revealed that the two transcripts arised from a single gene by alternative splicing using two different donor sites and a common acceptor. The exons were found 1.5 and 0.6 kb upstream of exon 2. RT-PCR method showed that each type was expressed in mouse brain. These results suggested that these transcripts could be regulated under control of independent promoters.

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

514.2

TARGETED DISRUPTION OF THE MURINE GLUTAMIC ACID DECARBOXYLASE (65 kDa isoform -- GAD65) GENE.

S. Kash, R. Johnson, L. Tecott, D. Lowenstein*, D. Hanahan and S. Baekkeskov.

The inhibitory neurotransmitter GABA is synthesized by one of two isoforms of the enzyme glutamic acid decarboxylase (GAD65 and GAD67), each of which is encoded by a separate gene. To address the role of GAD65 in development and central nervous system function, mice lacking GAD65 were generated. Insertion of a neomycin-resistance cassette in exon one created a nonsense mutation and functionally disrupted the protein. Homozygous mutant animals were born at a ratio that did not suggest embryonic lethality. Brains of homozygous mutant animals were shown to lack GAD65 by Western blot analysis and immunocytochemistry. Brain weights revealed the mutation had no effect on overall brain size. Animals were viable but there was a high frequency of spontaneous death (13% to date). Measurement of the threshold to seizure activity showed that mutant animals have a higher sensitivity to the GABAA-receptor antagonist pentamethylenetetrazole (metrazol). Continuous intravenous infusion of the convulsant showed significant differences in both the clonic seizure activity and lethal dose. There is a 30% decrease in the lethal dose, resulting from a 50% decrease in the clonic phase of seizure activity.

This work was supported by NIH Grant #P01 DK41822.

514.3

INCREASED EXPRESSION OF GLUTAMIC ACID DECARBOXYLASE (GAD) 65KD OR 67KD ISOFORMS LEADS TO INCREASED SYNTHESIS AND RELEASE OF GABA IN CULTURED NEURONS AND ASTROCYTES.

K. C. New[1]*, K. Gale[3], R. L. Martuza[2], S. D. Rabkin[1,2]. Departments of Microbiology & Immunology[1], Neurosurgery[2] and Pharmacology[3], Georgetown Univ. Med. Ctr., Washington, DC 20007.

Defective herpes simplex virus (HSV) vectors were used to express GAD65, GAD67, or β-galactosidase (β-gal) in primary cultures of cerebellar granule cell (CGC) neurons and astrocytes. We evaluated whether the increased expression of GAD leads to regulated release fo GABA. GABA release from neurons occurs through Ca^{++}-dependent vesicular release and Ca^{++}-independent reversal of the Na^{+}/GABA cotransporter. Defective vector (dv)GAD infected CGC neurons at 6 days in vitro (div) exhibited only Ca^{++}-independent GABA release, induced by glutamate or veratridine. In dvGAD infected CGCs (10 div), Ca^{++}-dependent GABA release was induced by K^{+} depolarization, and Ca^{++}-independent release by veratridine. Similar levels and mechanisms of GABA release were detected in dvGAD65 and dvGAD67 infected neurons. In primary cortical astrocytes, GAD vector infection resulted in isoform specific expression of GAD, and GABA levels were two-fold higher in GAD67 than GAD65 expressing cells. GABA was tonically released from dvGAD infected astrocytes, but no increase in release could be detected after treatment of the cells with K^{+}, veratridine, glutamate or bradykinin. β-gal vector infected or uninfected astrocytes expressed no GAD and contained no detectable GABA. These data demonstrate that GAD expressing defective HSV vectors are able to increase the synthesis and release of GABA in cultures of neurons and astrocytes. Neurologic disorders that result from decreased GABA levels are potential targets for gene therapy using these vectors.

Supported by a grant from the NINDS and an ARCS Foundation Scholarship

514.4

GABA TRANSPORTERS IN THE INFERIOR COLLICULUS OF RATS ARE MAINLY EXPRESSED BY ASTROCYTES.

C.E. Ribak*, W.M.Y. Tong and N.C. Brecha Dept. of Anat. & Neurobiol., Univ. of Calif., Irvine, CA 92717 and CURE, Wadsworth VA Med. Ctr., Los Angeles, CA 90073.

Previous studies that localized GABA transporters (GATs) showed that GAT-1 is found in axon terminals of GABAergic neurons whereas GAT-1 and GAT-3 are present in astrocytic processes. One brain region, the cerebellum, appeared to lack GAT-1 in one of its GABAergic cell types, Purkinje cells. Astrocytic processes compensated for this by isolating their axon terminals in the deep cerebellar nucleus and expressing large amounts of GAT-3. The present study was made to determine the localization of GAT-1 and GAT-3 in the inferior colliculus (IC), a brain region with several types of GABAergic neurons that play important roles in auditory processing. Both GAT-1 and GAT-3 were observed in punctate structures in the central nucleus, dorsal cortex and external nucleus of the IC. Electron microscopic preparations demonstrated that axon terminals forming symmetric synapses, the type previously labeled for GABA, lacked immunoreactivity for GAT-1. Instead, astrocytic processes that enveloped these axon terminals were densely labeled for GAT-1. Also, astrocytic processes as well as their cell bodies displayed GAT-3 immunoreactivity. These data indicate that most axon terminals of GABAergic neurons in the IC lack GATs. In addition, these results suggest that GABAergic neurons in the IC do not possess the ability to release GABA in a calcium-independent, non-vesicular manner, such as when neurons become overstimulated during high activity.

[Supported by NIH Grant NS 15669 to CER.]

514.5

GABA-ERGIC NEURONS IN CULTURES OF FREEZE-STORED EMBRYONIC RAT NERVOUS TISSUES : A MORPHOMETRIC ANALYSIS USING IMMUNOCYTOCHEMISTRY. M.C.Calvet * and D.Petite. INSERM U 336. Univ. Montpellier II. 34095. Montpellier. France.

Based on prior work on cryopreservation and subsequent culturing of neural tissue, we have applied the principles of these techniques to embryonic rat cerebral cortex and mesencephalon. Blocks of fresh tissue were dissociated after freezing and storage at -196°C and the cells were cultured for 2-4 weeks before immunocytochemical staining with anti-GABA antibodies. A comparative morphometric analysis of the GABA-immunoreactive (IR) neurons allowed the individualization of two similar populations in both frozen and unfrozen materials : small and large neuritic field neurons. These two types of GABA-IR neurons could be evidenced as well in all the studied cultures with significant quantitative morphological differences linked to their original anatomical structure. Thus, GABA-ergic phenotype is expressed similarly in fresh and frozen cultured neurons with intrinsically programmed morphological features : small and large neuritic field GABA-IR neurons represent respectively local and long range circuits of inhibition indicating that the role of GABA as a major inhibitory transmitter is fundamental to regulate excitatory processes.

(Supported by grants from INSERM, AFM, DRET and IRME.)

514.6

ACUTE RISPERIDONE DECREASES EXTRACELLULAR GABA LEVELS IN THE GLOBUS PALLIDUS AND PRODUCES CATALEPSY. Jeffrey W. Grimm* and Ronald E. See. Department of Psychology, Washington State University, Pullman, WA. 99164-4820

Haloperidol, a typical antipsychotic drug which is known to produce extrapyramidal side effects (EPS), has been shown to increase while clozapine, an atypical antipsychotic drug which has a low EPS profile, has been shown to decrease extracellular levels of GABA in the globus pallidus. The present study investigated the effects of risperidone, a putative atypical antipsychotic drug with a low EPS profile, on GABA levels in the globus pallidus. Female, Sprague Dawley rats were surgically implanted with stainless steel guide cannulae through which 2 mm length microdialysis probes were inserted. Probes were perfused with modified Ringer solution and samples were collected at 20 min intervals. Following a 1 hr baseline, rats were injected with risperidone (3 mg/kg sc) and samples were collected for the next 5 hr. Bar and paw test catalepsy measures were made 30 min, 1 hr, 2hr, and 5 hr after drug treatment. Risperidone treatment significantly reduced extracellular GABA levels between 100 min and 220 min following treatment. Rats displayed significant bar and paw test catalepsy throughout the measurement period. Front and back paw measures were not significantly different. Risperidone-induced catalepsy did not appear qualitatively identical to haloperidol-induced catalepsy in that risperidone-induced catalepsy was not marked with extreme rigidity. Vehicle treated rats did not show altered GABA levels, nor did they show any signs of catalepsy. These results show that risperidone produces decreases in pallidal GABA similar to previously reported effects found after acute clozapine. Differential pallidal GABA modulation may be a marker of the typical or atypical profile of an antipsychotic drug. The effects of alternate doses of risperidone on pallidal GABA levels and catalepsy will be discussed. This research was supported by USPHS Grant DE-09678 (RES) and the state of Washington.

514.7

EFFECTS OF STRESS ON EXTRACELLULAR GABA IN THE MEDIAL PREFRONTAL CORTEX. J.M. Finlay* and Veronica L. Neff, Dept. of Neuroscience, University of Pittsburgh, Pittsburgh, PA, 15260

Previous studies demonstrated that acute stress increases the activity of dopamine- and glutamate-containing projections to the medial prefrontal cortex (mPFC). Because the postsynaptic targets of these projections include the intrinsic γ-aminobutyric acid (GABA) interneurons it is also of interest to determine the impact of stress on these neurons. We have examined the impact of acute tail shock on GABA efflux in the mPFC and begun to characterize the origin of extracellular GABA in this region as determined by *in vivo* microdialysis. Approximately 18 hr after implantation of a microdialysis probe, basal extracellular GABA in the mPFC of freely moving rats was 59 ± 7 pg/20 µl (n=5). Thirty min of acute tail shock elicited a biphasic change in extracellular GABA. During application of the tail shock extracellular GABA increased to 85 ± 7 pg/20 µl. Fourty-five min after termination of the stress extracellular GABA had declined to 39 ± 8 pg/20 µl and subsequently returned to basal levels 90 min following termination of the stress. Local application of tetrodotoxin (1 µM) or 0 Ca^{++} perfusion solution delivered via the microdialysis probe decreased extracellular GABA levels by 66% and 56%, respectively. The present data demonstrate that acute stress alters extracellular GABA in the mPFC. Additional experiments will determine whether dopamine-containing projections modulate the effects of stress on extracellular GABA in the mPFC. *[Supported by NARSAD and USPHS grants MH 45156 and MH43947]*

514.8

STABLE OPA/SULFITE DERIVATIVE OF GABA IN CELLULAR INCUBATION MATRIX, CSF AND TISSUE HOMOGENATES VIA HPLC WITH ELECTROCHEMICAL DETECTION. **S. R. Bossi *[1], S. A. Morrison[2] and T.J.Maher[3]. [1]ESA Inc. Chelmsford MA, [2] CytoTherapeutics Inc. Providence RI, and [3]Massachusetts College of Pharmacy Boston MA.**

HPLC with electrochemical (EC) detection has been employed reliably to determine low level concentrations of amines and amino acids from brain tissue homogenates and CSF. Several of these methods employ the use of o-phthalaldehyde (OPA) with β-mercapto-ethanol to derivatize the amino functional group of these primary alkylamines to form an unstable alkylisoindole (variable stability observed vs excess OPA) molecular structure which supports EC and fluorescence detection. In this study we used OPA in the presence of sodium sulfite to form a more stable isoindole-sulfonate derivative form of Gamma-aminobutyric acid (GABA). Rat insulinoma (RIN) cells, which are known to secrete GABA (CytoTherapeutics, Inc.) were incubated in Hanks' Balanced Salt Solution buffered with 10 mM Hepes pH 7.4 for 1-3 hrs. Tissue homogenates were prepared from frozen rat brain cortex, cerebellum, striatum and brainstem via sonication with 4°C 0.1M perchloric acid followed by centrifugation and filtering. Human CSF was prepared by filtration with a 0.2 uM filter. All sample matrices were derivatized 1:1 with the OPA/sodium sulfite solution and maintained at 4°C prior to injection. Degradation of derivatized samples (n=6) at 7 and 13 hours was 1.7% and 7.6%. This method allowed for isocratic detection of GABA, via coulometric detection, with elution time of less than 6 minutes. Our linear range was 1-50 ng/mL with the lowest quantitation level of 19.5 pg. Intraday variability was < 3% (n=10). Interday variability was < 5% (n=24). This method provides a stable, sensitive and rapid assay for GABA in a variety of biological matrices.

514.9

GABA IMMUNOREACTIVE CELLS AND TERMINALS DECREASE IN CEREBRAL AND CEREBELLAR CORTEX OF RATS EXPOSED TO HYPERGRAVITY: PRELIMINARY FINDINGS I. Polyakov*, F. D'Amelio, N. Daunton, R. Fox, M. Corcoran, and L. Wu. Gravitational Research Branch, NASA Ames Research Center, Moffett Field, CA and San Jose State University, San Jose, CA.

Recent behavioral studies have shown that chronic hypergravity exposure produces changes in righting, locomotion, and orientation of centrifuged rats. To correlate those findings with morphological and chemical changes in the CNS we have investigated GABA immunoreactivity in the cerebral cortex and cerebellum of rats exposed to 3G for 14 days. Tissue was obtained from some rats immediately (within 2 hr) following centrifugation and from others after behavioral recovery (44 days after centrifugation). Vibratome sections of perfused brain tissue were processed for staining with anti-GABA serum, and reaction product was visualized by means of the avidin-biotin immunoperoxidase method. In the animals perfused immediately after centrifugation there was a marked decrease in immunoreactive GABAergic cells and terminals in the somatosensory cortex at the level of the hindlimb representation. GABA-IR terminals surrounding somas of large pyramidal cells in Layer V were decreased in the experimental group as compared with controls. In the cerebellum local circuit GABAergic cells ("basket" cells) and terminals in the 3G animals were decreased at the level of the molecular layer. GABA-IR terminals surrounding the Purkinje cells were also decreased. These results indicate that altered gravity can affect the local circuit GABA neurons that modulate the discharge of "output" neurons (pyramidal and Purkinje cells) of "command centers" (cerebral and cerebellar cortex). We also found that neuroglial cells (astrocytes) of the cerebellar gray (von Bergmann cells) and white matter of the 3G animals exhibited hypertrophy, suggesting that astrocytes may participate in the observed phenomena. After behavioral recovery, these alterations were still detectable, suggesting that chronic altered gravity exposure may result in long-term changes in the CNS. Supported by NASA: Task #199-16-12-01, NCC2-449 and National Research Council Associateship.

514.10

SEROTONIN 5-HT$_2$ RECEPTOR-MEDIATED MODULATION OF GABA NEURONS IN THE RAT PREFRONTAL CORTEX. W. M. Abi-Saab, A. C. Grobin, J. F. Cubells*, and A. Y. Deutch. Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06511, and Department of Psychiatry, VAMC, West Haven, CT 06516.

Anatomical studies have shown that serotonin (5-HT) axons synapse with cortical GABA neurons in the rat. In the piriform cortex, *in vitro* electrophysiological studies indicate that 5-HT depolarizes GABA neurons via a 5-HT$_{2a}$ receptor. In order to investigate *in vivo* the serotonergic regulation of the GABA neurons in the prefrontal cortex (PFC), we used microdialysis in awake, freely-moving rats. Local administration of 5-HT through the dialysis probe increased extracellular GABA levels in the PFC. Similarly, the 5-HT$_{2a/2c}$ agonist DOI increased in a dose-related manner extracellular GABA levels in the PFC. In order to determine if GABA neurons are targets for DOI, we examined the ability of DOI to induce c-*fos* expression in cortical interneurons. Preliminary data suggest that DOI induces Fos-like immunoreactivity in parvalbumin-containing interneurons in the PFC, but calbindin-containing interneurons were not affected. Taken together, these data suggest that 5-HT increases extracellular GABA levels through a 5-HT$_2$ receptor-mediated event, and may selectively target certain parvalbumin-containing GABA neurons in the PFC.

This work is supported by the VA Fellowship in Neuroscience, MH 45124, National Centers for Schizophrenia and PTSD at the VAMC, West Haven, CT, and the National Parkinson Foundation.

PEPTIDE RECEPTOR STRUCTURE AND FUNCTION I

515.1

MOLECULAR CLONING AND THE CHARACTERIZATION OF THE RAT NEUROTENSIN RECEPTOR GENE. H. Maeno[1]*, K. Tanaka[1], H. Kiyama[2] and K. Wada[1] . 1:Dept. Degen. Neurological Dis. Nat'l. Inst. Neurosci NCNP. Tokyo 187, Japan. 2:Dept. Neuroanatomy, Biochem.. Res. Center Osaka University Medical School., Osaka 565, Japan

. The expression of the neurotensin receptor is spatiotemporally controlled. In order to understand the mechanisms controlling the expression NTR gene, it is important to characterize its promoter. The 5'-terminal region of the rat neurotensin receptor gene was isolated and characterized. Genomic Southern analysis revealed that the NTR gene occurs as a single copy in the rat haploid genome. Primer extension showed that transcription is initiated from -236, -237, -319, and -374 upstream to an adenosine residue on the initiating methionine codon of the rat neurotensin receptor gene. The upstream putative promoter region did not contain canonical TATA or CAAT boxes but has a consensus sequence for the transcription factor Sp1. This promoter is embedded in a large G+C-rich domain with characteristics of a CpG island. Transfection experiments using neurotensin receptor-luciferase fusion genes demonstrated that the 5'-flanking sequence functions as a strong promoter in the NG-108-15 cell. Deletion analysis suggested the presence of a core promoter (-470 to -662) that drives the basal expression of the neurotensin receptor gene.

This work was supported in part by grants from the Ministry of Health and Welfare of Japan, the Ministry of Education and Culture of Japan, the Science and Technology Agency of Japan.

515.2

DIFFERENTIAL MOLECULAR REGULATION OF NEUROTENSIN RECEPTORS BY ENDOGENOUS NEUROTENSIN. W. Rostène, M. Azzi, H. Boudin, D. Gully§, D. Pélaprat* and A. Bérod. INSERM U339, Hôpital Saint-Antoine, 184 rue du Faubourg St Antoine, 75012 Paris, § Sanofi Recherche,31036 Toulouse, France.

Neurotensin (NT), a 13 amino acid peptide heterogeneously distributed in the central nervous system of the rat brain, has been described to act as a neurotransmitter or neuromodulator.

In the present study, we examined the role of endogenous NT in the regulation of its central receptors following chronic blockade of NTergic transmission with a new selective non-peptide NT receptor antagonist, SR 48692. Administration of SR 48692 for 5 or 15 days with 1 mg/kg i.p. enhanced the expression of NT receptor mRNA as shown by quantitative in situ hybridization in the ventral mesencephalon, respectively by 40% and 100%. After a 15-day treatment, the increase of the signal was extended to numerous areas of the brain, such as the anterior cingulate, perirhinal and retrosplenial cortices, the suprachiasmatic nucleus and the posterior cortical nucleus of the amygdaloid complex. Moreover, this treatment revealed the expression of NT receptor mRNA in several nuclei of the diencephalon where it could not be detected in basal conditions. Immunoblot analysis with a specific antibody directed against the rat cloned NT receptor showed an increase in NT receptor protein in SR 48692-treated rats for 5 or 15 days, correlating well with the increase in NT receptor mRNA levels. However, if a 5-day treatment with SR 48692 enhanced the number of NT binding sites determined on brain membrane homogenates or in vitro autoradiography, the same treatment for 15 days did not alter the number and the affinity constant of the NT binding site.

These results demonstrate that chronic treatment with a specific NT antagonist induces an up-regulation of NT receptors at the level of its mRNA and protein. Furthermore, the lack of changes in the number of NT binding sites after a long blockade of NTergic transmission suggests an adaptative regulation of the receptor at the level of its binding sites.

515.3

***In vivo* differential regulation of neurotensin receptor mRNA expression by SR48692 in rat brain and peripheral tissues.**

Milagros Méndez, Frédérique Souazé, § Makoto Nagano, § Paul A. Kelly, William Rostène* and Patricia Forgez.
INSERM U339 Hop. St-Antoine 75012 Paris France § INSERM U344, Faculté de Médecine Necker, 75015 Paris, France

Neurotensin (NT) is widely distributed in the central nervous system and peripheral tissues and its actions are mediated by a specific G protein-coupled receptor, the neurotensin receptor (NTR). In this study, we have measured the levels of NTR gene expression by quantitative RT-PCR. In the brain, the highest quantities of NTR mRNA were found in ventral mesencephalon and the hypothalamus, followed by intermediate levels in the prefrontal cortex and striatum, and scant levels in the cerebellum. In peripheral tissues, the highest levels of NTR mRNA were detected in the colon, followed by the liver, and then the duodenum and pancreas.

A specific and potent non-peptide NTR antagonist, SR48692, was used to block *in vivo* neurotensin transmission in order to evaluate the effect on NTR gene regulation. Chronic SR48692 administration (1 mg/kg, ip for 15 days) induced an increase of NTR mRNA (50 to 80 %) in the prefrontal cortex, ventral mesencephalon, hypothalamus, and colon, suggesting that endogenous NT exerts a tonic inhibitory control on NTR gene expression. In contrast, substantial reductions of NTR mRNA were observed in the duodenum and pancreas. We suggest that the differential NTR expression is the result of distinct promoters or, alternatively , different NTR subtypes.

515.4

PHARMACOLOGICAL ACTIVITIES OF SR 142948A, A NEW POTENT AND SELECTIVE NEUROTENSIN RECEPTOR ANTAGONIST. D. Gully, J. Maruani, B. Labeeuw, F. Donat, A. Bachy, R. Steinberg, J.P. Chambon*, P. Kitabgi, J.P. Maffrand and P. Soubrié. Sanofi Recherche, 195, route d' Espagne, 31036 Toulouse, France.

SR142948A 2-{(5-(2,6-dimethoxyphenyl)-1-(4-(N-(3-dimethylaminopropyl)-N-methylcarbamoyl)-2-isopropyl-phenyl)-1H-pyrazole-3-carbonyl)amino}-adamantane-2-carboxylic acid, hydrochloride, a new extremely potent and selective neurotensin (NT) receptor antagonist was characterized in comparison to SR 48692. SR 142948A displays subnanomolar affinities for the human NT receptor (IC_{50}=0.3 nM) and equally recognizes the high- and the low-affinity (levocabastine-sensitive) binding sites described in murine species. *In-vitro*, it antagonizes classical NT effects, i.e. NT-stimulated IP1 formation in HT29 cells (IC_{50}= 3.9 nM) or NT-induced [Ca^{2+}]i mobilization in CHO cells transfected with the receptor cloned from HT29 cells. It dose-dependently (from 0.037 to 640 µg/kg, p.o.) inhibits circling behaviour induced by unilateral intrastriatal injection of NT in mice with the biphasic profile described for SR 48692. At 100 µg/kg (i.p.) it completely antagonizes NT-evoked ACh release measured by microdialysis in the rat striatum. In contrast to SR 48692, SR 142948A (p.o.) blocks both hypothermia (ID_{50} = 1.7 mg/kg in rat, 3.6 mg/kg in mice) and analgesia (1.4 mg/kg in mice) induced by i.c.v. injection of NT. In summary, the novel and extremely potent NT receptor antagonist SR 142948A retains the properties of the lead compound (no intrinsic agonist activity, oral bioavailability, long duration of action and good brain access), reveals a wider spectrum of activity than SR 48992 likely through inhibition of NT receptor subtypes and thus represent an additional tool for exploring further the therapeutical uses of this new class of compounds.

515.5

THE THIRD INTRACELLULAR LOOP OF THE HIGH AFFINITY NEUROTENSIN RECEPTOR IS IMPLICATED IN THE ADDRESSING OF THE RECEPTOR TO THE PLASMA MEMBRANE. Boudin H., Botto J.M., Chabry J., Rostene W., Vincent J.P., Beaudet A.* and Mazella J. Montreal Neurological Institute, Montreal, H3A 2B4, Canada; CNRS, 06560 Valbonne, France; INSERM U339, Hopital St Antoine, 75012 Paris, France.

Most of the physiological effects of neurotensin (NT) in mammalian brain are exerted through interaction with a high affinity receptor which was cloned (Tanaka et al., 1990) and shown to correspond to a seven trans-membrane domain, G-protein-coupled receptor. In order to identify amino acid sequences implicated in the addressing of this receptor to the plasma membrane, COS-7 cells were transfected with cDNA encoding deletion mutants of the receptor. Deletion of a large segment of the third intracellular loop (Δ273-306) abolished specific binding of ^{125}I-NT to transfected whole cells but not to membrane homogenates. In the former, however, confocal immunohistochemical localization of the receptor using an antibody directed against a sequence of the second extracellular loop of the receptor revealed a pile up of the immunoreactivity within intracellular organelles reminiscent of Gogi saccules. COS-7 cells incubated with an antibody directed against a sequence of the third intracellular loop of the receptor were immunonegative, confirming the efficiency of the deletion. Shorter deletions of the third intracellular loop, including deletion of segments Δ273–282, Δ287–296, Δ284–299 variably reduced, but never completely abolished ^{125}I-NT binding. We conclude that the third intracellular loop of the NT receptor plays a critical role in the addressing of the receptor from the endoplasmic reticulum/Golgi apparatus to the plasma membrane. The identity of the amino acids implicated in this function and their relationship to those involved in the binding of the receptor to G-protein remains to be established. Supported by CNRS, INSERM, MRC and NATO grants.

515.6

NEUROTENSIN INTERNALIZES VIA THE LOW AFFINITY, LEVOCABASTINE-SENSITIVE RECEPTOR IN TRANSFECTED COS-7 CELLS, BUT NOT IN GLIAL CELLS. D. Nouel*, J.M. Botto[1], P. Sarret[1], J.P. Vincent[1], J. Mazella[1] and A. Beaudet. Montreal Neurol. Inst., Montreal, Qué., Canada, H3A 2B4, [1] C.N.R.S., 06560 Valbonne, France.

We previously demonstrated the presence of specific, levocabastine-sensitive neurotensin (NT) binding sites in astrocytes in culture (Nouel et al., '94). The aim of the present study was to determine wether these NT binding sites correspond to the low affinity NT receptor (NTRL) recently cloned by Mazella et al. ('96). RT-PCR made on RNA extracted from astrocytes prepared from cerebral cortex of newborn rats demonstrated the expression of NTRL by these cultured glial cells. To compare the pharmalogical properties of the native glial NTRL with that of NTRL expressed in transfected COS-7 cells, both cell types were labeled for 45 min, at either 4° or 37°C, with 10 nM of a BODIPY conjugate of NT (fluo-NT), in the presence or absence of 1μM levocabastine. After labeling, cells were air-dried and examined by confocal microscopy before or after a hypertonic acid wash to strip off surface-bound ligand. Both astrocytes and COS-7 cells exhibited specific, levocabastine-sensitive fluo-NT labeling. This labeling was displaceable by a hundredfold excess of the NT antagonist SR-48692 in transfected, but not in glial cells. At 4°C, the labeling was exclusively membrane-bound in both types of cells. By contrast, at 37°C, the labeling remained strictly extracellular in glial cells but was mainly intracellular in transfected ones. This intracellular labeling was prevented by co-incubation with the endocytosis inhibitor, phenylarsine oxide and was concentrated within small endosome-like particles, suggestive of receptor-mediated internalization. These results demonstrate that the NTRL is expressed by glial cells and that this native form of the receptor has pharmacological properties distinct from that of the transfected receptor. Supported by the MRC of Canada.

515.7

CONFOCAL IMAGING SHOWS THAT NEUROTENSIN (NT) INTERNALIZATION IN CAT STELLATE GANGLION IS DENDRITIC AND INFLUENCED BY TARGET-DERIVED AND TRANSYNAPTIC FACTORS. B. Bachoo, M.P. Faure, C. Polosa* and A. Beaudet. Montreal Neurol. Inst., 3801 University St., Montreal, QC, Canada H3A 2N4.

Preganglionic axons innervating the cat stellate ganglion contain and release neurotensin. Slices of the cat stellate ganglion, incubated with fluorescent NT (fluo-NT) at 37 °C and examined by confocal microscopy, showed selective fluorescent labeling of a sub-population of TH-positive cells retrogradely labeled by WGA applied to the inferior cardiac nerve. Internalization occurred at concentrations as low as 2 nM fluo-NT, was prevented by an excess of native NT (1 uM), did not occur at 4 °C and was not blocked by levocabastine, indicating that it was mediated through the high affinity NT receptor. For each ganglion cell, internalization was initiated at the level of a single dendrite, within small vesicular organelles. After 10 min, some of the internalized vesicles had moved to the perinuclear region of the perikaryon. The centripetal movement of the labeled vesicles was microtubule dependent since it was blocked by nocodazole. Double labeling with NT-antibodies showed the neurons internalizing NT to be surrounded by a network of NT-IR varicosities. Incubation of ganglion slices in 25 mM K+, known to cause release of NT from preganglionic axon terminals, prevented internalization of fluo-NT, presumably through competition by endogenous NT for the binding sites. Fluo-NT internalization was markedly reduced in ganglion cells which had either been denervated or axotomized 7 days previously. The data suggest that following interaction with its receptor, the NT-receptor complex is rapidly internalized by an endocytic process. The effects of chronic denervation or axotomy suggest that the NT receptor distribution, the uptake process, or both are controlled by a presynaptic and a retrograde, target derived influence. Supported by the MRCC.

515.8

INTERNALIZATION OF FOUR OF THE FIVE SOMATOSTATIN RECEPTOR SUBTYPES. A. Roth, H.-J. Kreienkamp, R. Nehring, W. Meyerhof, D. Richter* Institut für Zellbiochemie und klinische Neurobiologie, Universitäts-Krankenhaus Eppendorf, Universität Hamburg, Martinistr. 52, 20246 Hamburg

The neuropeptide somatostatin exists in two isoforms, SST-14 and SST-28. So far, five different G protein-coupled somatostatin receptor subtypes (SSTR1-5) have been identified in mammals. Although the physiological functions of the five SSTRs have not been resolved yet, the receptor subtypes differ in their pharmacological and functional properties when expressed in mammalian cell lines. Unlike other G protein-coupled receptors, little is known about desensitization and internalization of SSTRs. We investigated internalization of the five rat somatostatin receptor subtypes as well as their recycling in stably transfected HEK cells by biochemical ligand binding assays and confocal microscopy using the labeled ligands, ^{125}I-Tyr^{11}-SST-14 or rho-Ala^{1}-SST-14, and antibodies raised against native or epitope tagged SSTRs, respectively. In contrast to SSTR1-3 which are rapidly internalized in the presence of SST-14 and SST-28, SSTR5 only internalized upon SST-28 stimulation. Phenylarsine oxide and hyperosmolar sucrose blocked internalization. In the case of SSTR4 no receptor endocytosis was observed regardless of the ligand used. In colocalization experiments SSTR3 and transferrin were found in the same intracellular compartment. After removal of agonists receptors reappeared on the cell surface whereas the labeled ligand rho-Ala^{1}-SST-14 remained inside the cell. Reappearance of the receptors was blocked by brefeldin A but not by cycloheximide, indicating that this process was due to receptor recycling and not to *de novo* synthesis. In this study we showed for the first time that somatostatin receptors internalize and recycle in an agonist-dependent manner. These data may also be of clinical importance as SSTR scintigraphy is used for detecting SSTR positive tumors prior to radioguided surgery.

Supported by Deutsche Forschungsgemeinschaft (SFB 232/B4)

515.9

THE LINEAR PEPTIDE BIM 23055 IS AN ANTAGONIST AT SOMATOSTATIN RECEPTOR RAT *SST5*. G. Liapakis*, A.- M. O' Carroll† and T. Reisine. Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, 36th Str and Hamilton Walk, Philadelphia PA 19104 and † Lab. of Cell Biology, Building 36, National Institute of Mental Health, NIH, Bethesda MD 20892.

Somatostatin (SRIF) induces its multiple physiological actions via five receptor subtypes. While a large number of SRIF agonists are available, there are no antagonists. The linear peptide BIM 23055 binds to the cloned rat *sst5* (*rsst5*) with an affinity of 3.3 nM (Raynor et al.,1993, Mol. Pharmacol. 43, 838 - 844). It has limited ability (15±5% inhibition, n=10) to inhibit forskolin stimulated cAMP accumulation in CHO cells expressing *rsst5*. In contrast, SRIF and the linear peptide BIM 23052, full agonists at *rsst5*, inhibit cAMP accumulation by 57±3% (n=7) and 70±2% (n=8), respectively. BIM 23055 competitively blocks the inhibition of cAMP accumulation by BIM 23052, showing it is an antagonist. It does not alter the effects of SRIF, suggesting that SRIF and BIM 23052 may interact differently with *rsst5*, and BIM 23055 competes for the same site as BIM 23052. The capability of BIM 23055 to block the actions of BIM 23052, a selective *rsst5*, agonist, indicates that BIM 23055 or its analogs could be useful *rsst5*, antagonists.

515.10

An Interhelical Hydrogen Bond Network In G Protein Coupled Receptors May Stabilize Conformations For Both Intracellular Transport And Multiple Functional Pathways. R.M. Brodbeck, B.S. Sachais, V.V. Karpitskiy, and J.E. Krause*. Washington University School of Medicine St. Louis, MO 63110

The substance P (SP) or neurokinin-1 type (NK-1) tachykinin receptor is a member of the G protein coupled receptor superfamily. G protein coupled receptors are predicted to have seven transmembrane helical domains, and are functionally expressed at the cell surface where they bind to multiple ligands and couple to one or more second messenger pathways. Molecular modeling of the rNK-1 receptor suggests an interhelical hydrogen bond network in the rNK-1 receptor involving residues located in the putative transmembrane domains, several of which are conserved within the GPCR family. Such a network may play a major role in stabilizing the receptor in multiple functional conformations. Site-directed mutagenesis was utilized to construct point mutants of the rNK-1 receptor which remove the potential for selected putative hydrogen bonds within the predicted network. Binding data from clonally isolated CHO cells stably transfected with the receptor constructs indicate that several of these NK-1 receptor mutants fail to bind ^{125}I-Y^{-1}-SP at levels comparable to that of wildtype receptor (E78Q, E78Q-N301D, T124D, Y305A). Further studies examining phospholipid turnover, cAMP production, and arachidonic acid release from CHO cell lines expressing mutant receptors which have both a subnanomolar affinity for SP and a B_{max} comparable to wildtype, suggest that the configuration of the putative hydrogen bonding network may selectively stabilize receptor conformations which enhance or decrease coupling to specific response pathways. Further analysis of the NK-1 receptor bearing CHO cell lines by immunofluorescence indicates that while the receptor mutants which efficiently bind SP are clearly expressed at the cell surface, variants which are deficient in SP binding appear to be intracellularly localized. These data indicate that the hydrogen bond network predicted for the NK-1 receptor and other GPCRs may have multiple structural roles. Hydrogen bonds may stabilize specific receptor conformations for the efficient folding, processing and transport to the cell surface, high affinity ligand binding, and coupling to multiple second messenger pathways. (Supported by NIH NS21937 & NS07129)

515.11

STRUCTURALLY ANALOGOUS DOMAINS OF NK1 AND NK2 RECEPTORS ARE FUNCTIONALLY DISTINCT IN INOSITOL PHOSPHATE METABOLISM, ARACHIDONIC ACID RELEASE, AND cAMP PRODUCTION. J.M. Nave Mentzer*, S.K. Schmidt, J.R. de Wet[1] and J.E. Krause. Dept. of Anatomy and Neurobiology, Washington U. School of Medicine, St. Louis, MO 63110; [1] Dept. Molecular Sciences, Central Res. Division, Pfizer Inc., Groton, CT 06340

Tachykinin receptors have been shown to mediate PI hydrolysis, arachidonic acid release and cAMP production. Activation of the AA cascade may be the mechanism whereby tachykinins mediate inflammatory and nociceptive responses in vivo. The goal of this study was to ascertain if structurally analogous domains of rat NK1 and NK2 receptors are functionally distinct with regard to mechanisms mediating activation of these three effector molecule systems. We used stably transfected CHO cell lines expressing receptors that had either their putative third cytoplasmic loop (C3) or carboxy tail (CT) replaced with the equivalent domain of the other receptor (Blount and Krause, 1993). Agonist binding affinity for truncated and chimeric receptors was similar to that for wildtype receptors. Replacement of the C3 domain of the NK1 receptor with the analogous region of the NK2 receptor (NK1/NK2.C3) resulted in a receptor which possessed a weak IPs response and was unable to produce a cAMP or AA response. By contrast, the complimentary chimera (NK2/NK1.C3) was hyperresponsive (compared to wildtype NK2 receptors) for all three responses tested. The truncated NK1 receptor (NK1Δ325) was hyperresponsive for IPs production and AA release, but produced a normal cAMP response. However, the truncated NK2 receptor (NK2Δ347) exhibited weak AA, IPs and cAMP responses. These data indicate that structurally analogous domains of NK1 and NK2 receptors are functionally distinct in terms of second messenger generation. (Supported in part by NIH grant NS21937 and Pfizer Inc.)

515.12

CHARACTERIZATION OF THE BINDING AND ACTIVITY OF A HIGH AFFINITY, PSEUDOIRREVERSIBLE MORPHOLINO NEUROKININ-1 RECEPTOR ANTAGONIST S. J. Sadowski*, L.-L. Shiao, E. Ber, S. G. Mills, J.J. Hale, M. MacCoss, T. M. Fong and M. A. Cascieri, Merck Research Laboratories, Rahway, NJ 07065

L-742,694 is a selective morpholino NK-1 antagonist that inhibits the binding of ^{125}I-substance P to the human NK-1 receptor with a K_d = 37 pM. Increasing concentrations of L-742,694 added to cells 15 minutes prior to agonist progressively increase the apparent EC_{50} of substance P for inducing the synthesis of inositol phosphate in human NK-1/CHO cells and decrease the maximal level of stimulation observed. In contrast, addition of substance P and L-742,694 to the cells at the same time results in an increase in the EC_{50} for substance P with no decrease in the maximal level of stimulation. The compound also decreases the apparent number of binding sites for ^{125}I-substance P observed by Scatchard analysis. The observation that altering the order of addition of antagonist and substance P attenuates the effect of the antagonist on the maximal activation suggests that L-742,694 is a competitive antagonist that can behave as a pseudo-irreversible antagonist under some experimental conditions. L-742,694 has reduced affinity for NK-1 receptors in which alanine has been substituted for Gln^{165}, His^{197} or His^{265} in transmembrane helices 4, 5 and 6, respectively. These three residues have previously been shown to be present in the binding site of NK-1 antagonists of several structural classes. In addition, L-742,694 inhibits binding of the quinuclidine antagonist, [^{125}I] L-703,606 with the same affinity as it inhibits binding of ^{125}I-substance P. These data indicate that L-742,694 binds to the same site within the transmembrane domain of the receptor as previously described competitive antagonists.

Merck Research Laboratories

515.13

Neurokinin-3 receptors in rat dopamine neurons: evidence for somatodendritic and terminal localization and heterogeneity of expression. C.J. Whitty* and M.J. Bannon. Depts. of Psychiatry and Behavioral Neurosciences and Pharmacology, Wayne State University, Detroit, MI 48201.

The rat midbrain contains the highest amount of substance P and related neurokinins in the brain and application of neurokinins activates midbrain dopamine (DA) cells. We have previously localized neurokinin-3 (NK3) (but not NK1 nor NK2) receptor mRNA to DA cells of the rat midbrain. In order to better understand the role that neurokinins may play in modulating various DA systems and their targets, NK3 receptor expression in the rat midbrain was examined in greater detail. Significant heterogeneity of NK3 receptor mRNA abundance was seen within and between the DA cell-containing nuclei of the rat midbrain. The ventral tegmental area contained the highest amount of NK3 receptor mRNA, with almost every DA cell NK3 receptor positive. Within the substantia nigra pars compacta, 50% of the DA cells contained little or no NK3 receptor mRNA. The majority of the DA cells in the substantia nigra pars reticulata and pars lateralis were NK3 receptor mRNA negative, although non-DA cells in the most lateral aspect of the pars lateralis were labeled. Since DA neurons of the VTA and dorsal tier of the pars compacta project to the striatal matrix compartment, while DA neurons of the pars reticulata, pars lateralis, and ventral tier of the pars compacta project to the striatal patch compartment, NK3 receptor expression may provide a novel marker for DA cells projecting to striatal patch vs matrix compartments. Neurokinin-3 receptors on DA cell nerve terminals may account for a significant proportion of all striatal NK3 receptors, as evidenced by a significant decrease of striatal NK3 ligand binding after DA cell lesions. Neurokinins may therefore play an important role in basal ganglia function through effects on DA cells at somatodendritic and terminal sites of action. Supported by NS34935.

515.14

Effect of Neurokinin-1 Receptor Antagonists on Stress-induced c-fos Immunoreactivity in the Rat Locus Coeruleus. M.K. Hahn* and M.J. Bannon. Departments of Psychiatry and Behavioral Neuroscience, and Pharmacology, Cellular and Clinical Neurobiology Program, Wayne State University, Detroit, MI 48201

The locus coeruleus (LC) noradrenergic cell group of the brain is implicated in animal models of stress and anxiety and in human anxiety disorders. The neurokinin substance P is localized to the rat LC and has excitatory effects on LC neuronal firing rate. The rat LC possesses neurokinin-1 (NK-1) receptor mRNA and binding sites. It is known that various stimuli that increase LC excitability result in expression of the immediate early gene c-fos. We explored whether stress-induced increases in LC c-fos levels could be attenuated by antagonism of NK-1 receptors. Rats received an i.p. injection of the selective NK-1 receptor antagonist RP 67580 (10 mg/kg, Rhône-Poulenc Rorer) or vehicle. Twenty min following the injection some rats were placed in a restraining tube for 1 hr. Rats were anesthetized and perfused 2 hr after cessation of restraint, or 3 hr 20 min after injection for non-restrained animals. Forty μm sections at the level of the LC were processed by free-floating immunocytochemistry using a polyclonal antibody to c-fos and LC cells expressing the brown-black 3,3'-Diaminobenzidine (DAB) reaction product were counted. Control rats that received neither injection nor restraint showed no c-fos-positive cells in the LC (0±0, n=3). Restraint resulted in a significant increase in the number of c-fos-positive LC cells compared to control (45±10, n=6; $p<0.05$). Rats injected with vehicle prior to restraint were not different from restraint alone (66±11, n=3). In contrast, the number of c-fos-positive LC cells in rats injected with RP 67580 prior to restraint was significantly increased compared to restraint alone (91±13, n=6; $p<0.05$). Injection of RP 67580 alone resulted in a small number of c-fos-positive LC cells (14±4, n=3). In a separate experiment, another selective NK-1 receptor antagonist, SR 140333, (0.3-1.0 mg/kg, Sanofi Recherche) was also unable to block a restraint-induced LC c-fos activation. Thus, NK-1 receptor antagonists do not attenuate stress-induced activation of the LC. These results may have implications for the possible use of NK-1 antagonists as anxiolytic agents. Supported by NIH NS 34935.

515.15

ADRENOMEDULLIN RECEPTORS ARE PRESENT ON C6 GLIOMA CELLS. T.W. Moody*, M.J. Miller, A. Martinez, E. Unsworth and F. Cuttitta. Biomarkers & Prevention Research Branch, NCI, 9610 Medical Ctr. Dr., Rockville, MD 20850

Adrenomedullin (AM) is a 52 amino acid peptide with structural homology to calcitonin-gene related peptide (cGRP). The receptor for AM is a G-protein coupled receptor with 7 transmembrane domains (Kapas et al., JBC 270:25344 (1995)). Previously, we found the AM is biologically active in MCF-7 breast cancer cells and here the effects of AM on C6 rat glioma cells were investigated. Using a cDNA probe for the AM receptor, a major 1.5 kb band was observed by Northern analysis. Using primers specific for the AM receptor, a 471 bp band was observed by RT-PCR. ^{125}I-AM bound with high affinity (Kd = 3 nM) to a single class of sites (Bmax = 5,000/cell). Specific ^{125}I-AM binding was inhibited with high affinity by AM, cGRP, cGRP(8-37) and amylin amide (IC_{50} values of 3, 10, 30 and 100 nM respectively) but not by proAM N-20 peptide 1(PAMP) or AM(22-52). AM elevated the cAMP and the increase in cAMP caused by AM was reversed by monoclonal antibody (MoAb-G6). These data suggest that AM receptors are biologically active on glioma cells.

515.16

CENTRAL AND PERIPHERAL ACTIONS OF THE NEW BOMBESIN-LIKE PEPTIDES, PHE^{13} BOMBESIN AND SAP-BOMBESIN: EVIDENCE THAT A NOVEL BOMBESIN RECEPTOR SUBTYPE MAY MEDIATE THE HYPOTHERMIC EFFECTS OF BOMBESIN. E.R. Spindel*[1], A. Cowan[2], V. Martinez[3], Y. Tache[3] and B. Barry[1]. [1]Div. Neuroscience, Oregon Rgl. Primate Research Ctr., Beaverton OR 97006; [2]Dept of Pharmacology, Temple University, Phil., PA 19140 and [3]Ctr. for Ulcer Research, UCLA, LA, CA 90073.

Recently our laboratory reported that bombesin occurs in multiple forms. For example, the frog *Bombina* orientalis expresses three bombesin-like peptides: Leu^{13} bombesin (prototypic bombesin), Phe^{13} bombesin and SAP bombesin (J. Biol Chem 271:7731,1996). The receptor for Phe^{13} bombesin has been characterized and shown to constitute a distinct subclass of bombesin receptor (BB4). To characterize the biological effects of these peptides, they were administered to rats intravenously and intraventricularly and the effects on temperature regulation, grooming and pentagastrin-induced gastric acid secretion was measured. Phe^{13} bombesin has roughly similar potencies for the GRP and NMB receptors as does Leu^{13} bombesin. Consistent with this, Phe^{13} bombesin had similar effects on grooming and acid secretion as did Leu^{13} bombesin. SAP bombesin which has 10-fold weaker affinities for the GRP and NMB receptors was proportionately less potent. Strikingly, however, despite similar affinities as Leu^{13} bombesin for the known mammalian bombesin receptors, doses of Phe^{13} bombesin ten-fold higher than effective doses of Leu^{13} bombesin had no effect on hypothermia. Therefore the failure of Phe^{13} bombesin to induce hypothermia despite its high affinity for the GRP and NMB receptors suggests that an as yet undescribed bombesin receptor subtype may mediate the effects of bombesin-like peptides on temperature regulation. This *research supported by NIH grants CA39237, RR00163 and by Biomeasure Inc.*

515.17

BIM 26292 IS A POTENT AGONIST FOR THE BOMBESIN BRS-3 RECEPTOR. R. P. Searles*[1], S. Kim[2], J.E. Taylor[2], E.R. Spindel[1]. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton OR 97006 and [2]Biomeasure Inc., Milford MA 01757.

Four subtypes of bombesin receptors have been characterized to date. The BB1 receptor has highest affinity for neuromedin B, the BB2 receptor has highest affinity for GRP and the BB4 receptor has highest affinity for the amphibian peptide Phe^{13} bombesin. To date however no natural or synthetic high affinity ligands for the BRS-3 (BB3) receptor have been described. As part of our ongoing studies to discover the endogenous ligand of the BRS-3 receptor we have established an assay for BRS-3 ligand activity and screened the known natural bombesin-like peptides and a battery of synthetic agonists. To measure BRS-3 activity, *Xenopus* oocytes were injected with RNA encoding the human BRS-3 receptor (cDNA kindly provided by J. Battey) and then injected with the calcium photoprotein aequorin. 24 hours later oocytes were challenged with ligands. Ligands interacting with the BRS-3 receptor cause an increase in intracellular calcium that can then be measured luminometrically. The most potent natural ligand for the BRS-3 receptor was ranatensin. Of synthetic compounds screened, BIM 26292 (Phe-Gln-Trp-Ala-Val-betaAla-His-Phe-NorLeu amide) was most potent. A response to as little as 5 nM BIM26292 could be detected. Substitution of Leu for the penultimate Phe caused a decrease in potency. The importance of this Phe combined with the potency of ranatensin suggests the natural BRS-3 ligand will also contain a penultimate Phe. The identification of an agonist will now allow tests to determine biological functions of the BRS-3 receptor. *This research supported by NIH grants HD 30272, CA39237, RR00163 and by Biomeasure Inc.*

PEPTIDE RECEPTOR STRUCTURE AND FUNCTION II

516.1

CYTOCHEMICAL IDENTIFICATION AND CLONING OF A G-PROTEIN COUPLED GROWTH HORMONE SECRETAGOGUE RECEPTOR. D. Hreniuk, A. Howard, C. Rosenblum, O. Palyha, C. Diaz, D. Cully, P. Paress, K. McKee, M. Dashkevicz, J. Arena, K. Liu, J. Schaeffer, R. Nargund, R. Smith, L.H.T. Van der Ploeg and S. Feighner*. Merck Research Laboratories, Rahway, NJ, 07065

Growth hormone (GH) is secreted in a pulsatile manner by somatotroph cells of the anterior pituitary, under the control of growth hormone releasing hormone and somatostatin. A class of small synthetic molecules (i.e., GHRP-6 and non-peptidyl growth hormone secretagogues (GHSs), exemplified by MK-0677) can also mediate GH release. To understand the mode of action of these GHSs, we characterized their receptor by whole-cell binding in rat primary pituitary cell cultures and by cloning of the GHS-receptor (GHS-R). Using biotinylated GHSs and avidin conjugated fluorophores, we were able to show that a subpopulation of GH-containing cells also express the GHS-R. Co-localization of GH and GHS binding sites with dual fluorophore labeling indicated that GHS binding was restricted to a subpopulation of somatotrophs: 33% of rat primary pituitary cells stained positive for GH, while only 50% of these immunocytochemically identified somatotrophs exhibited GHS binding. GHS binding could be displaced by biologically active GHSs, but not with an inactive enantiomere or with gonadotropin releasing hormone.

GHS-R cloning was accomplished using a *Xenopus* oocyte expression system. We injected *Xenopus* oocytes with porcine pituitary mRNA. To enhance the sensitivity of the assay, we co-injected aequorin cRNA rather than aequorin protein, and G-protein subunit α11 cRNA leading to the reproducible identification of MK-0677 mediated Ca^{2+} release. We now report the cloning of a rare mRNA from human and swine pituitary which encodes the GHS-R. The cDNA has a predicted open reading frame of 366 amino acids, with a transmembrane topology typified by the G-protein coupled receptor family.

Merck & Co., Inc.

516.2

NON-RADIOACTIVE, PHARMACOLOGICAL BINDING STUDIES USING FLUORESCENT PEPTIDES SK Lau, C Desjardins*, A Gagné, M-P Faure Advanced Bioconcept Inc. 1440 Ste-Catherine Ouest, Suite 424, Montreal, QC H3G 1R8, Canada.

Several fluorescent peptides with a high degree of retention of biological activity have recently been described (e.g Faure et al., 1994). These fluorescent peptides have been shown to be useful tools in receptor imaging, flow cytometry, as well as in cell sorting. Using distinct fluorescence detection instruments, we have compared binding parameters (Kd and IC_{50}) using fluorescent peptides with those obtained in conventional radioactive binding studies. Fluorescein-labeled neurotensin and Bodipy(504/570)-labeled D-trp^{11} somatostatin (1-14) have previously been shown to displace corresponding radioactive peptide binding with an IC_{50} of 0.67 nM and 1.2 nM, respectively. Pooled COS membranes transfected with either the neurotensin or somatostatin receptor (NTR and SSTR2), were used in distinct instruments. First, fluorescent peptide binding was performed in non-equilibrium conditions in 96-well filter plates (Millipore, MA) and relative fluorescence intensity values were derived from scanned images of the filter plate using a FluoroImager (Molecular Dynamics Inc.,CA). In this system, saturation binding of fluo-neurotensin™ to known concentrations of COS-NTR-transfected membranes yielded Kds which were similar to those obtained using radioactive peptides, (0.35 nM fluo-neurotensin™ versus 0.55 nM for radioactive neurotensin assayed in similar conditions). Second, homogeneous fluorescent peptide binding assays were performed using the Beacon Fluorescence Polarization System (PanVera, WI) and the SLM 48000 (Spectronic Instrument, NY). Preliminary studies indicate several limitations to the use of PanVera's instrument for pharmacological characterization of receptors using fluorescent peptides. However, initial results using SLM 48000 indicate significant increases in fluorescence polarization (4-5 fold over basal values) upon addition of fluo-somatostatin™ to a solution containing 13.2 fmol of transfected receptor membranes. The change in polarization can be reversed by the addition of 500 fold excess of unlabelled peptide. One of the advantages of using fluorescence polarization instruments is the elimination of the separation step of bound from free ligand. The ability to quantify fluorescent peptide binding using both the FluoImager and fluorescence polarization suggests that either approach may potentially be adapted for high-throughput drug screening. Supported by Advanced Bioconcept Inc.

516.3

***p*-(4-Hydroxybenzoyl)phenylalanine: An Iodinatable Photoreactive Amino Acid Analog:** Application to Substance P Receptor. Carol J. Wilson, S. Shaukat Husain, Evelyn R. Stimson, Lawrence J. Dangott, Keith W. Miller, Frederique Popitz-Bergez*, and John E. Maggio. BCMP Dept.; Dept. of Anesth., MGH; Dept. of Neurobiol.; Dept. of Anesth., BWH; Harvard Med. Sch., Boston, MA 02115.

Benzoylphenylalanine, a photoreactive phenylalanine analog that can be incorporated into a peptide during solid phase synthesis, is a useful probe for investigating the interactions of peptides with their receptors. This probe, however, lacks versatility because it cannot be labeled with radioiodine, requiring radiolabeling of the peptide ligand at a position distal to the photoreactive amino acid. The separation along the primary sequence of the radioisotope and photoaffinity labels limits identification of the photoinsertion site to a peptide fragment rather than a specific amino acid of the receptor protein.

We have now synthesized *p*-(4-hydroxybenzoyl)phenylalanine (HBPA) to overcome these disadvantages. HBPA was incorporated at a discrete site into substance P (replacing Phe^{8}) giving an 11-mer peptide that binds with high (nM) affinity and specificity to the substance P receptor. Radioiodination of the substance P analog resulted in the incorporation of ^{125}I at the photoreactive amino acid residue yielding a photoreactive probe of high (~2000 Ci/mmol) specific activity. Subsequent photolysis of the radiolabeled peptide in the presence of substance P receptor caused covalent attachment of the peptide to the receptor with high photoinsertion yield (~14%); photolabeling was abolished in the presence of excess unlabeled SP or a specific SPR antagonist CP-96,435. The novel amino acid retains *p*-benzoylphenylalanine's high insertion yield and low reactivity with water, but places the radiolabel and the photoactive moieties within the same residue. HBPA is likely to be useful in the elucidation of the interaction of a variety of bioactive peptides with receptors and other macromolecules.

Supported by Public Health Service Grant GM-15904.

516.4

A SPECIFIC ANTIBODY RECOGNIZES RAT PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE RECEPTORS M.Li, T.Kozicz, Y.Shuto, A.Somogyvari-Vigh, S.Vigh, H.Onda#, D.Hurley* and A.Arimura. U.S.-Japan Biomed. Res. Labs., Tulane Univ. Hebert Ctr., Belle Chasse, LA 70037; #Takeda Chem. Ind., Ltd., Tsukuba, Japan.

Pituitary adenylate cyclase activating polypeptide (PACAP) is a new member of the secretin/VIP family of peptides. The specific receptor for PACAP was cloned in rat, human and bovine. PACAP also binds to VIP-1 and VIP-2 receptors, but VIP does not bind to PACAP receptors. Although expression of the transcripts of PACAP receptor gene in tissues was studied using *in situ* hybridization, distribution of receptor proteins needs to be examined to evaluate functional receptors. Thus, we generated rabbit antisera against a 25 residue synthetic peptide corresponding to the C-terminal intracellular domain of the rat PACAP receptor. Membrane and soluble fractions of CHO cells stably transfected with rat PACAP receptor cDNA were examined in Western blot analysis. Three bands were demonstrated in both membrane and soluble fractions from the transfected CHO cells (57 kDa the most prominent band corresponding to the size of cloned rat PACAP receptor, a larger, less intense band and a weak, smaller than 57 kDa band), but no bands were found in the similar preparations from the non-transfected cells. All these bands disappeared or diminished when the antiserum was preabsorbed with the immunogen peptide, suggesting that all of these bands are related to the PACAP receptor protein. The membranes from the transfected CHO cells bound to ^{125}I-PACAP27 and the ligand/protein complex approximated 60 kDa, corresponding to the sum of 57 kDa and molecular size of PACAP27. There were no additional bands. The results indicate that both the larger band, probably the precursor, and the smaller band, degraded product, do not bind the ligand. Unlabeled PACAP27 and PACAP38, but not VIP, displaced the binding, suggesting that the receptors expressed in CHO cells are specific PACAP receptors. Immunohistochemistry with this antiserum showed a distribution of PACAP receptor like-immunoreactivity similar to that of the mRNA in the rat brain. The results indicate that the antibody is sufficiently specific for PACAP receptors and the related proteins, and suitable for immunohistochemical studies. (Supported in part by NIH grant DK09094).

516.5

PHARMACOLOGY OF MELANOCORTIN RECEPTORS: CHARACTERIZATION OF RECEPTOR-LIGAND INTERACTIONS. J. Oosterom, R. A. H. Adan, J. P. H. Burbach, P. R. Bär* and W. H. Gispen. Rudolf Magnus Institute for Neurosciences, Utrecht University, Utrecht, The Netherlands, 3508 TA.

Melanocortins have various physiological roles in the nervous system. The cloning of so far three neural melanocortin (MC) receptor subtypes allows the development of new and selective agonists. The aim of this study is to characterize the molecular interactions between peptide and MC-receptor. We investigated the activity of peptides, analogous to the core sequence of α-MSH, on cloned MC_3, MC_4 and MC_5 receptors as well as on mutated receptors expressed in 293 HEK cells. These data revealed that several residues in the peptide are involved in selective receptor activation. Modifications of the core sequence of α-MSH led to the identification of MC-receptor selective antagonists: [D-Arg^8]ACTH-(4-10), [$Pro^{8,10}$,Gly^9]ACTH-(4-10), [Phe-I^7]ACTH-(4-10) and [Ala^6]ACTH-(4-10). The first three peptides slectively antagonized the MC_4 receptor. In addition, mutation of a histidine residue in transmembrane domain 6 of the MC_4 receptor showed that this residue is important for binding of α-MSH.The *in vitro* analysis of the molecular interaction of MC-receptors and melanocortin peptides will lead to the design of more potent and selective agents. Then, *in vivo* application may lead to a better understanding of the role of MC-receptors in (patho)physiological processes. Source of funding: Netherlands Organisation for Scientific Research (N.W.O.).

516.6

IN VIVO ANTISENSE OLIGONUCLEOTIDES REVEAL THE STRUCTURE/FUNCTION PROPERTIES OF ANGIOTENSIN RECEPTORS CONTROLLING INGESTIVE BEHAVIOR. L.Y. Ma*, S. Chow, R.R. Sakai, J.F. Hines, S. Ho#, P.R. Hartig and S.J. Fluharty. Departments of Animal Biology, Pharmacology and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104 and #DuPont-Merck Pharmaceutical, Wilmington DL 19880.

The behavioral actions of angiotensin II (AngII) are mediated by two distinct classes of receptors referred to as the Type I (AT_1) and Type 2 (AT_2) receptors. AngII receptors (AngII-Rs) have been cloned previously and we have used homology-based cloning strategies to isolate their cDNAs from a neuronal cDNA library. The availability of AT_1 and AT_2 receptor clones affords the opportunity to investigate the molecular mechanisms underlying the behavioral actions of AngII. Previously, we have demonstrated that antisense oligonucleotides block the expression of AngII-R subtypes in rat brain and significantly attenuate thirst mediated by the renin-angiotensin system. In the present study, we utilized the known structural features of AngII-Rs to design highly selective antisense oligonucleotides (ASONs) targeted for specific regions of AngII-R mRNAs. Intracerebroventricular administration of ASONs (18 mer) extending from the ATG initiation region and upstream of the amino terminus inhibited the behavioral actions of AngII and significantly reduced the binding of ^{125}I-AngII to brain membranes. In contrast, ASONs targeted for the carboxy terminus also inhibited AngII mediated ingestive responses but did not decrease ^{125}I-AngII binding presumably because ASONs resulted in the expression of truncated receptor unable to couple to G-proteins. Collectively, these results demonstrate the utility of ASONs for investigating the molecular mechanisms of AngII action in the brain.

NS23986, MH43787 and DK48061

516.7

INTRACEREBRAL ANTISENSE OLIGONUCLEOTIDE REDUCE MINERALOCORTICOID-INDUCED INGESTIVE BEHAVIORS, R.R. Sakai*, L.Y. Ma, P. Itharat and Fluharty, S.J. Departments of Animal Biology, Pharmacology and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

The physiological, endocrine and behavioral actions of mineralocorticoids are mediated by specific receptors (MR), located in peripheral tissues and the brain. In an effort to study the regulation and function of these receptors, we have developed short MR antisense s-oligonucleotides sequences that were 15-18 mer in length and given in a dose ranging from 250 ng - 2 μg/day. Three daily interacerebroventricular (ICV) injections of MR antisense significantly attenuated sodium appetite aroused by daily injections of the mineralocorticoid deoxycorticosterone (DOCA) whereas scrambled, sense sequence antisense oligonucleotide or an antisense sequence directed to the glucocorticoid receptor (GR) were ineffective. These antinariorexigenic effects were dose dependent, reversible and specific to DOCA-induced sodium appetite. That is sodium appetite aroused after adrenalectomy which is thought to be generated by marked elevations in angiotensin II activity was unaffected. Radioligand binding analysis of selected brain regions revealed that ICV antisense oligonucleotide treatments produced decreases in the appropriate receptor targeted. Further, the regional distribution and uptake of injected antisense oligonucleotides was further validated by localization of biotinylated probes and immunohistochemistry. The effects of MR and GR antisense oligonucleotide treatment had no effect on the animals daily food intake or body weight. The effects of antisense oligonucleotide treatment on diurnal glucocorticoid secretion and response to a novel stressor was also examined. The results demonstrate that *in vivo* administration of antisense oligonucleotides attenuate mineralocorticoid receptor expression and function associated with DOCA-induced sodium intake.

DK48061 and MH43787

516.8

VASOPRESSIN-INDUCED MOBILIZATION OF INTRACELLULAR CALCIUM IS LINKED TO ELEVATION OF CYCLIC AMP (cAMP)

S.M. Rezazadeh, A. Dibas, M.W. Martin, A.J. Mia and T. Yorio* Department of Pharmacology, U. of North Texas Health Science Center at Ft Worth, TX 76107.

Two G-protein-coupled receptors for vasopressin have been characterized. The V_1 subtype stimulates phospholipase C whereas the V_2 subtype activates adenylyl cyclase. In this study, the effects of arg-vasopressin (AVP) on intracellular free calcium ($[Ca^{2+}]_i$) and cAMP was investigated in LLC-PK_1 pig kidney epithelial cell line. Using digital imaging fluorescence microscopy of fura-2 loaded cells, we observed that AVP increased $[Ca^{2+}]_i$ in a dose and time dependent manner. Thapsigargin (10 μM) abolished this AVP response by depleting Ca_i pools. Addition of dibutyryl cAMP increased $[Ca^{2+}]_i$ and this prevented the subsequent AVP-induced increase in $[Ca^{2+}]_i$. The AVP-induced increases in $[Ca^{2+}]_i$ may be mediated by cAMP and activation of protein kinase A (PKA), since inclusion of H-7, an inhibitor of PKA, abolished the AVP effect on $[Ca^{2+}]_i$. We further examined the effect of AVP on cAMP accumulation. Using Dowex-alumina column chromatography, ^{3}H-cAMP accumulation was measured after prelabelling cells with ^{3}H-adenine. Cells loaded with ^{3}H-adenine were incubated with respective drugs for 10 min in the presence of the PDE inhibitor RO20-1724. Forskolin (FSK) or AVP elevated cAMP levels in a concentration dependent manner. Addition of both FSK and AVP resulted in synergistic responses. The AVP-induced accumulation of cAMP was reversed by the V_2 selective antagonist, [$d(CH_2)_5$, D-Ile^2-Ile^4, Arg]-vasopressin (10 μM). The results suggest that AVP-induced increases in $[Ca^{2+}]_i$ in LLC-PK_1 cells are predominantly mediated by cAMP/PKA, and involve the release of Ca_i from a thapsigargin-sensitive pool. (Supported by DAMD 17-95-5086 and UNTHSC Grants).

516.9

DESIGN OF POTENT AND SELECTIVE DYNORPHIN A-(1-9) ANALOGUES DEVOID OF SUPRASPINAL MOTOR EFFECTS IN MICE.

Simon Lemaire*[1], Hoang-Thanh Le[2], Robert Michelot[2], Michel Dumont[1], Vijay Kumar Shukla[1], Michel Mayer[2] and Kim Phi-Phung Nguyen[2]. [1]Department of Pharmacology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H- 8M5 and [2]Institut de chimie des substances naturelles, Centre national de la recherche scientifique, Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France.

Four analogues of dynorphin (Dyn) A-(1-9) incorporating D-Leu in position 8 alone or in combination with the nonhydrolysable psi[CS-NH] thiopeptide bond surrogate between positions 6 and 7 were tested in vitro for their ability to compete with the binding of selective κ, μ and δ opioid ligands using membrane preparations of guinea pig cerebellum (κ) and rat brain (μ and δ), for their ability to block the electrically-induced contractions of the guinea pig ileum (GPI), and for their in vivo antinociceptive (writhing test) and motor (motor dysfunction assay) activities in mice. [D-Leu^8]Dyn A-(1-9) displayed an affinity and a selectivity for the κ opioid receptor that were comparable to those of Dyn A-(1-9). The potencies of [D-Leu^8]Dyn A-(1-9) in the GPI, writhing and motor dysfunction assays were markedly enhanced (8-12 fold) as compared with those of Dyn A-(1-9). [$^6\Psi^7$(CS-NH),D-Leu^8]Dyn A-(1-9), [Lys^6, $^6\Psi^7$(CS-NH), D-Leu^8]Dyn A-(1-9) and [Leu^6, $^6\Psi^7$(CS-NH), D-Leu^8]Dyn A-(1-9) were somewhat less potent than [D-Leu^8]Dyn A-(1-9) in all opioid assays. However, the pseudopeptides were more potent analgesic than Dyn A-(1-9) (ED_{50} of 29.5, 23.9 and 15.5 nmol/mouse, respectively, as compared with 90.7 nmol/mouse for Dyn A-(1-9)) and caused no or little motor impairment at analgesic doses. Supported by the MRC (Canada) and INRS (France).

516.10

SPATIAL DISTRIBUTION AND TEMPORAL REGULATION OF TYPE I PACAP-SELECTIVE RECEPTOR ISOFORM EXPRESSION IN PRIMARY RAT ASTROCYTE CULTURES. D.M. Jaworski* and K.M. Braas. Dept. of Anatomy and Neurobiology, Univ. of Vermont College of Medicine, Burlington, VT 05405.

The type I pituitary adenylate cyclase activating polypeptide (PACAP)-selective receptor belongs to the family of seven transmembrane-domain G protein coupled receptors. Splice variants of the type I receptor differ in the presence or absence of two 84 bp cassettes, termed HIP and HOP, in the region of the gene encoding the third cytoplasmic loop, a primary site of interaction between the receptor and G proteins. The six splice variants of the type I receptor exhibit different patterns of adenylyl cyclase and phospholipase C stimulation, suggesting a cell specific mechanism for differential intracellular signaling. Rat brain astrocytes exhibit PACAP-selective binding, thus the present studies were undertaken to identify the PACAP receptor isoforms expressed by primary cultures of rat brain astrocytes. To distinguish the specific type I PACAP receptor isoforms expressed by astrocytes, the presence or absence of either one or both of the 84 bp cassettes was examined using reverse transcription PCR with primers adjacent to the cassette insertion site. The predominant molecular form of PACAP receptor mRNA in primary rat cortical, brainstem and cerebellar astrocyte cultures from postnatal day one (P1) animals contained neither insert; low expression of a one cassette variant was also observed. In contrast, astrocyte cultures from the same brain regions from P15 animals exhibited lower receptor mRNA levels and equivalent expression of the short and one cassette isoforms. Southern blot analysis with sequence specific hybridization for the HIP and HOP cassettes will determine the identity of the insert. Combined *in situ* hybridization histochemistry-immunohistochemistry will be used to determine whether the PACAP receptor isoforms are expressed by distinct astrocyte subtypes. Supported by HD27468.

516.11

CHOLECYSTOKININ RECEPTORS IN BOVINE ADRENAL CHROMAFFIN CELLS. P.J. Vainio, A.M. Aarnisalo, P.T. Männistö[1], E. Vasar[2] and R.K. Tuominen*. Dept Pharmacol Toxicol, Inst Biomedicine, Univ Helsinki, FIN-00014, Finland; [1]Dept Pharmacol, Univ Uppsala, Sweden; [2]Dept Physiol, Univ Tartu, Estonia

Cholecystokinin (CCK) regulates several functions in gastrointestinal tract, and it is the most abundant neuropeptide in the brain. It participates in the control of feeding, analgesia and anxiety. The two subtypes of CCK receptors, CCK_A and CCK_B, are both activated by a decapeptide, caerulein. CCK_A and CCK_B receptors can be blocked by devazepide and L-365.260, respectively. We have used primary cultured bovine adrenal medullary (BAM) cells as a model of nerve cells.

In these cells, caerulein (0,1–100 nM) increased accumulation of inositol phosphates in a concentration dependent manner. The effect of caerulein (100 nM) was antagonized by devazepide (1 μM), but not by L-365.260 (1 μM). CCK-4, a CCK_B receptor agonist, did not induce accumulation of inositol phosphates. Caerulein (30 min, 100 nM) also increased membrane-associated protein kinase C (PKC) activity, as determined by histone phosphorylation assay and immunoblot against PKC α isoenzyme.

In conclusion, primary cultured BAM cells possess CCK_A receptors linked to phosphoinositidase C signal transduction pathway. The functional significance of CCK_A receptors in adrenal medulla remains unknown.

Supported by: The Academy of Finland, Yrjö Jahnsson Foundation

516.12

BASIC AMINO ACIDS AT THE C-TERMINUS OF THE THIRD INTRACELLULAR LOOP ARE REQUIRED FOR THE ACTIVATION OF PHOSPHOLIPASE C BY CCK_B RECEPTORS. H. L. Wang*, J. F. Chen and M. K. Sun. Department of Physiology, Chang Gung College of Medicine and Technology, Kwei-San, Tao-Yuan, Taiwan, R.O.C.

Recent studies suggest that charged amino acids of the third intracellular segment are required for the G-protein activation. The third intracellular loop of CCK_B receptor also contains three basic amino acids (K333/K334/R335) at the C-terminal end. To test the importance of conserved charged amino acids in the G-protein activation and the stimulation of phospholipase C (PLC), we mutated these amino acid residues and expressed mutant CCK_B receptors in COS cells and *Xenopus* oocytes. Subsequently, the ability of mutant receptor to activate PLC was investigated by measuring the formation of inositol phosphates (IP) in COS cells and recording Ca^{++}-activated chloride currents from *Xenopus* oocytes.

Site-directed mutagenesis was performed to mutate three basic amino acids, K333/K334/R335, to non-charged amino acids, M333/T334/L335. When the resulting mutant CCK_B receptors were expressed in COS cells and *Xenopus* oocytes, CCK-8 failed to induce IP formation in COS cells and evoke Ca^{++}-dependent chloride currents from oocytes. We also mutated each basic amino acid (K333→M333; K334→T334; R335→L335). Each of these mutations resulted in a significant reduction in the activation of PLC by CCK-8. Interestingly, substituting conserved basic amino acids, K333/K334/R335, with another three charged amino acids, R333/R334/K335, did not change the maximal CCK-8-simulated IP formation and the amplitude of CCK-8-evoked Ca^{++}-activated chloride currents. These findings propose that basic amino acids at the C-terminus of third cytoplasmic loop are required for the signal transduction by CCK_B receptors. (This work was supported by Chang Gung Medical Research Foundation)

516.13

Role of $Asp^{2.61(98)}$ in Agonist Complexing with the Human Gonadotropin-Releasing Hormone Receptor. Vladimir Rodic[1], Colleen Flanagan[2], Robert Millar[2], Karel Konvicka[3], Harel Weinstein[3], Stuart C.Sealfon*[1]. [1]Fishberg Research Center for Neurobiology and [3] Department of Physiology and Biophysics, Mount Sinai School of Medicine, NY 10029, [2]Department of Chemical Pathology, University of Cape Town Medical School, Observatory 7925, South Africa.

Previous studies have identified an acidic residue in the third extracellular loop of the GnRH receptor that confers specificity for the Arg^8 containing mammalian receptor (J. Biol Chem. 269:22636-41, 1994) and a Lys in helix 3 required for high affinity agonist interactions (J. Biol Chem.370,18853-18857,1995). In the present study, the role of $Asp^{2.61(98)}$ in agonist and antagonist interactions was explored. Mutation of this site to Glu, Ala, Asn and Val eliminated detectable agonist binding. Competition of labeled antagonist by GnRH showed that the affinity of GnRH for the Glu mutant receptor is reduced ~235-fold. All receptor mutants studied were capable of mediating agonist-stimulated phosphoinositol turnover. The EC_{50} values obtained with most agonists, including GnRH, were increased several orders of magnitude in comparison with the wild-type receptor. Interestingly, the EC_{50} for Trp^2 GnRH was equivalent in the wild-type and $Glu^{2.61}$ mutant, whereas the EC_{50} of GnRH was increased ~70-fold with this mutation. Thus the interaction of Trp^2-GnRH with the receptor does not appear to be dependent on the length of this side chain. These results show that high affinity GnRH interaction depends on $Asp^{2.61(98)}$, possibly due to a direct interaction with His^2 in the ligand. Supported by grants NIH DK44943 KO5 DA 00060.

516.14

ROLE OF THE AMINO TERMINUS IN LIGAND BINDING FOR THE ANGIOTENSIN RECEPTOR FAMILY. D.K. Yee*, J.N. Heerding, M.Z. Krichavsky, and S.J. Fluharty. Departments of Animal Biology, Psychology, Pharmacology, and Institute of Neurological Sciences. University of Pennsylvania, Philadelphia, PA 19104.

There are at least two main subtypes of angiotensin II (AngII) receptors, termed Type 1 (AT_1) and Type 2 (AT_2). Comparison of their amino acid sequences shows a relatively low level of homology (34%), which is lowest in their extracellular domains. Mutagenesis studies have previously demonstrated that the AT_1 amino terminus plays an important role in the binding of AngII; some mutations of this protein domain reduced the affinity for the peptide by more than 5000-fold [Hjorth *et al.*, 1994, JBC **269**:30953]. The key residues identified by these AT_1 mutagenesis experiments, however, are not conserved on the AT_2 amino terminus. Since the two subtypes bind AngII with identical affinities, we constructed and characterized AT_1 and AT_2 amino terminal exchange mutants in order to further investigate the role of this protein domain in ligand binding for the AngII receptor family. When expressed in COS-1 cells, the affinities of these chimeric receptors for ^{125}I-AngII were essentially identical to that of the wild type receptors. These data suggest that: *(i)* the AT_2 amino terminus is important in the binding of AngII and *(ii)* despite great differences in their amino acid sequences, the two amino termini can compensate for one another. In addition, pharmacological analyses have demonstrated that the replacement of the AT_2 amino terminus with that of the AT_1 did not affect the specificity of AT_2 selective compounds CGP42112A and PD123319. This suggests that specific epitopes that are responsible for the AT_2 selective binding of these compounds do not reside in the AT_2 amino terminus. Supported by NS23986 and MH43787.

516.15

THE EFFECT OF POINT MUTATIONS ON LIGAND BINDING FOR THE ANGIOTENSIN TYPE 2 RECEPTOR. J.N. Heerding*, D.K. Yee, L.A. Rogers, S. Jacobs, and S.J. Fluharty. Departments of Animal Biology, Psychology, Pharmacology, and Institute of Neurological Sciences. University of Pennsylvania, Philadelphia, PA 19104.

There are at least two main subtypes of angiotensin II (AngII) receptors, termed Type 1 (AT_1) and Type 2 (AT_2). Although they share a relatively low level of homology (34%), both subtypes possess nearly identical affinities for AngII. Thus far, mutagenesis studies of this receptor family have focused solely on the AT_1 receptor subtype. These experiments have identified several amino acid residues that are important in AngII binding [Yamano *et al.*, 1994, JBC **269**:14024; Hjorth *et al.*, 1994, JBC **269**:30953]. In order to establish the level of similarities in ligand binding that may exist in this receptor family, we have been mutating residues conserved in the AT_2 subtype that were identified in the AT_1 mutagenesis experiments as important in AngII binding. Analyses of the point mutants lysine 215 to glutamine (K215Q) and aspartate 280 to asparagine (D280N) have been completed. When expressed in COS-1 cells, the K215Q mutant possessed greatly reduced affinity for both ^{125}I-AngII and ^{125}I-Sar^1,Ile^8-AngII and was unable to bind the nonpeptide ^{125}I-PD122979. In contrast, the D280N mutant bound ligands with wild-type affinities. These data indicate that despite a relatively low homology, some commonalities in the binding mechanism for AngII may exist between the two subtypes. Additional point mutants are being characterized to more fully determine the extent of these similarities. Supported by NS23986 and MH43787.

516.16

OVEREXPRESSION OF HUMAN GONADOTROPIN RELEASING HORMONE RECEPTOR PROTEIN IN TRANSFECTED Sf9 CELLS. S.S. Kakar, S. Nath, D.C. Tucker*, N.S. Reddy and L. Jennes. Dept. Physiology and Biophysics, and Dept. Physiological Optics, University of Alabama at Birmingham, Birmingham, AL 35294 and Dept of Anat. & Neurobiology, Univ. Kentucky, Lexington, KY 40536.

Gonadotropin releasing hormone (GnRH) receptors belong to the family of G protein-coupled receptor proteins and have been localized to the anterior pituitary, brain, reproductive organs as well as many steroid-dependent tumor tissues. In order to generate sufficient receptor protein for detailed structural analyses and for the generation of specific antibodies, the full length human GnRH receptor cDNA was subcloned into baculovirus transfer vector pVL 1392 and, after homologous recombination, insect Sf9 cells were transfected with the resulting virus. After several rounds of amplification, the transfected cells exhibited binding for GnRH and its analogs with ligand specificities similar to the pituitary GnRH receptor, however, the affinities were slightly lower. Thus, for example, the GnRH agonist $D\text{-}Ala^6$-GnRH displaced ^{125}I-Buserelin with an EC_{50} of 3 nM compared to 0.2 nM in pituitary. Metabolic labeling of transfected Sf9 cells with ^{35}S-methionine and ^{35}S-cysteine resulted in the appearance of 56 kDA protein band which is similar to the molecular weight of glycosylated pituitary GnRH receptor proteins. Together, the results suggest that human GnRH receptor protein can be overexpressed in transfected Sf9 cells and that the resulting protein retains its binding specificities. Supported by NIH CA60871 (SSK) and HD 24697 (LJ).

516.17

CALORIE RESTRICTION DELAYS THE ALTERATIONS OF GROWTH HORMONE-RELEASING FACTOR RECEPTOR BINDING SITES OBSERVED IN AGING RAT PITUITARY. P. Gaudreau*, N. Girard, L. Boulanger and G. Ferland. Neuroendocrinology Laboratory, Notre-Dame Hospital Research Center and Departments of Medicine and Nutrition, University of Montreal, Montreal, Canada H2L 4M1.

Growth hormone (GH) secretion is regulated by two hypothalamic peptides, growth hormone-releasing factor (GRF) and somatostatin (SRIF). These peptides exert opposing actions on somatotroph cells by triggering specific G_s- and G_i-protein-coupled receptors. In middle age and old mammals, several changes occur along the hypothalamo-pituitary GH axis, including alterations of the pituitary GRF receptor binding sites. This leads to a diminution of GH and IGF-1 circulating levels and contributes to aging processes. Because the anti-aging effects of calorie restriction ressemble those of GH, we studied GRF binding in this rat model of successful aging. Interestingly, in 18-month-old rats submitted to a moderate calorie restriction from 8 to 18 months of age, high and low affinity GRF binding site parameters remain similar to those of 2-month-old control rats. On the contrary, high affinity GRF binding sites are blunted in 18-month-old *ad libidum* fed rats. These results suggest that some of the anti-aging effects of calorie restriction are related to the maintenance of pituitary GRF receptor integrity. Functional studies will however be needed to assess the physiological relevance of such nutritional interventions to delay GH somatopause. Supported by MRC (PG) and NSERC (GF) Grants.

PEPTIDE RECEPTOR STRUCTURE AND FUNCTION III

517.1

EXPRESSION OF CALCITONIN RECEPTORS IN HUMAN NEUROBLASTOMA CELLS. G.Campana, S.Cacciaguerra, M. Canossa, S.Ferri and S.Spampinato*.Department of Pharmacology, University of Bologna, Irnerio 48, 40126, Bologna, Italy.

The 32-amino acid peptide calcitonin (CT) influences calcium homeostasis binding to receptors expressed in osteoclasts and in the kidney. CT also acts on binding sites in the central nervous system, where exogenous CTs modulate appetite, body temperature and pain perception (Spampinato et al., 1984, Neurosci. Lett., 45:135-139). CT receptors have been cloned from peripheral tissues of several mammalians including humans. However, to date CT receptors expressed in the central nervous system have been poorly investigated. We have examined a series of human neuroblastoma cell lines with the aim to detect, by polymerase chain reaction, any mRNA coding for the calcitonin receptor using two primers complementary to an unique sequence of a cDNA (Kuestner et al., 1994, Mol. Pharmacol., 46: 246-255). Electrophoretic analysis of reverse-transcribed total RNA, provided evidence that mRNA coding for this receptor occurs in IMR 32 cells. Receptor binding studies using ^{125}I-salmon CT revealed a high affinity binding site for this peptide (B_{MAX}=4.66 x 10^{-10} M) and human CT competes with binding at this site (K_i=1.44 x 10^{-10} M). In cells, loaded with fura-2, human CT (IC_{25}=1.58 x 10^{-7} M) and salmon CT (IC_{25}=1.62 x 10^{-7} M) antagonized Ca^{2+} entry evoked by high extracellular K^+ (50 mM). Therefore, IMR 32 cells represent an useful tool to investigate the gene expression and molecular interactions of CT receptors in neuronal cells.

Supported by CNR grant n° 05.02937.CT14

517.2

CLONING AND EXPRESSION STUDY OF A CGRP RECEPTOR FROM MOUSE UTERUS. M. Howard, G. P. Dahl and L. M. Dickerson*, Dept. of Physiology and Biophysics, Univ. of Miami Sch. of Med., Miami, FL 33136

CGRP-IR neurons have been identified in the uterus and CGRP inhibits spontaneous contractions in the uterus and fallopian tubes, suggesting that CGRP may play an important role in regulation of smooth muscle activity. Using degenerate PCR, we have determined the primary structure of the mouse homologue of the CGRP-receptor component protein (CGRP-RCP) recently isolated from guinea pig cochlea (Luebke et al. 1996, PNAS, 93:3455-3460). The 1.8 kb cDNA of the mouse CGRP-RCP contains a 444 bp open reading frame (ORF) preceded by a Kozak ranslation initiation consensus sequence. An oocyte expression system using the cystic fibrosis transmemberane conductance regulator (CFTR) as a sensor of intracellular cAMP levels was used to monitor CGRP responsiveness of the protein encoded by the ORF. Uterus mRNA was reverse transcribed and PCR amplified using primers that flank the ORF. The resulting RT-PCR product was transcribed *in vitro* and co-injected into the *Xenopus* oocytes with CFTR cRNA. Under voltage clamp conditions, oocytes expressing the protein encoded by the ORF produced an inward current when incubated with CGRP. We have carried out northern analysis, western analysis and immunohistochemistry in order to map the expression of CGRP-RCP in the mouse uterus during pregnancy. This work was supported in part by, AHA (FL affiliate) Grant-In-Aid #9401236 (IMD) and NIH #GM48610 (GPD).

517.3

TISSUE DISTRIBUTION OF CLONED CGRP RECEPTORS. A.E. Luebke*, M. Howard, I.M. Dickerson. Department of Physiology and Biophysics, Univ. of Miami Sch. of Med., Miami, FL 33101.

We have recently identified a cDNA from guinea pig cochlea that confers CGRP responsiveness in an oocyte expression assay (Luebke et al. 1996, *PNAS*, 93:3455-3460). The deduced protein is a small hydrophilic molecule that does not contain an obvious membrane-spanning domain. We hypothesize that this protein is a receptor for CGRP, or is a component of a complex of proteins that together constitute the CGRP receptor. We have mapped the distribution of this CGRP-receptor component protein (CGRP-RCP), and it is colocalized in the cerebellum with neurons that immunostain for CGRP. Two orphan receptors, RDC1 and GPRN1, have recently been implicated as CGRP receptors (Law and Rosenzweig,1994, *BBRC* 201:458-465; Kapas and Clark , 1995, *BBRC* 217:832-838). As a first step in determining the composition of a CGRP receptor, we have begun to map the distribution of CGRP-RCP, RDC1, and GPRN1. Northern analysis and immunohistochemistry have mapped the distribution of CGRP-RCP, and this distribution is being compared to that for RDC1 and GPRN1 in brain, heart and lung determined by *in situ* hybridization and Northern analysis. This work was supported in part by AHA (FL Affiliate) Grants-In-Aid #9401236 (IMD) and #9501349 (AEL).

517.4

CALCITONIN GENE-RELATED PEPTIDE (CGRP) RECEPTOR EXPRESSION IN THE RAT BRAIN. AN IN SITU HYBRIDIZATION STUDY, Y. Tong*[1,2], A.J.L. Clark[3], S.H. Shen[2] and R. Quirion[1]. [1]Douglas Hospital Res. Ctr, Dept. of Psychiatry, McGill Univ., Montreal, Que. H4H 1R3; [2]Biotechnology Res. Inst., NRCC, Montreal, Que., [3]Dept. of Clin. Endo. The Medical College of St. Bartholomew's Hospital, London, U.K.

The existence of at least two classes of CGRP receptors has been proposed on the basis of the differential potency of CGRP fragments and analogues in *in vitro* bioassays (Dennis *et al*, J. Pharmacol. Expt. Ther. 251:718, 1989) and behavioral tests (Quirion *et al*, Ann. N.Y. Acad Sci. 657:88, 1992). The $CGRP_1$ subtype is particularly sensitive to the antagonistic properties of the fragment $CGRP_{8-37}$ while the $CGRP_2$ receptor is less affected in addition to being activated by the linear agonist [Cys(ACM)^{2,7}]hCGRPα. Recently, the cloning of a $CGRP_1$-like receptor has been reported (Kapas and Clark, Biochem. Biophys. Res. Comm. 217:832, 1995). It is a seven-transmembrane G-protein coupled receptor highly sensitive to both CGRP and $CGRP_{8-37}$. Additionally, another CGRP binding protein has just been cloned and shown to have a single transmembrane domain (Luebke *et al*, PNAS, 93:3455, 1996). In the present study, we used a ^{35}S-labelled rat cRNA probe to investigate the discrete expression of the cloned $CGRP_1$-like receptor in the rat brain. Probes were used at a concentration of 1x10^7 cpm/ml using a method described in detail elsewhere (Tong *et al*, Neuropeptides 20:33, 1991). Hybridized sections were exposed for 2 (films) to 6 (emulsion) weeks before development. Specific moderate to strong hybridization signals were observed in the anterior olfactory nuclei, nucleus accumbens, dentate gyrus, and linings of ventricles and choroid plexus. At higher microscopic resolution, many CGRP receptor expressing neurons were diffusely distributed in various hypothalamic, thalamic and brainstem nuclei. Such a localization is reminiscent of that of [^{125}I]CGRPα binding sites in the rat brain. Supported by the MRCC.

517.5

COMPARATIVE EFFECTS OF [Cys(ACM)^{2,7}]hCGRPα AND [Cys(Et)^{2,7}]hCGRPα IN $CGRP_1$ AND $CGRP_2$ IN VITRO BIOASSAYS: CHARACTERIZATION OF A MORE POTENT AND SELECTIVE $CGRP_2$ AGONIST. A. Fournier[1*], Y. Dumont[2], S. St-Pierre[1] and R. Quirion[2]. [1] INRS-Santé, Université du Québec, Pointe-Claire, Qc, Canada, H9R 1G6. [2] Douglas Hospital Research Center and Dept Psychiatry, McGill University, 6875 LaSalle Blvd, Montréal, Qc, Canada, H4H 1R3.

The existence of $CGRP_1$ and $CGRP_2$ receptor subtypes has been proposed on the basis of the differential potency of $hCGRP_{8-37}$ to antagonize the effect of hCGRPα in the guinea pig atria and in the rat vas deferens (pA_2 of 7.6 and 6.2, respectively; Dennis et al., JPET, 254: 123-128, 1990). Moreover, the linear analogue, [Cys(ACM)^{2,7}] hCGRPα, was shown to be more effective to inhibit the rat vas deferens twitch response than to induce a positive inotropic effect in the guinea pig atria (Dennis et al., JPET, 251: 718-725, 1989). Taken together, these results suggested the existence of at least two classes of CGRP receptors, one being more sensitive to the antagonistic properties of $CGRP_{8-37}$ ($CGRP_1$) while the other is activated by moderate to high nM concentrations of the linear analogue ($CGRP_2$). However, the rather low potency of [Cys(ACM)^{2,7}]hCGRPα (ED_{50} of 70nM in the rat vas deferens) limits its usefulness. We postulated that modifications of the linear analogue should lead to more potent and selective $CGRP_2$ agonists. Among them, [Cys(Et)^{2,7}]hCGRPα was developed and tested in the guinea pig atria and rat vas deferens, representing prototypical $CGRP_1$ and $CGRP_2$ bioassays, respectively. [Cys(Et)^{2,7}]hCGRPα inhibited the rat vas deferens twitch response with an ED_{50} of 2.6 ± 1.2 nM (50 times more potent than [Cys(ACM)^{2,7}]hCGRPα) while in the guinea pig atria, this analogue induced only a slight contraction at the high concentration of 1 μM (20 to 30 % of the maximal response induced by hCGRPα). [Tyr^0, Cys(Et)^{2,7}]hCGRPα and [Cys(Et)^{2,7}]hCGRPα also competed with high affinity (low nM range) for [^{125}I][Tyr^0]hCGRPα sites in rat brain homogenates. These results suggest that [Cys(Et)^{2,7}]hCGRPα is the most potent and selective agonist of the $CGRP_2$ receptor subtype developed thus far. This molecule could prove most useful to address the functional role of this CGRP receptor class. Supported by the MRC of Canada.

517.6

CALCITONIN GENE-RELATED PEPTIDE-INDUCED FORMATION OF SECOND MESSENGERS IN CULTURED NEONATAL RAT SPINAL CORD. A.M. Parsons* and V.S. Seybold. Graduate Program in Neuroscience, Univ. of Minnesota, Minneapolis, MN 55455.

The signal transduction pathway for calcitonin gene-related peptide (CGRP) receptors in the spinal cord is not known. We investigated the formation of second messengers (cAMP, cGMP and inositol phosphates) formed in response to CGRP in primary cultures prepared from spinal cords of 1-2 day post natal rats. The cultures were maintained for 10-14 days in vitro prior to experiments. Cellular cAMP and cGMP levels were determined by radioimmunoassay. Inositol phosphate formation was determined by recovery of [^{3}H]inositol phosphate. Basal levels of cAMP were 50 pmol/mg protein. CGRP increased the level of cAMP with the concentration-response curve showing an intermediate plateau at 180 pmol cAMP/mg protein in response to 0.01 to 0.1 nM CGRP and a maximal plateau of 850 pmol cAMP/mg protein at 300 nM CGRP. This biphasic curve (EC_{50}'s of 0.7 pM and 22 nM) suggests activation of high and low affinity receptors for CGRP. Both neurons and non-neuronal cells contributed to increased cAMP levels in response to CGRP. The CGRP receptor blocker, $CGRP_{8-37}$, antagonized the response. A high concentration of CGRP (1 μM) moderately increased the formation of cGMP (180% above basal), whereas CGRP had no effect on the formation of inositol phosphates at any of the concentrations tested (0.1 - 1 μM). In summary, these results suggest that CGRP-induced responses in spinal cord are predominately via the formation of cAMP. The observation that both neurons and non-neuronal cells respond to CGRP indicate that this peptide may have multiple actions in the spinal cord. (This study was funded by NIH NS17702 and NIDA T32DA07234.)

517.7

CHARACTERIZATION OF THE GALANIN RECEPTOR IN THE CHP 212 HUMAN NEUROBLASTOMA CELL LINE, L.-L. Shiao , M. A. Cascieri* and K.A. Sullivan. Molecular Pharmacology and Biochemistry, Merck & Co., Rahway, NJ 07065.

Biological functions of galanin are mediated via membrane bound high-affinity receptors. In order to identify and characterize galanin receptor subtypes, we have detected specific ^{125}I-human galanin binding in the CHP 212 human neuroblastoma cell line. The rank order of potency in the inhibition of ^{125}I-human galanin binding by galanin and the chimeric galanin peptides: [h-Gal = p-Gal = h-Gal(1-19) = M35 >M40 >p-Gal(1-16) = M15>C7 = r-Gal(2-29)], indicates that this cell line displays the same pharmacology as the Bowes melanoma cell line. Scatchard analysis reveals a Kd of 0.07 nM that is in close agreement with the hGALR1 expressed in stable CHO cell line as well as in Bowes melanoma cell line from which hGALR1 was cloned. Western blot analysis using an antisera raised against hGALR1 crossreacts with a species of approximately 70 kDa in Bowes melanoma membranes which is also observed in a stable CHO cell line expressing hGALR1. Furthermore, Northern blot analysis demonstrated an ~3 kb mRNA in both CHP 212 and Bowes melanoma cells. These data suggest that the human galanin receptors expressed in CHP212 are the same subtype as the cloned GALR1 from the Bowes melanoma cells.

Merck Research Laboratories

517.8

PHARMACOLOGICAL CHARACTERIZATION OF THE RECOMBINANT HUMAN AND RAT GALR1 GALANIN RECEPTOR C. Forray*, K. Smith, C. Gerald, P. Vaysse, R. Weinshank, and T. Branchek Synaptic Pharmaceutical Corporation, Paramus N.J. 07652.

We have studied the pharmacological properties of the recombinant GALR1 receptor, in an attempt to understand its role in mediating the effects of galanin (Gal) in the CNS. LM(tk-) cells stably transfected with either the cDNA of the rat or human GALR1 receptor displayed high affinity binding for [^{125}I]porcine galanin. Competition binding assays using a series of truncated galanin analogs indicated that both rat and human GALR1 receptors had similar profiles. The rank order of affinities for C-terminal truncated analogs was: $Gal_{(1-29)} \geq Gal_{(1-16)} > Gal_{(1-12)} >> Gal_{(1-9)}$. Conversely, the N-terminal truncated analogs $Gal_{(3-29)}$, $Gal_{(9-29)}$, and $Gal_{(17-29)}$ were inactive (> 100 μM). Human and rat GALR1 also showed similar binding affinities for chimeric Gal antagonists with the following rank order: M35 ≥ M32 > M40 > C7 ≈ galantide.

Functional activity of these receptors was assessed by measuring inhibition of cAMP formation stimulated by forskolin. In LM(tk-) cells expressing the human or rat GALR1 receptors, porcine $Gal_{(1-29)}$ inhibited cAMP formation by 98 and 75% respectively. The galanin "antagonists" C7, M40, M35 and M32 were all found to inhibit cAMP formation to the same extent as porcine $Gal_{(1-29)}$, indicating that in this "in vitro" assay these chimeric peptides are full agonists at the GALR1 receptor subtype. This observation suggests that the GALR1 subtype is not likely to mediate the effects of galanin on nociception or feeding behavior, since these effects have been found to be antagonized by either C7, M40, M35 or M32. These findings remain to be validated using non-peptide galanin antagonists, antisense studies or transgenic animals.
(Supported by Synaptic Pharmaceutical Corporation)

517.9

NEUROANATOMICAL DISTRIBUTION OF A NOVEL RAT GALANIN RECEPTOR SUBTYPE. D. O'Donnell*, S. Ahmad, P. Walker, C. Wahlestedt. Astra Pain Research Unit, Montreal, Quebec, Canada H4P 2R2.

Recent molecular cloning studies by our laboratory have identified the existence of a novel rat galanin receptor subtype, GALR2. RT-PCR analysis has revealed that GALR2 mRNA is expressed in various tissues including dorsal root ganglia, spinal cord, brain and certain peripheral tissues. In this study, we further examined the precise neuroanatomical distribution of GALR2 mRNA using *in situ* hybridization. Frozen rat brain and spinal cord sections with dorsal root ganglia attached were hybridized with ^{35}S-labeled riboprobes. In sagittal brain sections, the highest levels of GALR2 mRNA were detected in the dorsal hippocampus with dentate gyrus granule cells expressing slightly higher levels than pyramidal neurons of Ammon's horn. High levels of labeling were also detected in the mammillary bodies and cerebellum, particularly in some Purkinje cells as well as in numerous cells throughout the molecular layer. Moderate levels of GALR2 were observed in the pontine nucleus and in specific cranial nuclei. Moderate to weak hybridization signal was also present in the superficial layers of the cerebral cortex. Other cephalic areas such as the thalamus and basal ganglia were relatively devoid of labeling. In sections taken at the level of the spinal cord, very high levels of GALR2 mRNA expression were observed in dorsal root ganglia with large, intermediate and small diameter primary sensory neurons being specifically labeled. In contrast, only diffuse labeling was detected throughout the dorsal and ventral horns of the spinal cord.

This novel yet distinct pattern of GALR2 distribution within the rat CNS differs considerably from that of the GALR1 and therefore may partially account for the more widespread distribution of galanin binding sites. Moreover, the particularly high levels detected in sensory dorsal root ganglia neurons suggest that GALR2 is likely to play an important role in mediating galanin's antinociceptive actions.

518.1

PARTIAL SEQUENCE OF A PRODYNORPHIN cDNA CLONED FROM THE BRAIN OF THE MARINE TOAD, *BUFO MARINUS*. David Walker, Philip Danielson and Robert M. Dores*. University of Denver, Department of Biological Sciences, Denver CO 80208.

In mammals, the precursor, Prodynorphin, contains the sequences of three endogenous opioids, α-neoendorphin, dynorphin A, and dynorphin B. Although Prodynorphin-related products have been detected in all of the classes of tetrapods, Prodynorphin has not been characterized in any species of non-mammalian vertebrates. In an effort to analyze the evolution of the Prodynorphin gene, we attempted to clone and sequence Prodynorphin cDNA isolated from the brain of the anuran amphibian, *Bufo marinus*. Using a 3′ RACE procedure we were able to sequence a 450 base pair fragment of *Bufo* Prodynorphin. This region of the cDNA contained the sequences of dynorphin A, dynorphin B, the C-terminal of *Bufo* Prodynorphin, a stop sequence, and a 300 base pair 3′ untranslated region. A comparison of mammalian and amphibian dynorphin sequences will be discussed. This research was supported by NSF grant: 9406967.

518.2

FEEDBACK REGULATION OF A POST-TRANSLATIONAL PEPTIDE α-N-ACETYLTRANSFERASE PARTIALLY PURIFIED FROM BOVINE NEUROINTERMEDIATE PITUITARY. A.B. Manning* and W.R. Millington. Division of Molecular Biology and Biochemistry, University of Missouri-Kansas City, Kansas City, MO 64108.

Post-translational α-N-acetylation of the pro-opiomelanocortin-derived peptides β-endorphin (β-End) and ACTH(1-13)NH_2 alters their biological functions, eliminating β-End(1-31)'s analgesic potency, yet potentiating many of ACTH(1-13)NH_2's behavioral effects. Previous studies in our laboratory have indicated a single post-translational α-N-acetylating activity modifies both β-End and ACTH(1-13)NH_2. Here we report that post-translational peptide α-N-acetylating activity is inhibited by its reaction end-products. Enzyme activity was partially purified by using DEAE Sepharose and Cibacron Blue 3GA chromatography and measured using ^{3}H-acetyl coenzyme A and ACTH(1-13)NH_2 or β-End as substrates. Kinetic analysis showed K_m values for β-End and ACTH(1-13)NH_2 of 80 μM and 60 μM, respectively. Peptide end-product inhibition studies revealed N,O-diacetyl-α-MSH inhibited β-End α-N-acetylation (K_i=213 μM); N-acetyl-β-End(1-27) also inhibited α-N-acetylation of ACTH(1-13)NH_2 (K_i=309 μM). Inhibition was concentration-dependent and non-competitive and K_i values were within the estimated range of vesicular peptide concentrations. Coenzyme A, the other reaction end-product, also inhibited ACTH(1-13)NH_2 α-N-acetylation in a concentration-dependent manner (IC_{50}=53 μM) while bromoacetyl coenzyme A (250 μM) inhibited irreversibly. To determine whether inhibition was attributable to the peptides's N-terminal residues, N,O-diacetyl-seryl-tyrosine was tested; the dipeptide inhibited ACTH(1-13)NH_2 α-N-acetylation minimally (11 %) and only at high concentrations (600 μM). Similarly, L-tyrosine-glycine-glycine (600 μM), β-End's N-terminal tripeptide, inhibited ACTH(1-13)NH_2 α-N-acetylation by only 22 %. Cyclo(seryl-tyrosine) (600 μM), a cyclic derivative of seryl-tyrosine capable of penetrating biological membranes, partially inhibited α-N-acetylating activity (34 %). Thus, α-N-acetyltransferase reaction end-products clearly inhibit enzyme activity although the N-terminal peptide fragments, both des- and α-N-acetylated, have minimal inhibitory effects. (NIDA DA-04598)

518.3

PROCESSING SITE BLOCKADE RESULTS IN MORE EFFICIENT CONVERSION OF PROENKEPHALIN TO ACTIVE OPIOID PEPTIDES. K. Johanning, J.P. Mathis and I. Lindberg*. Dept. of Biochemistry and Molecular Biology, LSUMC, New Orleans, LA 70112.

Prohormones are known to be processed at various cleavage sites in a defined temporal order, suggesting the possibility of sequential unfolding of processing sites. To investigate whether sequential processing at predefined sites is in fact required for proper processing, site-directed mutagenesis was performed to block known initial cleavage sites within proenkephalin. Pulse-chase/immunoprecipitation experiments were employed to analyze mutant and native proenkephalins in stably transfected AtT-20 cells. While processing did not occur at blockaded sites, surprisingly, overall processing of mutant proenkephalins proceeded efficiently, and alternative sites were chosen. Processing of mutant proenkephalins occurred more slowly at early stages and more quickly at later stages. Endoglycosidase H experiments indicated that the early slow processing of mutant proenkephalins may be due to delays in intracellular transport. More efficient production of bioactive opioids occurred in all four site blockade mutants examined. We conclude that efficient processing of prohormone precursors does not require a specific temporal order of processing events. Mutant proenkephalins may provide a route for more efficient production of opioid peptides in applications for chronic pain treatment. (DA05084)

518.4

CHRONIC EXPOSURE TO CAFFEINE INCREASES OPIOID NEUROPEPTIDE mRNA LEVELS IN THE NEOSTRIATUM AND TYROSINE HYDROXYLASE mRNA IN THE MESENCEPHALON OF THE RAT BRAIN. U. Datta, P.-A. H. Noailles, M. Kraft, Y. Zhang and J. A. Angulo*. Hunter College and the Graduate School of the City University of New York, Department of Biological Sciences, NY NY 10021.

Rats were injected ip with caffeine (20, 40 and 80 mg/kg of body weight) twice daily for nine consecutive days in order to assess the effect of this adenosine receptor antagonist that displays psychomotor stimulant properties, on the expression of preproenkephalin (PPE) and preprodynorphin (PPD) mRNA expression in the neostriatum. We also assessed tyrosine hydroxylase (TH) mRNA levels in the substantia nigra compacta (SNc) and the ventral tegmental area (VTA). Chronic caffeine elevated TH mRNA in the SNc (64% relative to controls) and the VTA (33%). These increases were blocked by concurrent administration of the non-competitive N-Methyl-D-Aspartate receptor antagonist MK-801. In the neostriatum, PPE and PPD mRNA levels were increased (up to two-fold) in all aspects of the caudate-putamen. Concurrent administration of caffeine and MK-801 attenuated the increases observed with caffeine alone. Preliminary observations show that chronic caffeine treatment decreases mu receptor levels in the striatum and accumbens.

518.5

IDENTIFICATION OF MULTIPLE FORMS OF ORPHANIN F Q (OFQ) IMMUNOREACTIVITY IN MOUSE HYPOTHALAMIC EXTRACTS. D.I. Quigley, R.G. Allen*, G. Zhang, J.A. McDougall and D.K. Grandy, Vollum Inst., Oregon Health Sciences Univ., Portland OR 97201.

Recently, a peptide tentatively named Orphanin FQ (OFQ) that binds the opioid-like orphan receptor LC 132 was isolated from porcine hypothalamic extracts. Behavioral studies suggest that OFQ possesses anti-opioid activity. The synthetic peptide was used to produce a polyclonal anti-OFQ antibody in rabbits that does not cross-react with dynorphins, or the multiple forms of β-endorphin contained in mouse pituitary extracts. This antiserum has been used in the development of an OFQ radioimmunoassay (RIA). In initial experiments we determined the levels of OFQ in mouse hypothalamic extracts to be 0.265 pmol/hypothalamus +/- 0.028, S.E.M. (N=4). Additionally, we found very low, to undetectable levels of OFQ immunoreactivity in striatum, spinal cord, hippocampus and the VTA/SNc, with the VTA/SNc extracts containing the highest amount of OFQ outside of the hypothalamus. Since the cDNA clone suggests that OFQ is contained within a larger precursor molecule, we fractionated hypothalamic extracts using reversed phase HPLC. Multiple peaks of immunoreactivity were detected by RIA suggesting that OFQ is contained within a discrete set of biosynthetic intermediates, as are the opioid peptides. This work was supported by NIH grants HD30236 (RGA) and DA08562 (DKG).

518.6

ALTERATIONS IN THE BLOOD LEVELS OF HEMORPHIN-7 AND β-ENDORPHIN IN PATIENTS SUBJECTED TO OPEN-HEART-SURGERY. E. Nyberg[1], K. Sanderson[1], N. Majid[1], P. Andrén[1], A. Adem [2], K. Karlsson[3], E. Sandström[4] and L.-O.D. Koskinen[5] [1]Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden, [2]Department of Clinical Neuroscience & Family Medicine, Huddinge Hospital, Huddinge, Sweden, [3]Departments of Clinical Chemistry, [4] Thoracic Surgery and [5]Neurosurgery, NUS, Umeå, Sweden. (Spon: SONA)

The hemorphins are opioid active peptides enzymatically released from the β-chain of the blood protein hemoglobin. Previous studies have shown that the level of hemorphin-7 is increased in plasma samples collected from human subjets following long distance running. Furthermore, this increase was parallelled by an elevation of plasma levels of β-endorphin.

In this presentation we describe a study of hemorphin-7 and β-endorphin in patients subjected to open-heart surgery using a heart-lung machine. In the arterial blood flow the level of hemorphin-7 was found to increase with time, whereas β-endorphin remained unaltered. In the blood collected from the jugular venous bulb, however, the level of β-endorphin was increased, whereas no significant changes were found in the hemorphin-7 concentration. It is suggested that hemorphin may produce a release of β-endorphin from the pituitary. It is known that patients undergoing cardiopulmonary bypass generally have a sense of well beeing the first postoperative day. This could be due to the released peptides. However, it could not be excluded that the release of the peptides may be involved in the minor brain injuries often seen in conjunction with open heart surgery.

The study also describes the characterization of the measured hemorphin-7 immunoreactivity using the Pharmacia-SMART system for reversed phase separation. The chromatographic instrument was used to isolate the immunoreactive material for a subsequent analysis by mass-spectrometry, where the identity between the detected activity and the authentic peptide was confirmed.

518.7

CHARACTERIZATION OF A NOVEL SOLUBLE PROTEIN FACTOR WITH NON-OPIOID DYNORPHIN A-BINDING AND CORVERTASE ACTIVITIES. T. Yakovleva*, M. Melzig, K. Hjertman, I. Nylander, J. Silberring, G. Bakalkin and L. Terenius. *Department of Clinical Neuroscience, Karolinska Institute, S-17176 Stockholm, Sweden.*

A novel protein factor with non-opioid dynorphin A-binding sites was characterized in human neuroblastoma SK-N-MC cells and in the rat spinal cord. The factor binds dynorphin A-(1-13), A-(1-17), dynorphin A-(2-17) and the proenkephalin fragment peptide E, but not other opioid and nonopioid peptides, morphine, etorphine, naloxone or benzomorphans. The IC_{50} for dynorphin A-(1-17) and dynorphin A-(2-17) was about 50 and 5 nM respectively. Bound labelled dynorphin A cannot be easily displased by a nonlabelled peptide and seemed to undergo conversion to the shorter fragment Leu-enkephalin which remained bound to the protein. SH groups of the factor were apparently involved in the binding of dynorphin A, since PHMB inhibited this process. The dynorphin A-binding factor has an apparent molecular mass of about 70 kDa and was found in a nuclear extract and in cytosol. These data suggest that the factor functions as a dynorphin A convertase interfering with the activity of this peptide. Supported by grants from the Swedish Medical Research Council (Project 3766) and NIDA (DA-05186-04).

518.8

REGULATION OF OPIOID PEPTIDE GENE EXPRESSION IN RAT HIPPOCAMPUS: ROLE OF PROTOONCOGENES. J. S. Won, Y. H. Kim, D. K. Song, and H. W. Suh*, Department of Pharmacology, College of Medicine, Institute of Natural Medicine, HallymUniversity, Chunchon, 200-702, S. Korea.

The effect of cycloheximide (CHX), a protein synthesis inhibitor, on the regulation of proenkephalin (proENK) mRNA level and proto-oncogenes, such as c-fos, 35 kDa-fra, and c-jun mRNA and the levels of their products induced by kainic acid (KA) in rat hippocampus. The proENK mRNA level was markedly increased 1 and 8 hr after administration with KA (10 mg/kg). The proENK mRNA elevation was inhibited by the pretreatment with CHX (15 mg/kg). The intracellular proENK protein levels was not changed by neither KA. The increase of proENK mRNA level was well correlated with c-Fos, 35 kDa-Fra, and c-Jun protein levels. C-Fos, 35 kDa-Fra, and c-Jun proteins were detected at 1 hr after KA administration, increased gradually, and reached a peak after 8 hr. The increases of c-Fos, 35 kDa-Fra, and c-Jun protein levels elevated by KA were inhibited by the pretreatment with CHX. KA administration increased c-fos and c-jun mRNA levels in 1-2 hr and the levels of these proto-oncoproteins were potentiated by CHX. Furthermore, CHX alone increased the c-jun mRNA level without altering c-fos mRNA level. The AP-1 and ENKCRE-2 DNA binding activities were also increased by KA and were attenuated by CHX. We found that KA-induced AP-1 and ENKCRE-2 DNA binding activities were diminished by the antibodies against c-Fos, 35 kDa-Fra, and c-Jun proteins. Furthermore, the cross-competition studies revealed that AP-1 proteins actively participated in ENKCRE-2 DNA domain. In addition, CREB antibody attenuated KA-induced ENKCRE-2 DNA binding activity. The results imply that newly synthesized c-Fos, 35 kDa-Fra, and c-Jun proteins, which bind to AP-1 as well as ENKCRE-2 domain, may play important roles in the regulation of proENK mRNA expression induced by KA in rat hippocampus (supported by grant 95-0403-19-01-3 from KOSEF).

OPIOIDS: ANATOMY, PHYSIOLOGY, AND BEHAVIOR—ANATOMY

519.1

PROTEIN KINASE C ACTIVATOR PDBu BLOCKS THE INCREASE OF GTPase ACTIVITY INDUCED BY δ-OPIOID RECEPTOR AGONIST AND δ-RECEPTOR BINDINGS IN THE MOUSE SPINAL CORD. M. Narita[1], M. Narita[1], H. Mizoguchi[1], R. M. Quock[2], and L. F. Tseng[1]. [1]Dept. of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226, [2]Dept. of Biomedical Sciences, University of Illinois College of Medicine, Rockford, IL 61107.

We found in the previous study that intrathecal (i.t.) administration of a protein kinase C (PKC) activator, phorbol 12,13-dibutyrate (PDBu), dose-dependently attenuated the antinociception induced by i.t.-administered δ-opioid receptor agonist. The present study was then designed to investigate the effect of PDBu on the increase of high affinity GTPase activity induced by a selective δ-opioid receptor agonist, [D-Ala^2]deltorphin II, and δ-opioid receptor bindings in the spinal cord of ICR mice. In GTPase assay, suspensions of the spinal membrane were incubated with [^{32}P]GTP and D-Ala^2]deltorphin II in the presence or absence of PDBu. Low K_m GTP hydrolysis in the spinal cord was stimulated in a concentration-dependent manner by [D-Ala^2]deltorphin II (0.1 to 1 μM). This effect was completely blocked by co-incubation with a selective δ-opioid receptor antagonist, NTB (0.1 μM). PDBu (0.1 to 10 μM), which had no effect on basal GTPase activity, dose-dependently attenuated the increase of low K_m GTP hydrolysis induced by [D-Ala^2]deltorphin II (1 μM). In the receptor binding experiment, incubation of the spinal membrane with PDBu (1 μM) caused approximately 20 % inhibition of both the [^{3}H]DSLET (δ-agonist) and [^{3}H]NTI (δ-antagonist) bindings. On the other hand, DSLET (10 μM) did not interfere acutely with [^{3}H]PDBu binding, indicating that PDBu did not cause a simple displacement of δ-ligands on spinal membranes. These findings suggest the possibility that the down-regulation of δ-opioid receptor induced by the activation of PKC by PDBu is involved in the PDBu-induced desensitization of δ-opioid receptors in the mouse spinal cord (Supported by NIH grant, DA 03811).

519.2

THE SPATIAL RELATIONSHIP BETWEEN PREPRODYNORPHIN IMMUNOREACTIVITY AND KOR1 IMMUNOREACTIVITY IN GUINEA PIG BRAINSTEM S. Shuster[1-2] M. Riedl[2], L. Vulchanova[1-2], J. Wang[2], and R. Elde*, [1-2]. [1]Graduate Program in Neuroscience, [2]Department of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

A complementary spatial relationship between a receptor and its putative endogenous ligand is one important criterion in conferring a biological interaction *in vivo*. For the kappa opioid receptor and its putative ligand, dynorphin, this relationship has been inferred, for example, by comparing receptor autoradiography and *in situ* hybridization with dynorphin immunohistochemistry. In order to elucidate their spatial relationship with greater resolution, antisera were raised to the C-terminus of preprodynorphin (ppDYN) and kappa opioid receptor (KOR1, previously described). The specificity of the preprodynorphin was determined by absorption control with its cognate peptide and by simultaneous two color immunofluorescence microscopy with anti-dynorphin A (1-8). Double labeled sections of guinea pig brainstem, imaged using confocal laser microscopy, revealed both ppDYN immunoreactivity (-ir) and KOR1-ir in many areas including periaqueductal gray, ventral tegmental area, and various n. of the trigeminal complex. The degree of overlap of ppDYN- and KOR1-ir was variable within a given region. This relationship also held true at higher magnification. In some instances ppDYN-ir was found in close apposition to KOR-ir elements. In addition, instances in which ppDYN-ir was not immediately adjoined by KOR1-ir were also evident. Supported by grants from NIDA.

519.3

ACUTE AND CHRONIC EFFECTS OF INTRAPERITONEAL (IP) MORPHINE SULFATE (MS) ON C-FOS EXPRESSION IN THE NUCLEUS OF THE SOLITARY TRACT (NTS) AND AREA POSTREMA R.D. Hofbauer, E.R. Stevens, A.M. Marshall, D.A. Simone, B.K. Hartman, and P.L. Faris*. Div. of Neuroscience Research in Psychiatry, Univ. of Minnesota, Mpls, MN 55455

The present study was designed to determine if an analgesic dose of MS, reported to induce analgesia via both peripheral vagal and central mechanisms, activates a discrete population of NTS/AP neurons. Rats (n=3) were injected IP with 0 or 10 mg/kg MS. One hour later, animals were sacrificed for subsequent immunohistochemical visualization of the proto-oncogene c-fos using a commercially available antibody (Cambridge). Sections at five levels of the NTS/AP were digitally mapped to numerically quantify and anatomically localize the populations of activated neurons. C-fos positive nuclei were discretely localized to the commissural and medial subdivisions of the NTS. Immunoreactive neurons were also present in AP, but to a lesser extent. The second experiment sought to replicate and extend this finding by determining the effect of morphine tolerance on c-fos activation by IP morphine. Three animals were rendered tolerant to the analgesic effects of morphine (determined by tail-flick latencies) by twice daily injections of 10 mg/kg MS. Three additional animals received nociceptive testing and twice daily saline injections. One hour prior to sacrifice all six animals received a challenge dose of 10 mg/kg IP. The induction of prior morphine tolerance was accompanied by a marked reduction in c-fos compared to the non-tolerant control group. The involvement of these morphine-activated neurons in mediating analgesia is yet to be determined. Supported by NS31223 and MH47189.

519.4

NOCICEPTIN CAN MODULATE EVOKED ENKEPHALIN RELEASE FROM THE MYENTERIC PLEXUS. A.R. Gintzler*, I.D. Adapa, L.Toll, V.M. Medina and L. Wang. SUNY Health Sciences Center at Brooklyn, Brooklyn N.Y. 11203 And SRI International, Menlo Park, CA, 94025.

Nociceptin is an endogenous peptide agonist for the ORL_1 receptor that bears striking homology to opiate receptors. Initial reports claimed that this peptide had hypoalgesic effects following i.c.v. or i.t. administration. The present study demonstrates that in the absence of opiate receptor blockade, nociceptin can substantially reduce the magnitude of the stimulated release of methionine-enkephalin from the guinea pig myenteric plexus. Nociceptin has low to moderate affinity for opiate receptors (μ > k > d) which, under appropriate conditions, should be sufficient to permit interactions with multiple opiate receptor types. Indeed, the nociceptin dose response curve for inhibition of enkephalin release is substantially altered by naloxone. Following opiate receptor blockade, inhibition of enkephalin release is observed in response to I and 10 nM but a facilitation of release is manifest at higher concentrations of nociceptin. Dose responsiveness for nociceptin modulation of enkephalin release is complicated by the fact that this peptide modulates enkephalin release via naloxone-resistant and naloxone-sensitive receptors, both of which exhibit a concentration-dependent bimodality (albeit in opposite directions). Thus, determination of i.c.v. or i.t. nociceptin dose-responsiveness over several orders of magnitude is suggested before concluding the effect of this peptide on pain thresholds

519.5

ORPHANIN FQ/NOCICEPTIN-IMMUNOREACTIVITY IN THE CNS OF ADULT RATS AND ITS RELATIONSHIP TO ENKEPHALIN- AND DYNORPHIN-IMMUNOREACTIVITY. M. Riedl*, S. Shuster, L. Vulchanova, T. Olson, J. Wang, and R. Elde. Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

Recently, two laboratories identified a 17 amino acid peptide that is a likely ligand for an orphan opioid receptor. Termed orphanin FQ or nociceptin, this endogenous peptide is similar in structure to dynorphin A yet lacks the canonical Tyr-Gly-Gly-Phe of other opioid ligands. This study examined some of the distributional similarities between orphanin FQ/nociceptin and two other endogenous opioid ligands, dynorphin and enkephalin, using multicolor immunofluorescent techniques. Antisera raised in both rabbits and guinea pigs revealed prominent orphanin FQ/nociceptin immunoreactivity (-ir) in peptidergic-like fibers in spinal cord dorsal horn, lateral spinal n., dorsal gray commissure, n. of the trigeminal complex, n. of the solitary tract, periaqueductal gray, dorsal raphe n., lateral habenula, paraventricular n. of hypothalamus, various amygdaloid n., lateral septum, bed n. of stria terminalis, and endopyriform n. While tissue from normal animals revealed very little immunoreactivity contained in cell bodies, colchicine treatment resulted in dense staining of vesicle-like structures within cell bodies. As is true for dynorphin- and enkephalin-ir, dorsal rhizotomy did not greatly reduce the staining in spinal cord dorsal horn, suggesting intraspinal or supraspinal synthesis of this peptide. In fact, the distribution of orphanin FQ/nociceptin-ir closely paralleled that of dynorphin- and enkephalin-ir in the spinal cord yet instances of colocalization were rare. In other regions of the CNS, the distribution of these three peptides was more widely disparate. Supported by grants from NIDA.

519.6

MOLECULAR CLONING AND CHARACTERIZATION OF *N23K/N27K* (NOCICEPTIN/ORPHANIN FQ GENE) AS DEVELOPMENTALLY REGULATED MOLECULE. Y. Saito*, K. Maruyama ☆, T.C. Saido, S. Kawashima Dept. Molec. Biol., Tokyo Metropol. Inst. Med. Sci., Bunkyo-ku, Tokyo-113, Japan. ☆Lab. Neurochem., National Inst. Physiological Sci., Okazaki, Aichi-444, Japan.

We used a subtraction cloning approach to isolate cDNAs of *N23K* and its splicing form, *N27K*, whose mRNA and protein are transiently increased during both neuronal differentiation in mouse NS20Y cells and development in mouse brain. Unexpectedly, the amino acids sequence of the *N23K/N27K* are identical to that of the newly discovered rat nociceptin/orphaninFQ precursor in its C-terminal region, although the rat nociceptin precursor cDNA sequence is incomplete at the 5'-terminus. Using antibodies against each C-terminal peptide of the *N23K/N27K* proteins, a punctate structure in the perinuclear region and areas near the tip of neurites is visualized in neurally differentiating NS20Y cells. The time of maximal expression correlates with periods of neurite extension, and those expression decrease as the neuritic network develops. This redistribution suggests that the *N23K/N27K* protein function not only as neuropeptide precursors but also as important components in a process in neurite outgrowth and nervous system development. (Supported by the Ministry of Education, Science and Culture of Japan to Y.S. and by Uehara Memorial Foundation to Y.S).

519.7

LONG TERM EFFECTS OF KAINIC ACID-INDUCED SEIZURES ON THE EXPRESSION OF OPIOID PEPTIDES AND AP-1 TRANSCRIPTION FACTORS: I. MORPHOLOGICAL STUDIES. J. Simpson*+, G. Bing, Z. Feng, B. Wilson and J. Hong. Neuropharmacology Section, Systems Toxicology Branch, NIEHS/NIH, Research Triangle Park, NC 27709, +Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC.

Although the acute effects of kainic acid (KA) on the expression of various members of the AP-1 transcription factor family and the opioid peptides, dynorphin (DYN) and enkephalin (ENK), have been well characterized, little is known about the long-term effects of KA on these transcription factors and opioid peptides. In hippocampal sections of male Wistar 344 rats at progressive time points following KA administration (7.25 mg/kg), we examined (1) the extent of KA-induced neuronal damage (Nissl stain); (2) the development of KA-induced mossy fiber sprouting using the neo-Timm stain for zinc and immunocytochemistry for DYN and ENK; (3) the level of proenkephalin (PENK) mRNA using *in situ* hybridization histochemistry; and (4) the expression of KA-induced AP-1 transcription factors using immunocytochemistry for c-Fos, Fos B, FRA, c-Jun, Jun B, and Jun D. KA induced a profound loss of hilar interneurons and CA1/CA3 pyramidal neurons. Sprouting of opioid peptide-containing mossy fibers into the inner molecular layer of the dentate was observed as early as 1-2 w following KA and continued to increase in density and width in the inner molecular layer over time. In addition, the level of mossy fiber ENK-IR was elevated at all time points following KA. The progression of mossy fiber sprouting was accompanied by a second, prolonged induction of PENK mRNA beginning 2-3 w after KA. Whereas KA caused a rapid and transient induction of hippocampal c-Fos, Fos B, c-Jun, and Jun B, a prolonged induction of FRA and Jun D was observed in the dentate granule cells up to 1 year following KA injection. These results indicate that KA causes a prolonged alteration in hippocampal opioid peptide distribution. The development and maintenance of mossy fiber sprouting and/or the prolonged alteration of hippocampal ENK following KA injection may be mediated by the long-term increase in an AP-1 complex containing FRA and Jun D. Supported in part by NS 07166.

519.8

LONG-TERM EFFECTS OF KAINIC ACID-INDUCED SEIZURES ON THE EXPRESSION OF OPIOID PEPTIDES AND AP-1 TRANSCRIPTION FACTORS. II. BIOCHEMICAL STUDIES Z. Feng*, W. Zhang#, G. Bing, J. Simpson+, P. Hudson, and J. Hong. Neuropharmacology Section, System Toxicology Branch, NIEHS/NIH, Research Triangle Park, NC 27709, #Department of Physiology, DaLian Medical University, DaLian, P.R. China, +Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599,

Studies from our laboratory have shown that kainic acid (KA) injection elicits time-dependent increases in proenkephalin mRNA in the hippocampus. The initial increases in mRNA levels peaked at around 3-6 h and then returned to control levels (short-term increase), and the second phase of increases began at 2-3 weeks after KA injection and lasted for up to at least 5 months (long-term increase). The short-term increases in proenkephalin mRNA levels were closely associated with an increase in AP-1 DNA-binding activity. However, the specific transcription factors regulating the long-term increase in opioid peptide expression are not clear. To determine the possible involvement of AP-1 transcription factors in the regulation of the long-term increases in proenkephalin mRNA levels and in the formation of sprouting, gel-retardation assay, Western blot assays and antisense oligonucleotides were used after systemic injection of KA. The results showed that two AP-1 complexes, AP-1-Upper complex and AP-1-Lower complex occur during the acute stage (0-24 h after KA treatment). However, only the AP-1-Lower complex is seen during the long-term stage (from 3 days to several months after KA treatment). The long-term increase in AP-1 DNA-biding activity is associated with the long-term increases of proenkephalin mRNA and last for at least 5 months. Also, the 35-37-kDa Fra and 28-kDa Jun-D proteins were induced and expressed at high levels. These results suggest that these AP-1 transcription factors play an important role in the regulation of increase in proenkephelin mRNA and the development of mossy fiber sprouting.

519.9

EXPRESSION OF Δ FOSB IN THE RAT HIPPOCAMPUS AND STRIATUM AFTER SYSTEMIC ADMINISTRATION OF KAINIC ACID. G. Bing*, W. Wang, Q. Qi, Z. Feng, P. Hudson, L. Jin, and J. Hong. Neuropharmacology Section, Systems Toxicology Branch, NIEHS/NIH, Research Triangle Park, NC 27709.

Systemic administration of kainic acid (KA), an analog of glutamic acid, causes limbic seizures and pathophysiological changes in adult rats that are very similar to human temporal lobe epilepsy. One of the earliest changes in gene expression after treatment with KA is the induction of immediate-early genes. The *fos* and *jun* families are frequently studied immediate-early genes which are induced by KA. Several groups, including ours, have recently reported that a 35-kDa Fos-related antigen (FRA) is induced for a protracted time by various stimuli. It has been suggested that this FRA is Δ FosB which has a molecular weight around 35-kDa. The present study characterizes the long-term expression of FRA and Δ FosB after systemic treatment with KA. Immunocytochemistry and Western blot analysis using an antibody that cross-reacts with all known FRAs showed that a 35-kDa FRA was induced at high levels in both the hippocampus and striatum for up to 1 month by KA. A semi-quantitative PCR analysis showed that Δ FosB was induced by KA, but its expression lasted for only 6 hours. This result was also verified by Northern blot analysis. These results suggested that the long-term elevated 35-kDa FRA seen with Western blot analysis and immunocytochemistry is a new species of the FRA and not Δ fosB. In addition, this study also indicates that seizure activities induced Δ FosB, and not FosB as reported in earlier studies. The long-term expression of FRA in both the hippocampus and striatum may be associated with the pathophysiological changes after KA administration.

519.10

STRESS-INDUCED ALTERATIONS IN THE OPIOIDS CONCENTRATION AND PREPROENKEPHALIN mRNA EXPRESSING CELLS IN THE LAMB BRAIN. K. Pierzchała-Koziec* and M. Dziedzicka-Wasylewska[1]. Dept. Anim. Physiol. Agric. Univ. Cracow, Poland, [1]Polish Acad. Sci. Cracow, Poland.

The biosynthesis and release of the enkephalins can be modified with different environmental and experimental procedures. The localization of enkephalins mRNA is prerequisite to investigate their expression and regulation. Therefore, as a part of study dealing with the opioids importance in sheep growth and development, the present experiment was carried out to investigate the effects of stress on the Met-enkephalin (irMET) concentration and the proenkephalin synthesis at the cellular level in the several areas of ram and ewe brains. Animal studies were performed on 3 months old sheep divided into control and stressed by 30 min of isolation group. Brains were removed within 5 min, dissected to obtain the blocks from hypothalamus, hippocampus, striatum, nucleus accumbens, cortex, cerebellum, pons, pituitary, pineal and infundibulum. Small fragments of the areas were taken for radioimmunoassay of immunoreactive Met-enkephalin. The mRNA expression for preproenkephalin was measured by in situ hybridization according to Young et al. (1986). After 28 days of exposure, quantitative analysis of autoradiographic film with an image system show the highest degree of mRNA expressing cells in pituitary followed by nucleus accumbens and pineal. Much lower optical density was noticed in the cortex. The concentration of irMET varies fom 2.4 ± 0.19 in cortex to 112.4 ± 11.2 pmol/g in infundibulum in ewes and from 3.4 ± 0.31 in cerebellum to 110.3 ± 11.9 pmol/g in pineal in rams. Of considerable interest, isolation potentiated the synthesis of proenkephalin in all tested areas by 15 - 70% and altered the irMET in several fragments. Unexpectedly, there were clear sex differences in the localization of mRNA, concentration of irMET and stress responses. Thus, the synthesis of preproenkephalin appears to be sex and stress dependent and dissociated from the releasing and processing in some brain areas. Supported by KBN Grant No: 052/PO6/95-08.

519.11

Ultrastructural relationship between neurons containing leu-enkephalin and GABA labeling in the ventromedial nucleus of the rat hypothalamus. K.G. Commons*[1], T.A. Milner[2] and D.W. Pfaff[1] [1]Lab. of Neurobiology and Behavior, Rockefeller U., and [2]Cornell U.M.C. NY, NY 10021.

The ventromedial nucleus of the hypothalamus (VMN) is thought to regulate a number of neuroendocrine and behavioral functions. The signaling molecules enkephalin and GABA are abundant in parts of VMN, and, in other brain regions, are known to commonly interact with each other. Thus, we sought to determine relationship between leu-enkephalin (enk) and GABA containing neurons in both the ventrolateral (vl) and dorsomedial (dm) subnuclei of VMN in male rats by dual labeling immunohistochemistry and electron microscopy. Enk antisera raised in rabbit localized with the immunoperoxidase method, was used in combination with a GABA antisera raised in rat detected with the immunogold-silver technique. Enk-labeled terminals were abundant and similar between the two subdivisions of VMN. 85-90% of the synapses formed by enk-containing terminals were symmetric (inhibitory type) (41/48 in vl; 19/22 in dm), and ~10% of enk containing terminals also contained GABA-labeling (22/173 in vl; 8/69 in dm). Enk-GABA double labeled terminals accounted for one quarter to a third of all GABA terminals (22/66 in vl; 8/32 in dm). A few dendrites (7) and somata (7) also contained enk labeling; however they were only found in the vl subnucleus of VMN. In single section analysis, enk-labeled dendrites or soma received few synaptic contacts; however many were from GABA-labeled terminals. In both subdivisions of VMN, GABA-labeled dendrites were found, many of which were contacted by enk-containing terminals. These data suggest that there are many possible mechanisms through which neurons containing enk and GABA may interact, and some of these may involve a nested series of inhibitory circuits. Supported by: DK07313-18 (KC) and DA08259 (TM).

519.12

LOCALIZATION OF α-N-ACETYL-β-ENDORPHIN IMMUNOREACTIVITY AND PRO-OPIOMELANOCORTIN mRNA IN CARDIOVASCULAR NUCLEI OF RAT AND HUMAN BRAIN. D.W. Rosenthal[1], A.B. Manning[1], G.E. Resch[1]*, M.J. Manalo[2] and W.R. Millington.[1] [1]Division of Molecular Biology and Biochemistry and [2]Department of Anesthesiology, University of Missouri-Kansas City, Kansas City, MO 64108.

β-Endorphin (β-End), a pro-opiomelanocortin (POMC)-derived opioid peptide, is synthesized by neurons in the hypothalamic arcuate nucleus (ArcN) and the nucleus of the solitary tract (nTS) which project to cardiovascular regulatory sites in the medulla. Chromatographic studies have shown that brainstem β-End is predominantly α-N-acetylated, a modification that abolishes β-End's bradycardic and hypotensive effects. Whether brainstem β-End is α-N-acetylated by POMC neurons in the ArcN, nTS or both nuclei is unknown, however. In the present study, we examined the localization of β-End and α-N-acetyl-β-End immunoreactivity (IR) in rat brain and assessed whether the POMC gene is expressed in the nTS of the human brain, as it is in rat. Consecutive tissue sections were incubated with two antisera: one that recognizes all β-End peptides equally and a second that selectively detects α-N-acetyl-β-End derivatives. Consistent with previous studies, β-End IR axons were detected in the ArcN, nTS and throughout the brainstem and subcortical forebrain structures. α-N-Acetyl-β-End IR was largely restricted to the nTS and ventrolateral medulla, although a small number of α-N-acetyl-β-End IR fibers was also detected in the ArcN and nucleus accumbens. β-End, but not α-N-acetyl-β-End, IR axons were present in the midbrain which contains caudal projections from the ArcN, but not nTS, POMC neurons indicating brainstem α-N-acetyl-β-End IR is derived from the nTS and not the ArcN. Reverse transcriptase PCR, using specific primers for the rat and human POMC gene, confirmed that POMC mRNA is present in rat nTS and further demonstrated that POMC mRNA is localized in the human nTS although the transcript size was larger than the rat gene product. These data provide evidence that brainstem α-N-acetyl-β-End is derived from the nTS, but not ArcN, POMC neurons. (NIDA DA-04598 and St. Luke's Foundation)

519.13

CIRCADIAN RHYTHM RELEASE OF IR-OPIOID PEPTIDES AND IR-SYNENKEPHALIN IN THE AMYGDALA OF PENTYLENETETRAZOL-KINDLED RATS. **M. Asai, G. Matamoros-Trejo, M. Zubieta, G. Linares, P. Agustin, and F. Antón-Tay*. Instituto Mexicano de Psiquiatría. *Universidad Autónoma Metropolitana. México**

Epileptiform activity has been reported to activate the Endogenous Opioid System (EOS). Release of opioid peptides after seizure, has been associated with a protective action against another seizure. Several studies have shown that both, the opiate receptors and the enkephalin tissue content, displayed a circadian rhythm. Their maximal values are observed during the dark phase, when higher sensitivity to pain stimulus and epileptiform activity occurs. In the present study, we analyzed around the clock, the release and concentration of Immunoreactive (IR)-opioid peptides and of IR-Synenekaphalin (the non-opioid portion of Proenkephalin) in the amygdala of pentylenetetrazol (PTZ) kindled rats. Animals were housed with 12 h-light: 12 h-dark cycles, the dark period started at 18:00 h. PTZ-kindling was induced in male Wistar rats (220-260 g) by daily i.p. injection of 35 mg/kg of the convulsant agent. Measurements were conducted every 4 h. Opioids IR-(Met, Leu, MERF, MERGL, and Syn) tissue concentration and release were assessed by RIA in both, amygdala slices of PTZ-kindled rats and sham controls. The results present herein show that, there were increased both concentration and release of all opioid peptides, measured in PTZ-rats from 16:00 h until 04:00 h. IR-Syn was only detected at 16:00 and 20:00 h. At 16:00 h the opioid peptides with IR-Syn release and their tissue content increased. Our results show that besides the circadian rhythmicity described on both, opiate receptors and enkephalin tissue content, a daily rhythm in IR-opioid peptides and synenkephalin release occurs. Thus it is suggested that EOS is activated before the dark phase.

519.14

RELEASE OF DYNORPHIN A IN THE RAT PERIAQUEDUCTAL GRAY (PAG) AFTER NOXIOUS COLD STIMULATION. L. Xin, E. B. Geller,* M. R. McCaffery, G. H. Sterling and M. W. Adler. Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140

We reported previously that noxious cold stimulation to the rat's tail caused an increased release of substance P (SP) from the PAG and that either noxious cold or PAG microinjection of a high dose of SP increased the release of the endogenous opioid peptide ß-endorphin (ß-E) from the PAG. These findings indicated that endogenous ß-E was involved in supraspinal SP antinociception during noxious cold. Since the results from our lab also showed that pretreatment with either μ- or κ-opioid receptor agonists suppressed the SP release during noxious cold, we investigated whether the endogenous κ-opioid peptide dynorphin A (Dyn) could be released after noxious cold. Artificial CSF was microdialyzed into the PAG of freely moving S-D rats. Samples were collected every 30 min for 4 hrs and analyzed for Dyn level by radioimmunoassay. The rat's tail was immersed in a ~10 °C water bath 30 sec/min for 5 min. Baseline release of Dyn in the PAG was 0.2-0.6 fmol/fraction. Noxious cold stimulation induced a 60% - 130% increase in Dyn release over baseline, occurring mostly in the samples collected 30 and 60 min after cold stimulation. In contrast, warm water (35 °C) stimulation to rat's tail did not cause any significant change in Dyn release. These data indicate that endogenous Dyn in the PAG may also be involved in supraspinal SP antinociception during noxious cold. (Supported by NIH grant DA 00376 from NIDA)

519.15

CHANGES IN THE ANATOMICAL DISTRIBUTION OF OPIOID PEPTIDES, PROLACTIN AND ACTH IN HYPOPHYSIS OF AXOLOTL, *Ambystoma mexicanum* DURING METAMORPHOSIS. M. León-Olea, M. Sánchez-Alvarez, E. Sánchez-Islas, C. Clapp* and F. Pellicer. Lab. de Histología, Instituto Mexicano de Psiquiatría. Av. Mex. Xochimilco 101, Mex. D.F. 14370.

The study of opioid peptides is relevant in neurosecretory and hormonal processes related to ontogeny. Therefore, we used the amphibian *Ambystoma mexicanum*, since its different developmental stages can be manipulated. Metamorphosis was experimentally induced by a unique intraperitoneal injection of T4 (2.5μg/g). Histological and immunohistochemical techniques were carried out to study the anatomical distribution of opioid peptides, prolactine and ACTH in hypophysis of animals at different stages of metamorphosis. Immunoreactivity (IR) to enkephalins (LE and ME) in cells of intermediate and anterior hypophysial lobes presented changes that comprehend its absence in stage I, an important increase in its intensity in stage II, followed by its disappearance in stages III and IV. The IR of other opioid peptides, PRL and ACTH included in this study did not present significant changes during metamorphosis. The existence of IR to opioid peptides in neurons of anterior and intermediate hypophysial lobes, as well as in fibers of median eminence and neural lobe, and the changes in IR intensity of these peptides during metamorphosis suggest a paracrine or endocrine modulatory physiological role of these substances during maturation and developmental processes involved in metamorphosis. Financed by IMP projects 3290 and 3290a, and CONACyT 1183-N9203.

519.16

NEUROANATOMICAL DISTRIBUTION AND REGULATION ACROSS PREGNANCY OF THE mRNA FOR THE LONG FORM OF THE PROLACTIN RECEPTOR IN RAT. J.C. Bakowska and J.I. Morrell[1]*, IAB, [1]CMBN, Rutgers University, Newark, NJ 07102.

Prolactin by acting on its own receptors in the brain promotes many behavioral and neuroendocrine changes, including the induction of maternal behavior. Prolactin receptors exist in two forms, a short and a long form. Others have demonstrated the regional distribution in the brain of the mRNA of both forms of the receptor (Chiu&Wise, J.Neuroend, '94). Analysis of brain homogenates reveals that only levels of the long form varies across pregnancy (Sugiyama et al., Endo, '94). As part of our ongoing investigation of the neurochemical basis of maternal behavior, we have examined the neuroanatomical distribution of the mRNA from which the long form of the prolactin receptor (LPRL-R mRNA) is translated, and examined its regulation across pregnancy. A 290-basepair ^{33}P cRNA probe, complementary to a cytoplasmic domain of the long form of the prolactin receptor mRNA, was synthesized using T3 polymerase. Following *in situ* hybridization procedures, the slides were coated with Kodak emulsion. Large populations of neurons expressing the LPRL-R mRNA were found in the bed nucleus of the stria terminalis, the medial preoptic area, the supraoptic, paraventricular, the ventromedial, and the arcuate nuclei of the hypothalamus as well as in the medial and central nuclei of amygdala. Overall these nuclei contained more neurons that expressed the prolactin receptor and had higher levels of expression per neuron in brains from females on day 21 of pregnancy than on day 2. For example, relative levels of LPRL-R mRNA in the medial preoptic area were four times higher on day 21 of pregnancy compared to day 2. *(Supported by HD 22983 to J.I.M).*

519.17

REPRODUCTIVE EXPERIENCE DIFFERENTIALLY ALTERS PROOPIOMELANOCORTIN (POMC) GENE EXPRESSION IN THE MEDIAL BASAL HYPOTHALAMUS (MBH) OF RATS DURING PREGNANCY AND LACTATION, P.E. Mann*, B.S. Rubin, and R.S. Bridges. Dept. Comp. Med., Tufts Univ. Sch. of Vet. Med., N. Grafton, MA 01536 and Dept. Anat. Cell. Biol., Tufts Univ. Sch. Med., Boston, MA 02111

In situ hybridization (ISH) was used to determine if pregnancy (PREG) and lactation (LACT) affect MBH POMC gene expression. In addition, the effects of reproductive experience on POMC mRNA were evaluated. POMC mRNA levels in the MBH were assessed during PREG (days 7, 12, and 21) and LACT (day 12) in rats with and without prior reproductive experience and compared with age-matched, cycling, nulliparous females. ISH was performed using a digoxigenin-labelled riboprobe complementary to 837 base pairs of the POMC gene. The number of cells expressing POMC mRNA in the MBH decreased in primiparous rats on day 12 of LACT when compared with nulliparous rats in diestrus. In addition, POMC cell number in multigravid rats was significantly less than in the nulliparous group on days 7 and 21 of PREG, and day 12 of LACT. Reproductive experience affected the number of POMC mRNA positive cells; there were fewer cells expressing POMC mRNA in the multigravid females on day 7 of PREG and an increase in the number of POMC cells in the multiparous group on day 12 of LACT compared to the primiparous rats. Optical density analysis revealed an increase in reaction product in individual cells on all days of PREG and a decrease on day 12 of LACT in the multiparous group. The results of the present study indicate that POMC gene expression changes across pregnancy and lactation and that repeated reproductive experience has long-term, possibly permanent, effects on the endogenous opioid system. (NSF [IBN 94-07305] to PEM and a NIH grant [HD29782] to RSB)

519.18

GONADAL STEROIDS REGULATE ENKEPHALIN IN SOCIAL BEHAVIOR CIRCUITRY IN THE MALE SYRIAN HAMSTER A. G. Holt* and S. W. Newman Dept. of Anatomy and Cell Biology and Neuroscience Prog., Univ. of Michigan, Ann Arbor, MI

In the male Syrian hamster, enkephalin is distributed throughout neural pathways that control aggression and mating, and both behaviors are dependent upon gonadal steroids in this species. To test the hypothesis that sex steroids regulate enkephalin production, each of twenty male Syrian hamsters was castrated and was immediately implanted subcutaneously with silastic capsules (Dow Corning; 3.18 mm ID, 1.98 mm OD) . The capsule contained either 10mm of crystalline testosterone (T) (Sigma; n=5), 2mm of crystalline estradiol-17β (E) (Steraloids Inc.; n=5), or 5mm crystalline dihydrotestosterone (DHT), a non aromatizable androgen, (Sigma; n=5) or nothing, (n=5). An additional five intact animals were implanted with empty silastic capsules. After twelve weeks all animals were injected intracerebroventricularly with 200μg of colchicine in 2μl of .9% saline and 48 hours later were perfused transcardially with 4% paraformaldehyde. The brains were removed and 40μm sections were immunostained using a met enkephalin antibody from Incstar Inc. Enkephalin immunoreactivity (Enk-ir) was visualized using the avidin biotin technique (Vectastain Elite, Vector Labs). Analysis of variance indicates that castration, is significantly reduced in the medial septum (MS), anterior dorsal preoptic nucleus (ADPN), horizontal limb of the nucleus of the diagonal band (HLNDB), anterior medial bed nucleus of the stria terminalis(BNSTam), anterior lateral BNST (BNSTal), posterior medial BNST(BNSTpm), posterior intermediate BNST (BNSTpi), medial preoptic nucleus (MPN), anterior nucleus (AH), paraventricular nucleus (PVN), and ventromedial nucleus (VMH) of the hypothalamus. Some of these areas maintain enkephalin production after implantation of only T, (BNSTpi, BNSTpm, MPN), or E (AH, PVN), while in others T and E were equally effective (HLNDB, BNSTam, VMH), and in (MS, ADPN, BNSTal), T, E, and DHT were all effective. These results suggest that Enk productions regulated by gonadal steroids within circuitry underlying social behaviors in the male hamster and that this production is differentially regulated by testosterone and its metabolites. (Supported by NIH GM13553 and NS20629)

OPIOIDS: ANATOMY, PHYSIOLOGY, AND BEHAVIOR—PHYSIOLOGY

520.1

OPIOID SIGNALING: INVOLVMENT OF KINASE CASCADES. HB Gutstein*, EA Rubie, A Mansour, H Akil, JR Woodgett. Dept. Anesth. and MHRI, U. of Michigan, and Ontario Cancer Institute, Toronto, ON Canada

The signal transduction mechanisms that opiates use to mediate cellular adaptive responses are not clear. The MAP kinase cascade is important in transducing proliferative signals. Recently, homologous kinase cascades have been discovered (SAPK and p38/RK) that relay information about events that alter cellular homeostasis. Our aim was to determine whether opioid effects on cellular function could be mediated by these cascades.

C6 glioma cells stably expressing mu, delta and kappa opiate receptors were generated. Cells were stimulated with DAMGO, DPDPE, U50,488, and/or naloxone and lysed. Cascade activation was measured either by kinase assay using c-jun or MBP as substrates, or by western blotting with anti-phospo MAPK or MKK3 (RK pathway). After SDS-PAGE electrophoresis, gels were analyzed using Image Quant software, while blots were developed by ECL.

Mu and delta opiates activated MAPK as strongly as the positive control PMA, while kappa showed moderate activation by western blotting. No significant SAPK or RK cascade activation was observed following opiate agonist treatment in any of the C6 cell lines expressing the 3 opioid receptors.

This study provides evidence that mu opiate effects may be mediated in part by the MAP kinase cascade. These cascades function to link extracellular signals to cellular changes by regulating expression of target genes. The reasons for differential MAPK activation by opiate receptor subtypes in C6 cells are not clear. Further studies are underway to determine how the opiate receptor interacts with the MAPK cascade. Further clarification of the mechanisms and functional significance of these interactions could have important implications for understanding mechanisms underlying analgesic responses and narcotic tolerance and dependence. Support : NIDA and MRC

520.2

THE OPIOID MODULATION OF IPSPS INDUCED IN LUMBAR MOTONEURONS BY STIMULATION OF THE NUCLEUS RETICULARIS GIGANTOCELLULARIS DURING CARBACHOL-INDUCED ACTIVE SLEEP. M.-C. Xi*, R.-H. Liu, J. Yamuy, F.R. Morales and M.H. Chase. Department of Physiology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The present study was undertaken to test the hypothesis that opioid peptides may modulate the glycinergically-mediated postsynaptic inhibition of motoneurons during active sleep. Opioid peptides have been shown to modulate synaptic transmission in cat spinal motoneurons (*Yaksh, Handb. Exp. Pharm., 104/II: 53-90, 1993*). In addition, the opioid enkephalin has been reported to selectively increase the spontaneous release of glycine in the rat striatum (*Arneric et al., Neurosci. Lett., 66:73-78, 1986*). We were therefore interested in determining whether glycinergic IPSPs, which are evoked in lumbar motoneurons by stimulation of the medullary nucleus reticularis gigantocellularis (NRGc) during active sleep or carbachol-induced active sleep, would be affected by opioid peptides. To carry out these studies, intracellular recordings were obtained from lumbar motoneurons in 4 cats anesthetized with α-chloralose, in which active sleep was induced by the microinjection of carbachol into the nucleus pontis oralis (NPO) (*López-Rodríguez et al., Brain Res., 699:201-207, 1995*). Utilizing this preparation, an opioid peptide receptor agonist and antagonist were juxtacellularly applied to the recorded cells. Electrical stimulation of the NRGc elicited large amplitude, long-latency IPSPs in lumbar motoneurons (amplitude: 1.6 ± 0.3 mV (S.E.M.); latency to onset: 29.1 ± 1.4 ms; latency to peak: 53.3 ± 1.4 ms; n = 9). Microiontophoretic applications of morphine, an opiate μ receptor agonist, reversibly increased by 23.1% the mean amplitude of these NRGc-induced IPSPs ($n = 5$; $p < 0.05$). In contrast, naloxone, a non-selective opiate receptor antagonist, reversibly reduced by 34.6% the mean amplitude of these IPSPs ($n = 7$; $p < 0.01$). Both morphine and naloxone did not have any detectable effects on either the resting membrane potential or the input resistance of the recorded motoneurons. These data indicate that opioid peptides have an amplificatory effect on NRGc-induced IPSPs during carbachol-induced active sleep. We therefore suggest that opioid peptides act as state-dependent neuromodulators to regulate inhibitory glycinergic synaptic transmission at the level of the motoneuron during active sleep.

Supported by USPHS grants MH 43362, NS 23426 and NS 09999.

520.3

OPIOID REGULATION OF SEROTONIN RELEASE FROM RAT VENTRAL SPINAL CORD J. Franck[1]*, I. Nylander[2], and A. Rosén[1] Dept of Physiology and Pharmacology[1], and Clin Neuroscience[2], Karolinska Institute, S-171 77 Stockholm, Sweden.

The effect of met-enkephalin (met-ENK) and opioid receptor antagonists on the electrically evoked release of endogenous serotonin (5-hydroxytryptamine, 5-HT) was studied in superfused slices of the rat ventral lumbar spinal cord. 5-HT was assayed by high performance liquid chromatography, using electrochemical detection. Two electrical stimulation periods (300 pulses, 30 mA, 2 ms, 2 Hz) were given with 40 min interval. Drugs were added to the medium 10 min prior to the second stimulation, and the effect of the drug calculated from the ratio of 5-HT release during each stimulation period (S2/S1 ratio). Met-ENK ($1x10^{-8}$M--$1x10^{-6}$M) reduced the evoked 5-HT release in a concentration dependent fashion. The selective δ agonist, DPDPE, concentration-dependently reduced 5-HT release whereas DAMGO, a selective μ agonist, was ineffective. The selective *k* receptor agonist, (-)-U-50488 ($1x10^{-6}$M) had no effect on the evoked 5-HT release. The inhibitory effect of met-ENK ($5x10^{-7}$M) was completely abolished by the selective δ-receptor antagonist ICI-174,864 ($1x10^{-6}$M). In contrast, neither a selective μ_1 antagonist (naloxonazine; $1x10^{-6}$M), nor a *k* antagonist (nor-binaltorphimine; $1x10^{-6}$M) had any effect on the inhibitory action of met-ENK on 5-HT release. The antagonists used had no effect on their own on evoked 5-HT release. To study the possible coexistence of 5-HT and met-ENK in the rat ventral spinal cord, the tissue concentrations of 5-HT, noradrenaline (NA) and met-ENK were measured in rats treated with the selective serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT). Two weeks after intracisternal injection of 5,7-DHT, the tissue concentration of 5-HT was reduced by 97%, whereas the concentration of NA was reduced by only 5%. The tissue concentration of met-ENK, as measured by radioimmunoassay, was not significantly reduced. The results suggest that met-ENK is present in the rat ventral spinal cord mainly in non-serotonergic nerve terminals and exerts an inhibitory action on 5-HT release via δ opioid receptors. (Supported by the Swedish Alcohol Research Fund)

520.4

EFFECT OF OPIOID RECEPTOR AGONISTS ON INTRACELLULAR CALCIUM IN DORSAL HORN NEURONS. S.D. Hocherman*, R.Cerne and M. Randic. Dept. of Veterinary Physiol. and Pharmacol., Iowa State University, Ames, IA 50011.

The synapses between primary afferents and neurons of laminae I-III in spinal cord display a considerable degree of plasticity. Both opioids and calcium have been implicated in this process in central neurons. We examined the effects of mu, delta and kappa opioid receptor agonists on the basal level of calcium ($[Ca^{2+}]_i$) and on NMDA-induced calcium transients in freshly dissociated laminae I-III neurons of the dorsal horn (DH). Fura-2 AM was loaded into the cells and calcium transients were acquired using an intensified CCD camera. A Y-tube system was used for the application of different agents. The presence of calcium transients elicited by NMDA was used as a criterion for accepting a cell for analysis.

Results: a. Basal level of calcium: In the presence of the kappa-receptor agonist U-69,593 (10 μM), an increase in $[Ca^{2+}]_i$ was observed in 48% of the cells tested (n=50). The delta-receptor agonist DPDPE (1 μM) also increased $[Ca^{2+}]_i$ in 29% of the cells (n=35); with 10 μM DPDPE, the increase in $[Ca^{2+}]_i$ was larger and was observed in 42% of the cells (n=19). The mu-receptor agonist DAMGO (10 μM) has produced an increase in 1 out of 6 cells tested.

b. NMDA-induced transients: Changes in NMDA are reported as ratio (r) between the transient produced during or immediately following opioid application and a control transient. In control runs (no opioids applied) r=1.04±.18 (SD, n=11). Both U-69,593 and DAMGO (10 μM) produced a decrease in NMDA-induced transients (r=.90±.44, n=47 for U-69,593; r=.75±.25, n=12 for DAMGO). Following the application of DPDPE (10 μM) some increase in the average response was observed (r=1.23±.57, n=11). These data and the larger SD values associated with the presence of opioids suggest that there is a subpopulation of DH neurons in which opioids modulate the basal level of calcium and calcium transients induced by NMDA in a complex manner. *Supported by the NINDS and the NSF.*

520.5

KAPPA OPIOID MEDIATED INHIBITION OF BRAINSTEM PAIN MODULATING NEURONS IN RATS *IN VITRO*. Z. Z. Pan* & H. L. Fields, Departments of Neurology & Physiology and the keck Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143.

Neurons in the nucleus raphe magnus (NRM) in the medulla are implicated in pain modulation. In the brainstem slice there are two types of NRM neurons: the primary cell and the secondary cell. Primary cells are indirectly excited by μ opioids through presynaptic inhibition of GABA transmission and functionally correspond to NRM off-cells that inhibit spinal pain transmission. Secondary cells, which correspond to NRM on-cells, are directly hyperpolarized by μ opioids and facilitate spinal nociceptive transmission. In this study using infrared video-microscopy, whole cell patch clamp recordings were made from NRM neurons in slice preparations. The κ agonist U69593 (100-300nM) produced an outward current (or a hyperpolarization) exclusively in a subpopulation of primary cells which, as shown previously, had no postsynaptic response to the μ/δ agonist met-enkaphalin (10 μM). The secondary cells that were hyperpolarized by enkaphalin were not affected by the κ agonist. The outward current induced by U69593 was selectively blocked by the κ antagonist nor-BNI (100nM) and reversed its polarity near the potassium equilibrium potential. Furthermore, increasing the extracellular potassium concentration shifted the reversal potential to less negative potentials, suggesting that the κ-mediated outward current results from an increase in a potassium conductance. In addition to the hyperpolarization, U69593 also increased a hyperpolarization-activated current (I_h) in these primary cells. The κ-mediated inhibition restricted to primary cells in the NRM indicates that κ opioids in the NRM would have a hyperalgesic effect and/or may counteract the μ opioid-mediated antinociception. Supported by the USPHS grant DA1949 and the National Headache Foundation.

520.6

ORIGIN OF LOCAL OPIOID-SENSITIVE GABAERGIC AND GLUTAMATERGIC NEURONS PROJECTING TO SEROTONERGIC NEURONS OF THE RAT DORSAL RAPHE IN VITRO. T. Jolas* and G.K. Aghajanian. Depts. of Psychiatry & Pharmacology, Yale School of Medicine, New Haven, CT 06508.

We recently reported that opioids suppress spontaneous and NMDA-induced GABAergic IPSCs and glutamatergic EPSCs in serotonergic (5-HT) neurons of the rat dorsal raphe nucleus (DRN) in brain slices. The purpose of the present study was to localize, in the slice, local opioid-sensitive GABAergic and glutamatergic cells projecting to 5-HT neurons, combining voltage-clamping of 5-HT neurons at -90 mV, with KCl (2 M) electrodes, and focal iontophoretic application of NMDA. After establishing that bath perfusion of NMDA (20 μM) induced PSCs in a 5-HT neuron, focal 30 sec iontophoretic applications of NMDA (10 nA) were given at different positions of the slice until the PSC induction could be reproduced in the 5-HT neuron. The iontophoretic pipette was considered to be at the site of the cell projecting to the 5-HT neuron when PSCs frequency increased within 10 sec. The nature of the projecting neuron was determined by the ability of the AMPA antagonist LY 293558 (3 μM) or the $GABA_A$ antagonist bicuculline (30 μM) to block the NMDA-induced response. Opioid-sensitive glutamatergic cells projecting to 5-HT neurons were found near the border of the DRN and the periaqueductal gray (PAG). Opioid-sensitive GABAergic cells projecting to 5-HT neurons were scattered in the DRN as well as in the PAG. No projecting cells were found beyond the PAG and the DRN.

These results provide evidence that GABAergic and glutamatergic neurons mediating the actions of opioids on local afferents to 5-HT neurons are in the DRN and PAG. *Supported by MH17871 & DA08227.*

520.7

FEDOTOZINE INJECTED INTRAVENOUSLY INHIBITS THE INCREASED ACTIVITY OF LOCUS COERULEUS (LC) NEURONS INDUCED BY COLORECTAL DISTENTION IN RATS. H.Kosoyan* & Y.Taché, UCLA: CURE/Digestive Diseases Research Center., VA Medical Ctr. & Dept. of Medicine & Brain Res. Inst., Los Angeles, CA 90073.

Background: Fedotozine, a newly developed peripherally acting *K*-opioid agonist, inhibits visceral pain induced by duodenal and colonic distention (Eur. J. Pharmacol., 271; 65 & 245; 1994). Several findings indicate that the pontine noradrenergic nucleus, LC, is activated by visceral stimuli including bladder and colorectal distention (Br. Res. 375: 117, 1986; Ann. N.Y. Acad. Sci., 697:173, 1994; Soc. Neurosc. 21: 90, 1995). **Aim:** To assess the influence of systemic fedotozine on repetitive colorectal distention (CRD)-induced activition of LC neuronal discharge rate. **Method:** Male SD rats (280-320g) were anesthetized with chloral hydrate (400 mg/kg, im) and implanted with an intrajugular catheter for iv (0.3 ml) injection. A catheter ending in a compliant balloon (4 cm) was inserted through the anus-rectum. The distal colon and rectum were distended by delivering into the balloon 3 or 4 ml of air over 1-3 sec for 30 sec. Extracellular single cell activity recording from the LC was performed by glass micropipette with 1.0 μm diameter tip (10 MΩ), filled with 1 M NaCl in saturated Fast Green Dye. Nerve signals were sent to a window discriminator and counted on a computer. Recording from LC neurons was assessed by established criteria of pattern of firing rate, steady rhythm and shape of the spike. At the end of each experiment recording sites were verified histologically. Maximum 3 min peak changes for CRD and max 5 min peak changes for saline and fedotozine were calculated and expressed as mean ± SEM peak changes from baseline. **Results:** In 9 rats, iv injections of vehicle and fedotozine (5 mg/kg) 8.1 ± 1.2 min after vehicle had no significant effect on LC neuronal activity (98.4 ± 3.1%, and 111.4 ± 8.3% respectively) and there was no significant increase in LC activity (111.5 ± 5.6%, 99.9 ± 2.1%, 97.1 ± 3.1% and 100.2 ± 2.7%) induced by 3 repeated 3 ml CRD and a 4 ml CRD applied within 7.3 ± 0.5 min, 15.3 ± 1.8 min, 22.7 ± 2.7 min and 35.3 ± 6.4 min respectively after fedotozine injection. In 7 rats not treated with fedotozine two consecutive CRD (3 ml) induced increase LC activity to 136 ± 4.6% and 121.2 ± 2.4%, respectively ($P<0.05$). **Conclusion:** Systemic injection of fedotozine inhibits the LC neuronal activity induced by repetitive CRD (3-4ml) suggesting a role of kappa agonist in the modulation of visceral sensory stimuli activating noradrenergic A6 neurons. Supported by NIH grant DK33061.

520.8

ACTIVATION OF RAT LOCUS COERULEUS NEURONS IN OPIOID WITHDRAWAL IS REDUCED BY INHIBITION OF NITRIC OXIDE SYNTHASE *IN VIVO*. J. Pineda* and L. Ugedo. Dept. of Pharmacology, Fac. of Medicine, Univ. of the Basque Country, E-48940 Leioa, Vizcaya, Spain.

Biochemical, electrophysiological and behavioral studies have suggested that locus coeruleus (LC) nucleus plays a role in the pathophysiology of opioid withdrawal. The present study examined, using electrophysiological techniques in anesthetized rats, the effect of the nitric oxide synthase (NOS) inhibitor L-NAME (30 mg/kg) on the activation of LC neurons in opioid withdrawal. Opioid withdrawal was precipitated by administration of naloxone (5 mg/kg) in rats that had been pretreated with increasing doses of morphine (10-100 mg/kg/8 h) for five days. In control animals, LC neurons discharged spontaneously at ~2 Hz. In morphine-dependent animals, naloxone precipitated a withdrawal syndrome associated with an increase in LC cell firing rate (~4 Hz). L-NAME, which was administered 1 h before naloxone in morphine-dependent rats, markedly attenuated opioid withdrawal syndrome and reduced the elevation in firing rate of LC neurons (~2.5 Hz). In control animals, L-NAME failed to affect the LC cell firing rate. We conclude that the naloxone-precipitated activation of LC neurons in morphine-dependent rats is reduced by NOS inhibition. This effect might be responsible in part for the effect of NOS inhibitors on opioid withdrawal syndrome. (Supported by SAF96-0071. J.P. is a fellow from the Basque Government.)

520.9

INCREASED PROBABILITY OF GABA RELEASE LINKS ADENYLYL CYCLASE AND CHRONIC OPIOID TREATMENT. A.Bonci*[1,2], G.Bernardi[2] and J.T.Williams[1]. 1:Vollum Institute for Advanced Biomedical Research, Portland OR, USA. 2:Clinica Neurologica, Università di Roma "Tor Vergata" and IRCCS S. Lucia, Rome, Italy.

The Ventral Tegmental Area (VTA) play a key role in modulating the self-administration of drugs of abuse.The opioid receptors located on the VTA interneurons inhibit GABA-mediated synaptic transmission to disinhibit dopamine projection neurons. In spite of the pivotal role that the VTA plays in the maintenance of drug abuse, little is known about how this GABA synapse is affected after chronic morphine treatment. The present study show that the activation of adenylate cyclase with forskolin and the cAMP dependent protein kinase Sp-cAMPS, produce a larger increase in the GABAA IPSCs after the chronic treatment with morphine compared to controls. Similarly, the frequency of the spontaneous miniature events was increased after the chronic morphine treatment.Conversely, the kinase inhibitor staurosporin and Rp-CPT-cAMPS, decreased the GABAA IPSCs to a greater extent after drug treatment. Our results indicate that chronic morphine treatment increases the probability of GABA release and that this effect is mediated by an upregulation of the cAMP dependent cascade.

Funding source: IRCCS S.Lucia, via Ardeatina 306, 00179 Rome, Italy.

520.10

OPIOID MODULATION OF THE $GABA_B$ IPSP IN DOPAMINE NEURONS OF THE VENTRAL TEGMENTAL AREA. J.M. Delfs* and J.T. Williams. Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201.

GABAergic afferents from the nucleus accumbens (NA) synapse on dopamine neurons of the ventral tegmental area (VTA). Release of GABA from NA afferents causes a $GABA_B$-mediated IPSP in these dopamine neurons. Anatomical studies have demonstrated the presence of mu, delta and kappa opioid receptors on the GABAergic afferents to the VTA. The aim of this study was to examine the modulation of the $GABA_B$ IPSP in dopamine neurons by the mu agonist DAMGO, the kappa agonist U-69,593, and the delta agonist DPDPE. Evoked GABA synaptic potentials were recorded from dopamine neurons in horizontal slices of guinea pig midbrain using intracellular recording techniques. The $GABA_B$ IPSP was inhibited by the opioid agonists DAMGO (1nM-10μM) and U69,593 (1nM-10μM) in a dose-dependent manner by 40-60%. The delta agonist DPDPE (1nM-10μM) resulted in a slight inhibition of the $GABA_B$ IPSP (<10%) only at the highest doses. All of the opioid agonist effects were reversible with the non-selective opioid antagonist naloxone (1 μM). These results demonstrate that the $GABA_B$ IPSP in dopamine neurons of the VTA is modulated by activation of mu and kappa opioid receptors, but not delta opioid receptors. Supported by NIDA grants DA07262 & DA08163.

520.11

OPIOID MODULATION OF VENTRAL PALLIDAL RESPONSES TO ACTIVATION OF DOPAMINERGIC PROJECTIONS FROM THE VENTRAL TEGMENTAL AREA. I. Mitrovic* and T.C. Napier. Dept. of Pharmacol. and Neurosci. and Aging Inst. Division for Research on Drugs of Abuse, Loyola Univ. Chicago, Sch. of Med., Maywood, IL 60153.

The ventral tegmental area (VTA) is a major source of dopaminergic innervation to the ventral pallidum (VP). Electrical activation of the VTA elicits early-onset (latency ≤12 ms) inhibitions in the activity of VP neurons that are attenuated by microiontophoresis of dopaminergic antagonists. These results are consistent with a monosynaptic dopaminergic projection from the VTA to the VP. Microiontophoretic application of dopamine or opioid agonists alters spontaneous firing of VP neurons. Using chloral hydrate-anesthetized rats, the present study investigated the ability of iontophoretically applied μ and κ opioid agonists to modulate dopaminergic neurotransmission within the VP elicited by electrical stimulation of the VTA. VTA stimulation (1 Hz, 0.1-1.4mA, 0.1ms) evoked responses in 95% of 21 neurons tested. An early-onset inhibition was encountered in 66% of the neurons responding to VTA stimulation. Fifty-four percent of these neurons were inhibited by the μ opioid agonist DAMGO, and 60% were inhibited by the κ opioid agonist U50488H. One third of the neurons tested with DAMGO and U50488H agonists were sensitive to both. Application of the opioid agonists concomitant with VTA stimulation altered early-onset evoked inhibitions; DAMGO attenuated this response, and U50488H potentiated it. These data suggest that opioids may be able to modulate dopaminergic neurotransmission within the VP, and the mode of this interaction may be opioid receptor subtype-dependent. Work supported by DA05255 to TCN.

520.12

EFFECTS OF MORPHINE ON RAT VENTRAL PALLIDAL NEURONS RECORDED *IN VITRO*. J.X. Liao* and T.C. Napier. Dept. of Pharmacology, Neurosci. and Aging Inst. Div. for Research on Drugs of Abuse, Loyola Univ. Chicago, Stritch Sch. of Med., Maywood, IL 60153.

The ventral pallidum (VP) is a brain region that contains high concentrations of opioid receptors and likely is involved in opioid abuse. The present study was designed to determine the effects of morphine on intracellularly recorded electrophysiological characterisitics of VP neurons from sagittial basal forebrain slices. In the course of these experiments, we found that a lowered osmolar perfusion of artificial cerebrospinal fluid (ACSF) was helpful in obtaining a stable intracellular recording (the concentration of NaCl was decreased from 124 mM to 111 mM). The effects of low NaCl on the electrophysiological characteristics of neurons in VP (n = 43) were determined and compared to those characteristics obtained from recordings conducted in normal NaCl (n = 14). Low NaCl did not alter the resting membrane potential, input resistance, *tau*, threshold to fire, spike duration and height, or the neuronal response to morphine; therefore, the data obtained with morphine were pooled for low and normal NaCl ACSF. Morphine (10 μM) hyperpolarized 16 of 20 tested VP neurons by 4.1±1.0 mV (mean ± SEM) and inhibited the activity of 6 of 9 spontaneously firing neurons. Morphine generally did not alter neuronal input resistance, *tau*, spike duration and height, or threshold to fire. However, in 4 of 19 neurons, the input resistance was decreased by more than 35 MΩ. All morphine-induced responses were blocked by the opioid antagonist naloxone (1-10 μM). The results suggest that morphine most consistently alters spontaneous firing in the VP by hyperpolarizing the cell. Work supported by DA05255 to TCN.

520.13

PROTEIN KINASE INHIBITION REVERSES TOLERANCE ASSOCIATED WITH CHRONIC MORPHINE EXPOSURE IN HYPOTHALAMIC NEURONS OF THE GUINEA PIG. E.J. Wagner* and M.J. Kelly. Dept. of Physiology & Pharmacology, Oregon Health Sciences Univ., Portland, OR 97201.

Tolerance to the effects of morphine with chronic administration has been shown in many different paradigms, and involves an attenuation of the inhibitory effects of morphine in both the peripheral and central nervous systems. In hypothalamic neurons, tolerance is manifested, in part, by a reduction in the potency of the μ-opioid receptor agonist DAMGO. Recent evidence suggests that the cAMP-dependent protein kinase pathway is up-regulated with chronic morphine treatment. The purpose of the present study, therefore, was to determine if the protein kinase inhibitor staurosporine could reverse the tolerance associated with chronic morphine treatment in hypothalamic neurons. To this end, intracellular recordings were made from the arcuate nucleus in hypothalamic slices prepared from ovariectomized female guinea pigs treated for 4 days with either placebo or morphine pellets (4x75mg on day 0; 6x75mg on day 2; implanted s.c.). As expected, chronic morphine treatment elicited a significant reduction in DAMGO potency (EC_{50}=99.0±16.1nM; n=10) compared to placebo-treated controls (EC_{50}=41.7±3.3nM; n=10). A 30 min perfusion of staurosporine (100nM) completely abolished the reduction in DAMGO potency (EC_{50}=38.7±8.1nM; n=3). These results indicate that the tolerance induced by chronic morphine administration in hypothalamic neurons is due, at least in part, to increased protein kinase activity. (This work was supported by Grants DA05158, DA00192 and 5T32 DA07262).

520.14

A SUBPOPULATION OF THALAMIC RETICULAR NEURONS IS INHIBITED BY OPIOID PEPTIDES. J. Brunton and S. Charpak*. Lab. of Physiology, ESPCI, 10 rue Vauquelin, 75005 Paris France.

Previous work has revealed a widespread opioidergic inhibition of cells located in thalamic relay and intralaminar nuclei (Neurosci. Abstr. 372.1, 1995). In the present work, we investigated if this inhibition extended to the thalamic reticular (RE) nucleus. RE neurons were studied using the whole cell patch-clamp recording technique in brain slices from young rats. Their intrinsic membrane properties were similar to those seen in other preparations, displaying the characteristic accelerating-decelerating low threshold burst pattern with a calcium spike crowned by 5-10 action potentials, followed by a pronounced after-burst hyperpolarization. In several cases, sustained periods of oscillatory behaviour were observed in response to a depolarizing pulse from a hyperpolarized potential, or as a rebound from a hyperpolarizing pulse. Cells were tested with U50488, DPDPE, and DAGO, the respective agonists for the κ-, δ-, and μ-opioid receptor subtypes. 40 % of RE cells were inhibited by DAGO while none responded to either U50 or DPDPE. The effect of DAGO, which persisted in TTX, resulted in a hyperpolarization accompanied by a decrease in membrane input resistance with a reversal potential at about E_K. This opioidergic inhibition has therefore enabled us to distinguish two populations of RE neurons which display similar electrophysiological and anatomical (preliminary results) features.
Supported by the Swiss National Science Foundation.

520.15

Opioids suppress GABAergic IPSCs in medial septal/diagonal band neurons via μ receptors. M. Alreja* Dept. of Psychiatry, Yale Univ. Sch. of Med., CMHC, New Haven, CT 06508.

The rat medial septal/diagonal band complex (MSDB) contains a high density of μ and δ receptor binding sites and receptor mRNAs. Both inhibitory and excitatory effects of enkephalin have been reported in *in vivo* and *in vitro* extracellular studies. Intracellular and pharmacological studies on the effects of opioids on MSDB neurons are lacking. In the present study, the effects of opioids on electrophysiologically characterized cholinergic and GABA-type MSDB neurons were studied in an *in vitro* rat brain slice preparation using extracellular, intracellular and whole-cell recordings.

In whole-cell voltage-clamp recordings with K gluconate-containing electrodes, bath-applied met-enkephalin (ENK; 3-100 μM) decreased the frequency of spontaneously occurring bicuculline and TTX-sensitive IPSCs in a subpopulation of GABA-type and cholinergic-type MSDB neurons (n=28). The μ agonist DAMGO (10 nM-1 μM) mimicked the inhibitory effect of ENK and produced a dose-dependent decrease in the frequency of spontaneously occurring IPSCs (n=8). In contrast, the δ agonist, DPDPE (1 μM) had little or no effect on GABAergic IPSCs (n=3). The inhibitory effect of enkephalin and DAMGO on IPSCs was blocked by prior treatment with the selective μ antagonist CTOP (1 μM). Consistent with these findings, in intracellular recordings, a subpopulation of GABA-type neurons in the MSDB responded to enkephalin (n=6) and DAMGO (n=4) with a TTX-insensitive outward current, whereas DPDPE had little or no effect (n=3). In conclusion, opioids inhibit a subpopulation of GABAergic neurons in the MSDB via the μ receptor and these neurons make local synaptic contacts with both cholinergic and other GABA-type MSDB neurons. *Supported by NIH R 29 DA 09797.*

520.16

NALOXONE INHIBIITS CA3 MOSSY FIBER LTP IN RAT HIPPOCAMPAL SLICES W. Jin*, and C. Chavkin Dept. of Pharmacol., Univ. of Wash., Seattle, WA 98195

Mossy fibers constitute the principal source of dynorphin and enkephalin in the hippocampus. It has been shown that endogenous opioid peptides, via activation of μ opioid receptors, facilitate mossy fiber long term potentiation (LTP) in anesthetized rats (Derrick et al 1994). Presently little is known about how the endogenous opioids mediate the excitatory effect. The goal of this study is to understand the mechanism underlying opioid-mediated facilitation of mossy fiber LTP. Field excitatory postsynaptic potentials (fEPSP) were recorded in CA3 stratum lucidum of rat hippocampal slices, and mossy fibers were stimulated by placing a stimulating electrode in the stratum granulosum of the dentate gyrus. The tetanic stimuli (100Hz for 1s repeated once every 20 sec. for 4 times at $S_{1/3}$) elicited a potentiation of the fEPSP amplitude to 217±20% (n=11) of baseline amplitude measured 25 to 30 min after tentanus; this potentiation was maintained for 60 min. Application of the opioid antagonist (-) naloxone (10 μM) prior to and after tetanic stimulation significantly reduced the amplitude of LTP measured at 50 to 60 min (213±24% for control slices, n=11 vs. 136±15%, n=10, P<0.05). To test the hypothesis that endogenous opioids facilitate LTP by depressing $GABA_A$ inhibition (disinhibition), bicuculline, a $GABA_A$ receptor antagonist, was used. The inhibitory effects of naloxone were still observed in the presence of bicuculline; naloxone significantly suppressed the potentiation immediately following the tetanus under this condition, from 320±50% (control, n=10) to 203±13% (n=9, P<0.05), measured 1 to 5 min after tentanus; the LTP measured at 50 to 60 min was also reduced from 243±30% (control, n=10) to 194±33% (naloxone treated slices, n=9). (+) Naloxone (10 μM), an inactive stereoisomer, had no effect in these experiments. Our results demonstrated that naloxone inhibited the induction of CA3 mossy fiber LTP in rat hippocampal slices, consistent with the previous reports from in vivo studies, and our data support the hypothesis that endogenous opioids might have a direct excitatory effect on the induction of LTP in the mossy fiber synapses. Supported by DA04123.

520.17

DYNORPHIN AUGMENTS THE POTASSIUM M-CURRENT IN CA1 HIPPOCAMPAL PYRAMIDAL NEURONS *IN VITRO*. G.R. Siggins*, S.G. Madamba and P. Schweitzer. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Previous intracellular studies in our laboratory (Moore et al., J. Neurosci. 14: 809, 1994) showed that opioids have direct postsynaptic actions on rat CA3 hippocampal pyramidal neurons (HPNs): agonists selective for κ opiate receptors (KORs) enhanced the voltage-dependent K^+ conductance I_M, whereas δ agonists reduced I_M. However, several reports have suggested that neither CA1 nor CA3 HPNs show direct postsynaptic opiate effects. Although previous physiological and binding studies suggested that opiate receptors (mostly μ and δ) were located primarily on elements presynaptic to CA1 HPNs, a recent ultrastructural study (Commons and Milner, Proc. INRC, 1996) found profuse postsynaptic DOR localization on dendrites of CA1 HPNs. Therefore, we re-evaluated the role of opioids in CA1 by superfusing a variety of opiates onto rat hippocampal slices while recording CA1 HPNs intracellularly in discontinuous voltage-clamp mode. In contrast to our findings in CA3, 1-8 μM of the δ agonists D-Pen^{2,5}-enkephalin, [D-Ala^2]-deltorphin-II or DADLE (or the μ agonist DAMGO) had no significant effect on membrane currents, including I_M, in CA1 HPNs. Surprisingly (because there is little evidence yet for KORs on CA1 HPNs), 0.5 μM dynorphin A significantly increased I_M, to a mean of 131-160% of controls (n = 9); the κ agonist U50,488h also increased I_M, but less consistently. Superfusion of the KOR selective antagonist nor-binaltorphimine (1 μM) or naloxone (1-2 μM) prevented the dynorphin effects (n = 5). These results support our previous CA3 data showing clear postsynaptic (somatic) effects of dynorphin on K^+ conductances in HPNs, but now in CA1, and suggest that further anatomical studies on KORs in CA1 are warranted. We speculate that the previous inability to observe postsynaptic actions may have resulted from the use of whole-cell clamp or other methodological differences.

Supported by NIH grants DA03665 and MH 44346.

520.18

OPIOID STIMULATION OF NA,K-ATPASE IN CRUDE SYNAPTOSOMES ISOLATED FROM SELECTED REGIONS OF RAT BRAIN. S.C. Corey Specht* and C. Torres-Silva. Dept. Pharmacology, Institute of Neurobiology, Univ. Puerto Rico, San Juan, PR 009801.

The μ-opioid agonist Methadone (10^{-3}M) stimulated the Na,K-ATPase activity in crude synaptosomes isolated from selected regions of rat brain up to a maximum of 20%. Na,K-ATPase was assayed as ouabain-inhibited, K-stimulated *p*-nitrophenylphosphatase activity. The greatest opioid stimulation was obtained in cortex, superior colliculus, thalamus and sub-cerebellar medulla. Lesser stimulation was obtained with striatum, hindbrain, and hippocampus. There was no opioid-stimulated activity in cerebellum. Methadone did not stimulate purified Na,K-ATPase, suggesting that intracellular mediators are required. Pertussis toxin blocked the stimulatory effect of methadone, thus confirming the participation of G_i. Forskolin inhibited the basal K-stimulation as well as blocking the effect of methadone, suggesting that cAMP-mediated signalling inhibits the Na,K-ATPase. The potential role of intracellular kinases and phosphatases is under further investigation.

520.19

PHARMACODYNAMICS OF REMIFENTANIL, A NOVEL ULTRA-SHORT-ACTING OPIOID ANESTHETIC, USING EEG IN MALE SPRAGUE-DAWLEY RATS. SH Haidar , JE Moreton*, ZM Liang, JF Hoke[2], KT Muir[2] and ND Eddington. Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD 21201. [2]Glaxo-Wellcome, RTP, NC 27709.

Remifentanil (**R**) is an ultra-short-acting-opioid which binds μ receptors. **R** is rapidly metabolized by non-specific esterases in the blood and other tissues. We evaluated the pharmacokinetics and the EEG response of **R** in the rat. Male Sprague-Dawley rats (N=7, Wt.=325±15g) were surgically implanted with stainless steel cerebrocortical EEG electrodes three days before the study. Each rat was infused with **R** through an external jugular vein cannula (dose=15μ g/min/kg) for 21 minutes. Serial blood samples (N=6) were collected from a femoral artery cannula prior to, during, and after the end of infusion. Blood samples were extracted with acetonitrile and methylene chloride and analyzed by a validated GC-MS assay. EEG was captured and subjected to power spectral analysis (0.1-50 Hz) for edge frequency using a MacIntosh-based software package (Neurodata). EEG was collected continuously from 15 minutes before infusion until return to baseline after infusion. A linked sigmoid Emax model was used to describe the concentration-effect relationship. Mean (±S.D.) parameter estimates are listed below. Edge frequency of 97 was used in this study.

Eo (Hz)	EC50 (ng/mL)	Emax (Hz)	Ke0 (min^{-1})	$t_{1/2}$ Ke0 (min)	Gamma
39.9 (0.98)	24.7 (8.2)	19.9 (5.3)	0.41 (0.09)	1.7 (0.37)	11.4 (4.9)

The equilibration half-life ($t_{1/2}$ Ke0) between blood and the effect compartment was comparable to findings of clinical studies evaluating the pharmacodynamics of **R**. (This work is supported by Glaxo-Wellcome.)

OPIOIDS: ANATOMY, PHYSIOLOGY, AND BEHAVIOR—BEHAVIOR

521.1

COMPARISON OF THE DISCRIMINATIVE AND ANALGESIC EFFECTS OF MORPHINE AND ITS GLUCURONIDE METABOLITES AFTER CENTRAL AND SYSTEMIC ADMINISTRATION K.W. Easterling* and S.G. Holtzman Emory University, Department of Pharmacology, Atlanta, GA 30322

This study represents an initial characterization of the potential of centrally or systemically delivered morphine-3-β-d-glucuronide (M3G) and morphine-6-β-d-glucuronide (M6G) to substitute for morphine (MOR) in a drug discrimination task. For comparison, the effects of these same drugs were measured in the tail-flick test of analgesia. Rats with a cannula in the lateral cerebral ventricle were trained to discriminate between injections (3 mg/kg, SC) of morphine and saline using a discrete-trial avoidance/escape procedure, then were tested twice-weekly with randomized doses of either ICV- or SC-administered SAL, MOR, M3G and M6G. Response latency in the tail-flick test was measured just before the discrimination session began. As previously reported, M6G (≥ 0.3 μg) given ICV was 10-fold more potent than MOR (ICV) in the tail-flick test whereas M3G (3.0 μg;ICV) was inactive. Similarly, M6G was 10X more potent than morphine in occasioning MOR-appropriate responding in the drug discrimination task, whereas M3G was inactive. In contrast, when given SC, MOR (3.0 mg/kg) was 3-fold more potent than M6G (SC) in inducing complete analgesia, and M3G (10 mg/kg) induced partial analgesia (68%). MOR SC also was 3-fold more potent than M6G in the discrimination task, however, M3G (3.0-30 mg/kg, SC) was inactive. In both behavioral assays, as compared to SC administration, M6G was approximately 3,500-fold more potent when delivered ICV, whereas MOR was only 300-fold more potent by this route.

(Supported in part by NIH Grant DA00541 and Research Scientist Award K05DA00008)

521.2

ORPHANIN FQ IS A SUPRASPINAL ANTI-OPIOID PEPTIDE THAT BLOCKS MU, DELTA AND KAPPA ANTINOCICEPTION. J.E. Grisel*, J.S. Mogil, G. Zhang, J.K. Belknap and D.K. Grandy. Dept. of Behavioral Neuroscience, Oregon Health Sciences University, Portland, OR 97201.

We have recently shown that orphanin FQ (OFQ), the newly isolated endogenous agonist of the orphan opioid receptor, LC132, does not produce hyperalgesia as originally reported but rather acts as an anti-opioid peptide. Intracerebroventricularly (i.c.v.), OFQ blocks opioid-mediated stress-induced antinociception and systemic morphine antinociception in mice. The present studies were designed to further characterize the anti-opioid activity of OFQ. Nociceptive sensitivity of Swiss-Webster mice was assessed in the 49°C tail-withdrawal (TW) test, before and after drug injection. In the first experiment, cocktails of morphine (0, 1, 10 or 50 μg) and either vehicle (artificial CSF) or OFQ (10 nmol) were injected (under light halothane anesthesia) either i.c.v. (into the lateral ventricle) or intrathecally (i.t.; between L4 and L5) in a volume of 2.5 μl. TW latencies were assessed 15, 30 and 60 min later. Dose-dependent morphine antinociception was observed by both routes. OFQ significantly attenuated i.c.v. morphine antinociception, but did not affect i.t. morphine antinociception. In the second experiment, cocktails of either vehicle or OFQ (10 nmol) combined with opioid receptor agonists--DAMGO (μ; 0, 0.02, 0.05, or 1.0 nmol), DPDPE (δ; 0, 10, 25, or 50 nmol), or U-50,488H (κ; 0, 100, 200, or 1000 nmol)--were injected i.c.v. and tested as described. All agonists produced dose-dependent antinociception that was completely blocked by OFQ. In a third experiment, we demonstrated that OFQ does not appreciably bind to μ, δ, or κ receptors expressed in CHO cells. These findings support and extend our contention that OFQ is a supraspinal anti-opioid peptide acting at the LC132 receptor. Supported by grants from NIAAA (JEG), NIDA (DKG) and the VA (JKB).

521.3

ORPHANIN FQ IS A FUNCTIONAL ANTI-OPIOID PEPTIDE THAT DOES NOT CAUSE HYPERALGESIA. J.S. Mogil*, J.E. Grisel, M.A. Hunter, R.A. Juarez, J.K. Belknap and D.K. Grandy. Vollum Institute and Dept. of Behavioral Neuroscience, Oregon Health Sciences University, Portland, OR 97201.

The present studies were designed to further investigate the role in nociceptive processing of orphanin FQ (OFQ)/nociceptin, the recently isolated endogenous agonist of the orphan opioid receptor, LC132 (ORL-1, XOR). Naïve adult Swiss-Webster mice of both sexes (20-35 g; n=8-11 per group) were used. Nociceptive sensitivity was assessed in the 46°C tail-withdrawal (TW) test. Following measurement of baseline TW latencies, mice were given subcutaneous (s.c.) injections of saline (10 ml/kg) or naloxone (1 mg/kg), followed by intracerebroventricular (i.c.v.) administration (by direct injection under halothane anesthesia) of vehicle (2.5 µl artificial CSF) or OFQ (1-25 nmol). Post-injection TW latencies were measured 10, 20 and 30 min later. In contrast to previous reports, no hyperalgesia--defined relative to baseline latencies--was observed. We believe that the original conclusion that OFQ produces hyperalgesia was confounded by the existence of stress-induced antinociception (SIA) related to the halothane anesthesia/i.c.v. injection protocol, which we observed in vehicle-treated animals. Both OFQ and/or naloxone completely abolished this apparent SIA. Parallel results were obtained using two other nociceptive assays, the hot-plate and writhing tests. We thus propose that OFQ is a novel endogenous anti-opioid peptide, which is further supported by our observation that OFQ dose-dependently reverses systemic morphine antinociception (5 mg/kg, s.c.) in the 49°C TW test (half-maximal antagonism by 7.5 nmol OFQ), and attenuates morphine-induced hypothermia (20 mg/kg, s.c.) and respiratory depression (15 mg/kg, s.c.). Supported by grants from NINDS (JSM), NIAAA (JEG), NIDA (DKG) and the VA (JKB).

521.4

MOTIVATIONAL AND MOTORIC EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF THE NOVEL NEUROPEPTIDE ORPHANIN FQ. D. P. Devine*, R. Reinscheid, F. Monsma, O. Civelli, O. and H. Akil University of Michigan, Mental Health Research Institute, Ann Arbor, Michigan, 48109-0720 USA and CNS Research, Pharma Division, Hoffman-La Roche AG, CH-4002 Basel, Switzerland.

We used an unbiased Conditioned Place Preference procedure to determine if the newly-identified neuropeptide orphanin FQ (O-FQ) produced motivational effects after intracerebroventricular (i.c.v.) microinjections in rats. O-FQ (0.1-100 nmoles) failed to produce conditioned place preference or aversion, but a pronounced motor impairment was observed during conditioning sessions with the highest doses. Thus, it appears that O-FQ lacks motivational effects when administered at behaviourally active doses. We characterized the locomotor effects of i.c.v. O-FQ using a photocell apparatus, and we characterized O-FQ-induced motor-impairment using a battery of motor skill tests. Acute administration of O-FQ (0.1-10 nmoles) produced hypolocomotion, and repeated administration produced tolerance to this effect. During motor skill tests, rats exhibited a profound O-FQ-induced disruption of balance and motor control. Muscle tone was flaccid, particularly in the hindlimbs, and rats exhibited pronounced gnawing or vacuous oral movements. We are currently assessing which anatomical loci may mediate the observed effects, and we are examining the possibility that O-FQ participates in learning and memory processes. This work was supported by grants DA02265 and DA08920. D.P.D. was supported by a fellowship from Fonds de la Recherche en Santé du Québec, Canada

521.5

MODULATION OF SPINAL OPIOID ANTINOCICEPTION BY NEUROPEPTIDE FF-LIKE ENDOGENOUS PEPTIDE. K. Jhamandas*, M.Sutak, H.-Y.T. Yang[1]. Dept. of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada K7L 3N6. [1]NIMH Neuroscience Ctr. at St. Elizabeths, Washington, DC. USA 20032.

Neuropeptide FF (FLFQPQRF-NH_2), designated as morphine modulatory peptide, enhances spinal opioid antinociception and produces antinociceptive effects. Recently, a NPFF-like peptide - SLAAPQRF-NH_2- has been isolated from the rat brain and spinal cord. To study potential opioid modulatory action of this peptide, its action was evaluated in animals bearing chronic intrathecal catheters. Intrathecal injections of SLAAPQRF-NH_2 (11.2 and 22.4 nmol) produced weak antinociceptive action in the tail flick and paw pressure tests. At sub-effective doses (0.06 - 0.6 nmol) SLAAPQRF-NH_2 increased the duration of antinociception produced by intrathecal morphine (6.6 nmol) or [D-Ala^2]-deltorphin I (20 nmol) in both tests. At 24 and 48 hours following injections full recovery was observed. SLAAPQRF-NH_2 administered alone or in combination with opioids did not affect motor function. The results indicate that SLAAPQRF-NH_2, like NPFF, may play a role in modulation of nociceptive signals at the spinal level. [Supported by MRC Canada].

521.6

TYR-PRO-LEU-GLY-NH_2 (Tyr-MIF-1) ENHANCES THE ANALGESIA INDUCED BY REPEATED INJECTIONS OF MORPHINE IN RATS. W. L. Nores*, R. L. Bell, A. L. Vaccarino, A. J. Kastin, J. E. Zadina, G. A. Olson, & R. D. Olson. Dept. of Psychology, Univ. of New Orleans & VA Medical Center., New Orleans, LA.

Findings from a pilot study in our laboratory suggested that the endogenous peptide, Tyr-MIF-1, can act synergistically with morphine to produce a more potent analgesic response to thermal pain. Accordingly, we studied the effects of Tyr-MIF-1 on morphine-induced analgesia in rats. Analgesia was measured by subjecting rats (n=10) to a water-immersion tail-flick test 30 min. after receiving an ip injection of either diluent, morphine (10.00 mg/kg), or morphine+Tyr-MIF-1 (10.00 mg/kg+0.01 mg/kg). The latency of each rat to curl or withdraw its tail (tail-flick) from water maintained at 48°C was recorded. Each rat was tested in this fashion on alternating days for a total of 12 test sessions. A 3 x 12 (Treatment x Session) mixed ANOVA, with session as the repeated measure, yielded a significant Treatment x Session interaction, $p<.05$. Post hoc analyses revealed that the diminished analgesic response of morphine from session 1 to session 2 was attenuated by Tyr-MIF-1. Furthermore, the analgesic response to morphine was observed until session 5, whereas analgesia induced by morphine+Tyr-MIF-1 remained significant until session 6. The results suggest that Tyr-MIF-1 can enhance the analgesic effectiveness of repeated injections of morphine.

521.7

PROLACTIN RELEASE, DOPAMINERGIC NEURAL ACTIVITY AND TAIL FLICK LATENCY RESPONSES TO MET-ENKEPHALIN. J. Janik,* J. Tompkins, R. Weise, M. Gardon and P. Callahan. Miami Univ, Center for Neuroscience, Dept of Zoology, Oxford, OH 45056.

The role of met-enkephalin in the suckling-induced PRL increase was studied. Endogenous met-enkephalin was immunoneutralized by injecting anti-rat-met-enkephalin into the right lateral ventricle. The suckling-induced prolactin increase in post-partum, female Sprague Dawley rats was significantly attenuated by the 1.8 µg dose and totally abolished by the 3.6 µg dose of antiserum. Antiserum administration did not affect the activity of the tuberoinfundibular dopaminergic (TIDA) neurons. In order to further characterize the antiserum, we tested its effectiveness in reducing latency times in a tail flick test applying low heat stimulus (42°C) to female rats that did not receive any opiate agonist. Anti-met-enkephalin had no effect on baseline latencies. However, opiate antagonists, specifically β-Funaltrexamine (µ antagonist, 5 µg, ivt), Naltrindole (δ antagonist, 5 µg, ivt) or nor-Binaltorphimine (κ antagonist, 8 µg, ivt) significantly attenuated the baseline response to the heat stimulus.

These results indicate that met-enkephalin is necessary for the PRL secretory response to suckling, but that it does not mediate this increase through inhibition of TIDA neurons. Met-enkephalin may not play a role in maintaining tolerance to a mildly noxious stimulus, but the EOP are involved through actions at specific receptor subtypes. Supported by NIH HD 30375-01 to JJ and PC.

521.8

Gi/Go PROTEINS AND SMALL CONDUCTANCE Ca^{2+} ACTIVATED K^+ CHANNELS ARE INVOLVED IN SPINAL ANTINOCICEPTION INDUCED BY δ_2-OPIOID RECEPTOR AGONIST [D-Ala^2]DELTORPHIN II. H. Mizoguchi*, M. Narita and L. F. Tseng. Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226

The effects of the pretreatment with pertussis toxin (PTX) and various K^+ channel blockers on antinociception induced by δ_2-opioid receptor agonist [D-Ala^2]deltorphin II were studied in male ICR mice. [D-Ala^2]deltorphin II was administered intrathecally (i.t.) and the antinociception was measured by the tail-flick test 10 min after the injection. I.t. pretreatment with PTX (0.125-0.5 µg) for 96 hr dose-dependently attenuated the antinociception induced by i.t.-administered [D-Ala^2]deltorphin II. The antinociception induced by i.t.-administered [D-Ala^2]deltorphin II was blocked by apamin (5 ng, 10 min), a small conductance Ca^{2+} activated K^+ channel blocker, but not by glibenclamide (50 µg, 10 min), a ATP-sensitive K^+ channel blocker. In in vitro [^{3}H]DSLET (D-Ser^2, Leu^5]enkephalin-Thr^6) binding studies using crude synaptic membrane preparation of the mouse spinal cord, co-incubation of GTPγS (0.1-100 µM), a non-hydrolyzable GTP analog, inhibited [^{3}H]DSLET bindings. Our results indicates that δ_2-opioid receptors in mouse spinal cord couples to PTX sensitive Gi/Go proteins, and the antinociception induced by i.t.-administered [D-Ala^2]deltorphin II is mediated by small conductance Ca^{2+} activated K^+ channels. (Supported by NIH grant DA 03811).

521.9

CHARACTERIZATION OF SPINAL ANTINOCICEPTION INDUCED BY δ-1 OPIOID RECEPTOR AGONIST (-)TAN67. L. F. Tseng[1*], M. Narita[1], H. Mizoguchi[1] and H. Nagase[2]. [1]Dept. of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226, [2]Basic Res. Lab., Toray Industries Inc., Kamakura 248, Japan.

The aim of the present study was to characterize δ-1 opioid receptors for antinociception in the spinal cord of ICR mice using newly synthesized δ-1 agonist (-)TAN67. (-)TAN67 was given intrathecally (i.t.) and the antinociception was measured by the tail-flick test. I.t. administration of (-) TAN67 (5 to 50 μg) produced antinociception in a dose-dependent manner. The antinociception induced by i.t.-challenged (-)TAN67 was dose-dependently blocked by i.t. pretreatment with a highly selective δ-1 antagonist BNTX (0.03 to 0.33 μg), but not by a selective δ-2 antagonist NTB (1 μg), μ antagonist CTOP (50 ng) or κ antagonist nor-BNI (5 μg). In acute antinociceptive tolerance studies, mice tolerant to i.t.-administered (-)TAN67 did not produce cross-tolerance to antinociception induced by i.t.-administered selective δ-2 agonist [D-Ala^2]deltorphin II, μ agonist DAMGO or κ agonist U50,488, but it produced cross-tolerance to that produced by δ-1 agonist DPDPE given i.t. Thus δ-1-opioid receptors in the mouse spinal cord plays a specific role in antinociception induced by i.t.-administered (-)TAN67. I.t. pretreatment with pertussis toxin (PTX; 0.125 to 0.5 μg) 96 hr prior to i.t.-challenge of (-) TAN67 dose-dependently attenuated i.t.-administered (-) TAN67-induced antinociception. Furthermore, pretreatment with apamin (2 to 10 ng), a selective Ca^{2+}-activated K^+-channel of small conductance (BK_{Ca}) blocker, given i.t. dose-dependently attenuated antinociception induced by i.t.-administered (-)TAN67, whereas i.t. pretreatment with glibenclamide (50 μg), a selective ATP-sensitive K^+-channel (K_{ATP}) blocker, did not affect (-) TAN67-induced antinociception. Our results indicate that spinal δ-1 opioid receptors may be linked to PTX-sensitive Gi/Go-proteins and BK_{Ca}, but not K_{ATP}, is involved in spinal δ-1 opioid receptor-mediated antinociception in the mouse (Supported by NIH grant, DA 03811).

521.10

DECREASED BLOOD LYMPHOCYTE PROLIFERATIVE RESPONSES MEDIATED BY CENTRAL μ-RECEPTORS. R.D. Mellon* and B.M. Bayer. Dept. Pharmacology, Georgetown Univ., School of Medicine, Washington, D.C. 20007.

Acute administration of morphine to rats decreases whole blood mitogen induced proliferative responses of T-lymphocytes via central opioid receptors (Hernandez et al. 1993). These effects appear to be largely independent of either pituitary or adrenal factors (Flores et al. 1994). Previous studies indicate that opioids decrease natural killer cell cytolytic activity through central μ receptors (Band et al. 1992), however, the specific opioid receptors mediating blood lymphocyte proliferative responses have not been established.

Equimolar doses of morphine, saline, or specific opioid receptor agonists (20 nmol) were administered into the lateral ventricle of freely moving male Sprague-Dawley rats in a volume of 2.0 μl. Analgesia, as measured by tail-flick assay, was measured 30 minutes after injection. Two hours post-injection, whole blood, spleen, and thymus proliferative responses to the T-cell specific mitogen Concanvalin A (ConA) and plasma concentrations of corticosterone were determined.

Results indicate that both morphine and the μ specific agonist DAMGO decreased blood lymphocyte proliferative responses compared to either saline, the κ agonist U50488 or the δ agonist DPDPE. Spleen and thymus proliferation were unaffected by any treatment. Additionally, only morphine and DAMGO increased latency to tail-flick and corticosterone levels. Separate studies with DAMGO show a dose dependent effect on blood lymphocyte proliferation. Further, central administration of CTOP (5 μg/2.0 μl), a specific μ-antagonist, blocked the immunomodulatory effects of systemic morphine (6 mg/kg, sc).

These studies provide evidence that morphine acts through central μ receptors to modulate blood lymphocyte proliferation responses in the periphery.

This work was supported by NIH grant DA04358.

521.11

INTRACISTERNAL INJECTION OF MORPHINE INDUCES FACIAL SCRATCHING IN MICE. C. Tohda*, T. Yamaguchi, Y. Kuraishi. Dept. of Applied Pharmacol., Res. Inst. for Wakan-yaku, Toyama Med. & Pharm. Univ., Toyama 930-01, Japan.

Epidural and intrathecal administrations of morphine result in pruritus in humans, and the microinjection of opioid μ-agonists into the medullary dorsal horn produces facial scratching in monkeys and rats. The present experiments were conducted to determine whether morphine and μ-agonist, [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO) induced centrally itch-related behavior, that is, scratching in mice. Male ddY mice (4 -6 weeks old) were used. When intracisternally injected (5 μL), morphine (0.1 - 3 nmol) and DAMGO (0.03, 0.1 nmol) dose-dependently elicited scratching of face, but not ear and body trunk, by the hind paws for about 20 min after injection. The morphine (0.3 nmol)-induced face scratching was inhibited by pretreatment with naloxone (1 mg/kg, s.c., 15 min before). When injected intradermally, morphine (3, 30 nmol, 50 μL) into the rostral back didn't significantly elicit scratching of any areas of the body. These results suggest that opioids produce an itch sensation at least in part through opioid μ-receptors in the central nervous system in mice. The present experimental model is easy to use and seems to be available to clarify central mechanisms of itch.

521.12

Foraging Behavior in the Rat Following Manipulation of Reinforcer Palatability, Deprivational State, and Naltrexone and Butorphrenol Administrations

J. Cleary*, E. O'Hare, D.T. Weldon, P.J. Bartz, C.J. Billington and A.S. Levine
VA Medical Center and University of Minnesota, Minneapolis, MN 55417

Six male Sprague Dawley rats were trained to obtain all of their food nightly under a seven hour laboratory analog of foraging behavior. Subjects were trained to respond on one lever, in a two lever operant test chamber, under randomly assigned search costs of FR-2, FR-5 or FR-10. Completion of the search requirement resulted in activation of the other lever in the apparatus, the food-procurement lever. Activation of the food-procurement lever was preceded by either flashing of the house-light in the chamber, which indicated a procurement cost of FR-2, or flashing of a bank of colored lights above the levers, which indicated a procurement cost of FR-10. Completion of the procurement cost resulted in the delivery of one 45 mg casen-based (sweet) food pellet. Subjects could decline the procurement opportunity by either responding on the search lever, or failing to respond on the procurement lever for more than 15 seconds following procurement lever activation. When subjects successfully maintained stable body weight under the nightly foraging paradigm, experimental manipulations of reinforcer palatability (grain pellets vs sweet pellets), deprivational state (24 hours deprivation), and effects of naltrexone and butorphrenol administrations (0.3, 1.0 and 3.0 mg/kg) were evaluated. Data were collected at hourly intervals throughout the nightly feeding sessions and response profiles were compared with control data under each experimental manipulation. Analysis of the data indicated dissimilar response profiles for palatability, deprivation state and drug manipulations. These results suggest that the effects of thesedrugs on foraging behavior do not mimic environmental manipulations.

Supported by NIDA grant #DA-03999

521.13

DIFFERENTIAL SYMPTOMATIC PRESENTATION IN PREMENSTRUAL SYNDROME (PMS) AS A FUNCTION OF BETA-ENDORPHIN.

Robert H. Loiselle*, A. James Giannini, David Martin and Carlton Turner.
Northeastern Ohio Medical College, Boardman, Ohio 44512.

Twenty women, ages 19-28 with PMS-symptoms were studied. All had serum ß-endorphin levels on day 1 and day 20 of their menstrual cycle for three consecutive cycles. Symptoms were measured using DSM-III criteria.

Symptom presentation was then compared with ß-endorphin levels using linear regression. Specific symptoms including anxiety, cramping, cravings for food and physical discomfort were found to be associated with significant declines in ß-endorphin levels. As a result, a specific subgroup of PMS-patients with a distinctive tetrad of symptoms associated with ß-endorphin decline was postulated.

Funding: Wellesley Corporation, Youngstown OH

521.14

PRENATAL EXPOSURE TO NALTREXONE ALTERS SOMATIC AND NEUROBIOLOGICAL DEVELOPMENT IN RAT OFFSPRING. P.J. McLaughlin*, C.M. Lang, S.W. Tobias and I.S. Zagon. Depts. Neuroscience and Anatomy and Comparative Medicine, Penn State University College of Medicine, Hershey, PA 17033.

Little is known about the role of endogenous opioid systems in prenatal development. To examine whether opioids influence development *in utero*, time-mated nulliparous Sprague-Dawley rats were injected daily from conception to parturition with 50 mg/kg NTX. This drug regimen was found to block opioid receptors all day in the pregnant rat. No differences were noted between control (i.e., sterile water) (CO) and NTX-treated litters in terms of gestation time, litter size, infant mortality, or incidence of congenital malformations. Within 8 hr of birth, all pups were cross-fostered to untreated lactating females. Birthweights of NTX rats were increased 25% from CO levels, and remained 18% to 41% greater than CO values throughout the preweaning period. Crown-rump lengths for NTX-treated neonates were significantly greater than CO values. Wet, dry, and relative weights of selected organs in the NTX group were significantly increased from CO levels at birth, as well as on postnatal days 10 and 21. Brain weights and morphometric measurements of the cerebral cortex and the cerebellum were markedly greater in the NTX group relative to CO values at weaning. These data suggest that an endogenous opioid system functions during prenatal development in the rat, and that a native opioid peptide acts as a tonic negative regulator of growth. Supported by NIH grants HL53557 and NS20500.

521.15

TAPE STRIPPING-INDUCED HYPERALGESIA AS A MODEL FOR THE EVALUATION OF ANALGESIC AGENTS. L. Cortes Burgos* and D.L. DeHaven-Hudkins. Adolor Corporation, Malvern, PA 19355.

A model of inflammatory pain was developed which uses tape stripping to produce an abrasion injury by removal of the stratum corneum layer of the epidermis. Male Sprague-Dawley rats are anesthetized with ketamine-xylamine, and the right hind paw is treated with a depilatory agent and tape stripped by repeated application and removal of Scotch tape to the dorsal side of the paw. Hyperalgesia in the tape stripped paw is measured by recording the paw pressure thresholds using a Randall-Selitto apparatus. The degree of hyperalgesia observed is maximal after 20 tape strippings. Hyperalgesia is present by 1-2 hr following stripping, is maximal at 4-24 hr and subsides by 72 hr. Peak antinociception occurs at 5 min following intrapaw injection of fentanyl (3 μg), and at 15 min following treatment with morphine (300 μg) or spiradoline (300 μg), while injection of 300 μg of the δ agonist SNC-80 fails to produce analgesia. The ED_{50} values for reference opiate agonists in this model are: morphine, 631 μg, fentanyl, 3.2 μg, and spiradoline, 204 μg. The nonsteroidal antiinflammatory reference agents acetaminophen, acetylsalicylic acid, diclofenac, ibuprofen, indomethacin or naproxen are without significant effect. This model of hyperalgesia detects antinociception produced by μ and κ opiate agonists, and is useful for the assessment of pain resulting from abrasion injuries.

CATECHOLAMINE RECEPTORS: SECOND MESSENGER SIGNAL TRANSDUCTION

522.1

EFFECT OF THE STIMULATION OF INSULIN RECEPTOR ON D2 DOPAMINE RECEPTOR ACTIVITY IN FIBROBLAST CELLS. S. Maltais* and P. Falardeau. Centre de Recherche du CHUL and School of Pharmacy, Université Laval, Ste-Foy, Qc, Canada, G1V 4G2

Several observations reveal the importance of the close relationship between insulinergic and dopaminergic systems. Their respective contribution to the regulation of blood glucose level, blood pressure, motor and affective control, and on the regulation of each other activity has been shown. This was further demonstrated in animal studies indicating that diabetic rats are resistant to the locomotor and behavioral effects of the dopamine agonist amphetamine, and largely restored with chronic insulin therapy. Although the relationship between these two systems seems very important, little is known about the direct modulation of the dopamine receptor activity by the activation of the tyrosine kinase signaling pathway through the insulin receptor. In order to verify if the activation of the insulin receptor could modify the activity and the pharmacology of D2 receptor, Ltk- mouse fibroblasts expressing both insulin and human D2 receptors (1000fmol/mg protein) were used. In cells treated with 1 μM insulin for 1 hour, the proportion of receptors in its high affinity state raised from 33% to 50%, without change of the high or low affinity dissociation constants. This is an important evidence that the D2 dopamine receptor could be regulated by the activation of the insulin receptor. This regulation could involve, as for ß-adrenergic receptor (EMBO J. 1995 Nov 15; 14(22): 5542-9), a variation in the level of receptor phosphorylation . The variation of the D2 dopamine receptor phosphorylation, and the modulation of the receptor functionality following insulin treatment will be discussed.

Supported by a Medical Research Council of Canada grant.

522.2

FUNCTIONAL ANALYSIS OF THE HUMAN DOPAMINE D2 RECEPTOR VARIANTS. A. Cravchik[1], D.R. Sibley[2], R.M. Post* and P.V. Gejman[1]. [1]National Institute of Mental Health, [2]National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland.

The human dopamine D_2 receptor gene (DRD2) has three polymorphic variants which predict the amino acid substitutions Val→Ala96, Pro→Ser310, and Ser→Cys311 in the receptor protein. We have investigated the receptor binding and signal transduction properties of these D_2 receptor variants by stably expressing them in cultured mammalian cells. The Cys311 and Ser310 variants of the human D_2 receptor, which involve substitutions located in the third cytoplasmic loop, were markedly less effective in inhibiting cAMP synthesis than the most prevalent form (Pro310, Ser311). Additionally, the variants Cys311 and Ser310 were found to exhibit a higher proportion of high affinity agonist binding than the most prevalent form of the D_2 receptor. The impairment of the Cys311 and Ser310 variants to inhibit cAMP levels thus appears to result from a reduced ability of the receptors to activate the appropriate Gi-like protein. The demonstration of substantial functional differences between DRD2 gene variants found in the human population might have important pharmacological implications given the widespread use of D_2 receptor blocking drugs in the treatment of psychotic disorders.

This research is funded by the NIMH Intramural Program.

522.3

D-1 DOPAMINE RECEPTOR LINKED TO PI HYDROLYSIS MEDIATES TARDIVE DYSKINESIA IN RATS H.Rosengarten* and A.J. Friedhoff. Department of Psychiatry, Millhauser Labs, NYU School of Medecine, New York, NY 10016.

Long term neuroleptic therapy and D-1 stimulation by D-1 agonist SKF 38393 induces repetitive jaw movements in rats which are considered to be a rat model of tardive dyskinesia(TD)(1,2). The existence of multiple forms of the dopamine D-1 receptor is supported by behavioral, biochemical, electrophysiological and molecular biological evidence (3,4,5,6,7). More recently a D-1 receptor was identified which is not linked to c-AMP stimulation but to PI hydrolysis (8). This receptor can be stimulated by dopamine and the D-1 agonist SKF 38393, and inhibited by the D-1 antagonist SCH 23390. RJM can be also induced by SKF 38393 and inhibited by SCH 23390. It has been found in our laboratory that 20-30 % of the D-1 receptors labeled with SCH 23390 are resistant to EEDQ inactivation (9). Reduction of the number of functional D-1 receptors by 70-90% by administering the peptide coupling agent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), did not affect RJM scores which actually increased following EEDQ inactivation of most of the D-1 receptors. Suspecting that RJM behavior may be induced by a PI system we examined the PI system as a mediator of rat tardive dyskinesia - RJM. As predicted, based on the failure of EEDQ to reduce RJM scores, PI hydrolysis was unaffected by EEDQ, whereas binding analysis of the other caudate from each rat showed an expected 80% decrease of the binding of the D-1 antagonist SCH 23390. This demonstrates that about 20% of D-1 receptors producing RJM (TD) are linked to PI.

This study provides evidence that rat TD is elicited by the D-1 receptor pathway linked to second messengers derived from the hydrolysis of phosphoinositides.

1.Rosengarten et al. 1983; Rosengarten et al.1986; 3. Mailman et al. 1986; 4.Keyser et al.1989; 5. Clark and White, 1987; 6. Anderson et al. 1990; 7.Sunahara,1990; 8. Undie and Friedman, 1989; 9.Rosengarten et al. 1989.

522.4

THE ROLE OF THE D_1 RECEPTOR CARBOXYL TERMINUS IN DESENSITIZATION AND DOWN-REGULATION. Chun K. Mak* and David R. Sibley. Molecular Neuropharmacology Section, ETB/NINDS/NIH, Bethesda, MD 20892.

The D_1 dopamine (DA) receptor has a long carboxyl (C)-terminus containing consensus sequences for phosphorylation by protein kinases. In contrast, the D_2 DA receptor has a short C-terminus with no apparent phosphorylation sites. To assess the potential role of the D_1 receptor C-terminus in desensitization, we constructed a D_1/D_2 chimeric receptor in which the D_1 receptor C-terminus was replaced with that of the D_2. The cDNAs of the chimeric D_1/D_2 and wild type (WT) receptors were stably transfected into CHO or C6 cells. Cyclic AMP levels and [^{3}H]SCH-23390 binding assays were used as measures of receptor function and expression, respectively. Both the D_1 WT and D_1/D_2 chimera receptors exhibited similar levels of expression and produced similar cAMP responses in both CHO and C6 cells When pre-treated with DA, both the D_1/D_2 chimera and D_1 WT receptors in C6 cells approached complete desensitization within 1 hr, while those in the CHO cells showed moderate desensitization after several hours. In both cell lines, the D_1/D_2 chimera was desensitized to a greater extent than the D_1 WT receptor. In receptor binding experiments, pre-treatment of DA did not lead to significant receptor down-regulation within the first hour for either cell type. A moderate down-regulation was seen in 2 to 7 hours, and 90% down-regulation by 24 hours. There was no difference between the D_1 WT and D_1/D_2 chimeric receptors in this regard. It is concluded that the C-terminus of the D_1 receptor does not play a major role in G-protein coupling, desensitization or down-regulation. (Supported by NINDS/NIH).

522.5

HYDROXYLAMINE TREATMENT OF THE DOPAMINE D2 RECEPTOR RESULTS IN G-PROTEIN UNCOUPLING. S.P. Lee, G.Y.K. Ng, G.A. Cottrell*, M.R. Brann, S.R. George, and B. O'Dowd. Depts. of Pharmacology and Medicine, Univ. of Toronto, Toronto, ON, M5S 1A8, The Addiction Research Foundation, Toronto, ON, M5S 2S1, and the Depts. of Psychiatry and Pharmacology, Univ. of Vermont School of Medicine, Burlington, VT, 05405

Previous studies in our laboratory have shown that both the dopamine D2 receptor (D2R) monomer and dimer are palmitoylated. Palmitoylation may have a role in the desensitization or trafficking of receptors. In our current study, the effects of chemical depalmitoylation of the D2R were examined. D2R-expressing Sf9 cells (D2/cells) were metabolically labeled with [^{3}H]-palmitic acid and immunoprecipitated D2R were shown to be palmitoylated. Incubation of the palmitoylated D2R with 1M hydroxylamine removed the [^{3}H]-palmitate from the receptor indicating that palmitoylation occurs by a thioester bond to cysteine residues. We are currently investigating whether the cysteine in the carboxyl tail is the putative site for palmitoylation in the D2R. Treatment of crude membranes from D2/cells with hydroxylamine resulted in the loss of agonist-detected high affinity sites as detected by agonist competition binding analysis, suggesting that uncoupling from the G-protein occurs as a result of depalmitoylation of the D2R. Saturation binding with the benzamide antagonist [^{3}H]-YM-01951-2 and the butyrophenone antagonist [^{3}H]-spiperone on the hydroxylamine-treated membranes detected a significant decrease in receptor density versus control membranes. Subsequent immunoblot analysis of these tissues showed no significant change in the electrophoretic mobility or intensity of the immunoreactive monomer and dimer species. These data suggest that chemical depalmitoylation lowers receptor affinity for ligand by uncoupling from G-protein but does not change the size or the proportions of D2R dimers and monomers. This work was supported by the Medical Research Council of Canada and the National Institute of Drug Abuse.

522.6

DIHYDREXIDINE ACTIVATES D_2 DOPAMINE RECEPTORS COUPLED TO ADENYLYL CYCLASE, BUT NOT TO POTASSIUM ION CHANNELS
H.P. Smith[1*], G.S. Oxford[1], D.E. Nichols[2], R.B. Mailman[1] and C.P. Lawler[1]. Univ. of North Carolina[1], Chapel Hill, NC 27599, and Purdue Univ.[2], W. Lafayette, IN. 47907

Dihydrexidine (DHX) is a dopamine agonist that has high affinity to both "D_1-like" and "D_2-like" receptors. Functionally, DHX has equally high intrinsic activity at "D_1-like" receptors in rat/primate brain and in molecular systems. Surprisingly, however, there is significant data indicating that DHX has high intrinsic activity at "D_2-like" receptors coupled to inhibition of adenylyl cyclase (AC) activity, but antagonist activity (i.e., low intrinsic activity) at "D_2-like" receptors coupled to potassium channels. The studies using primary cultures of pituitary lactotrophs are consistent with this pattern. DHX (like dopamine) inhibits both forskolin- and VIP-mediated stimulation of adenylyl cyclase activity with a maximal inhibition of 50% and 80%, respectively. The D_2-selective antagonists, (-) sulpiride and (-) eticlopride, attenuate DHX- and dopamine-mediated inhibition of AC, whereas the D_1-selective antagonist, SCH23390, does not. In the same cells, DHX has low intrinsic activity at D_2 receptors coupled to the potassium inward rectifier (GIRK): DHX elicits peak potassium ion currents $\leq$ one-fourth the peak I_K elicited by dopamine, even at concentrations as high as 100 μM. DHX- and dopamine-mediated I_K are blocked by the D_2-selective antagonists, (-) sulpiride and domperidone, but not the D_1-selective antagonist, SCH23390. Pituitary lactotrophs express only the D_{2SHORT} and D_{2LONG} molecular isoforms of the receptor, to which DHX binds equally well. In cell lines transfected with either the D_{2S} or D_{2L} receptor, DHX has high intrinsic activity to inhibit D_2-coupled AC. Thus, the mechanism(s) underlying the effector selectivity of DHX likely involve(s) stoichiometric differences or conformational differences in D_2 receptor coupling to distinct G protein isoforms. This provides further evidence that drugs might have "functional selectivity" in their interactions with the same receptor isoform. (Supported by MH42705, MH40537, and MH33127 grants)

522.7

STRUCTURAL DETERMINANTS OF G-PROTEIN COUPLING IN THE DOPAMINE D2 RECEPTOR: CONTRIBUTION OF THE BBXXB MOTIF.
B. L. Wiens*, V. J. Watts, and K. A. Neve Oregon Health Sciences University and VA Medical Center, Portland OR, 97201.

Synthetic peptides corresponding to regions in the cytoplasmic domains of G-protein coupled receptors activate G-proteins in vitro. A sequence motif of BBXXB/F or BBXB (B=basic, F=aromatic, X=non-basic residue) at the C-terminal end of these peptides is required for activation of Gi. The dopamine D2 receptor contains two putative G-protein activator sequences with this motif in the third intracellular loop (LRSSR and HHGLH). To test the hypothesis that these sequences are necessary for agonist activation of G-proteins via the D2 receptor we substituted an alanine for the last basic residue of each putative Gi-activator sequence in the rat D2L receptor. These mutants, R230A and H316A, were characterized in HEK293 cells stably expressing similar levels (500-1000 fmol/mg protein) of mutant and wild-type (wt) receptors. The affinities (Kd) of mutant receptors for [3H]spiperone were similar to the wt receptor (approximately 30 pM), as were the apparent affinities (Ki) for the agonists dopamine, 7OH-DPAT, and quinpirole. The ability of H316A to inhibit forskolin-stimulated cAMP accumulation did not differ from the wt receptor, however, the IC50 values of dopamine and 7OH-DPAT inhibition at R230A (IC50=313 and 2 pM) were at least 10-fold lower than at the wt receptor (3500 and 670 pM). In addition, preliminary results indicate that dopamine pretreatment (2 hrs) sensitizes forskolin-stimulated cAMP accumulation via R230A to a greater extent than via H316A or the wt receptor, which exhibit similar levels of sensitization. Although these results do not support the hypothesis that the BBXXB elements in the D2L receptor are required for activation of Gi, the N-terminal region of the third intracellular loop appears to influence G-protein coupling as the mutant R230A exhibits enhanced functional activity. (Supported by NIDA Training Grant 5T32DA07262, MH45372, and the VA Merit Review Program)

522.8

INVESTIGATION OF cAMP-MEDIATED REGULATION OF THE RAT D_1 DOPAMINE RECEPTOR USING SITE-DIRECTED MUTAGENESIS. Dong Jiang* and David R. Sibley. Molecular Neuropharmacology Section, ETB/NINDS/NIH, Bethesda, MD 20892.

Previous investigations of D_1 receptor regulation have suggested a role for the cAMP-dependent protein kinase (PKA) in agonist-induced desensitization and down-regulation. Given the presence of four putative consensus recognition sites for PKA in the D_1 receptor protein, a reasonable hypothesis is that some of these effects are due to direct phosphorylation of the receptor. In order to investigate this possibility, we used site-directed mutagenesis techniques to alter each of the four potential PKA sites to determine the effects on agonist-induced regulation. Using PCR-based methods, we created a mutant D_1 receptor with the following amino acids substitutions: Thr135→Val135, Ser229→Ala229, Thr268→Val268, and Ser380→Ala380. Residue 135 is found within the 2nd intracellular loop of the receptor, residues 229 and 268 are present in the 3rd intracellular loop whereas residue 380 is located in the carboxyl terminus of the receptor. Characterization of the wild type (WT) and mutant receptors stably expressed in C6 glioma cells suggests that both receptors are expressed at similar levels. The mutations have no effect on receptor expression, antagonist or agonist affinities, or on functional coupling with Gs. Preliminary experiments indicate that dopamine (DA) preincubation of the C6 cells results in desensitization and down-regulation of both WT and mutant receptors. DA treatment induces a maximal 85% receptor down-regulation at ~3 hr with a $t_{1/2}$ of ~1 hr in an identical fashion for both WT and mutant receptors. In contrast, DA-induced desensitization is attenuated in the mutant receptor. After 1 hr of DA treatment, the WT receptor exhibits ~80% desensitization of the cAMP response whereas the mutant receptor is desensitized by only ~20%. These results support the hypothesis that direct phosphorylation of the D_1 receptor by PKA is involved in agonist-induced desensitization. (Supported by NINDS/NIH).

522.9

SEQUESTRATION OF DOPAMINE D2 RECEPTORS IS FACILITATED BY G-PROTEIN COUPLED RECEPTOR KINASES 2 AND 5.
K. ITO and T. HAGA*. Dep. of Biochemistry, Inst. for Brain Research, Univ. of Tokyo, Tokyo 113, Japan

We have examined the agonist-dependent sequestration of dopamine D2 receptors in COS-7 cells that had been transiently transfected with expression vectors encoding D2 receptors (the long isoform D2L or the short isoform D2S) and G-protein coupled receptor kinases (GRK2 or GRK5). Sequestration was assessed by two methods: 1) loss of [^{3}H]sulpiride binding activity from the cell surface, and 2) transfer of [^{3}H]spiperone binding activity from the membrane fraction to a light vesicle fraction, isolated by sucrose density gradient centrifugation. In COS-7 cells expressing D2 receptors alone, almost no sequestration was observed (less than 4%). When GRK2 was co-expressed with D2S, 47% of the receptors were sequestered by treatment with 10^{-4} M dopamine for 2 hours (EC_{50} = 2.2×10^{-7} M, $t_{1/2}$ = 25 min). In cells coexpressing GRK5, 25% of the D2S receptors were sequestered following the same treatment. Co-expression of a dominant-negative receptor kinase mutant (DN-GRK2) led to no change in sequestration. These results indicate that GRK facilitates sequestration of dopamine D2 receptors as well as muscarinic m2 and ß2 adrenergic receptors.
(Supported by grants from the Ministry of Education, Science, and Culture of Japan)

522.10

DOPAMINE RECEPTORS AND THE REGULATION OF TYROSINE HYDROXYLASE AND AROMATIC L-AMINO ACID DECARBOXYLASE. N.H. Neff*, S. Cho, B. Weiss[1] and M. Hadjiconstantinou. Dept. Pharmacol. and Psychiat., Ohio State Col. Med., Columbus OH, 43210 and [1]Div. Neuropsychopharmacol. Med. Col. Penn. Phila, PA 19129

Acute administered of the dopamine D2 receptor family of agonists to mice decreases the activity of tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AAAD) in striatum, while D2 antagonists increase these activities. In contrast, D1 receptor agonists or antagonists decrease or increase, respectively, AAAD activity without affecting TH activity. The changes of TH activity after acute treatment with the D2 family of drugs apparently reflect activation/deactivation of the enzyme, while the changes of AAAD appear more complex and include activation/deactivation as well as induction of the enzyme. Chronic treatment with D2 receptor agonists decreased AAAD activity only, while treatment with D2 antagonists increased TH and AAAD in striatum. Similarly, treatment with a D2 receptor antisense oligonucleotide increased TH and AAAD activity in striatum. D1 active drugs had no effect on TH, while D1 antagonists elevated AAAD activity. The increase of TH and AAAD after chronic treatment with D2 or D1 antagonists, and D2 antisense was accompanied by a rise of mRNA for both enzymes, suggesting de novo protein synthesis.

Grant: NS-34571

522.11

DISSOCIATION OF TYROSINE HYDROXYLASE AND ADENYLATE CYCLASE MODULATION BY D2 DOPAMINE AUTORECEPTORS. C.M. O'Hara, K.L. O'Malley, and R.D. Todd*. Depts. of Psychiatry, Genetics, and Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

D2 dopamine receptors have been shown to inhibit dopamine release and synthesis as well as adenylate cyclase in a number of systems. We have utilized a mouse mesencephalic cell line (MN9D) to show that transfected D2 dopamine receptors are capable of inhibiting dopamine synthesis and release as well as adenylate cyclase activity following agonist stimulation in a pertussis toxin (Ptx)-sensitive manner. Previously we have shown that MN9D cells express the Ptx-sensitive G-protein subunits $G\alpha_{i2}$, $G\alpha_{oA}$, and $G\alpha_{oB}$ but not $G\alpha_{i1}$ or $G\alpha_{i3}$. In the current study, we have determined the specific G-proteins involved in the inhibition tyrosine hydroxylase and adenylate cyclase in this cell line. MN9D cells were cotransfected with the long form of the D2 dopamine receptor and $G\alpha_{i2}$ or $G\alpha_{oA}$ which have been mutated such that they are no longer sensitive to Ptx. In this paradigm, if the specific mutant G-protein couples to the D2 receptor then inhibition should occur in the presence of Ptx. Cotransfected MN9D cells were grown to near confluence, treated overnight in the absence and presence of 100 ng/ml Ptx then assayed for agonist inhibition of forskolin-stimulated cAMP accumulation or dopamine synthesis. For synthesis, the cells were stimulated in the presence of high K^+ (60 mM) with the dopamine agonist quinpirole (10 μM); L-dopa accumulation was measured in the presence of the L-aromatic amino acid decarboxylase inhibitor, NSD1015(200 μM) by high performance liquid chromatography/electrochemical detection. Agonist stimulation of D2/$G\alpha_{i2}$ and D2/$G\alpha_{oA}$ cell lines inhibited dopamine synthesis following Ptx treatment while only the D2/$G\alpha_{i2}$ cell line inhibited cAMP accumulation. In this system, D2-mediated modulation of synthesis is coupled to two G-protein pathways while modulation of cyclase is limited to the $G\alpha_{i2}$ pathway. (Supported by MH31302, DA08818)

522.12

SPECIFIC COUPLING OF A CLONED HUMAN α2A-ADRENERGIC RECEPTOR TO A PHOSPHOINOSITIDE PATHWAY BY LOW DOSES OF OCTOPAMINE. C.N.Airriess[1]*, J.E.Rudling[1], T.R.Cheek[1], J.M.Midgley[2] and P.D.Evans[1]. [1]The Babraham Institute, Lab.Mol.Signalling, Dept.Zoology, Univ. Cambridge, Cambridge, CB2 3EJ, U.K. and [2]Dept.Pharmal.Sci., Univ. Strathclyde, Glasgow, G1 1XW, U.K.

Agonist-specific coupling of G-protein coupled receptors to different second messenger systems has been demonstrated for a number of receptors (see Evans *et al.*, 1995, Prog. Brain Res. 106:259-268). We have shown previously that *m*- and *p*-octopamine, which coexist in sympathetic neurons, can couple a cloned human α2A-adrenergic receptor (α-2AR) to multiple second messenger systems when expressed in a Chinese hamster ovary (CHO) cell line (Evans *et al.*, 1995, Neurosci. Soc. Abstr. 21:1613). Here we report that low doses of (±)-*m*-octopamine specifically will couple the α-2AR (expressed in CHO cells) to a phosphoinositide pathway. The ability of agonists to change intracellular levels of D-*myo*-inositol 1,4,5-trisphosphate (IP_3) was assessed using a specific binding assay (Amersham) and their ability to change cytosolic Ca^{2+} levels was assessed using ratio imaging techniques with the dye, fura-2. Very low concentrations (10^{-13} to 10^{-11}M) of (±)-*m*-octopamine were specifically able to increase IP_3 levels in CHO cells expressing α-2AR when exposed to agonist for 30 min in the presence of 10^{-3}M LiCl. At higher agonist concentrations this effect was much reduced. Parallel studies indicated that comparable low concentrations of (±)-*m*-octopamine, but not of the catecholamines or (±)-*p*-octopamine, can initiate cytosolic Ca^{2+} signals. Typically, such signals take the form of transient rises, which may or may not be accompanied by oscillatory changes, in intracellular Ca^{2+} levels. These studies provide further evidence for a specific functional role for *m*-octopamine (co-released with norepinephrine from sympathetic neurons) which may be mediated by phosphoinositide-induced changes in cytosolic Ca^{2+} levels through the activation of an α-adrenergic receptor.

Funded by BBSRC through the Babraham Institute (PDE) and by NSERC and AHFMR Fellowships (CNA).

522.13

ACTIVATION OF D5 DOPAMINE RECEPTORS ON CHROMAFFIN CELLS INHIBITS SECRETAGOGUE-STIMULATED Na^+ UPTAKE BY A cAMP-INDEPENDENT MECHANISM. M.K. Dahmer* and S.E. Senogles. Department of Biochemistry, University of Tennessee College of Medicine, Memphis, TN 38163.

Recent studies have demonstrated that D1-selective and D2-selective dopamine receptor agonists inhibit catecholamine secretion and Ca^{2+} uptake into bovine adrenal chromaffin cells by receptor subtypes which we have identified as D5, a member of the D1-like dopamine receptor family, and D4, a member of the D2-like dopamine receptor family. The purpose of this study was to determine whether activation of D5 or D4 receptors inhibits influx of Na^+ which could explain inhibition of secretion and Ca^{2+} uptake by dopamine agonists. Na^+ influx was measured by the uptake of $^{22}Na^+$ into cells. D1-selective agonists preferentially inhibited both dimethylphenylpiperazinium- (DMPP) and veratridine-stimulated $^{22}Na^+$ influx into cells. The D1-selective agonists, Cl-APB (100 μM) and SKF38393 (100 μM) inhibited DMPP-stimulated Na^+ uptake by 87.5 ± 2.3% and 59.7 ± 4.5%, respectively, while the D2-selective agonist, bromocriptine (100 μM), inhibited Na^+ uptake by only 22.9 ± 5.0%. Veratridine-stimulated Na^+ uptake was inhibited 95.1 ± 3.2% and 25.7 ± 4.7% by 100 μM Cl-APB or bromocriptine, respectively. The effect of CL-APB was concentration dependent. A similar IC_{50} (~18 μM) for inhibition of both DMPP- and veratridine-stimulated Na^+ uptake was obtained. The addition of 8-Br-cAMP (1 mM) had no effect on either DMPP- or veratridine-stimulated Na^+ uptake suggesting that D1-selective agonists are acting in a cAMP independent manner. (Supported in part by grants DK43152 (to M.K.D.) and NS28111 (to S.E.S.) from the NIH.)

522.14

G PROTEIN KNOCKOUT: EFFECTS ON D2 RECEPTOR COUPLING EFFICIENCY FOR DOPAMINE INHIBITION OF PROLACTIN (PRL) SECRETION. E. Meller,* K. Bohmaker, P. Albert,[1] P. Falardeau[2] and A. Zaremba. Dept. of Psychiatry, NYU Medical Center, New York, NY 10016; [1]Neuroscience Research Institute, Univ. of Ottawa, Ottawa, Canada K1H-8M5; [2]Dept. Genetiques Moleculaire, Le Centre Hôpital Univ. Laval, Ste-Foy, Quebec, Canada G1V-4G2.

GH_4C_1 cells transfected with pRSVneo express D2S receptors whose expression is under control of a metallothionein promoter. Expression of D2S receptors can easily be up- (by treatment with Zn^{2+}) or down- (by treatment with phenoxybenzamine; PBZ) regulated. Exposure to Zn^{2+} shifted the dose-response (DR) curve for dopaminergic inhibition of PRL release to the left, while irreversible receptor inactivation with PBZ shifted the DR curve to the right. Analysis of the DR curves indicated highly efficient receptor coupling (large receptor reserve). GH_4C_1 cells were stably transfected with pcDNA3 vectors containing the cDNA's for Giα3, Goα and Giα2 in antisense orientation. Clones have been isolated in which expression of Giα3 and Goα is selectively attenuated by 75-80%, as determined by Western blot; selection of Giα2 knockout clones is ongoing. Preliminary results show that knockout of Giα3 attenuates dopamine inhibition of PRL secretion and shifts the DR to the right. The effects of altering D2S receptor density, G protein α subunit identity/levels, or both, on DR curves for inhibition of PRL secretion are in progress and are expected to quantify the parameters which specify efficient receptor/effector coupling in this system. Supported by PHS grant NS 23618.

522.15

PHOSPHOLIPASE C-ß2 EXPRESSION SWITCHES INHIBITORY DOPAMINE D2 RECEPTOR SIGNALLING TO STIMULATION OF CALCIUM MOBILIZATION. Stephen J. Morris[1], Ronald Kriz[2] , and Paul R. Albert[1]*. [1]Neuroscience Research Institute, University of Ottawa, Ottawa, Ontario, K1H 8M5. [2]Genetics Institute, Cambridge, MA, 02140.

Previously, we had observed that dopamine D2S receptors expressed in GH4 pituitary lactotrophs couple in a pertussis toxin sensitive fashion to inhibition of VIP-stimulated cAMP formation, decreased basal [Ca++]i and to membrane hyperpolarization (Vallar *et al.* 1991). However, the same receptor when expressed in fibroblast Ltk- cells induced an increase in [Ca++]i which was associated with phosphoinositide turnover. The receptors retained their ability to inhibit cAMP formation and to cause membrane depolarization. All of these effects were sensitive to pertussis toxin suggesting that the receptors mediated their effects through the Gi/Go family of G-proteins. We have created stable cell lines expressing cDNAs for various phospholipase C (PLC) isoforms in the sense and antisense orientation and used them to address the question of how the same receptor expressed in two different cell types can have diametrically opposed effects on [Ca++]i.

GH4 cells stably transfected with D2S receptor and PLC-ß2 sense increased [Ca++]i upon agonist addition whereas Ltk- cells expressing D2S and the antisense of PLC-ß2 have a much reduced calcium flux compared to Ltk- cells expressing D2S alone. Transfection of cells with sense and antisense constructs of PLC-ß1 and -ß3 did not cause such effects. The Gßγ-coupled PLC-ß2 appears to be obligatory for coupling to increase [Ca++]i. The differential expression of effector molecules (eg. PLC-ß2) in various cell types can alter the signalling properties of G protein coupled receptors. Supported by NCI, Canada

522.16

MODULATION AND HETEROLOGOUS SENSITIZATION OF SPECIFIC ISOFORMS OF ADENYLATE CYCLASE BY D2L DOPAMINE RECEPTORS IN HEK 293 CELLS V. J. Watts,* and K. A. Neve Oregon Health Sciences University and VA Medical Center, Portland OR, 97201

In the present study we have created cells lines expressing the D2L dopamine receptor and type 1 (Ca++-stimulated) adenylate cyclase (AC-1/D2L) or the D2L receptor and type 2 (phorbol ester-stimulated) adenylate cyclase (AC-2/D2L). We examined the ability of D2L dopamine receptors to modulate cAMP accumulation acutely as wells the effects of D2 agonist pretreatment on drug-stimulated cAMP accumulation. In AC-1/D2L cells it was found that D2 dopamine receptor agonists inhibited Ca++-stimulated (A23187) cAMP accumulation, although agonist potency was reduced compared to inhibition of forskolin-stimulated cAMP accumulation in D2L-Hek 293 cells. In contrast, dopamine receptor agonists did not inhibit cAMP accumulation in AC-2/D2L cells. Under conditions where Gs was partially activated (100 nM isoproterenol), coincubation with D2 agonists resulted in a potentiation of cAMP accumulation in AC-2/D2L cells, consistent with the activation of AC-2 via release of βγ subunits. We also examined heterologous sensitization of adenylate cyclase by D2L receptors. AC-1/D2L or AC-2/D2L cells were pretreated with dopamine agonists for 2 hours, followed by stimulation of cAMP accumulation under a variety of conditions. The results from these studies demonstrated that D2 agonists sensitized AC-1 mediated cAMP accumulation (A23187-stimulated). The A23187-stimulated cAMP accumulation in dopamine-treated cells was 500 % greater than that in vehicle-treated cells. Similarly, it was found that in AC-2/D2L expressing cells, agonist pretreatment sensitized phorbol ester-stimulated cAMP accumulation approximately two-fold. Pretreatment with D2 agonists under conditions where cAMP accumulation is elevated (AC-2/D2L cells pretreated in the presence of isoproterenol) also resulted in sensitization of phorbol ester-stimulated cAMP accumulation. The results from the present studies provide additional information on the complex regulation of specific forms of adenylate cyclase and also provide clues regarding the mechanism for D2 receptor-mediated sensitization of adenylate cyclase activity. Supported by MH45372.

523.1

CHARACTERIZATION OF THE 7-HYDROXY-2-AMINOMETHYL CHROMAN WAY-133023 AND ITS ENANTIOMERS IN RODENT BEHAVIORAL TESTS FOR DOPAMINE D2 RECEPTOR ACTIVITY. J.A. Brennan, M.J. Piesla, A.T. Shropshire*, T.P. Wasik, R.A. Scerni, T.H. Andree, R.E. Mewshaw and K.L. Marquis. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

The dopamine D2 partial agonist WAY-133023 [2-(benzylamino-methyl)-chroman-7-ol oxalate] and its enantiomers (Mewshaw *et al.*, ACS, Orlando FL, 1996) were characterized in a battery of *in vivo* behavioral tests for presynaptic and postsynaptic agonism/antagonism potential at the D2 receptor. *In vitro* binding studies with WAY-133023 indicate selectivity and high affinity for this receptor (IC50 nM; D2ag=0.4, 5HT1A=104, α1=1145) and partial agonist intrinsic activity as indicated by preferential affinity for D2 agonist vs. antagonist binding. Locomotor activity (LMA) effects in non-habituated mice revealed that the *in vivo* agonist potential of WAY-133023 lies in the (+)enantiomer [(+)023]. Low doses (0.001-0.003 mg/kg sc) and high doses (0.3-3 mg/kg sc) of (+)023 decrease LMA while intermediate doses (0.01-0.03 mg/kg sc) were less effective. The distomer [(-)023], however, produced only a reduction in LMA (0.1-3 mg/kg sc). WAY-133023 and its enantiomers were also characterized in Ungerstedt's rat rotational behavior model and in antagonism or induction of stereotypic and climbing behaviors in mice. Whereas (+)023 induced stereotypy in mice at doses $\geq$ 0.3 mg/kg sc when co-administered with a D1 agonist and following reserpine pretreatment, and also induced climbing at 10 and 17 mg/kg sc in reserpinized mice, (-)023 was devoid of activity in these models at doses up to 10 mg/kg sc. Both the (+) and (-) enantiomers antagonized apomorphine-induced stereotypy and climbing in mice (MED= 1 and 10 mg/kg sc, respectively). These *in vitro/in vivo* results suggest that WAY-133023 represents a new generation of D2 partial agonists which uses the 2-aminomethyl chroman moiety as a completely novel scaffold having access to the D2 agonist pharmacophore.

523.2

DISRUPTION OF DOPAMINE D1 RECEPTOR GENE EXPRESSION ATTENUATES ALCOHOL-SEEKING BEHAVIOUR. S.R. George, M. El-Ghundi, J. Drago, T. Fan, T. Nguyen, H. Westphal, D. Sibley, P. Fletcher, J. Khanna* and B. O'Dowd. Add. Res. Fdn., Dept. Pharm., Univ. of Toronto, ONT, Canada, Dept. Anatomy, Monash Univ., Victoria, Australia, NIH-NICHHD, Bethesda, MD, USA

Dopamine actions are mediated through the D1-like (D1&D5) and D2-like (D2, D3 & D4) receptors. The contribution of these receptors in mediating brain rewarding properties of ethanol has been difficult to define by pharmacological studies using agonists or antagonists due to lack of selectivity of these drugs. We used dopamine D1 receptor deficient mice (D1-/-) to investigate the role of this receptor in alcohol-seeking behaviors. D1-/- mice reproduce and move normally but are smaller in size than their wild type (WT) and heterozygote (D1+/-) littermates. In a 2-tube free choice limited access paradigm, mice were exposed to water and increasing concentrations of ethanol (3%, 6% & 12% w/v) over 30 consecutive days. Ethanol consumption and preference over water were markedly reduced (85-90%) in D1-/- mice compared to WT and D1+/- controls. In support of the hypothesis that dopamine mediates the action of ethanol in the brain, elevation of synaptic dopamine levels by blocking MAOB with selegiline significantly reduced ethanol intake and preference in WT & D1+/- but had no effect on D1-/- mice. The involvement of D1 & D2 dopamine receptor systems in mediating alcohol drinking was further assessed by examining the effects of selective antagonists. Sulpiride caused a 30% reduction in ethanol intake and preference in WT and D1+/- mice, and completely abolished residual ethanol intake in D1-/- mice. SCH23390, was more effective in reducing ethanol intake (75-80%) in WT and D1+/- mice but had no effect in D1-/- mice suggesting that susceptibility to a higher ethanol intake and preference in WT & D1+/- mice are mediated by D1 receptors. These findings implicate both dopamine D1 and D2 receptor mechanisms in alcohol-seeking behaviour in mice, however, D1 receptors play a more important role than D2 receptors in this behavior. (Supported by MRC).

523.3

LEARNING DEFICIT IN DOPAMINE D1 RECEPTOR KNOCKOUT MICE. M. El-Ghundi, P. Fletcher, J. Drago, H. Westphal, D. Sibley, P. Seeman*, S.R. George and B. O'Dowd. Add. Res. Fdn, Clarke Inst. Psych., Dept. Pharm., Univ. of Toronto, Toronto, ONT, Canada, Dept. Anatomy, Monash Univ., Victoria, Australia, NIH-NICHHD, Bethesda, MD, USA

Dopamine D1 receptors (D1) are highly expressed in the hippocampus and prefrontal cortex, suggesting a role in cognitive processes. Mice lacking D1 receptors (D1-/-) were used to investigate the role of D1 receptors in learning. Spatial learning and memory of D1-/- and their wild type (WT) littermates were assessed using the Morris water maze. All mice were given trials in which they were required to locate a submerged platform within 90 s (acquisition) which was then changed to a different location (reversal). The platform was removed from the maze and mice were allowed to swim for 90 s and scored for the % of time spent in the quadrant where the platform was located, and the number of crossings through the previous platform position. The escape latency for both D1-/- and WT during the acquisition phase declined over subsequent trials indicating that they learned the position of the platform. However D1-/- mice learned significantly slower than WT. Probe trials revealed that D1-/- spent less time in the designated quadrant and had reduced numbers of crossings compared to WT. When mice were given trials to escape a visible platform to test their visual acuity and nonspatial learning, no significant difference was observed. The deficits seen in D1-/- were not due to motor impairment since these mice exhibited normal spontaneous locomotor activity, although rearing was reduced, compared to WT in an open field test. These results indicate that D1-/- mice are learning impaired, and acquire spatial learning slowly, suggesting that D1 receptors are involved in learning. (Supported by the Medical Research Council of Canada).

523.4

BIOCHEMICAL AND BEHAVIORAL EFFECTS OF A D_2 DOPAMINE RECEPTOR ANTISENSE RNA VECTOR TRANSFECTED IN MOUSE BRAIN. L.-W. Zhou*, G. Davidkova, S.-P. Zhang, M. Morabito and B. Weiss. Dept. Pharmacol., Med. Coll. PA and Hahnemann Univ., Philadelphia, PA 19129

A D_2 DA antisense plasmid vector (D_2 AS vector) has been shown to inhibit D_2 dopaminergic supersensitivity in mice with unilateral 6-OHDA lesions of the corpus striatum. To investigate in greater detail the *in vivo* effects of the D_2 AS vector, we have performed studies in two animal models: normal mice and mice treated with the selective, irreversibly-acting D_2 DA receptor antagonist fluphenazine-N-mustard (FNM). In normal mice, bilateral injections into the corpus striatum of D_2 AS vector (25 μg) complexed to DOTAP (10 μg) produced catalepsy within one day, an effect which lasted up to 40 days. This behavior was not observed after similar injections of an "empty" cloning vector, lacking the D_2 antisense sequence. In the FNM model, a single injection of FNM inactivates the total pool of D_2 DA receptors and produces catalepsy within 4 hr of treatment, after which the receptors and behavior return to normal within 8 days. Mice were given a single bilateral intrastriatal injection of the D_2 AS vector or the "empty" vector and 5 days later were injected with FNM (20 μmol/kg, i.p.).Catalepsy was scored and the levels of the D_2 DA receptors were measured at various time intervals thereafter. D_2 AS vector treatment significantly reduced the rate of disappearance of the cataleptic behavior induced by FNM. This correlated with an inhibition of the rate of recovery of D_2 receptors. The inhibitory effects of the single injection of the D_2 AS vector on D_2 receptor-mediated behaviors lasted about 50 days. Unilateral intrastriatal injections of the D_2 AS vector also significantly inhibited the recovery of quinpirole-induced rotations in mice with unilateral intrastriatal injections of FNM. These results suggest that a single injection of a D_2 AS vector into mouse brain inhibits the synthesis of D_2 DA receptors and induces long-term inhibition of D_2 DA receptor-mediated behaviors.

523.5

BEHAVIORAL CHARACTERIZATION OF DOPAMINE D_{1A} RECEPTOR KNOCKOUT MICE. D.R. Smith, C.D. Striplin, R.B. Mailman, J. Drago[1], C.P. Lawler, and M. Gallagher. Dept. of Psychology and UNC Neuroscience Center, Univ. of North Carolina, Chapel Hill, NC 27599 and [1]Neuroscience Unit, Monash University, Clayton 3168 Australia

Behavioral assessments sensitive to different forebrain systems innervated by dopamine (DA) neurons were conducted on mutant mice in which the D_{1A} dopamine receptor gene was inactivated. The knockout construct was expressed in Sv129 mice from which male chimeras were crossed with C57BL/6 (PNAS 91:12564, 1994). The genotype of each mouse was assessed using Southern analysis. Compared to wild type mice (+/+), homozygote mice (-/-) were impaired severely in a spatial navigation task sensitive to mesocortical DA function. In additional assessments, (-/-) mice were slower to initiate exploration and failed to orient to visual cues, functions associated with the mesolimbic and mesostriatal DA systems. Apart from the initiation of exploration, however, (-/-) mice were as active as, and habituated similarly to (+/+) mice. Finally, (-/-) mice learned to discriminate accurately odor cues, but intermittently failed to initiate performance on trials throughout testing. In contrast to (-/-) mice, heterozygotes (+/-) were not distinguishable from (+/+) mice on any test, with the exception of spatial navigation. In this latter assessment, the accuracy of the spatial bias acquired by the (+/-) mice was lower than that of the (+/+) mice, although (+/-) mice performed normally when learning was guided by a local cue. These results show that (-/-) mice have deficits indicative of a loss of function in multiple forebrain systems innervated by DA, whereas a more circumscribed impairment in mesocortical function is evident in (+/-) mice. (DS holds an APA Predoctoral Fellowship. Supported by MH42705, MH01149, HD03310, and MH33127)

523.6

THE EFFECTS OF RACLOPRIDE ON NON-HORMONAL AND HORMONE-INDUCED SENSITIZATION TO PUPS IN NULLIPAROUS RATS. Y.M. Clarke-Hall*, J. Rosenblatt and Ian Creese. Behavioral and Neural Sciences Graduate Program, Rutgers University, Newark, NJ 07102.

We have previously reported that antagonism of dopamine D2 receptors specifically interferes with several components of maternal behavior. To further explore the role of D2 receptors in maternal behavior in nulliparous rats, we examined the effects of raclopride, a selective D2 receptor antagonist, on both non-hormonal and hormone-induced sensitization to pups. In the first study, raclopride (10 mg/kg/day) or no drug was administered to rats in their drinking water for two days before their initial exposure to 2-day old pups. The rats were maintained for 10 days on raclorpide or normal drinking water and were constantly exposed to pups which were exchanged each day. Animals were considered sensitized to pups when they demonstrated retrieval of 3 pups and crouching/nursing posture when tested with new pups each day. Raclopride completely disrupted non-hormonal sensitization to pups which occurred in all control rats, without significantly affecting their spontaneous activity as measured in photocell cages. In a second study, estradiol (5 mm silastic tube~80 pg/ml serum) and progesterone (2 x 50 mm silastic tubes~80-160 ng/ml serum) were surgically implanted in ovariectomized and hysterectomized rats on day 0 and day 3, respectively. The rats were given an injection (sc) of estrogen benzoate (50 μg/kg on day 16) to activate rapid onset of maternal behavior. Raclopride (10mg/kg/day) was given for different periods of time throughout the study. Preliminary results demonstrated that raclopride, given from 0-16 days, interfered with hormone-induced sensitization to pups when these rats were tested on days 18-28. Together, these results suggest that dopamine and D2 receptors play a crucial role in mediating sensitization to pups. Supported by NIMH.

523.7

INCREASED POTENCY OF NEUROPEPTIDE Y TO ANTAGONIZE α_2-ADRENOCEPTOR FUNCTION IN THE NUCLEUS TRACTUS SOLITARII OF SPONTANEOUSLY HYPERTENSIVE RATS. S.-N. Yang[1], D.R. Fior[1], A.C. Hansson[1,3], P.B. Hedlund[1*], A. Cintra[1], M. Castellano[2], U. Ganten[3], D. Ganten[3], L.F. Agnati[1] & K. Fuxe[1]. [1]Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden; [2]Scienze Mediche, Università degli Studi di Brescia, Italy; [3]Max-Delbrück-Centrum für Molekulare Medizin, Germany.

The regulation by neuropeptide Y (NPY) of α_2-adrenoceptors in the nucleus tractus solitarii (NTS) was evaluated in the adult normotensive Wistar Kyoto rat (WKY) and the adult spontaneously hypertensive rat (SHR). The threshold dose (1 pmol/50 nl) of NPY-(1-36) for the vasodepressor response in the NTS of the WKY was five times higher than that (0.2 pmol/50 nl) in the SHR. NPY-(1-36) at 0.2 pmol/50 nl could significantly counteract the vasodepressor response to *l*-noradrenaline (800 pmol/50 nl) in the NTS of the SHR, but not in the WKY, in which 1 pmol/50 nl of NPY-(1-36) must be employed to counteract the vasodepressor response to *l*-noradrenaline (800 pmol/50 nl). The in situ hybridization and autoradiographical experiments showed that the α_{2A}-adrenoceptor mRNA levels and the B_{max} value of the α_2-adrenoceptor agonist ^{3}H-p-aminoclonidine binding sites in the NTS of the SHR were substantially lower than those in the WKY. The autoradiographical results showed that in the SHR, NPY-(1-36) at 1 nM led to a significant increase in the K_d value of ^{3}H-p-aminoclonidine binding sites. In the WKY, NPY-(1-36) produced this effect only at 10 nM. The present study provides evidence for an increase of the potency of NPY-(1-36) to antagonistically modulate α_2-adrenoceptors in the NTS of the SHR, which may contribute to the development of hypertension in the SHR.

Supported by a grant 04X-715 from the Swedish MFR.

523.8

TRANSFECTION OF D_2 DOPAMINE RECEPTOR ANTISENSE RNA PLASMID VECTOR *IN VITRO* AND *IN VIVO*. G. Davidkova, L.-W. Zhou, S.-P. Zhang*, M. Morabito and B. Weiss. Dept. Pharmacol., Med. Coll. PA & Hahnemann Univ., Philadelphia, PA 19129

To achieve long-term inhibition of the D_2 dopamine (DA) receptors we have developed eucaryotic plasmid vectors (pCEP4-D_2 AS and pCR3-D_2 AS) producing antisense RNA to the D_2 DA receptor. The vectors contain a 322 bp cDNA sequence of the mouse D_2 DA receptor (long isoform) in the antisense orientation relative to a CMV promoter. Vector pCEP4-D_2 AS was stably transfected into human embryonic kidney (HEK) 293_{DL} cells, expressing the mouse D_{2L} receptor isoform, by liposome-mediated transfection. Radioligand binding studies showed that these transfected HEK 293_{DL} cells exhibited markedly reduced levels of D_2 DA receptors. The feasibility of transferring a plasmid vector into mouse brain was determined by injecting mice intrastriatally with a CMV-E. *coli* LacZ vector complexed with DOTAP (25 μg DNA/10 μg DOTAP). Positive histochemical staining for β-galactosidase indicated that the reporter gene was expressed in the injected striatum. Successful transfection of the vector pCR3-D_2 AS into brain was confirmed by PCR amplification of DNA from the injected striata. A behavioral analysis of mice with unilateral 6-hydroxydopamine-induced striatal lesions showed that a single intrastriatal injection of pCR3-D_2 AS significantly inhibited contralateral rotational behavior in response to challenge injections with the D_2/D_3 agonist quinpirole but not to a D_1 or cholinergic agonist. This effect lasted about one month. These studies indicate the feasibility of delivering D_2 DA receptor antisense RNA into the CNS by means of a non-viral plasmid vector, and as a consequence producing long-term specific inhibition of D_2 dopaminergically mediated behaviors.

523.9

REGULATION OF DOPAMINE RELEASE AND SYNTHESIS IN DOPAMINE D_3R RECEPTOR KNOCKOUT MICE. D.C. Cooper [1]*,T.E. Koeltzow [1],M. Xu[2], S. Tonegawa[2], F.J. White[1] and M. E. Wolf[1]. [1]Dept. of Neuroscience FUHS/The Chicago Medical School, North Chicago, IL 60064. [2]Dept. of Cell Biology, Cincinnati College of Medicine, Cincinnati, OH 45267. [3]Ctr. Memory & Learning, MIT, Cambridge, MA 02139.

The role of dopamine D_3 receptors (D R) in autoreceptor-mediated regulation of dopamine (DA) synthesis and release was tested in D_3R knockout mice (MUT) and their wild type (WT) littermates. Regulation of DA synthesis by nerve terminal DA autoreceptors was assessed in vivo using the GBL model. Impulse flow in DA cells was eliminated by GBL and decarboxylation of DOPA was inhibited by NSD-1015. We examined the effect of PD128907, a D_3R/D_2R agonist, on DOPA accumulation in the ventral and dorsal striatum. There were no differences in basal DOPA accumulation (elicited by GBL and NSD alone) between WT and MUT mice. PD128907 dose-dependently inhibited DOPA accumulation in both regions equally well in MUT and WT mice. The contribution of D_3Rs to local regulation of DA release was assessed using in vivo microdialysis in the ventral striatum of freely moving mice. MUT mice had increased basal DA levels compared to WT mice. Reverse dialysis application of PD128907 at 100 nM and 1μM significantly reduced DA concentrations in WT mice. In MUT mice, 1μM PD128907 decreased DA levels, but the effect was significantly blunted compared to the WT response. These results support a role for D_3Rs in DA release regulation, although our results do not distinguish between DA autoreceptor-mediated and possible long-loop feedback effects. In conclusion, these results suggest that: 1) Autoreceptor-mediated regulation of striatal DA synthesis occurs primarily via D_2R receptors, and 2) D_3Rs play a role in DA autoreceptor-mediated regulation of DA release in the ventral striatum. Supported by DA07735 to M.E.W and DA04093 and DA00207 to F.J.W.

523.10

DOPAMINE SYNTHESIS IS INCREASED IN MICE LACKING DOPAMINE D4 RECEPTORS. T. A. Pugsley*, S. Whetzel, A. E. Corbin, D. H. Van Leeuwen, R. G. MacKenzie T. Heffner, M. Rubinstein[1], M. J. Low[1] and D. K. Grandy[1]. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105, [1]Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University L474, Portland, OR 97201.

Dopamine (DA) is a principal neurotransmitter in neuronal systems that project from midbrain nuclei to the striatum, limbic system, frontal cortex and hypothalamus. DA receptors are involved in a number of physiological actions, including movement control, generation of emotion and neuroendocrine functions. Alterations in dopaminergic activity and/or receptors are implicated in the pathogenesis of several CNS disorders including schizophrenia and Parkinson s disease. DA receptors have been subdivided into two families, the D1-like (D1 and D5) and the D2-like (D2, D3 and D4). Similar to D2 receptors, activation of D4 receptors inhibits adenylyl cyclase. However, the in vivo functional role for D4 receptors remains uncertain. Mice lacking functional D4 receptors were generated by gene targeting in embryonic stem cells. Mice with the disrupted D4 gene, both homozygous (-/-) and heterozygous (+/-), developed normally, were fertile and did not display any gross abnormalities. Biochemical studies indicated that basal DA synthesis was increased in mouse striatum but not in hippocampus of (-/-) as compared to (+/+). The Merck D4 receptor antagonist L-745,870 increased DA synthesis in striatum and hippocampus of (+/+), but was inactive in the same brain areas of (-/-) mice. The non-selective DA antagonist haloperidol increased DA synthesis in striatum and hippocampus of both the (+/+) and (-/-) mice. These findings suggest that D4 receptors may play a role in modulating DA synthesis in certain brain regions. (Supported by Warner-Lambert and NIDA Grant DA-09620.)

CATECHOLAMINES: DOPAMINE III

524.1

POSTURAL STRATEGIES DURING REACHING IN UNILATERAL DOPAMINE-DEPLETED RATS: USE OF FORCE PLATFORMS FOR MEASURING GROUND REACTION FORCES. E.I. Miklyaeva*, E.G. Nikiforov, G.J. Tompkins, M.E.Ioffe, N. C. Woodward, I.Q. Whishaw. Dept. of Psychology, Univ. of Lethbridge, AB, Canada, T1K 3M4

In previous experiments it has been shown that rats with unilateral dopamine (DA) depletions (hemiParkinson analogue rats) are impaired in using the contralateral (bad) limbs for skilled movements and for postural adjustments. It was proposed that the bad limbs may be unable to apply force to adjust posture and produce movement. The present study tests this idea by combining videorecording with force analysis. Ground reaction forces applied by each limb were measured with force platforms that were designed specifically for rats. Control rats and rats with unilateral DA-depletion, produced by 6-hydroxydopamine (6-OHDA) injected into the nigrostriatal bundle, reached for food with one limb, while vertical forces applied by each limb were measured. The results confirm that in hemiParkinson analogue rats the affected limbs can mediate relatively normal supporting reactions but cannot mediate normal adjustments in posture. To compensate for this impairment, rats use their good limbs to actively assist weight shifts. The results demonstrate that behavioral compensation can contribute to recovery and suggest that force analysis can assist in measuring DA-depletion-induced deficits.

Funded by Alberta Heritage Foundation for Medical Research

524.2

TRANSGENIC RESCUE OF SNAP-25 RESTORES DOPAMINE-MODULATED SYNAPTIC TRANSMISSION IN THE COLOBOMA MUTANT M.C. Wilson*, S.C. Steffensen, M. Kreifeldt, S.J. Henriksen, Scripps Research Institute, La Jolla, CA 92037

The mouse mutation coloboma (*Cm*) is a contiguous gene defect which includes the *Snap* gene encoding SNAP-25, an integral component of the synaptic vesicle docking/fusion core complex required for neurotransmitter release. In support of the involvement of SNAP-25 in the anomalous behavior of *Cm*/+ mice, expression of a transgene (*sp/sp*) encoding SNAP-25 ameliorates the hyperactivity, but not the head bobbing or opthalmic deformation attributed to the mutation. We have previously demonstrated that *Cm*/+ mice have increased recurrent inhibition, reduced theta rhythm by tail pinch and reduced long-term potentiation (LTP) in the hippocampal dentate gyrus compared to normal (+/+) littermates. The involvement of dopamine in these effects was hypothesized based on previous studies demonstrating that amphetamine suppresses the hyperactivity in *Cm*/+ mice as well as the involvement of mesolimbic dopamine in the induction of theta rhythm and the sensitivity of LTP to dopamine agonists. We sought to identify neurophysiological correlates for the rescued hyperactivity in *sp/sp•Cm*/+ mice. Hippocampal EEG and evoked field potential activity were recorded by staggered microelectrodes oriented in the dentate hilus of halothane-anesthetized mice. In contrast to differences between *Cm*/+ and +/+ mice, there were no significant differences in the duration or amplitude of theta rhythmic activity (4-6 Hz) induced by tail-pinch (10s), afferent-evoked field EPSPs/population spikes (PSs), paired-pulse pEPSP/PS responses or LTP recorded in the dentate gyrus of *sp/sp•Cm*/+ and *sp/sp•*+/+ mice. Amphetamine (3.0 mg/kg i.p.) produced disinhibition of dentate paired-pulse responses in both *sp/sp•Cm*/+ and *sp/sp•*+/+ mice but increased inhibition in *Cm*/+ mice. These findings support the hypothesis that alterations of dopamine synaptic transmission underlie the hyperactivity in *Cm*/+ mice, and suggest that dopamine neurotransmission is particularly sensitive to alterations in the expression of SNAP-25. Supported by PHS MH48989 to MCW.

524.3

CO-ADMINISTRATION OF A PHOSPHODIESTERASE (PDE) INHIBITOR ENHANCES THE INDUCTION OF BDNF mRNA BY ANTI-DEPRESSANT TREATMENTS. S.Morinobu[1]*, M.Takahashi[1], K. Fujimaki[1], N.Okuyama[1], S.Totsuka[1] and R.S.Duman[2] [1]Dept. of Neuro-Psychiatry, Yamagata Univ. Sch. Med., Yamagata, Japan and [2]Dept. of Psychiatry Yale Univ. Sch. Med., New Haven, CT. 06508

We have reported that chronic antidepressant administration increases the expression of BDNF in rat hippocampus. Expression of BDNF is also increased by chronic administration of a PDE inhibitor. Here we examine the regulation of BDNF mRNA by co-administration of a PDE inhibitor (rolipram or Ro 20-1724) with a selective norepinephrine reuptake inhibitor (SNRI) (desipramine or Org 4428) using northern blot hybridization. Acute (1 d) co-administration of a SNRI and a PDE inhibitor did not influence levels of BDNF mRNA. Chronic (21 d) co-administration of a PDE inhibitor with a SNRI resulted in a greater induction of BDNF mRNA in rat hippocampus relative to that observed with either treatment alone. Preliminary studies suggest that co-administration of a PDE inhibitor with desipramine for 11 days also results in a more rapid induction of BDNF mRNA. The results indicate that co-administration of a PDE inhibitor enhances the time course and maximal induction of BDNF mRNA in response to SNRI treatment. BDNF is reported to increase the survival and sprouting of monoamine neurons and to have antidepressant effects in animal models of depression. Moreover, stress is reported to decrease the expression of BDNF and can cause atrophy of vulnerable neurons in hippocampus. Taken together, the results suggest that increased expression of BDNF may play an important role in the therapeutic action of antidepressants.

524.4

INCREASED DOPAMINE NIGROSTRIATAL ACTIVITY FOLLOWING NEUROPEPTIDE ANTIBODY ADMINISTRATION TO NEONATAL RATS. M.L.de Ceballos*[1], M.Pernas[1] and J.A.Fontenla[2]. [1]Neurodegeneration Group, Cajal Inst., 28002 Madrid; [2] Dept.Pharmacology, Univ.Santiago de Compostela, Spain.

Different neuropeptides have a neuromodulatory role on dopamine (DA) nigrostriatal activity. Following neonatal (P2) administration of neuropeptide antibodies (IgGs, 500 µg/rat sc), endogenous levels and release (microdialysis *in vivo*) of DA and its metabolites were measured in adult animals. Spontaneous locomotor activity and hyperactivity induced by DA agonists (apomorphine 0.5 mg/kg sc and amphetamine 3 mg/kg ip) was assessed as well. Controls were given IgGs from preimmune serum. DA striatal levels were increased by anti-met-enk and anti-SP treatement. DA release was enhanced by anti-met-enk and anti-neurotensin administration, while anti-SP increased HVA and DOPAC release. All treatments enhanced (x2) spontaneous locomotor activity of rats, while DA induced hyperactivity was reduced (50%) compared to control rats. Increased DA levels and release correlates with increased spontaneous activity and may result in subsensitivity of postsynaptic DA receptors which mediate DA agonist behaviour. These results confirm the neuromodulatory role of neuropeptides in basal ganglia.

Supported by FIS (94 0403)

524.5

BIOCHEMICAL EFFECTS OF BUPROPION IN DEPRESSION Y. Z. Feng, S. Madakasira, N. Goldman, H. Zhu, A. E. Halaris* and J. E. Piletz. Dept. of Psychiatry and Human Behavior, Univ. of Mississippi Medical Center, Jackson, MS 39216

Bupropion is a structurally novel antidepressant whose mechanism of action remains unclear. Bupropion is a weak inhibitor of monoamine reuptake and is not known to block MAO. We investigated the effects of bupropion in 8 depressed patients after 6-8 weeks of treatment. Patients received 300-450 mg/day in divided doses. Last dose was at 18:00 hrs and blood drawing was at 9:00 hrs on the next day. Plasma concentrations of norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), 3-methoxy-4-hydroxyphenyl glycol (MHPG) and agmatine were determined by HPLC. Agmatine, decarboxylated arginine, is a novel neurotransmitter candidate. The concentration (mean ± SE) of DA was significantly higher ($p<0.05$) in post-treatment (241.5 ± 110.6 pg/ml) than in pre-treatment (46.6 ± 5.4 pg/ml) depressed patients. The concentration of 5-HT was also significantly higher ($p<0.01$) in post-treatment (1322.6 ± 105.3 pg/ml) than in pre-treatment (423.3 ± 117.8 pg/ml) depressed patients and in healthy control subjects (645.7 ± 142.6 pg/ml). The concentrations of NE, DOPAC, MHPG and agmatine were not significantly different among the pre-treatment, post-treatment and control groups. These results provide evidence that dopamine and serotonin may contribute to bupropion's antidepressant properties. Supported by NIMH grants MH49248 and MH42859.

524.6

EFFECTS OF ALTERATIONS IN PERIPHERAL CORTICOSTERONE ON MESOCORTICAL AND TUBEROINFUNDIBULAR DOPAMINERGIC NEUROCHEMICAL INDICES. S.E. Lindley*, T.G. Bengoechea, A.F. Schatzberg and D.L.Wong, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, MSLS P104, Stanford, CA 94305-5485

Disturbances in the hypothalamic-pituitary-adrenal (HPA) axis in psychotic depressed patients have been hypothesized to produce delusions, hallucinations, and other cognitive disturbances through interactions with central dopaminergic (DA) neurons. In investigating this relationship in the male rat, a 7 day elevation in peripheral corticosterone (CORT), achieved through implantation of a sustained-release CORT pellet (placebo 4.0 ± 1.4 vs. CORT pellet 17.6 ± 1.3 ng/ul plasma CORT), resulted in a decrease in medial prefrontal cortex (mPfx) and an increase in median eminence (ME) DA utilization (dihydroxyphenylacetic acid (DOPAC) to DA ratio) and was without effect in any other DA nerve terminal regions examined. This chronic elevation also decreased noradrenergic utilization in the mPfx. Removal of basal levels of corticosterone, achieved through adrenalectomy 7 days prior to sacrifice, resulted in an increase in DA utilization in the mPfx and a decrease in the ME. Taken together, these results suggest plasma corticosterone selectively alters mPfx mesocortical and tuberoinfundibular DA neuronal activity, and is consistent with the hypothesized role of HPA axis-DA interactions in the pathophysiology of psychotic major depression. (Research Supported by NIMH grant MH50604, a NARSAD Young Investigator Award and a DANA Research Fellowship)

524.7

PHENCYCLIDINE INCREASES MONOAMINE UTHLIZATION IN PRIMATE PREFRONTAL CORTEX: BLOCKADE OF EFFECTS WITH S-(-)HA966. RH Roth[1,2,*], JD Jentsch[3], DE Redmond Jr[1] and JD Elsworth[1,2]. Depts of [1]Psychiatry, [2]Pharmacology & [3]Neurobiology, Yale School of Medicine, New Haven, CT 06510.

Recent studies from this lab have demonstrated that phencyclidine (PCP) potently activates monoamine turnover in the rodent brain and that the effect of PCP on dopamine (DA) turnover in the prefrontal cortex (PFC) can be selectively blocked with the γ-hydroxybutyrate-like agent, S-(-)HA966. In order to determine the relevance of these effects to the human brain, the consequences of PCP exposure and S-(-)HA966 pretreatment on regional monoamine turnover in the primate brain were examined.

Young adult male vervet monkeys were treated with PCP (0.3 mg/kg i.m.) or saline. S-(-)HA966 (1-3 mg/kg i.m.) was administered 15 min prior to PCP when given. One hour after PCP, the animals were deeply anesthetized with sodium pentobarbital and were subsequently perfused transcardially with ice-cold heparinized saline. The brain was removed, prepared in 4 mm coronal slices and regionally dissected on an ice-cold platform.

PCP potently activated DA utilization (HVA/DA) in several regions of the frontal cortex (dorsolateral prefrontal, dorsomedial prefrontal, ventromedial prefrontal and cingulate cortex). Serotonin (5-HT) turnover (5-HIAA/5-HT) was also increased after PCP in all areas studied. S-(-)HA966 pretreatment blocked the effects of PCP on frontal cortical DA turnover.

This study demonstrates that PCP alters monoamine transmission in the primate PFC, an area involved in cognition and potentially dysfunctional in schizophrenia. Our lab has recently shown that increased DA turnover in the PFC impairs cognitive ability (Murphy et al., 1996), and as such, the present data suggest that at least some of the acute cognitive dysfunction associated with PCP adminstration may be related to a hyperdopaminergic state of the PFC and that S-(-)HA966 may be useful in preventing these effects.

This study was funded in part by Public Health Service Grants MH-14092 (RHR) and MH-00643 (DER), the Axion Research Foundation and the St Kitts Biomedical Research Foundation.

524.8

DIFFERENTIAL EFFECTS OF ACUTE AND SUBCHRONIC PHENCYCLIDINE ADMINISTRATION ON PREFRONTAL CORTICAL DOPAMINE: RELEVANCE TO SCHIZOPHRENIA. JD Jentsch[1,*] and RH Roth[2,3]. Depts of [1]Neurobiology, [2]Psychiatry & [3]Pharmacology, Yale School of Medicine, New Haven, CT 06510.

Phencyclidine (PCP) has been used in attempts to model psychosis since PCP can cause psychotomimetic behavior in normal humans. Acutely, PCP induces transient psychotoform states, while repeated exposure to PCP can lead to more long-term and robust schizophreniform symptomology. As the neurobiological consequences of PCP administration may have relevance to the pathophysiology of schizophrenia, we have investigated the neurochemical effects of acute and repeated PCP exposure.

Acute PCP treatment (rat: 10 mg/kg; monkey: 0.3 mg/kg) induced marked activation of monoaminergic systems in the rodent and primate brain. In particular, PCP potently increased dopamine (DA) turnover in the prefrontal cortex (PFC) of both species. The effect of PCP on PFC DA was blocked by S-(-)HA966 (3 mg/kg) and clonidine (0.1 mg/kg), drugs which prevent stress-induced increases in PFC DA turnover. The PCP-induced hyperdopaminergic state of the PFC may be the neurochemical substrate for the cognitive effects observed in humans following acute PCP administration as we have recently shown that increased DA turnover in the PFC can lead to cognitive impairment (Murphy et al., 1996).

After subchronic PCP treatment (10 mg/kg/day for 14days), basal DA turnover in the rat PFC was significantly depressed, while basal DA turnover in NAc and STR and basal 5-HT and NE turnover were unaltered. In addition, after subchronic PCP administration, the effects on PFC DA turnover of an acute PCP challenge were merely blunted, whereas complete tolerance developed in the NAc. Thus, there is a selective inhibition of basal and stimulated PFC DA turnover after subchronic PCP.

The depression of PFC DA activity after repeated PCP may be a more relevant neurochemical model of schizophrenia as a hypoactivity of the dorsolateral PFC associated with decreased DA transmission has been shown in schizophrenics (Weinberger et al., 1986, 1988). Therefore, the inhibition of PFC DA function after repeated PCP may be the most accurate model of schizophrenia yet studied.

These studies were funded by Public Health Service Grant MH-14092 (RHR).

524.9

DOPAMINE TERMINALS IN THE MONKEY PREFRONTAL CORTEX SELECTIVELY INNERVATE PARVALBUMIN-CONTAINING LOCAL CIRCUIT NEURONS. DA Lewis,* VA Hawrylak, DS Melchitzky, SR Sesack. Departments of Psychiatry and Neuroscience, University of Pittsburgh, PA 15213.

Complex modulatory actions of dopamine (DA) in the prefrontal cortex (PFC) are likely to involve physiological regulation of both pyramidal cells and local circuit neurons. Our previous work suggests that the latter effect is mediated synaptically by DA terminal inputs to GABA interneurons (*J Comp Neurol* 363:264). However, this innervation appears not to include the subclass of GABA cells that contain the calcium-binding protein calretinin (*Neurosci Lett* 200:9). We sought to determine whether DA terminals synaptically innervate the separate subset of GABA local circuit neurons that express parvalbumin (PV). Sections through area 9 of cynomolgus monkeys were labeled with immunogold-silver for PV and immunoperoxidase for tyrosine hydroxylase (TH) to label DA terminals. Electron microscopic examination of fields containing both markers revealed that TH-positive terminals were sometimes directly apposed to PV-labeled dendrites; of these contacts, approximately one third were symmetric synapses. Other TH-immunoreactive terminals contacted unlabeled dendrites in the vicinity of PV-labeled processes. Because PV is present in wide arbor and chandelier neurons, which specifically target pyramidal cell soma and axon initial segments, respectively, these findings indicate that DA terminals synaptically innervate one or both of these classes of local circuit neurons. Thus, DA's modulatory actions in the PFC involve selective effects on only certain interneuron populations, some of which mediate potent inhibitory actions on pyramidal cells. Supported by USPHS grants MH50314 and MH43784.

524.10

POSTSYNAPTIC TARGETS OF TYROSINE HYDROXYLASE-LABELED TERMINALS IN MONKEY ENTORHINAL CORTEX. S.L. Erickson*, S.R. Sesack and D.A. Lewis. Depts. of Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

The rostral portions of the entorhinal cortex (ERC) receive a dense dopamine (DA) innervation, and this innervation is significantly reduced in schizophrenic subjects. Knowledge of the postsynaptic targets of DA terminals in the ERC will shed light on the functional significance of the abnormal DA innervation of this region in schizophrenia. In other regions of cortex, DA terminals target pyramidal cell dendrites and spines, as well as the dendrites of subpopulations of GABA-containing local circuit neurons. In this study, antibodies against tyrosine hydroxylase (TH) and GABA were used to study the postsynaptic targets of DA terminals in the superficial layers of the rostral subdivision in the adult cynomolgus monkey ERC. TH immunoreactivity was visualized with a peroxidase label and GABA immunoreactivity was visualized with a pre-embedding gold-silver label. By electron microscopic examination, TH-positive axons were 0.15-0.2 μm in diameter, and were unmyelinated. TH-positive varicosities were as large as 0.7 μm, and most were apposed to unlabeled dendritic shafts or spines. Some TH-labeled varicosities were apposed to dendrites labeled for GABA. These findings suggest that DA may influence directly the activity of both excitatory and inhibitory neurons in the ERC. This work was supported by USPHS grant MH43784.

524.11

TYROSINE HYDROXYLASE-LIKE IMMUNOREACTIVE ASTROCYTES IN THE RAT BASAL GANGLIA. A.M. Cantuniari,* J. Patrickson and C. Moore. Dept. of Anatomy and Biochemistry, Morehouse School of Medicine, Atlanta, GA 30310. Catecholamines are ubiquitous neurochemicals implicated in a variety of neuronal functions including motor and neuroendocrine. Their presence in brain is primarily identified by immunohistochemical localization of tyrosine hydroxylase (TH) one of the enzymes involved in their synthesis, well known for a long time, as a specific marker for dopaminergic neurons. This is a pertinent extension of our previously described distribution of DA receptors subtype D2/3 in astrocytes of the rat SN (A. Cantuniari et al. Abstract 21, 3, 750.15, 1909, 1995, Society for Neuroscience).

A modified immunohistochemical ABC-DAB-α Naphthol method employing a mouse anti-TH antibody (Incstar) and a rabbit anti-GFAP (DACO) was used to identify the presence of TH in astrocytes. Although the TH-like expression within GFAP$^+$ astrocytes was heterogenous relative to the regional distribution a general pattern could be observed. TH-immunostaining extended from the cell body to the processes showing different intensity. Our data indicate that TH-like immunoreactivity is present in most (~ 80%) of the astrocytes in the rat basal ganglia. Among the GFAP$^+$ cells certain astrocytes appear more immunoreactive than others. The immunoreactivity of the astrocytes in the basal ganglia appears to be only partially correlated to the size and regional distribution of the cells. This could be an important factor in the rate of DA turnover and synthesis in astrocytes. Taken together with studies of F.G. Seil et al. Brain Res. 1992, Jan. 8, 569; 164-8 in cerebellar cultures our results suggest that TH cannot be considered anymore as a specific marker for dopaminergic neurons in the rat brain. Our data support the view that astrocytes should be considered catecholaminergic as long as they express TH.

Support: NIH grants S06GM08248 and 3G12RR03034.

524.12

PRESENCE OF CATECHOLAMINERGIC CALLOSAL NEURONS IN THE VISUAL CORTEX OF THE CAT. B. Jardon*, R. A. Ase, F. Lepore and T. A. Reader. *Centre de recherche en sciences neurologiques, Départements de Psychologie et Physiologie, Université de Montréal, Montréal (Québec) H3C 3J7 Canada.*

Until now, no data has shown any involvement of chemically-specified neurotransmitters in a possible direct inhibitory function of the corpus callosum (CC); therefore, we began to study the catecholaminergic profile of this large commissure. Initially, the CC of adult cats were dissected in three parts: the genu, the body and the splenium, and the latter division was used to measure its content in CA and some of their major metabolites by high-performance liquid chromatography. In a second series of experiments, callosal neurons were retrogradely labelled by either fluoro-gold or rhodamine-latex-microspheres, injected eleven days before into contralateral visual cortical areas. Brain sections containing the labelled callosal neurons were processed for tyrosine-hydroxylase (TH) and dopamine-β-hydroxylase (DBH) immunocytochemistry. The results show that measurable amounts of dopamine (DA) and noradrenaline (NA) and their main metabolites were present in the splenium of the CC, that the CA levels were significantly lowered following treatment of the animals with the synthesis inhibitor α-methyl-*p*-tyrosine, indicating the specificity of the method of measurement, and that numerous visual cortical neurons, of which some were identified as callosal neurons and visualized owing to the fluorescent retrograde tracer, showed immunoreactivity for TH, but not for DBH. From these data, TH-immunoreactive callosal neurons can be proposed to be at least partly responsible for the presence of DA in callosal fibers, and they may account for a direct inhibitory function of the CC.

[Supported by the FCAR , the NSERC and the MRC of Canada].

524.13

LOCALIZATION OF GTP CYCLOHYDROLASE IN MONOAMINERGIC BUT NOT NITRIC OXIDE PRODUCING CELLS. O. Hwang*, H. Baker, S. Gross and T. H. Joh. Cornell Univ. Med. Coll. at Burke Med. Res. Inst., White Plains N.Y. 10605 and Cornell Univ. Med. Coll., New York, N.Y. 10021

The first and rate-limiting enzyme in tetrahydrobiopterin (BH4) biosynthesis is GTP cyclohydrolase (GTPCH). BH4 serves as the essential cofactor for aromatic L-amino acid hydroxylases, such as tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), the enzymes responsible for monoamine synthesis, as well as for nitric oxide synthase (NOS), the nitric oxide synthesizing enzyme. We hypothesized that to provide access to the cofactor a close association exists between BH4 synthesizing and BH4-dependent enzymes. We sought to determine the relationship between GTPCH, neuronal NOS (nNOS) and TH in rat brain and adrenal gland using immunohistochemistry and in situ hybridization. Analyses of adjacent sections revealed specific localization of GTPCH in TH-containing cells in brain including substantia nigra, ventral tegmental area, hypothalamus and locus ceruleus, as well as chromaffin cells of adrenal medulla. GTPCH also was present in TPH-containing cells of the dorsal raphe nucleus and pineal gland. Thus, BH4 can be synthesized in all monoaminergic cells and is readily available for the enzymes requiring it. In contrast, analysis of adjacent sections showed that nNOS was not colocalized with GTPCH. Scattered nNOS positive cells were found in the cortex, striatum, cerebellum and olfactory bulb, all areas that receive monoaminergic innervation. The absence of GTPCH in nNOS cells suggests that the NO-producing cells may either obtain biopterin from monoamine containing processes which terminate in close proximity or take up biopterin released into the blood. (Supported by MH24285 and AG09686)

524.14

MONOAMINE OXIDASE (MAO)- B CONTAINING NEURONAL SYSTEMS IN MAO A DEFICIENT TRANSGENIC MICE, K. Ikemoto[1]*, K. Kitahama[1], J. L.Valatx[1], M. Jouvet[1], J. Seif[2], E. De Maeyer[2]. [1]Département de Médecine Expérimentale, INSERM U52, CNRS URA1195, Université Claude Bernard, Lyon, France. [2]Institute Curie, CNRS URA1343, Paris, France.

We studied type B momoamine oxidase (MAO B)-containing neuronal systems employing MAO histochemistry in aggressive MAO A deficient transgenic mice, Tg8 line, of which MAO A-activity was not observed in the locus coelureus cells. In contrast, the dorsal raphe and laterodorsal tegmental nuclei contained many MAO B-positive cells. In this mutant line, MAO B-containing cells were distributed in the tuberomammillary, and dorsomedial posterior hypothalamic nuclei. In the thalamus, stained cells were packed in the paraventricular, mediodorsal, central medial, ventrolateral, rhomboid and reuniens thalamic nuclei, as well as the habenular nucleus. In the forebrain, MAO B-containing cells were observed in the preoptic area, striatum, septal nucleus, major island of Calleja, diagonal band, ventral pallidum and amygdaloid nucleus. In this model, we are able to examine exclusively MAO B structures, which has been difficult to be differentiated from MAO A even with pharmacological suppression in normal animals.

525.1

PHARMACOLOGICAL PROPERTIES OF BURST-FIRING SEROTONERGIC NEURONES IN THE RAT DORSAL RAPHE NUCLEUS

M. Hajós[1], A.E.P. Villa*[2] and T. Sharp[1], [1]Oxford University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, U.K. and [2]Department of Physiology, University of Lausanne, Rue du Bugnon 7, Lausanne, Switzerland

We have recently reported a sub-population of presumed 5-HT neurones in the raphe nuclei of anaesthetised rats (Hajós et al. Neuroscience, 69: 189-197, 1995) which display all the electrophysiological features of 5-HT neurones, except that they discharge spike doublets or triplets with a short interspike interval (<10ms). In the present study we analysed some pharmacological properties of these neurones. Extracellular recordings were made in the dorsal raphe of anaesthetised male Sprague-Dawley rats using conventional electrophysiological methods. In some experiments the pharmacological properties of burst-firing 5-HT neurones were compared to single spiking, classical 5-HT neurones.

Both single spiking and bursting 5-HT neurones were inhibited by i.v. administration of 8-OH-DPAT, a 5-HT_{1A} receptor agonist or paroxetine, a selective 5-HT reuptake inhibitor, with equal sensitivity. Interestingly, in burst-firing neurones low doses of 8-OH-DPAT preferentially inhibited higher order spikes, i.e. spikes fired in burst. As with single spiking, classical 5-HT neurones, burst-firing neurones were inhibited by prazosin, an α1-adrenoceptor antagonist. In order to determine whether excitatory amino acid receptors are involved in the generation of bursting activity, MK-801 (0.4 mg/kg, i.v.), and kynurenic acid (0.1-0.5 µmol, i.c.v.) were administered. Both antagonists failed to alter significantly the bursting activity, although kynurenic acid significantly inhibited the overall firing activity. Microiontophoretic application of glutamate significantly increased the firing rate but did not induce burst activity in single spiking neurones.

Our findings demonstrate that both single spiking and burst-firing 5-HT neurones are modulated by α1-adrenoceptors and 5-HT_{1A} receptors, and indicate that excitatory amino acid receptors are not involved in the bursting activity.

(Supported by the Medical Research Council, U.K.)

525.2

ELECTROPHYSIOLOGICAL EFFECT OF ALNESPIRONE (S-20499) ON RAT LOCUS COERULEUS NEURONS - COMPARISON WITH BUSPIRONE Schmitt P[1]; Soulière F[1]; Lesourd M[2]; Renaud B[1]*, Chouvet G[1] [1] CJF INSERM 95-06 and Université Claude Bernard Lyon I, 8 avenue Rockefeller, 69373 Lyon, France [2]Institut de Recherches Internationales Servier, 6 place des Pléiades, 92415 Courbevoie Cedex, France

Alnespirone (S-20499), a new chroman derivative, is a selective 5HT_{1A} receptor agonist which displays a slight affinity for α_1 and α_2 receptors (ratio $K_{0.5}$ 5HT_{1A} - α_1/α_2 = 330). Pharmacological studies have shown its anxiolytic and antidepressant activity in several animal models following single or repeated intraperitoneal, intramuscular, subcutaneous and oral administration. Since buspirone, a chemically related 5HT_{1A} agonist, is also known to display *in vivo* α_2-adrenergic antagonist properties, in spite of its slight affinity for adrenergic receptors (ratio $K_{0.5}$ 5HT_{1A} - α_1/α_2 = 240), we have compared the effects of alnespirone and buspirone on the spontaneous activity of locus coeruleus (LC) noradrenergic neurons, before or after administration of the α_2-agonist clonidine. Extracellular recordings of LC neurons were performed on chloral hydrate-anesthetized rats with glass micropipettes (baseline = 1.9 ± 0.2 spikes/sec, n = 35) and were confirmed by subsequent histological analysis. Clonidine (40 µg/kg i.p.) induced a rapid inhibition of LC activity (100% inhibition in about 7 mn) that was reversed (92% recovery in 15 mn) by subsequent buspirone (10 mg/kg i.p.). In contrast, alnespirone (10 mg/kg i.p.) did not affect the typical and slow recovery to baseline activity after clonidine (17% recovery in 15 mn). On the other hand, clonidine-induced inhibition was prevented by prealable administration of buspirone (20% inhibition in 22 mn) but not alnespirone (100% inhibition in 7 mn, as in control group). These findings indicate that alnespirone, unlike buspirone, is devoid *in vivo* of significant α_2-adrenergic properties. As already described, the α_2-adrenergic antagonist properties of buspirone could be due to its main metabolite (1-PP), and such properties are clearly not shared by alnespirone or one of its metabolites.

525.3

INHIBITION OF DORSAL RAPHE CELL FIRING BY PUTATIVE 5-HT_{1A} ANTAGONISTS: 5-HT_{1A} PARTIAL AGONISM OR α-ADRENERGIC BLOCKADE? B.L. Waszczak*, L.P. Martin and D.M. Jackson. Dept. of Pharmaceut. Sci., Northeastern Univ., Boston, MA 02115 and Dept. of Behav. and Biochem. Pharmacol., Astra Arcus AB, Preclinical R & D, S-151 85, Sodertalje, Sweden.

Agonists at 5-HT_{1A} receptors inhibit the firing of serotonergic dorsal raphe (DR) neurons by an action on somatodendritic autoreceptors. Accordingly, 5-HT_{1A} antagonists should shift rightward the dose-response curve for inhibition of firing by highly potent and efficacious agonists such as 8-OH-DPAT while having no effect, or a stimulatory effect, on DR neuronal firing when given alone. Single unit recording studies were undertaken in chloral hydrate-anesthetized rats to assess effects on DR cell firing of several putative 5-HT_{1A} antagonists, including WAY 100635, NDL 249, and p-MPPI. As expected, pretreatment with a 10 µg/kg i.v. dose of WAY 100635 or NDL 249 caused significant shifts to the right of the dose-response curve for 8-OH-DPAT (ED_{50} for 8-OH-DPAT, 1.3±0.3 µg/kg; after WAY 100635, 16.9± 2.9 µg/kg; after NDL 249, 6.0±1.2 µg/kg). Surprisingly, however, i.v. administration of both WAY 100635 and NDL 249 alone caused dose-dependent and ultimately complete suppressions of DR cell firing (ED_{50}'s 0.6±0.2 and 0.7±0.3 µmol/kg, respectively). It should be noted that the inhibition by these drugs was seen only at high doses (>0.1 µmol/kg), whereas 8-OH-DPAT inhibited firing at 100-fold lower doses. In contrast, a third putative antagonist, p-MPPI, produced no significant changes in DR cell firing even at high (equimolar) doses. To assess the mechanism of the inhibition by high doses of WAY 100635 and NDL 249, we attempted reversal with *d*-amphetamine (AMPH, 3.2 mg/kg i.v.). The inhibition by both drugs was readily reversed by AMPH, whereas effects of 8-OH-DPAT were not. These results suggest that the inhibitory effects may be due to α-blockade, rather than 5-HT_{1A} agonism, and support the view that these drugs act as "silent" 5-HT_{1A} antagonists.

(Supported by Astra Arcus AB)

525.4

EFFECT OF REPEATED VERSUS SUSTAINED ADMINISTRATION OF THE 5-HT_{1A} AGONIST IPSAPIRONE: ELECTROPHYSIOLOGICAL STUDIES IN THE RAT HIPPOCAMPUS AND DORSAL RAPHE. J. Dong*, C. de Montigny and P. Blier. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada H3A 1A1.

The present study was aimed at examining the adaptation of presynaptic 5-HT_{1A} autoreceptors in the dorsal raphe (DR) and of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus (DH) during long-term administration of the 5-HT_{1A} agonist ipsapirone given either repeatedly or in a sustained fashion. Concurrent microiontophoretic application of ipsapirone did not attenuate the suppressant effect of 5-HT on 5-HT neurons but markedly decreased it when co-applied on CA_3 pyramidal neurons in the DH. Thus, ipsapirone acted as a full agonist in the DR and as a partial agonist in the DH. Ipsapirone (15 mg/kg/day, s.c. x 2 days) delivered by osmotic minipumps markedly decreased the firing activity of the DR 5-HT neurons. After 14 days of treatment, there was a complete recovery of their firing activity and a desensitization of their somatodendritic 5-HT_{1A} autoreceptors, as assessed using microiontophoretic applications of 5-HT and 8-OH-DPAT onto 5-HT neurons. The same degree of desensitization was obtained when ipsapirone was given repeatedly (7.5 mg/kg b.i.d., s.c. x 14 days). In contrast, the two modalities of ipsapirone administration left unaltered the responsiveness of CA_3 pyramidal neurons to microiontophoretic application of 5-HT and 8-OH-DPAT. In conclusion, long-term administration of ipsapirone most likely increases 5-HT neurotransmission by enhancing the tonic activation of postsynaptic 5-HT_{1A} receptors. In addition, the use of sustained release 5-HT_{1A} agonists should not alter their therapeutic effectiveness in anxiety and affective disorders since the same effects on 5-HT_{1A} receptor functions were produced in this rat model by the sustained and the repeated modes of administration of ipsapirone.

525.5

TERMINAL 5-HT AUTORECEPTOR DESENSITIZATION IN THE ORBITOFRONTAL CORTEX IS PRODUCED EXCLUSIVELY BY DRUGS EFFECTIVE IN OBSESSIVE COMPULSIVE DISORDER (OCD). V. G. Ducharme, C. Bouchard and P. Blier* Neurobiological Psychiatry Unit, McGill University, Montreal, Canada, H3A 1A1.

The only drugs so far demonstrated to be effective in the treatment of OCD are the ones that potently block the 5-HT transporter. These 5-HT reuptake inhibitors (SRI) enhance 5-HT release in the hippocampus and the hypothalamus by desensitizing terminal 5-HT autoreceptors. However, PET scan studies in humans have previously implicated the orbitofrontal cortex (OFC) in the mediation of OCD symptoms. We have shown that, after 8 weeks of treatment with the SRI paroxetine at a high dose (10 mg/kg/day, s.c. using minipumps), the electrically evoked release of [3H]5-HT from guinea pig preloaded brain slices was enhanced and the sensitivity of the terminal 5-HT_{1D} autoreceptor was attenuated, but not after fluoxetine at a low dose (5 mg/kg/day). In order to investigate the efficacy of other antidepressant treatments to alter 5-HT release in OFC, further studies were performed in male Hartley guinea pigs. Superfusion experiments were carried out to assess the evoked release of [3H]5-HT from preloaded brain slices. There was a significant enhancement of [3H]5-HT release in the OFC after 8 weeks of treatment with a high dose of the SRI fluoxetine (10 mg/kg/day) and the sensitivity of the autoreceptor was attenuated. In contrast, the inhibitor of type A monoamine oxidase moclobemide administered for 8 weeks (10 mg/kg/day) did not alter terminal 5-HT_{1D} autoreceptor sensitivity, and neither did 12 electroconvulsive shocks given over one month. In conclusion, the desensitization of the terminal 5-HT_{1D} autoreceptor requires higher doses of SRI, as is often the case clinically in OCD. Electroconvulsive shock treatment and moclobemide, which are effective in depression but not in OCD, do not alter the sensitivity of the terminal 5-HT_{1D} autoreceptor. Consequently, the desensitization of this autoreceptor in the OFC may be the substratum of the anti-OCD effect of SRI in humans. Supported by the Medical Research Council (MRC).

525.6

MODULATION OF GUINEA PIG DORSAL RAPHE CELL FIRING BY GR127935, A 5-HT_{1D} AUTORECEPTOR ANTAGONIST. L. Reynolds*, H. Rollema and J. Sprouse. Pfizer Central Research, Groton, CT 06340 USA

GR127935 is a selective antagonist of release-modulating 5-HT_{1D} autoreceptors on serotonergic terminals and as such would be expected to produce increases in extracellular 5-HT. The changes in 5-HT observed in guinea pig cortex are mixed, however (range = -65% to +65% relative to baseline, depending on investigator), possibly due to the presence of somatodendritic 5-HT_{1D} autoreceptors. Blockade of these autoreceptors elevates intra-raphe 5-HT which in turn activates somatodendritic 5-HT_{1A} autoreceptors and slows firing rate. The aim of the present study was to examine this mechanism by directly measuring changes in raphe cell firing following GR127935. In chloral hydrate anesthetized guinea pigs, dorsal raphe cell firing was unaffected by doses of GR127935 ranging from 20 to 20,000 µg/kg iv. 8-OH-DPAT, in these and similar neurons, produced the robust dose-dependent inhibitory response expected of a 5-HT_{1A} agonist (ED_{50} = 3.1 µg/kg iv; n = 2-9 cells per dose). Increases in extracellular 5-HT resulting from re-uptake blockade also suppressed unit activity (fluoxetine ED_{50} = 1.0 mg/kg i.v.; n = 3-4 cells per dose). Curiously, lower doses of GR127935 (0.002 - 2 µg/kg iv) yielded highly variable responses with some cells displaying no effect and others a brief excitation followed by a slowly developing inhibition. The most effective dose in this case appeared to be 0.02 µg/kg which produced an eventual 37 ± 17 % decrease in cell firing (n = 6), although this was not significantly different from baseline. The incomplete nature of this response suggests that (1) a competing receptor mechanism progressively limits the degree of inhibition produced by 5-HT_{1D} autoreceptor blockade, completely overcoming it at higher doses and/or (2) the increase in extracellular 5-HT obtained through blockade of somatodendritic 5-HT_{1D} autoreceptors is insufficient to completely inhibit cell firing.

525.7

DIFFERENTIAL EFFECT OF THE 5-$HT_{2C/2B}$ RECEPTOR ANTAGONIST SB 200646 AND THE SELECTIVE 5-HT_{2A} ANTAGONIST MDL 100907 ON MIDBRAIN DOPAMINE (DA) NEURONS IN RATS: AN ELECTROPHYSIOLOGICAL STUDY, [1]C.R. Ashby, Jr.* and [2]Y. Minabe. [1]PHS Dept., St. John's University, 8000 Utopia Parkway, Jamaica, NY 11439; [2]National Institute of Neuroscience, NCNP, Kodaira, Tokyo, 187, Japan.

In this study, we examined the effect of the acute and chronic administration of the 5-$HT_{2B/2C}$ antagonist SB 200646 (SB) and the 5-HT_{2A} antagonist MDL 100907 (MDL) on the number, as well as the firing pattern, of spontaneously active DA neurons in the A9 and A10 areas. This was accomplished using the technique of extracellular single unit recording in anesthetized, male Sprague Dawley rats. Animals received either 1 injection (acute) or 1 injection daily for 21 days (chronic) of SB (10 or 20 mg/kg, i.p.) or MDL (0.01, 0.03 and 0.1 mg/kg, i.p.). Experiments were conducted two hours after the last injection. The acute administration of 0.1 mg/kg of MDL significantly increased the number of spontaneously active A9 and A10 DA cells. The i.v. administration of MDL had no significant effect on the firing rate or pattern of either A9 or A10 DA neurons. In contrast, the acute administration of 20 mg/kg of SB significantly increased the number of spontaneously active A10 DA neurons and i.v. SB significantly increased the firing rate and burst firing of A10 DA neurons. The chronic administration of MDL, at all doses, significantly decreased the number of A9 and A10 DA neurons and this effect was not reversed by 50 μg/kg, i.v., of (+)-apomorphine (APO). The chronic administration of 20 mg/kg of SB selectively decreased the number of spontaneously active A10 DA neurons and this effect was reversed by i.v. APO. These results indicate that the activity of midbrain DA neurons in rats is differentially regulated by the antagonism of 5-HT_{2A} compared to 5-$HT_{2B/2C}$ receptors. Supported by NIMH R2952155-05.

525.8

Protein kinase C-mediated enhancement of glycine response in rat sacral dorsal commissural neurons by serotonin. N. Akaike*, T. Xu, J. Nabekura. Dept. Physiol., Kyushu Univ. Fac. Med., Fukuoka 812-82, Japan

Modulatory effect of 5-HT on strychnine-sensitive glycine response (I_{Gly}) was investigated in neurons acutely dissociated from rat sacral dorsal commissural nucleus (SDCN) using nystatin perforated patch recording under voltage-clamp. 5-HT potentiated 10^{-5}M Gly-induced Cl^- current in a concentration-dependent manner without affecting the affinity of Gly to its receptor. α-Methyl-5-HT mimicked and ketanserine blocked the 5-HT action, indicating that the 5-HT_2 receptor mediated the enhancement. Phorbol and OAG potentiated I_{Gly}. The 5-HT in the presence of these PKC potentiators or chelerythrine failed to potentiate I_{Gly}, suggesting an involvement of PKC in the pathway. This facilitation persisted in the pertussis toxin treatment for 6-8 hr. Neither PKA nor cAMP modulators had any effect on 5-HT potentiation of I_{Gly}. The 5-HT facilitation of I_{Gly} remained even when $[Ca^{2+}]_i$ was chelated by BAPTA. In conclusion, the activation of 5-HT_2 receptor coupled to a pertussis toxin-insensitive G-protein increases intracellular DAG formation which increases Ca^{2+}-independent PKC activity, resulting in the potentiation of the Gly response in the SDCN neurons. This potentiation might be one of analgesic mechanisms for nociceptive sensation in the spinal cord level.

525.9

Serotonergic amplification of apically-derived EPSPs in neocortical pyramidal cells via a persistent sodium current. G.J. Marek* and G.K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale School of Medicine, New Haven, CT 06508.

Active conductances are required for amplification of excitatory postsynaptic potentials (EPSPs) originating from distal apical dendrites of pyramidal cells. The purpose of the present experiments was to determine the effect of monoamines on amplification of EPSPs from the apical dendrites of pyramidal cells. Serotonin (5-HT) induced an increase in spontaneous PSPs in layer V pyramidals cells in many regions of the rat neocortex (e.g. prefrontal) that was blocked totally by the selective 5-HT_{2A} antagonist MDL 100,907 and the selective AMPA antagonist LY 293558, but not the $GABA_A$ antagonist bicuculline. Under voltage clamp, 5-HT and norepinephrine increased excitatory postsynaptic currents (EPSCs) by 6-fold and 2-fold, respectively while dopamine was ineffective. Focal (microiontophoretic) application of 5-HT enhanced EPSCs from the apical, but not basilar dendrites. The 5-HT-elicited EPSCs were blocked by focal application of the Na^+ channel blocker tetrodoxtoxin (TTX) to the apical dendrites. The Na^+ channels required for the 5-HT-mediated increase in EPSCs were intrinsic to the pyramidal cells since intracellular application of the quaternary lidocaine derivative QX-314 resulted in a progressive but delayed block of 5-HT-elicited EPSCs. Finally, slow depolarizing voltage ramps in pyramidal cells showed that 5-HT could enhance amplification of EPSCs by increasing a TTX-sensitive non-inactivating or persistent TTX-sensitive Na^+ current (I_{NaP}); both TTX and MDL 100,907 blocked enhancement of I_{NaP} by 5-HT. Thus, these studies indicate that 5-HT, via activation of 5-HT_{2A} receptors, amplifies EPSPs/EPSCs from the apical dendritic field of neocortical layer V pyramidal cells by increasing a persistent Na^+ current. *Supported by MH17871 and the VA.*

5-HT_{1A} RECEPTORS: BINDING

526.1

PURINERGIC RECEPTOR-MEDIATED REGULATION OF 5-HT_{1A} RECEPTOR FUNCTION. K.J. Evans*, K.A. Berg, J.D. Cropper, W.P. Clarke. Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

Previous work from our laboratory and others has demonstrated that 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated cAMP accumulation (FScA) in CHO cells is reduced by the phospholipase A_2-(PLA_2) arachidonic acid (AA) signaling cascade and by protein kinase C (PKC). CHO cells express naturally a purinergic receptor that couples to both phospholipase C (PLC) and PLA_2. However, activation of this purinergic receptor with ATP does not alter 5-HT_{1A}-mediated inhibition of FScA. In this study, we have investigated further the lack of effect of activation of the purinergic receptor on 5-HT_{1A} receptor function. In CHO cells expressing ≈140 fmol/mg of the human 5-HT_{1A} receptor, 5-HT_{1A} receptor function was evaluated by measuring inhibition of forskolin- (1 μM) stimulated cAMP accumulation after 15 min incubation with the selective 5-HT_{1A} agonist, dp-5-CT (10 nM; ≈EC_{50}). Incubation with ATP (1 mM) did not alter inhibition of FScA by dp-5-CT (27% ± 6% vs 29% ± 5% in the absence and presence of ATP respectively, n=4). However, in calcium-free medium (where ATP-mediated AA release is abolished) incubation with ATP enhanced 5-HT_{1A} function by approx. 50% (from 24% ± 3% to 37% ± 5%, n=3). This effect of ATP was mimicked by thapsigargin (300 nM) which releases calcium from intracellular stores (dp-5-CT-mediated inhibition was 36 ± 5%, n=3). In calcium-free medium, neither ATP nor thapsigargin altered FScA while, interestingly, in calcium-containing medium, thapsigargin decreased FScA by approx. 50%. Although in calcium-containing medium ATP did not alter FScA, downregulation of PKC with 24 hr phorbol ester treatment, resulted in an ATP-mediated inhibition of FScA by 50%. These data suggest that the lack of ATP-mediated effect on 5-HT_{1A} receptor function is the result of opposing actions of stimulatory (intracellular calcium) and inhibitory (AA and possibly PKC) factors on the 5-HT_{1A} receptor system. (Supported by USPHS grants HD 26437 and MH 48125).

526.2

CORTICOSTERONE ALTERS 5-HT_{1A} RECEPTOR AND G PROTEIN MEDIATED RESPONSES IN THE HIPPOCAMPUS. D.Y. Okuhara*, N.A. Muma and S.G. Beck Loyola University Medical Center, Maywood IL 60153.

Corticosterone (CT) alters the response elicited by the activation of several neurotransmitter receptor systems, including receptors which are linked to G proteins. We investigated the effects of CT on the 5-HT concentration response curve for the 5-HT_{1A} receptor, the outward current evoked by the activation of G proteins in subfield CA3 and G protein protein levels in the hippocampus. Four treatment groups were used: ADX, bilateral adrenalectomy; ALD, ADX with an aldosterone minipump implant; HCT, ADX with 200 mg CT pellet implant and SHAM. All treatments were for 2 weeks. Discontinuous single electrode voltage clamp data were collected using standard intracellular recording techniques (n=14-16 cells for all treatments).

CT altered the characteristics of the 5-HT concentration response curve for the 5-HT_{1A} receptor. The EC_{50} value obtained from cells from HCT animals (15.1 ± 5.5 uM, mean ± SD) was less compared to ADX (10.0 ± 3.5 uM). The Emax obtained from cells from HCT animals (360 ± 116 pA) was smaller than cells from ADX (540 ± 187 pA) animals. The magnitude of the current associated with the activation of G proteins with GTPγS was also modulated by CT. The amount of current required to voltage clamp the cell's membrane potential at -60 mV relative to it's RMP was smaller in cells from HCT animals when compared to ADX, ALD and SHAM, i.e., 46%, 34% and 22% respectively. Furthermore, the current in ADX cells was 30% higher than SHAM. In Western blot experiments, HCT increased the levels of G_s, $G_{i1\&2}$, and G_o α-subunit levels when compared to SHAM. Based on these results, we conclude that high levels of CT decreases the maximal current elicited by the activation of the 5-HT_{1A} receptor and this decrease maybe due to a decrease in the function of the G protein linked to the receptor. Supported by PHS NS28512, RSDA MH-00880, and Schmitt Foundation.

526.3

PEPTIDE MEDIATED UNCOUPLING OF THE HUMAN 5HT1A RECEPTOR. K.K. Parker*, N. Colson, M.-F. O'Connor, K. Hayataka, and J. Iverson. Dept. of Pharmaceutical Sciences, School of Pharmacy, University of Montana, Missoula, MT 59812-1075.

The human 5HT1a receptor is a member of the G protein-coupled, 7-transmembrane segment receptor superfamily. A 15 amino acid peptide (15 MER) derived from the known sequence of the transmembrane segment 5 (TM5)/intracellular loop 3 (i3) region of the receptor has been synthesized by solid state methods. This peptide produces inhibition (non-competitive) of agonist binding in both solubilized and membrane-bound preparations of the receptor, obtained in these experiments from cultured, transfected Chinese Hamster Ovary (CHO) cells (gift of Dr. John Raymond, Duke U.). The IC50 for the inhibitory effect of the 15 MER is about 0.1 uM. The 15 MER is also active against the 5HT1a receptor from rabbit brain. The bioactive peptide, bombesin, which has similar length but different sequence from the 15 MER, is inactive in agonist inhibition assays. Peptides from the N-terminal region of the i2 loop and from the C-terminal region of TM7 have also been characterized. These studies have been designed to better understand the biology of the 5HT1a receptor-G protein interface, and will be useful in elucidating the molecular pharmacology of the receptor-signal transduction boundary. Supported by the Montana Science and Technology Alliance, NSF-EPSCoR, and NIH RR10169.

526.4

AFFINITY AND EFFICACY OF NOVEL SEROTONIN (5-HT)$_{1D}$ LIGANDS AT HUMAN (h) RECOMBINANT 5-HT$_{1A}$ RECEPTORS. V. Audinot*, A. Newman-Tancredi, C. Conte, S. Lochon, L. Verrièle, C. Chaput and M.J. Millan, I.D.R.S., 125 Chemin de Ronde, 78290 Croissy, France.

We examined 5-HT$_{1D}$ ligands at h5-HT$_{1A}$ receptors expressed in CHO cells. Efficacies (% of stimulation by 5-HT) and Effective Concentration$_{50}$ (EC_{50}) were determined by [^{35}S]GTPγS binding[1]. K_is were determined with [^{3}H]8-OH-DPAT (h5-HT$_{1A}$) and [^{3}H]GR 125,743 (guinea pig striatal 5-HT$_{1D}$ receptors).

	← E_{max} (%)	h5-HT$_{1A}$ EC_{50} (nM)	→ K_i (nM)	5-HT$_{1D}$ K_i (nM)	K_i ratio (1A/1D)
Sumatriptan	100	6800	127	175	0.7
Dihydroergotamine	100	12	0.8	1.5	0.5
Methysergide	100	880	21.1	230	0.1
Alniditan	70	110	3.0	4.2	0.7
BMS 181,101	0	n.d.	18.5	6.3	2.9
GR 46611	77	35	1.0	0.11	9.0
GR 127,935	30	330	50.5	4.2	12
L 694,247	84	30	0.4	0.09	4.4

BMS 181,101, 5-fluoro-3-{3-[4-(5-methoxy-pyrimidin-4-yl)-piperazin-1-yl]-propyl}-1H-indole; GR 46,611, 3-[3-(2-dimethylamino-ethyl)-1H-indol-6-yl]-N-(4-methoxybenzyl)-acrylamide; GR 127,935, N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-2'-methyl-4'- (5-methyl-1,2,4-oxadiazol-3-yl)- biphenyl - 4 - carboxamide; L 694,247, (2-[5-[3-(4-methyl-sulphonylamino)-benzyl-1,2,4-oxadiazol-5-yl]-1*H*-indole-3-yl]ethylamine). n.d. = not determined

These data suggest that 5-HT$_{1A}$ receptors may play an important role in the action of several novel 5-HT$_{1D}$ ligands.

1. Newman-Tancredi et al., Neuropharmacol. 35, p.119-121, 1996

This work was supported by Servier Pharmaceuticals.

526.5

AGONIST AND INVERSE AGONIST EFFICACY AT HUMAN (h) RECOMBINANT SEROTONIN (5-HT)$_{1A}$ RECEPTORS AS A FUNCTION OF RECEPTOR:G-PROTEIN STOICHIOMETRY. A. Newman-Tancredi*, C. Conte, C. Chaput, L. Verrièle and M.J. Millan, I.D.R.S., 125 Chemin de Ronde, 78290 Croissy, France.

Serotonin$_{1A}$ receptor agonists such as buspirone act as partial agonists post-synaptically but as full agonists pre-synaptically. This may be due to differences in receptor reserve. We now report the effect of receptor:G-protein stoichiometry on efficacy. By growing a transfected CHO cell line in suspension or adherent conditions, we generated cultures which express either 1.6 pmol/mg or 5.5 pmol/mg of recombinant h5-HT$_{1A}$ receptor, respectively, as determined by saturation binding with the selective antagonist, [^{3}H]-4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine ([^{3}H]-S 15535[1]). However, there was no change in the total number of G-proteins activated by the full agonist 5-HT (1.3 pmol/mg, measured by [^{35}S]-GTPγS saturation binding) providing preparations in which the receptor:G-protein ratio was ≈ 1:1 (RGlow) or ≈ 4 : 1 (RGhigh). Agonist efficacy was measured by stimulation of [^{35}S]-GTPγS binding[2]. The E_{max} value for eltoprazine was 50 % at RGlow receptors but 100 % at RGhigh receptors, relative to 5-HT (=100%). In contrast, the neutral antagonist, WAY 100,635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)cyclo-hexane-carboxamide), did not significantly alter [^{35}S]-GTPγS binding from basal levels in either preparation. The inverse agonist, spiperone, decreased basal [^{35}S]-GTPγS binding by only 12 % in RGlow membranes but by 26 % in RGhigh membranes. In conclusion, an increase in receptor:G-protein ratios augments both agonist and inverse agonist efficacy at h5-HT$_{1A}$ receptors.

1. Newman-Tancredi et al., Soc. Neurosci. 21, 727.21, 1995
2. Newman-Tancredi et al., Neuropharmacol. 35, p.119-121, 1996

This work was supported by Servier Pharmaceuticals.

526.6

ALNESPIRONE (S 20499) AND 8-OH-DPAT, TWO 5-HT$_{1A}$ RECEPTOR AGONISTS, MODULATE THE *IN VIVO* STRIATAL D_1 AND D_2 SPECIFIC BINDING IN MICE - COMPARISON WITH BUSPIRONE P. Protais[1], M. Lesourd[2], E. Mocaër[2*] and E. Comoy[3] Laboratoires de Physiologie (VACOMED)[1] et de Biochimie[3], U.F.R. de Médecine-Pharmacie de Rouen, Institut de Recherches Internationales Servier[2], Courbevoie, France.

Alnespirone (S 20499) has previously been described as a potential anxiolytic drug through its agonist action at 5-HT$_{1A}$ receptors. Some data suggest that S 20499 could also be a weak D_2 receptor agonist on the basis of its *in vitro* D_2 affinity, prolactin release inhibition and yawning in rats. In order to test a possible interaction of S 20499 with DA receptors in vivo, we have studied the *in vivo* striatal [^{3}H]-SCH 23390 (D_1 receptor ligand-4 µCi) and [^{3}H]-raclopride (D_2 receptor ligand-4 µCi) binding in mice treated with intraperitoneal doses of S 20499. In the same mice, the levels of DA, 5-HT and their metabolites were measured by HPLC with electrochemistry detection. S 20499 decreased [^{3}H]-SCH 23390 binding at 5,10 and 40 mg/kg, and increased [^{3}H]-raclopride binding at 5 and 20 mg/kg. In these animals, the 5-HIAA/5-HT ratio was decreased at 5 - 40 mg/kg, whereas the HVA/DA ratio was unaffected at all the doses tested of S 20499. Similarly, 8-OH-DPAT decreased [^{3}H]-SCH 23390 binding at 0.25, 1 and 4 mg/kg, and increased [^{3}H]-raclopride binding at 1 and 4 mg/kg. All the tested doses of 8-OH-DPAT decreased the 5-HIAA/5-HT ratio but did not modify the HVA/DA ratio. In the same experimental conditions, buspirone (5 and 20 mg/kg) inhibited completely [^{3}H]-raclopride, but not [^{3}H]-SCH 23390 binding and increased the HVA/DA ratio, but did not modify the 5-HIAA/5-HT ratio. Apomorphine (3 mg/kg) decreased both in vivo striatal [^{3}H]-SCH 23390 and [^{3}H]-raclopride specific binding as well as striatal HVA/DA and 5 HIAA/5-HT ratios. These results indicate that S 20499 and 8-OH-DPAT do not seem to interact directly with DA receptors. In contrast, buspirone interacts directly with D_2, but not D_1, receptors.

526.7

CHARACTERIZATION OF BRAIN SEROTONIN 5-HT$_{1A}$ RECEPTORS USING A RADIOLABELED ANTAGONIST: COMPARISON WITH AGONIST RADIOLIGAND. M.T. Vilaró[1], J.F. López-Giménez[1], A. Raurich[1], J.M. Palacios[2], R. Cortés[1*], and G.Mengod[1]. [1]Dept. Neurochemistry, IIBB/CSIC, 08034 Barcelona, Spain.

5-HT$_{1A}$ receptor (5-HT$_{1A}$R) belongs to the 5-HT$_1$ receptor family and has potential role as a therapeutic target for affective disorders such as anxiety and depression. The use of radiolabeled agonist compounds such as ^{3}H-8-OH-DPAT for direct labeling of these receptors provides a partial view of the density and localization of these sites because they recognize only the high affinity state of receptors. The recent development of WAY 100635, a 5-HT$_{1A}$R antagonist, with an equal affinity for G protein-coupled and uncoupled forms of the receptor, provides a new tool to investigate these receptors in the mammalian brain. We have used ^{3}H-WAY 100635 to characterize and visualize (using quantitative autoradiographic techniques) 5-HT$_{1A}$R in monkey brain. The binding of ^{3}H-WAY 100635 was saturable, with comparable nanomolar affinity and with low non-specific binding in every monkey brain region examined. ^{3}H-WAY 100635 binding was competitively blocked by co-incubation with both unlabeled agonists and antagonists of the 5-HT$_{1A}$R. When compared with ^{3}H-8-OH-DPAT binding, the density of sites labeled by the radiolabeled antagonist was considerable higher in all the regions examined. Different ratios between antagonist and agonist binding site densities were observed for some brain regions . We have compared the distribution of the binding sites with that of the mRNA coding for 5-HT$_{1A}$ receptor and found a good correlation, confirming its somatodendritic localization.

[2] Permanent address: Reséarch Institute, Laboratorios Almirall, Barcelona.

Supported by FIS grant number 94/0864

526.8

IDENTIFICATION OF MULTIPLE FORMS OF THE 5-HT$_{1A}$ RECEPTOR USING STATE-DEPENDENT ANTIPEPTIDE ANTIBODIES **T.E. Anthony* and E.C. Azmitia Department of Biology, New York University, NY, NY 10003**

Discrepancies in the immunohistochemical labeling obtained using several different antipeptide antibodies against the 5-HT$_{1A}$ receptor led us to attempt characterization of these antibodies using western blotting to assess their specificity. The results of our characterization efforts have yielded useful insight into the properties of both the 5-HT$_{1A}$ receptor and the antipeptide antibodies themselves.

Two of these polyclonal antibodies generated against peptides derived from the third intracellular loop of the protein (S1A-258 - amino acids 258-274; L_5B_7 - amino acids 243-268 (gift of Dr. Michel Hamon)) have been shown to be highly specific for the receptor when expressed in both HEK 293 cells and COS-1 cells. However, the receptor itself is processed differently in each cell type, being more heterogeneously glycosylated in HEK 293 cells. Furthermore, when expressed in Ltk- mouse fibroblasts (gift of Dr. Paul Albert), the main receptor band of 70 kDa is not recognized by the S1A-258 antibody, but is labeled by the L_5B_7, which yields a labeling pattern very similar to that seen in HEK 293 cells. Examination of lysates from undifferentiated RN46A immortalized raphe neurons (gift of Dr. Scott Whittemore) has shown additional forms of the receptor not seen in the above mentioned cell types. Interestingly, hippocampal membrane preparations from animals at different stages of development have also shown heterogeneity in the form of the receptor expressed, implying that the modifications observed in culture may play a role *in vivo*. This data may explain the observation that the signal transduction coupling of the receptor varies according to the cell type it is expressed in. (NIA #1 PO1 AG10208)

526.9

Identification of Regulatory Elements in the 5' Flanking Region of the Rat 5-HT1A Receptor Gene. John M. Storring [1]*, Alain Charest[2] ,and Paul R. Albert [3] . Depts of Pharmacology [1] and Biochemistry [2], McGill University, Montreal Quebec H3G 1Y6 and Neuroscience Research Institute, University of Ottawa, Ottawa, Ontario, K1H 8M5 [3].

We have cloned and sequenced 3.4 kb of the 5' flanking sequence of the rat 5-HT1A gene. This intronless region contains a number of DNA elements believed to be important in the regulation of transcription. These elements include two AP-1 sites, two SP-1 sites, and several TATAA and CCAAT motifs. Using RNAase protection and primer extension analysis a major transcription start site was detected in rat tissue at -967 bp from the initial ATG codon. DNAase protection analysis using nuclear extract prepared from 5-HT1A expressing rat RN46A cells demonstrates a 60 bp DNA footprint consistent with the binding of TATAA binding complex located immediately upstream of this start site. To assess promoter activity, a series of 5' flanking region/ luciferase constructs has been prepared in the pGL-3 vectors (Promega). Using transient transfection, these constructs have been expressed in a variety of cell types which do (RN46A, SN48) and do not (Ltk-, COS 7, P19) express the 5-HT1A receptor. We have demonstrated that a non-specific promoter, active in all cell types, resides between -1188 bp and -972 bp of the initial ATG codon. A region located between -1378 bp and -1188 bp silences transcription in cell types which do not express the 5-HT1A receptor, but remains permissive for transcription in those cells which do express 5-HT1A. The basal promoter region contains typical TATAA and CCAAT boxes, while the silencer region contains a 52 bp poly GT repeat and an element which expresses 86% homology to the NRSE element known to silence the transcription of a number of neuron-specific genes in non-neural cell types.

Supported by the MRC, Canada.

526.10

HIGH-AFFINITY SEROTONIN 5-HT$_{1A}$ LIGANDS WITHIN A SERIES OF 6-SUBSTITUTED 1-INDANMETHYLAMINES. J.Perregaard,* J.Arnt, C.Sanchez. Research Departments, H.Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen-Valby, Denmark.

1-Indanmethylamines with a 6-hydroxy substituent are dopamine D_2 agonists. N,N-Dipropyl-6-hydroxyindanmethylamine binds to dopamine D_2 receptors (IC_{50}=11 nM, [^{3}H]-N-0437) and induces contralateral circling behaviour in the Ungerstedt 6-OHDA model in the rat (ED_{50}=0.32 µmol/kg, subcutaneously). Replacement of the phenolic OH group with 6-acylamino substituents eliminates dopaminergic activity. Instead very potent and selecive 5-HT$_{1A}$ agonists are obtained. 6-Acetylamino- and 6-formylamino-N,N-dipropylindanmethylamine bind with high affinity to 5-HT$_{1A}$ receptors (IC_{50}=0.89 nM and 4.1 nM, respectively, [^{3}H]8-OH-DPAT). In vivo these derivatives are full agonists which induce 5-HT syndrome after low doses. Proper replacements of the propyl substituents reduce 5-HT$_{1A}$ receptor efficacy and eliminate the 5-HT syndrome. 5-HT$_{1A}$ activity is retained in cyclic amine derivatives such as piperidino- and pyrrolidino compounds. Introduction of a 4-fluorophenyl substituent in the 4-position of the piperidine ring in piperidino derivatives results in combined affinities for both 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors. Such compounds even antagonise the 5-HT syndrome induced by the 5-HT$_{1A}$ agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT). Anxiolytic properties in isolation induced agression in mice and in footshock-induced ultrasonic vocalization in rats will be presented. Test results for buspirone and 8-OH DPAT will be shown for comparison.

526.11

STRUCTURE-ACTIVITY RELATIONSHIPS OF A SERIES OF 5-HT$_{1A}$ AGONISTS / D_2 ANTAGONISTS: POTENTIAL ANTIPSYCHOTICS.

E.K. Moltzen, J. Perregaard, and J. Arnt*, Research Departments, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen-Valby, Denmark.

Traditional treatment of schizophrenia mainly involves use of more or less selective dopamine D_2 receptor antagonists. However, all these drugs induce severe extrapyramidal side effects (EPS). Recently it was shown that 5-HT$_{1A}$ agonists are able to antagonize EPS induced by traditional neuroleptics in animal models. Compounds displaying both D_2 antagonism and 5-HT$_{1A}$ agonism might thus have potential as a new type of atypical neuroleptics.

During our work with a series of aryl piperazines and -piperidines exerting 5-HT$_{1A}$ affinity we discovered that certain structural modifications changed the pharmacological profile from selective 5-HT$_{1A}$ ligands to mixed 5-HT$_{1A}$/D_2 ligands. This series of compounds are exemplified by 3-cyclohexyl-1-[4-(4-(2,2-dimethyldihydro-7-benzofuranyl)-1-piperidinyl)-1-butyl]imidazolidin-2-one (IC_{50}(5-HT$_{1A}$) = 3.5 nM, IC_{50} (D_2) = 8.0 nM) and 3-cyclohexyl-1-[4-(4-(2-isopropyloxyphenyl)-1-piperazi-nyl)-1-butyl]imidazolidin-2-one (IC_{50}(5-HT$_{1A}$) = 1.8 nM, IC_{50}(D_2) = 6.4 nM). A structure-activity study showed that the branched alkoxy group and a length of the chain connecting the imidazolidinone ring with the piperidine- or piperazine ring of at least four carbon atoms are crucial for affinity for D_2 receptors, whereas the 5-HT$_{1A}$ affinity is less sensitive.

The 5-HT$_{1A}$ efficacy is highly dependent on the 3-substituent in the imidazolidinone ring as well as chain length. Compounds with aromatic substituents and chain length of at least four carbon atoms display most potent 5-HT$_{1A}$ agonistic effect as measured by the 8-OH-DPAT drug discrimination test.

526.12

EFFECTS OF AGONISTS AND ANTAGONISTS OF 5-HT$_{1A}$ RECEPTORS ON BINDING OF [^{35}S]GTPγS IN RAT HIPPOCAMPAL MEMBRANES. M.-P. Kung*, D. A. Stevenson, Z.-P. Zhuang and H. F. Kung, Departments of Radiology and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Recently, several selective and potent ligands for labeling 5-HT$_{1A}$ receptors, agonist (R)-8-OH-PIPAT and antagonists *p*-MPPF and *p*-MPPI, were reported (JPET 272:429-437, 1995, and Synapse, in press). To characterize further the agonist and antagonist activity of (R)-8-OH-PIPAT, *p*-MPPF and *p*-MPPI, the activation of 5-HT$_{1A}$ receptors by agonists to promote guanine nucleotide exchange, using [^{35}S]GTPγS binding, was evaluated in rat hippocampal membranes. Agonists, such as 5-HT, (R)-8-OH-DPAT, (R)-8-OH-PIPAT, 5-CT and DP-5-CT all stimulated [^{35}S]GTPγS binding in a dose-dependent manner resulting a maximum of 65-100% increase over basal level. The potencies of agonist stimulation (pEC_{50}) are in good agreement with the pIC_{50} binding affinities. Unexpectedly, the iodinated agonist, (R)-8-OH-PIPAT, unlike other agonists evaluated in this study, exhibits a [^{35}S]GTPγS binding profile consistent with binding to multiple sites. These data suggest that different types of G-protein activation may be involved with different agonists. The stimulation of [^{35}S]GTPγS binding by (R)-8-OH-DPAT can be antagonized by *p*-MPPI or *p*-MPPF (while tested alone did not affect the basal level binding of [^{35}S] GTPγS). The 5-HT$_{1A}$ agonist-stimulated binding is inhibited by Gpp(NH)p or GTPγS, but not by ATPγS. Homologous displacement by GTPγS of [^{35}S]GTPγS binding to measure the maximal binding sites, which can be stimulated by (R)-8-OH-DPAT, resulted in a K_d of 2.71 ± 0.68 nM and a B_{max} of 93.7 ± 31.0 fmol/mg protein. Using [^{35}S]GTPγS binding assay in native tissue preparations provides a useful system to investigate the pharmacology of agonists and antagonists of 5-HT$_{1A}$ receptors. In addition, relative potency of agonists can be directly measured with this binding assay. (Supported by MH-48125 and NS-35120)

526.13

AGONIST-PROMOTED DESENSITIZATION IN TWO NATURALLY-OCCURRING VARIANTS OF THE HUMAN 5-HYDROXYTRYPTAMINE$_{1A}$ RECEPTOR. A. Rotondo, B. Nakhai, D. Goldman and D. A. Nielsen*. Laboratory of Neurogenetics, NIAAA, National Institutes of Health, Rockville, MD 20852.

We recently reported two naturally-occurring polymorphisms of the human 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor that resulted in substitutions of glycine to serine at amino acid 22 (gly→ser) and isoleucine to valine at amino acid 28 (ile→val) in the extracellular amino-terminal domain of the receptor. To study the pharmacological and functional properties of these variants, the wild type and each variant 5-HT$_{1A}$ receptor was stably expressed in CHO cells. Both the wild type 5-HT$_{1A}$ receptor and the variants gly→ser and ile→val displayed similar saturable high-affinity binding to the selective 5-HT$_{1A}$ agonist [^{3}H]-8-OH-DPAT. Essentially identical pharmacological profiles in competition experiments with 8-OH-DPAT, serotonin and WAY-100478 were observed. Agonist-promoted down-regulation of receptor expression was tested by 24 h exposure to 100 µM 8-OH-DPAT (or 100 µM 5-HT). The wild type and the ile→val variant 5-HT$_{1A}$ receptors underwent a 62±2% and 57±8% reduction in receptor density, while the gly→ser variant underwent a 22±12% reduction. Pre-treatment for 24 h with 100 µM 5-HT diminished 5-HT induced inhibition of cAMP by 28±5% for the wild type and 19±7% for the ile→val variant, but only by 6±1% for the gly→ser variant.

Our results suggest that a mutation at amino acid 22 in the extracellular amino-terminus of the human 5-HT$_{1A}$ receptor decreases agonist-promoted desensitization of receptor expression.

Source of support: NIAAA/NIH Intramural Program

526.14

A MOLECULAR ANALYSIS OF THE STRUCTURAL REQUIREMENTS FOR INTERNALIZATION OF THE HUMAN 5-HT1a RECEPTOR

N. M. Page., D. McNab and K. Shaw* School of Animal and Microbial Sciences, University of Reading and *Department of Physiology and Pharmacology, University of Southampton, UK

The 5-HT1a receptor (5-HT1aR) undergoes desensitization in response to a 10 minute stimulation with its specific agonist, 8-hydroxy DPAT. In part, this response is characterized by a loss of high affinity binding sites, suggesting that the receptor may internalize. Residues within the 2nd and 3rd intracellular loops, carboxy terminal tail and 7th transmembrane domain of some G protein-coupled receptors (GPCRs) have been implicated in the internalization process. We have used a PCR-based strategy to (a) truncate the carboxy terminal tail after D406, (b) create two substitutions, Y399F or Y399A in the NPVIY motif in the 7th transmembrane domain and (c) create substitutions in 4 putative PKC phosphorylation recognition sequences. Moreover, in order to track the fate of internalized receptors by confocal microscopy, we have engineered an epitope tag (PPEPET) into the amino terminal sequence of the 5-HT1aR which may be recognized by the KT3 monoclonal antibody. In all cases, recombinant constructs have been used to stably transfect CHO-K1 cells. Receptor expression was confirmed by saturation binding analysis with [^{3}H] 8-OH-DPAT. Bmax values were in the range 250-500 fmol/mg protein. Agonist affinities for the mutant receptors did not significantly differ from the wild type receptor (Kd= approximately 2nM). Following confirmation of functional expression, this system will be used to assess the structural determinants for 5-HT1aR internalization. *Support from the Wellcome Trust (UK) is gratefully acknowledged.

527.1

CORTICOSTERONE ALTERS FUNCTIONAL AND BIOCHEMICAL PARAMETERS CHARACTERISTIC OF SEROTONERGIC NEUROTRANSMISSION ASSOCIATED WITH 5-HT$_{1A}$ RECEPTORS. *A. Czyrak*, M. Bijak, K. Tokarski, M. Maćkowiak, K. Wędzony. Institute of Pharmacology, PAN, Krakow, 12 Smetna street, Poland.*

In the present study we investigated the impact of repeated administration of corticosetrone (COR, 10 mg/kg, twice a day, for 7 days) on behavioral, electrophysiological and biochemical parameters characteristic of the 5-HT neurotransmission associated with 5-HT$_{1A}$ receptors. It was found that COR attenuated the 8-OHDPAT evoked disruption of the prepulse-induced inhibition of acoustic startle response and decreased the 8-OHDPAT-induced inhibition of hippocampal CA1 neurons *in vitro*. Both those effects indicated that chronic COR decreased the functional responsiveness to stimulation of 5-HT$_{1A}$ receptors. On the other hand, we observed that COR increased the number of 5-HT$_{1A}$ receptors in the hippocampus, prefrontal cortex and raphe nuclei, as measured by the autoradiographic and saturation binding studies ([^{3}H]8-OHDPAT as a ligand). The hippocampal levels of 5-HT and 5-HIAA were also increased. It is concluded that chronic occupation of glucocorticosteroid receptors by COR attenuates the functional responsiveness to stimulation of 5-HT$_{1A}$ receptors. In contrast to the effects observed in functional studies, the density of 5-HT$_{1A}$ receptors is increased.

Supported by KBN grant # 4.P05A 004.09

527.2

TIME-COURSE OF FLUOXETINE AND PAROXETINE DESENSITIZATION OF HYPOTHALAMIC 5-HT$_{1A}$ RECEPTORS: REDUCED NEUROENDOCRINE RESPONSES TO 8-OH-DPAT AND REDUCED LEVELS OF G_i AND G_o PROTEINS. Q. Li*, N.A. Muma, G. Battaglia and L. D. Van de Kar. Dept. Pharmacol. Sch. Med., Loyola Univ. Chicago, 2160 S. 1st. Ave. Maywood , IL. 60153

The aim of the study was to examine the time-course of desensitization of hypothalamic 5-HT$_{1A}$ receptors induced by 5-HT uptake blockers. Rats were injected daily with the 5-HT uptake blockers fluoxetine (10 mg/kg, ip for 0, 3, 7, 14 or 22 days) or paroxetine (10 mg/kg, ip for 0, 1, 3, 7 or 14 days). Daily injections of either fluoxetine or paroxetine gradually decreased the ACTH, corticosterone and oxytocin responses to 8-OH-DPAT. A partial reduction of the hormone responses to 8-OH-DPAT occurred after 3 days and a maximum inhibition occurred after 14 daily injections of both 5-HT uptake blockers. To determine the mechanism of the desensitization, we examined the effects of the 5-HT uptake blockers on the density of 5-HT$_{1A}$ receptors and their signal transduction system. Autoradiography of ^{3}H-8-OH-DPAT binding revealed that neither fluoxetine nor paroxetine changed the density of 5-HT$_{1A}$ receptors in the hypothalamus or other brain regions at any treatment time. Furthermore, Gpp(NH)p-induced inhibition of ^{3}H-8-OH-DPAT binding was not altered by repeated injections of fluoxetine in any nuclei of the hypothalamus or other brain regions. We further determined the levels of G_i and G_o proteins in the hypothalamus, midbrain and frontal cortex using immunoblots. The hypothalamic levels of G_{i1} and G_{i3} proteins were significantly reduced after 7 and 14 days of fluoxetine injections (the paroxetine data will be shown at the meeting). The time-course of the G protein reduction is similar to the time-course of fluoxetine-induced reduction in hormone responses to 8-OH-DPAT. The levels of G_o and G_{i2} proteins in the midbrain were significantly decreased after 3 days of fluoxetine injections and remained low until 22 days. Fluoxetine did not change the levels of G_i and G_o proteins in the frontal cortex. In conclusion, repeated injections of 5-HT uptake blockers produce a delayed and gradual desensitization of hypothalamic 5-HT$_{1A}$ receptors, which may in part be due to a decrease in the G_{i1} and G_{i3} protein levels. (Supported by NS34153)

527.3

THE 5-HT$_{1A}$ ANTAGONIST WAY-100635 INHIBITS 8-OH-DPAT INDUCED INCREASE IN ACTH, CORTICOSTERONE AND OXYTOCIN BUT NOT PROLACTIN A.Vicentic*, Q.Li, F.Garcia, W.Pinto, Y.Li, G.Battaglia and L.D.Van de Kar. Dept.Pharmacol. Loyola Univ.Chicago, Sch.Med., Maywood, IL 60153

The objective of the present study was to examine whether 5-HT$_{1A}$ receptors are involved in the 8-OH-DPAT-induced secretion of ACTH, corticosterone, oxytocin and prolactin. Male rats (N=8) received either saline or WAY-100635 (0.1, 1, and 10 mg/kg, sc), 1hr before sacrifice. Rats were challenged with saline or 8-OH-DPAT, (0.5 mg/kg, sc) 15 min before sacrifice. WAY-100635 at the dose of 0.1 mg/kg significantly inhibited the 8-OH-DPAT induced secretion of oxytocin but not corticosterone or ACTH. At the dose of 1 mg/kg WAY-100635 completely blocked the oxytocin response to 8-OH-DPAT and maximaly inhibited the ACTH and corticosterone responses to 8-OH-DPAT. For all three hormones, the 10 mg/kg dose of WAY-100635 produced the same maximum achieved at the 1 mg/kg dose. The lack of inhibition by WAY-100635 (0.1 mg/kg) of 8-OH-DPAT-induced elevation of ACTH and corticosterone suggests a greater receptor reserve for ACTH and corticosterone than for oxytocin. The basal levels of ACTH, corticosterone and oxytocin were not altered by any dose of WAY-100635. In contrast to the other hormones, the increase in prolactin by 8-OH-DPAT was not inhibited by any dose of WAY-100635. Surprisingly the highest dose of WAY-100635 (10 mg/kg) elevated basal prolactin levels. These data indicate that 8-OH-DPAT elevates oxytocin, ACTH and corticosterone levels via activation of 5-HT$_{1A}$ receptors while prolactin secretion may be mediated via non 5-HT$_{1A}$ receptors. (supported by USPHS NS34153 to LVdK).

527.4

EFFECT OF ESTROGEN ON SEROTONIN TURNOVER AND THE REDUCTION IN SEROTONIN TURNOVER IN RESPONSE TO 8-OH-DPAT W. P. Clarke* & R.J. Thielen Department of Pharmacol ogy University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

It was previously reported that estrogen enhances the response of 5-HT$_{1A}$ receptors in hippocampal slices from female rats. A recent report also suggests the reduction in hypothalamic 5-HT turnover in response to 8-OH-DPAT varies with the stage of the estrous cycle. The purpose of the present study was to examine the effect of estrogen on 5-HT turnover in a region containing 5-HT cell bodies and in some areas receiving 5-HT projections. Female rats were ovariectomized and two weeks later silastic capsules containing cholesterol (CHOL) or 10% estradiol-17β in cholesterol (EST) were implanted subcutaneously into each rat. Five days later, rats were treated with 0.17 mg/kg, 8-OH-DPAT (DPAT) or saline (SAL) and decapitated 60 min later. The frontal cortex (FCX), anterior striatum (STR), nucleus accumbens (ACB) and a section containing the dorsal raphe (DRN) were rapidly dissected on ice and stored at -70 °C. 5-HT and 5-HIAA contents were measured by HPLC with electrochemical detection, and their ratio was used as an index of the turnover of 5-HT. Turnover of 5-HT in rats receiving SAL was significantly increased in EST compared with CHOL treated rats in the DRN (1.12 ±0.01, EST; 1.02 ±0.02 CHOL) and FCX (0.71 ± 0.01, EST; 0.80 ± 0.03, CHOL). Similar increases were seen in the ACB and STR, but these did not reach significance. Treatment with DPAT reduced 5-HT turnover in all the brain regions examined; however, the magnitude of the reduction in turnover induced by DPAT was significantly increased in the DRN (30.8 ± 1.7% vs. 20.0 ±3.7%) and ACB (43.7 ± 4.2% vs. 27.8 ± 3.9%) of EST compared to CHOL treated rats, respectively. No differences were found in the STR or FCX. These data suggest that EST treatment may selectively enhance somatodendritic 5-HT$_{1A}$ autoreceptor responses to DPAT in the DRN.
(Supported by Research Funds USPHS Grant MH48125 and HD26437).

527.5

RELATIVE IMPORTANCE OF 5-HT REUPTAKE INHIBITION AND 5-HT$_{1A}$ AUTORECEPTOR BLOCKADE IN THE CITALOPRAM PLUS WAY100635-INDUCED ELEVATION OF EXTRACELLULAR 5-HT IN THE RAT BRAIN *IN VIVO*. S. Hjorth*, D. Westlin and H.J. Bengtsson. Inst. of Physiology & Pharmacology, Dept. of Pharmacology, Univ. of Göteborg, Medicinareg. 7, S-413 90 Göteborg, SWEDEN.

We, and others, have previously shown that blockade of release-inhibiting 5-HT$_{1A}$ autoreceptors augments the rise in extracellular 5-HT (5-HT$_{ec}$) caused by SSRI, like *e.g.* citalopram. This study assessed the relative importance of 5-HT reuptake inhibition vs. 5-HT$_{1A}$ autoreceptor blockade in this interaction. Citalopram (CIT; 0.5 or 5.0 mg/kg SC) and the selective 5-HT$_{1A}$ receptor blocker WAY100635 (WAY; 0.01-0.3 mg/kg SC) were given in different combinations, and 5-HT$_{ec}$ in the ventral hippocampus was sampled by *in vivo* microdialysis. CIT induced a dose-dependent rise in 5-HT$_{ec}$, maximally doubling the initial baseline values within 60 min after injection. Subsequent administration of WAY further augmented, in a dose-dependent manner, the high-dose CIT response (to ≈ 4-5 x the pre-CIT baseline); in this regard, the 0.03, 0.1 and 0.3 mg/kg doses were all similarly effective. For comparison, the low-dose CIT response was but mildly, but not significantly, potentiated by WAY (0.3 mg/kg). WAY given alone did not alter 5-HT$_{ec}$. The data agree with previous findings that 5-HT$_{1A}$ autoreceptor blockade reverses the SSRI-induced release suppression, thereby further elevating 5-HT$_{ec}$. In addition, adequate 5-HT reuptake blockade appears to be the primary prerequisite for this interaction to occur. New drugs and/or treatment regimes based on the SSRI/5-HT$_{1A}$ autoreceptor blocker concept should thus emphasize the former property.

Supported by the Swedish MRC (#7486) and Sv. Lundbeck-fonden.

527.6

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE 5-HT1A RECEPTOR TO SEROTONIN AND NON-SEROTONERGIC NEURONS IN RAT BRAINSTEM RAPHE NUCLEI. T. D. Patel* and F. C. Zhou. Program in Medical Neurobiology and Department of Anatomy. Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

Receptor autoradiography studies reveal a high density of 5-HT1A binding sites in the raphe nuclei. Functional studies demonstrate that these 5-HT1A receptors are "somatodendritic" autoreceptors. However, to date, there is no direct visual evidence confirming localization of the 5-HT1A receptor protein to 5-HT neurons. We used double label immunocytochemistry (ICC) to examine the neurotransmitter profile of raphe 5-HT1A-immunopositive cells. For 5-HT1A ICC, polyclonal antisera was generated against a fusion protein containing a portion of the amino acid sequence of the third cytoplasmic loop of the rat 5-HT1A receptor protein. The distribution in rat brain obtained by ICC with this antibody correlates well with the binding site distribution from receptor autoradiography using [3H]-8-OH-DPAT. Specifically, in the hippocampus, the laminar receptor distribution pattern from ICC and receptor autoradiography are superimposable.

In the raphe nuclei, cell body 5-HT1A immunoreactivity (5-HT1A-IR) was localized at the periphery in a patchy perisomatic fashion with little cytoplasmic staining, and immunoreactive proximal dendritic branches could be visualized extending for some distance. Dense, fiber-like 5-HT1A-IR was also found, especially in the dorsal raphe nucleus. In double labeled sections, we found that most but not all 5-HT and 5-HT1A coregistered. Some 5-HT positive neurons did not exhibit 5-HT1A-IR. It is not clear if these cells were understained for 5-HT1A or whether 5-HT1A was localized on more distal dendritic processes of these 5-HT positive cells. Finally, some scattered 5-HT negative cells exhibited 5-HT1A-IR, suggesting that non-serotonergic raphe neurons might also express functional 5-HT1A receptors. The neurotransmitter profile of these cells is currently under examination.

(Support: R24HD30508 and AA08553)

527.7

NEUROCHEMICAL PROFILE OF THE SELECTIVE 5-HT1A RECEPTOR (±)5-ME-8-OH-DPAT AND ITS ISOMERS: AN IN VIVO MICRODIALYSIS STUDY. A-C Trillat[1]*, I. Malagié[1], M. Langlois[2], M. Mathé-Allainmat[2], B. Brémont[2], C. Jacquot[1] and A.M. Gardier[1]. 1-Lab. Neuropharmacol. JE DRED 92-372. 2-BIOCIS URA CNRS 1843. Fac. Pharmacie, Univ. Paris-Sud, 92296 Châtenay-Malabry, France.

Numerous selective 5-HT$_{1A}$ receptor ligands have been described as being either full or partial agonists at the 5-HT$_{1A}$ receptor site in the brain. Few reports described highly selective and silent (compound having no intrinsic activity) 5-HT$_{1A}$ receptor antagonists making extremely difficult to perform the pharmacological characterization of the 5-HT$_{1A}$ receptor and its involvement in various pathologies and behavior. A new 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, the 5-HT$_{1A}$ reference agonist) derivative compound, the racemic mixture (±) 5-Me-8-OH-DPAT was synthetized {Langlois et al., *BioMed. Chem. Lett.* 3 (1993) 2035-2038}, then separated in (-) and (+) isomers that all have a high affinity for 5-HT$_{1A}$ receptors (Ki (nM): 6.5; 39.2 and 28.9, respectively). Here, we studied the effects of these compounds at the somatodendritic 5-HT$_{1A}$ receptors by using *in vivo* microdialysis in freely-moving rats to measure serotonin (5-hydroxytryptamine, 5-HT) release at nerve terminals located in the ventral hippocampus (vHi) (±) 5-Me-8-OH-DPAT, (-) and (+) isomers (all being subcutaneously administered at a 10 mg/kg dose) had no significant effect on extracellular 5-HT levels in vHi suggesting that they are devoid of 5-HT$_{1A}$ receptor agonist properties on this test. Conversely, 8-OH-DPAT (0.1 mg/kg s.c.) significantly decreased hippocampal 5-HT. Pretreatment with either (±) 5-Me-8-OH-DPAT or (-) 5-Me-8-OH-DPAT (10 mg/kg s.c. each) significantly attenuated the effect of 8-OH-DPAT suggesting that these compounds have antagonistic properties at somatodendritic 5-HT$_{1A}$ autoreceptors. A dose-dependant effect of (-) 5-Me-8-OH-DPAT was also observed. Thus, the present *in vivo* study suggests that (±) 5-Me-8-OH-DPAT is a silent and selective receptor antagonist at the somatodendritic 5-HT1A receptor with activity residing in the (-) enantiomer. (-) 5-Me-8-OH-DPAT is likely to be a useful tool to further investigate the 5-HT$_{1A}$ receptor function.

527.8

EVIDENCE FOR FUNCTIONAL PLASTICITY OF MOTONEURON 5-HT$_{1A}$ RECEPTORS AFTER 5,7-DHT LESION. N.M. Kheck[1]*, P.J. Gannon[2], B.L. Jacobs[3], T.E. Anthony[1] and E.C. Azmitia[1] [1]Dept. of Biology, New York Univ., NY NY 10029; [2]Dept. of Otolaryngology, Mt. Sinai Sch. Med., NY, NY 10029; [3]Prg. Neuroscience, Princeton University, Princeton, NJ 08544

The "5-HT syndrome" was used to characterize the functional plasticity of putative motoneuron 5-HT$_{1A}$ receptor populations in response to selective serotonergic spinal denervation. We used the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT to induce the 5-HT syndrome, a motor behavioral paradigm which results from serotonergic facilitation of brainstem and spinal motoneuron activity. This model provides a measure of postsynaptic 5-HT$_{1A}$ receptor activation. We have previously reported the localization of 5-HT$_{1A}$ receptors on motoneurons using antipeptide antibodies directed against several epitopes. Rats (n=21) were injected with 8-OH-DPAT (1mg/kg, s.c.), or saline (n=8) and assessed for components of the 5-HT syndrome by three observers. Seven days later, following pretreatment with desimipramine (25 mg/kg, i.p.), rats were lesioned by stereotaxic injection of 5,7-DHT (100 µg of free base in 10 µl vehicle; 0.2% ascorbic saline) into the cisterna magna, at 1 µl/minute. At 11 and 14 days postlesion, rats were again rated (under double-blind condition) for the 5-HT syndrome after injection of 8-OH-DPAT. We observed a marked enhancement of motor parameters, suggesting an upregulation of postsynaptic 5-HT$_{1A}$ receptor activity (denervation supersensitivity). Significant increases were noted in: tremor (84%), hindlimb abduction (73%), Straub tail (45%), reciprocal forepaw treading (27%), and flattened head/body posture (38%). The magnitude of spinal 5-HT lesion was determined by ^{3}H-paroxetine binding (which demonstrated >90% loss of 5-HT terminals). We are currently analyzing the anatomical changes in 5-HT$_{1A}$ receptor-IR in spinal cord sections at 14 days after 5,7 DHT lesion. The behavioral data will be correlated to these postlesion regulatory changes in 5-HT$_{1A}$ receptor expression in spinal motor nuclei. (Research supported by NIA AG 10208)

527.9

CHRONIC ANTIDEPRESSANT ALTERATION OF AGING HIPPOCAMPAL SEROTONIN TRANSPORTER AND RECEPTORS. J.E. Smith*, B.J. Keck, P. Deshmukh, and J.M. Lakoski. Departments of Pharmacology and Anesthesia, Pennsylvania State University College of Medicine, Hershey, PA 17033.

The clinical efficacy of antidepressants is, in part, mediated by ability to bind to the serotonin transporter (5-HTT). While the mechanism of action of antidepressants is presumed to be the same for all ages, the consequences of age-related changes in the 5-HTT are not yet fully understood. Alterations in presynaptic serotonergic transporter (5-HTT) function in the aging hippocampus were studied using electrophysiological techniques to evaluate the sensitivity of pyramidal neurons to serotonin (5-HT) after chronic exposure to antidepressants selective for the 5-HTT. Localization of the hippocampal 5-HTT and 5-HT$_{1A}$ receptors following chronic antidepressant administration was determined using quantitative autoradiographic techniques.

Female Fischer 344 rats ages 3-8, 11-12, 17-22 and >24 mo were injected (10mg/kg/day x 14 days, i.p.) with duloxetine, fluoxetine, desipramine, or vehicle control. After a 24 hr washout, extracellular recordings were performed using an *in vivo* chloral hydrate anesthetized preparation and drug solutions (0.01-0.04 M, pH 5.0-6.0) of 5-HT, fluoxetine, desipramine, and duloxetine were microiontophoretically applied to hippocampal pyramidal neurons in CA1 and CA3 subfields. Chronic vehicle treatment did not alter the electrophysiological profile of hippocampal pyramidal neurons. Following chronic duloxetine administration, a decrease in sensitivity (87.7% of IT$_{50}$ value) of hippocampal neurons to 5-HT was noted in the 17-22 mo group as compared to the age-matched vehicle group. [^{3}H]Paroxetine and [^{3}H]8-OH-DPAT were used to label 5-HTT and 5-HT$_{1A}$ sites, respectively, and comparisons across age and drug treatment investigated autoradiographic localization of presynaptic and postsynaptic receptors in the hippocampus. Changes in the density of these 5-HT sites may underlie the age-related decrease in sensitivity to 5-HT observed using electrophysiological techniques.

Publication No. 57A supported by P01 AG10514 (JML) and T32 AG00048 (JES)

527.10

REGION-DEPENDENT RECOVERY OF SEROTONIN 5-HT$_{1A}$ RECEPTORS FOLLOWING INACTIVATION BY EEDQ. B.J. Keck* and J.M. Lakoski. Depts. Pharmacology & Anesthesia, Penn State Univ. Coll. of Med., Hershey, PA 17033.

The neurotoxin EEDQ (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) was used to irreversibly inactivate serotonin 5-HT$_{1A}$ receptors in the hippocampus and frontal cortex to investigate region specific characteristics of receptor turnover. Adult male Sprague-Dawley rats (530-1070 g) were treated with EEDQ (6 mg/kg, s.c.) or vehicle (ETOH/propylene glycol/H$_2$O, 1:1:2, s.c.) and sacrificed at 1, 2 or 8 days following injection in order to evaluate the time-course of recovery for 5-HT$_{1A}$ receptors. Scatchard analyses using [^{3}H]8-OH-DPAT were conducted and receptor turnover parameters (rate of receptor synthesis, *r*; degradation rate constant, *k*; and receptor half-life, $T_{1/2}$) were detrmined for each brain region.

EEDQ-dependent decreases in 5-HT$_{1A}$ receptor density were observed at day 1 for the hippocampus (88%) and frontal cortex (55%) compared to vehicle-controls; complete recovery (94%) of the receptor density occurred by day 8 in the hippocampus, but not frontal cortex (73% recovery). The rate of 5-HT$_{1A}$ receptor synthesis was greater for hippocampus (r = 36.4 fmol/mg protein/day) than for frontal cortex (r = 4.2 fmol/mg protein/day), and receptor degradation rate constants (k) and half-life values ($T_{1/2}$) were also different for these brain regions (k = 0.38 day^{-1} and k = 0.09 day^{-1}, respectively; $T_{1/2}$ = 1.8 days and $T_{1/2}$ = 7.7 days, respectively). Affinity of the 5-HT$_{1A}$ receptor was decreased following EEDQ treatment in the frontal cortex at day 1 (5.5-fold decrease) but returned to the vehicle-control value by day 8. No change was observed in the K$_D$ values for 5-HT$_{1A}$ hippocampal receptors subsequent to EEDQ administration at any time point. Overall, these data revealed region-dependent differences in 5-HT$_{1A}$ receptor turnover properties following EEDQ administration. Studies of regional differences in 5-HT$_{1A}$ receptor recovery will be expanded to include analyses of receptor turnover as a function of age. *Pub. No. 55A supported by PO1 AG 10514 (JML), F32 AG 05728-01 and T32 DA 07277-05 (BJK).*

527.11

EFFECTS OF CHRONIC INTERFERON ADMINISTRATION ON SEROTONERGIC RECEPTORS IN RAT BRAIN

S. Abe[1], T. Hori[1]*, A. Baba[1], T. Suzuki[1], H. Shiraishi[1] And T. Yamamoto[2] [1]Dept. of Psychiat., The Univ. of Tsukuba, Ibaraki, 305,Japan [2]Lab. of Mol. Recog., Yokohama City Univ.,Kanagawa

Interferon (IFN) has been reported to cause depressive state. Serotonin (5-HT) plays a key role in the pathophysiology of affective disorders, however, the mechanism IFN produces depressive state is not clear. In this study,we investigated effects of chronic IFN (recombinant human IFN-α A/D) administration on 5-HT receptors in rat brain using a radioreceptor assay.IFN was injected for 2 weeks at a dose(100,000 I.U./kg, i.p.,daily) in male Wistar rats.Membrane preparations of individual cortices and hippocampi were prepared.

IFN failed to alter binding of [^{3}H]ketanserin (5-HT$_{2A}$ receptor) and [^{3}H] paroxetine binding (5-HT transporter). The Scatchard plots of [^{3}H]8-OH-DPAT binding (5-HT$_{1A}$ receptor) to rat cortical and hippocampal membranes were curvilinear, which suggested the presence of high- and low-affinity sites. IFN significantly increased both Kd and Bmax values of [^{3}H]8-OH-DPAT binding to cortical membranes at low-affinity site, but not at the high-affinity site. These results suggest the possibility IFN affects low-affinity site of 5-HT$_{1A}$.

(Supported by a grant from Univ. of Tsukuba Res. Project.)

527.12

THE *(S)*-UH-301 INDUCED INHIBITION OF RAT BRAIN SEROTONIN AND DOPAMINE SYNTHESES DO NOT SEEM TO BE MEDIATED BY 5-HT$_{1A}$ RECEPTORS. H. Yu*, B. Backlund Höök, U. Hacksell and T. Lewander. Department of Psychiatry (Ulleråker), S-750 17 and Department of Organic Pharmaceutical Chemistry, Uppsala University, S-751 23 Uppsala, Sweden

(R)-8-Hydroxy-2-(di-*n*-propylamino)tetralin (*(R)*-8-OH-DPAT; 0.32 mmol/kg, s.c.), a 5-HT$_{1A}$ agonist, induces a decrease in 5-HT (5-hydroxytryptamine) synthesis, measured as a decrease in the 5-hydroxytryptophan accumulation after inhibition of aromatic amino acid decarboxylase, and 5-HT turnover, measured as the ratio of 5-hydroxyindoleacetic acid over 5-HT, in hippocampus and striatum in the rats. *(S)*-5-Fluoro-8-hydroxy-2-(di-*n*-propylamino)tetralin (*(S)*-UH-301), a 5-HT$_{1A}$ receptor antagonist, dose-dependently (10 - 100 mmol/kg, s.c.) decreased 5-HT synthesis in the hippocampus, but not in the striatum, whereas it did not change 5-HT turnover. *(S)*-UH-301 (32 mmol/kg, s.c.) blocked the reduction of 5-HT turnover induced by *(R)*-8-OH-DPAT (1.0 mmol/kg, s.c.). However, *(S)*-UH-301 only partially antagonised the decrease in 5-HT synthesis in the hippocampus after *(R)*-8-OH-DPAT, indicating that *(S)*-UH-301 might be a partial 5-HT$_{1A}$ agonist. The decrease in 5-HT synthesis after *(R)*-8-OH-DPAT was completely antagonised in both brain regions by pretreatment with WAY-100635 (0.55 mmol/kg, s.c.), a new selective 5-HT$_{1A}$ receptor antagonist. However, WAY-100635 had no effect on the *(S)*-UH-301 (64 mmol/kg, s.c.) induced decrease in 5-HT synthesis in the hippocampus. In addition, WAY-100635 did not antagonise the previously reported *(S)*-UH-301 induced decrease in dopamine synthesis in the striatum. In conclusion, *(S)*-UH-301 inhibits 5-HT synthesis in the hippocampus and dopamine synthesis in the striatum by mechanisms that do not seem to be mediated by 5-HT$_{1A}$ receptors. Supported by the Swedish Board for Technical and Industrial development.

527.13

AUGMENTATION OF THE ANTIDEPRESSANT ACTION OF SSRIS BY PINDOLOL MAY NOT BE EXPLAINED BY 5-HT1A RECEPTOR ANTAGONISM

H.C. Jackson, K.N. Hewitt, L.J. Hutchins, S.C. Cheetham and D.J. Heal (SPON: Brain Research Association) Knoll Pharmaceuticals Research & Development, Nottingham, NG2 3AA, U.K.

Pindolol induces a dramatic and rapid improvement in the antidepressant efficacy of SSRIs - a response which has been explained by selective antagonism of 5-HT1A somatodendritic autoreceptors (Artigas et al., 1994, Arch. Gen. Psych. 51, 248-251; Blier & Bergeron, 1995, J. Clin. Psychopharm. 15, 217-222). This study compares the effects of combinations of pindolol (0.3-6 mg/kg ip) and fluoxetine (1-30 mg/kg ip) in the Porsolt test (PS) - an animal model of depression. The 5-HT1A receptor antagonist WAY100635 (0.03-1 mg/kg sc) was used for comparison. Male CD1 mice (20-30g; n=10-20) were injected with drug and 60 min later placed in water for 6 min. Mobility was measured using a Doppler recording system. Mobility scores of mice given pindolol (1 mg/kg) plus fluoxetine (10 mg/kg) were significantly greater than those of mice given vehicle, fluoxetine or pindolol. However, there was no evidence that WAY100635 potentiated the effects of fluoxetine in the PS. WAY100635 reversed the increase in mobility induced by 8-OH-DPAT confirming its antagonist action at post-synaptic 5-HT1A receptors. Furthermore, WAY100635 significantly increased 5-HTP levels in mouse brain demonstrating antagonism of pre-synaptic 5-HT1A receptors. In contrast, pindolol did not block the 8-OH-DPAT response in the PS and had no effect on 5-HTP levels. These results, highlighting important differences in the pharmacological actions of pindolol and WAY100635, show that 5-HT1A receptor antagonism cannot explain pindolol's potentiation of the effects of fluoxetine in the PS and may not account for its augmentation of the antidepressant actions of SSRIs in man. One alternative explanation is that pindolol is a partial agonist at 5-HT1A receptors. In this context, pindolol (6 mg/kg) increased mobility in the PS in comparison to WAY100635 which was inactive at all doses tested.

Financial support provided by Knoll Pharmaceuticals.

527.14

SYSTEMIC ADMINISTRATION OF THE 5-HT$_{1A}$ RECEPTOR ANTAGONIST p-MPPF ENHANCES THE EFFECT OF FLUOXETINE ON EXTRACELLULAR LEVELS OF 5-HT IN THE RAT STRIATUM.

J. M. Chou*, M.-P. Kung, H. F. Kung and I. Lucki. Departments of Psychiatry, Pharmacology and Radiology, Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Previous research suggests that the ability of selective serotonin (5-HT) reuptake inhibitors (SSRIs) to increase extracellular 5-HT may be restrained by the activity of somatodendritic 5-HT$_{1A}$ autoreceptors. Systemic administration of the new, selective 5-HT$_{1A}$ receptor antagonist, p-MPPF (Zhuang, ZP, Kung, MP, and Kung, HF. J. Med. Chem. 1994, 37: 1406-1407), was used to examine the effect of blockade of 5-HT$_{1A}$ receptors in combination with systemic administration of the 5-HT uptake inhibitor fluoxetine on extracellular levels of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the striatum. Extracellular levels of 5-HT and 5-HIAA were measured using *in vivo* microdialysis with electrochemical detection in awake unrestrained rats. On the day of the experiment, dialysate samples were collected for 120 min to establish baseline values. Fluoxetine was injected (20 mg/kg, i.p.) and dialysis samples were collected for 60 min. p-MPPF (30 mg/kg, i.p.) was then injected and dialysis samples collected for an additional 3 hours.

Systemic administration of fluoxetine increased striatal extracellular 5-HT levels to 300% above baseline values. The administration of p-MPPF further increased 5-HT levels, up to a maximum of 700% above baseline. These results indicate that the 5-HT-elevating capacity of SSRIs may be restrained by the inhibitory action of the 5-HT$_{1A}$ receptor and that 5-HT$_{1A}$ receptor antagonists may play an important role in the development of future antidepressant drugs. ***Supported by USPHS grant MH 48125.***

527.15

LACK OF LONG-TERM EFFECTS OF ALNESPIRONE (S 20499) AFTER CHRONIC TREATMENT IN RATS.

C. Munoz[1], C. Duyckaerts[2], M. Lupart[1], M.C. Rettori[3], M. Lesourd[3]. [1]Biologie Servier, 905 route de Saran, Gidy, France. [2]Laboratoire de Neuropathologie, Hopital de la Salpetrière, Paris, France. [3]Institut de Recherches Internationales Servier, Courbevoie, France

Alnespirone (S 20499), a new chroman derivative, is a selective pre- and postsynaptic 5HT$_{1A}$ receptor agonist. It displays anxiolytic and antidepressive activities in rats. Since this drug is intended for long-term treatment in humans, we have searched for possible central effects on the serotonergic system. Male Wistar rats were treated by repeated oral dose (100 mg.kg^{-1}.j^{-1}) for 13 weeks, control rats having received the vehicle. The assessment was carried out using a first-tier screen, consisting in a functional observational battery (FOB) and an automated test of motor activity (Figure 8-maze), a measure of glial fibrillary acidic protein (GFAP) in different brain areas and histology for neuronal density (Dirichlet tessellation). No significant difference was observed between the treated and control rats in any of the behavioral tests related to different functional domains of the central and autonomic nervous system and motor activity (horizontal activity and rearings). The treatment did not modify GFAP levels in any tested areas of the brain, particularly in the mesencephalon (dorsal raphe nucleus), hippocampus and cortex, the main pharmacological targets of S 20499. The light microscopic examination of the brain did not reveal any apparent cellular loss and the total number of neurons was not modified in the dorsal raphe nucleus, the major site of action of S 20499. In conclusion, S 20499 did not show any deleterious central effect in rats after chronic treatment.

527.16

8-OH-DPAT ELEVATES EXTRACELLULAR SEROTONIN LEVELS VIA A TRANSPORTER DEPENDENT MECHANISM.

D. S. Lorrain, C.J. Smith* & E.M. Hull. State University of New York at Buffalo, Buffalo, NY 14260.

Using in vivo microdialysis, we have shown that application of 8-OH-DPAT into the medial preoptic area (mPOA) dose dependently increased extracellular levels of dopamine and serotonin (5-HT). Although 8-OH-DPAT is a selective 5-HT$_{1A}$ receptor agonist, it also may bind to D_2 receptors, to α_2 receptors and to the 5-HT transporter. The present experiments examined whether 5-HT-$_{1A}$ stimulation or 5-HT transporter inhibition were responsible for 8-OH-DPAT's ability to increase extracellular 5-HT in the mPOA.

In experiment 1, 5-HT-$_{1A}$ receptor stimulated serotonin release was tested using in vivo microdialysis. Adult male rats with probes aimed at the mPOA received either 8-OH-DPAT (500μM) alone in the dialysate or a combination of 8-OH-DPAT plus the specific 5-HT-$_{1A}$ antagonist MPPI (500μM/500μM). Serotonin levels increased significantly following application of 8-OH-DPAT and this effect was not blocked by co-administration of MPPI. In experiment 2, transporter mediated 8-OH-DPAT effects were tested. Adult male rats received either vehicle, the serotonergic neurotoxin 5,7-DHT (6μg) alone, or a combination of 8-OH-DPAT plus 5,7-DHT (8μg/6μg) directly into the mPOA. Five hours following infusion, animals were sacrificed and the brains removed. Dissected mPOA tissue pellets were homogenized and then spun in a centrifuge. The supernatant was injected onto a capillary column for HPLC analysis of 5-HT content. Treatment with 5,7-DHT significantly depleted mPOA 5-HT content when compared to vehicle treated animals. Co-administration of 8-OH-DPAT prevented depletion of 5-HT by 5,7-DHT. Since 5,7-DHT is a transporter-dependent neurotoxin, and since 8-OH-DPAT blocked the neurotoxic effects, these data suggest that increases in extracellular 5-HT after local application of 8-OH-DPAT may be mediated by uptake inhibition. Research supported by NIMH grant MH-40826 to EMH.

527.17

DIFFERENT RESPONSE TO ANTIDEPRESSANTS ON DRL 72-S IN HOLTZMAN AND HARLAN SPRAGUE-DAWLEY RATS: A NEW APPROACH TO TREATMENT RESISTANT DEPRESSION?

L.S. Seiden*, M. Balcells-Olivero, J. Richards and G. Vosmer. University of Chicago, Department of Pharm/Phys Sci., Chicago, Il. 60637.

Several antidepressant-like compounds were tested on the Differential-Reinforcement-of-Low-Rate 72-Second (DRL 72-s) schedule, a widely used behavioral screen to determine putative antidepressants. Two outbred stocks of rats, Harlan Sprague Dawley (Hsd:SpragueDawley^{R} SD^{R}) and Holtzman (HsdHot: Holtzman SD^{R}) were trained. A dose-response determination for the tricyclic antidepressant imipramine, the 5-HT2 antagonist ketanserin, the β agonist clembuterol and the 5-HT1A agonist 8-OH-DPAT was assessed in both stocks. The two stocks of rats differed in baseline performance on the DRL 72-s schedule. Harlan rats obtained more reinforcements per hour and showed lower response rate than Holtzman rats. In Holtzman rats, imipramine, ketanserin and clembuterol had an antidepressant-like effect on the DRL 72-s schedule (increase number of reinforcements with little change in response rate) confirming previous studies . However, in Harlan rats, these drugs did not show an antidepressant-like effect. These compounds disrupted the DRL 72-s performance at low doses and did not increase the number of reinforcements over baseline as seen in Holtzman rats. 8-OH-DPAT had no significant antidepressant effect on Holtzman or Harlan rats. Difference in tissue levels of NE, DA and 5HT in different brain regions were also found between both stocks of rats. The present results suggest that behavior response to DRL 72-s as well as response to different antidepressants may be genetically mediated. Since the Harlan rats did not show an antidepressant-like response to any drugs tested, this difference between the stocks may be useful in exploring the genetic bases of treatment resistant depression which does not respond to common antidepressant drugs. Supported by PHS MH-11191, RSA MH-10562.

527.18

THE NEUROTENSIN ANTAGONIST, SR48692, POTENTIATES RAT MEDIAN RAPHE SEROTONERGIC NEURAL ACTIVATION BY STRESS, INTERLEUKIN-1β AND CRF.

K. Corley*, T. Phan, W. Daugherty and M.C. Boadle-Biber, Physiol. Dept, VA Commonwealth Univ., Richmond, VA 23298. Acute random, inescapable loud sound activates serotonergic (5-HT) neurons of the median (MRN) but not dorsal raphe nucleus (DRN) (*Neurosci. Lett.* (1995) **199**: 78-80). This response is mimicked by interleukin-1β (IL-1β) and CRF, blocked by neurotensin (NT), and potentiated by the NT antagonist SR48692 (*Faseb J.* (1996) **10**: A131 and A132; *Soc. Neurosci. Abstr.* (1995) **21**: 864). We now report that MRN 5-HT neurons are also activated by swim stress (15 min at 30°C) and tail shock (30 min, 1 mA, 100 msec shocks, VI-1 min). The index of 5-HT neuronal activity was *in vivo* accumulation of 5-hydroxytryptophan (5-HTP) after NSD 1015 (m-hydroxybenzylhydrazine, 100 mg/kg i.p.), an inhibitor of aromatic amino acid decarboxylase, given prior to stress and 30 min before decapitation. MRN but not DRN levels of 5-HTP (determined by HPLC-EC in 2 mm punches) were increased significantly by stress over levels in sham-stressed controls as follows: swim - 16 $\pm$ 0.5 versus 9 $\pm$ 0.4 and shock - 18 $\pm$ 0.4 versus 9 $\pm$ 0.1 ng 5-HTP/mg protein $\pm$ SEM (N=4 for each group; $P < 0.01$). SR48692 (80 μg/kg, i.p. 30 min prior to stress) further increased MRN 5-HTP levels to 22 $\pm$ 0.4 (swim) and 22 $\pm$ 0.5 (shock) ng 5-HTP/mg protein $\pm$ SEM over stress alone ($P < 0.01$). SR48692 also potentiated the activation of MRN 5-HT neurons by IL-1β (10 ng ICV) and CRF (3 ug ICV), increasing 5-HTP levels by 35% over IL-1β and 24% over CRF alone ($P < 0.01$). SR48692 did not alter basal 5-HTP levels. (Supported by NIH Grant NS14090 to MCBB and a generous gift of SR48692 from Dr. D. Gully, Sanofi Recherche, France)

527.19

ENHANCEMENT OF SSRI EFFECTS BY 5-HT$_{1A}$ ANTAGONISTS. REGIONAL SELECTIVITY AND ROLE OF SOMATODENDRITIC AUTORECEPTORS. F. Artigas', L. Romero. Dept. of Neurochemistry, C.S.I.C. Barcelona, Spain.

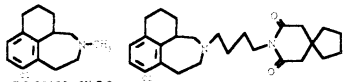
5-HT$_{1A}$ autoreceptor antagonists may accelerate the clinical onset of antidepressants by preventing a negative feedback action of serotonin (5-HT) at somatodendritic level. We have examined the effects of the blockade of 5-HT$_{1A}$ autoreceptors on extracellular 5-HT (5-HT$_{ext}$) during the treatment with paroxetine, a selective 5-HT reuptake inhibitor (SSRI). Paroxetine (3 mg/kg) elevated 5-HT$_{ext}$ in frontal cortex (FC), dorsal striatum (STR) and dorsal hippocampus (DHPC) by 250-300 %. Ten mg/kg paroxetine did not elevate 5-HT$_{ext}$ further in FC and induced a small additional increment in VHPC. Yet, the concurrent blockade of 5-HT$_{1A}$ autoreceptors with 1 mg/kg WAY-100635 dramatically potentiated the increment of 5-HT$_{ext}$ induced by 3 mg/kg paroxetine in FC and STR (maximal elevation to 1800 % of basal in STR). A moderate potentiation was observed in DHPC. The 5-HT-enhancing effect of WAY-100635 is likely due to its interaction with somatodendritic 5-HT$_{1A}$ receptors, as 1) its application in the DRN also potentiated paroxetine effects, 2) its systemic administration fully reversed the reduction of cortical 5-HT release that followed the activation of 5-HT$_{1A}$ autoreceptors by local application of citalopram in the raphe nuclei, and 3) it attenuated the reduction of 5-HT release in FC caused by the systemic administration of paroxetine when the terminal 5-HT transporter was blocked. These results indicate that 5-HT$_{1A}$ antagonists preferentially potentiate the elevation of 5-HT$_{ext}$ produced by SSRIs in brain areas receiving serotonergic afferents from the DRN. This effect results exclusively from the interaction of WAY-100635 with somatodendritic 5-HT$_{1A}$ autoreceptors. *Supported by FIS 95/266.*

527.20

ATYPICAL AGONISM AT 5-HT$_{1A}$ RECEPTORS BY RS-30199

M. Spedding*, A. Newman-Tancredi, M.J. Millan, C. Dacquet, B. Vickery', D. Tallentire', 'Roche Bioscience, Palo Alto, IdR SERVIER, 125 Chemin de Ronde, 78290 Croissy sur Seine, France.

RS-30199 has been shown to have atypical interactions at 5-HT$_{1A}$ receptors in that it has similar affinity (pKi 7.9) as RS-64459 (pKi 8.1), but RS-30199 is anxiogenic, and *devoid* of agonist or antagonist effects at the 5-HT$_{1A}$ receptor in guinea-pig ileum, whereas RS-64459 is a partial agonist (Small et al., 1990). The buspirone side chain may modify receptor coupling. At the human 5-HT$_{1A}$ receptor expressed in CHO cells, the two compounds had similar affinities (K_is against [^{3}H]-8-OH-DPAT, 27 and 22 nM respectively), but RS-30199 was almost a full agonist in GTPγS binding assays, whereas RS-64459 was a partial agonist (Emaxs 90.5% ; 49.1%, EC50s 2150 nM ; 385 nM), resembling buspirone. Unlike 8-OH-DPAT, RS-30199 (0.1, 1, 10 mg/kg sc) did not induce the 5-HT behavioural syndrome, but was a potent inhibitor of sexual activity in male rats at 0.1, 1 and 10 mg/kg sc, (methods: Tallentire et al., 1996). Unlike 8-OH-DPAT, RS-30199 prolonged intromission latency. RS-30199 (10 mg/kg sc) fully inhibited the facilitation of sexual behaviour caused by the α_2-adrenoceptor antagonist, delequamine (0.4 mg/kg, po). In conclusion, RS-30199 is an atypical agonist at 5-HT$_{1A}$ receptors.



RS-30199 pKi 7.9 RS-64459 pKi 8.1

Small et al. (1990). Br. J. Pharmacol. 104. 519-525
Tallentire et al. (1996). Br. J. Pharmacol. in press

funding, industry

5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1F}$ RECEPTORS

528.1

5-HT$_{1B}$ RECEPTORS ARE EXPRESSED IN RAT SENSORY NEURONS IN CULTURE J. J. Chen*, M. R. Vasko, T. P. Staeva, M. Baez and D. L. Nelson. CNS Res., Lilly Res. Labs, Indianapolis, IN 46285; Dept. of Pharmacol. & Toxicol., Indiana U., Sch. of Med., Indianapolis, IN 46202

One potential site of 5-HT's actions on nociception at the level of the spinal cord is the terminals of sensory neurons. To determine what subtypes of serotonin receptors mediate the effect of 5-HT on sensory neurons, we examined the subtypes of 5-HT receptors in these neurons using ligand binding and RT-PCR. Dorsal root ganglia cells from E15-E17 rat embryos were dissociated with trypsin and grown in culture. Receptor binding was measured using ^{3}H-5HT, ^{3}H-Lysergic acid diethylamide (LSD) or ^{3}H-5-carboxamidotryptamine (5-CT) in membranes prepared from neurons cultured for 14 days. The expression of serotonin receptor genes was investigated by RT-PCR analysis of total RNA isolated from the sensory neurons. Equilibrium binding of ^{3}H-5-HT or ^{3}H-LSD revealed highly specific binding sites. 5-CT displaced the ^{3}H-5-HT binding with an IC_{50} of 2.3 nM, pointing toward the possible expression of 5-HT$_1$ family, 5-HT$_{5B}$ or 5-HT$_7$ receptors in sensory neurons. The low affinity of ritanserin and Way100635 in the binding assays suggested the absence or minimal amount of 5-HT$_2$ family and 5-HT$_{1A}$ receptors in these neurons. Further experiments using cyanopindolol displacement of ^{3}H-5-CT binding revealed two binding sites with IC_{50}'s of 1.0 nM and >100 nM, respectively. This demonstrated that at least two subtypes of serotonin receptors are located in sensory neurons. The high affinity of the predominant binding site for cyanopindolol suggested that it could be the 5-HT$_{1B}$ subtype. RT-PCR analysis using primers specific for different subtypes indicated the expression of 5-HT$_{1B}$ mRNA as well as 5-HT$_{1D}$ message. These results demonstrate the expression of multiple subtypes of serotonin receptors in isolated sensory neurons, and the 5-HT$_{1B}$ receptor appears to be the predominant type of serotonin receptor present in these cultures. (Supported by Eli Lilly & Co. and DA07176)

528.2

AXONAL LOCALIZATION OF THE SEROTONIN 5-HT1B RECEPTOR IN RAT BRAIN. M. Riad* [1], N. Jodoin [1], S. Garcia [1], X. Langlois [2], M. Darmon [2], M. Hamon [2] and L. Descarries [1]. [1] Dép. pathologie et CRSN, Univ. Montréal, Montréal, Qc, Canada; [2] Neurobiologie Cellulaire et Fonctionnelle, INSERM U288, Paris, France.

A pre-embedding protocol was developed for the light and electron microscopic immunocytochemical visualization of 5-HT1B receptor in adult rat brain, using diaminobenzidine (DAB) and gold as markers. Specific polyclonal antibodies raised against a synthetic peptide corresponding to a highly selective portion of the third intracytoplasmic loop of the rat 5-HT1B receptor were used [Langlois et al., *J. Neurochem.* 65: 2671-2681, 1995]. Among several fixation procedures tested, perfusion with 4% paraformaldehyde yielded the best results. DAB or gold labeling was mainly observed in the substantia nigra and globus pallidus. This labeling essentially involved fine unmyelinated axons, but spared axon terminals. On the basis of these ultrastructural data, it was concluded that the 5-HT1B receptor of adult rat CNS is mostly located on preterminal unmyelinated axons. Quantitative analysis of the distribution of the gold particles should reveal whether this preferential localization is axolemnal and/or axoplasmic. Lesion and double labeling experiments are planned to determine to what extent it might correspond to an autoreceptor versus heteroreceptor localization (5-HT versus striato-nigral or -pallidal axons). [Supported by the FCAR, FRSQ and MRC grant MT-3544].

528.3

MODIFIED LIGAND BINDING TO THE NATURALLY OCCURRING PHE-124-CYS VARIANT OF THE HUMAN 5-HT$_{1B}$ RECEPTOR M. Bühlen, M. Brüss, H. Bönisch and M. Göthert*, Inst. Pharmacol. Univ. Bonn, Reuterstrasse 2b, 53113 Bonn, Germany

Single strand conformation analysis for DNA sequence variation of the 5-HT$_{1B}$ receptor gene from healthy individuals revealed a coding mutation in nucleotide position 371 (T → G substitution), leading to an amino acid exchange (Phe → Cys) in position 124 of the receptor protein (in the third transmembrane domain close to the junction with the first extracellular loop; Nöthen et al., Biochem. Biophys. Res. Comm. 205, 1194-1200, 1994). Since this substitution may lead to the formation of a disulfide bond with an extracellular Cys, the ligand binding properties of the mutant receptor may be modified. Genomic DNA from an individual, who was heterozygous for the mutation, was used as a template to clone (by PCR) the coding sequence of the wild-type and mutant receptor into the eukaryotic expression vector pSG5. COS-7 cells were transfected with the cDNA of the wild-type or the variant 5-HT$_{1B}$ receptor, and binding of [^{3}H]5-carboxamidotryptamine ([^{3}H]5-CT) to the plasma membranes from the cultured transfected cells was determined. In competition experiments, the concentration of [^{3}H]5-CT was 1 nM (specific binding, defined by 10 μM 5-HT: $\geq$ 90%). The full or partial 5-HT receptor agonists, dihydroergotamine, 2-[5-[3-(4-methylsulfonylamino) benzyl-1,2,4-oxadiazol-5-yl]1H-indole-3-yl]ethylamine (L-694,247), 5-CT, 5-HT, sumatriptan, 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl) 1H indole (RU24969) and methysergide inhibited specific [^{3}H]5-CT binding to the mutant and wild-type receptor. The inhibitory potencies at the mutant receptor (pK_i: 11.0, 9.8, 8.8, 8.4, 7.7, 7.5 and 7.5, respectively) were 0.3-0.5 log units higher than for the wild-type receptor. In conclusion, the naturally occurring Phe-124-Cys substitution in the human 5-HT$_{1B}$ receptor leads to an increase in the affinity of 5-HT$_{1B}$ receptor agonists. In vivo, this may cause pharmacogenetic differences in the action of human 5-HT$_{1B}$ receptor agonists. (Supported by a grant from the DFG: SFB 400)

528.4

GENOMIC CLONING AND HETEROLOGOUS EXPRESSION OF A RECOMBINANT GUINEA PIG SEROTONIN 5-HT$_{1D\beta}$-RECEPTOR. W.H.M.L. Luyten*, I. Van de Weyer, G. Nobels, A. Parker, P. Van Gompel, M. Ercken, A. Lesage and J.E. Leysen. Dept. Biochem. Pharmacol., Janssen Research Foundat., Beerse, 2340 Belgium.

Significant differences exist between the pharmacological properties of 5-HT$_{1D}$-receptors (Rs) in various species, rendering extrapolation of e.g. rodent data to humans hazardous. Human 5-HT$_{1D\alpha}$- and $_{1D\beta}$-R subtypes are very similar pharmacologically, but the ligand-binding properties of the latter differ significantly from those of their rat or mouse homologues, which are therefore called 5-HT$_{1B}$-Rs. A single amino acid difference (Thr355Asn) accounts for most of the pharmacological dissimilarity (Nature **360**: 161-163 (1992)). Guinea pigs are also used frequently for pharmacological studies but their 5-HT$_{1D\beta}$-R has not yet been cloned or expressed.

PCR-primers, based on regions conserved across human 5-HT$_{1D\beta}$-R as well as rat and mouse 5HT$_{1B}$-R sequences, but different from known 5-HT$_{1D\alpha}$-R sequences were used to amplify from a commercial guinea pig genomic library (Clontech) a ±770 bp fragment of coding region. This fragment was cloned into pBS SK+, and the sequence showed ±85% identity with that of human 5-HT$_{1D\beta}$-R. The purified PCR product was labelled with ^{32}P-dCTP by random-priming and used to screen a λEMBL-3 guinea pig genomic library (750,000 plaques; ±3.5 genome equivalents). One positive plaque was identified and purified, and the insert of this λ-clone was sequenced partially by primer-walking (cycle-sequencing followed by resolving reaction products on an ABI 373 automated fluorescent sequencer). The sequence of the open reading frame was 83% identical to that of the published human 5-HT$_{1D\beta}$-R sequence. Significantly, a Thr was found at position 355, just as in the human 5-HT$_{1D\beta}$-R sequence but different from the Asn found at the homologous position in rat and mouse 5-HT$_{1B}$-R. The coding region was subsequently PCR-amplified with *Pfu* (Stratagene), subcloned in the *SmaI* site of pBluescript (Stratagene) and the sequence verified once more. The insert was subcloned in the pcDNA3 expression vector (Invitrogen) for transient as well as stable expression for pharmacological characterization by radioligand binding and signal transduction. **Supported by** the Janssen Research Foundation.

528.5

DISTRIBUTION OF 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$ AND 5-HT$_{1F}$ RECEPTORS IN HUMAN BRAIN USING ^{3}H-SUMATRIPTAN AS A LIGAND

M.E. Castro, T. Romón, C. del Arco, J. Pascual, A. Pazos*
Dept. Physiol. and Pharmacol., Unit of Pharmacol., University of Cantabria, Santander, Spain

The autoradiographic distribution of ^{3}H-sumatriptan binding sites in postmortem brain tissue sections from 11 subjects was analysed. Ten μM 5-HT (to define specific binding), 0.1 μM 5-Carboxamidotryptamine (5-CT) (to examine binding to 5-HT$_{1F}$ receptors) and 0.2 μM ketanserine (to explore labelling to 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$) were added to 3 consecutive sections. Relevant densities of ^{3}H-sumatriptan binding sites were seen in: visual area = s. nigra> g. pallidus> frontal cortex (internal)> hippocampal subiculum = n. tractus solitarius> n. trigeminalis caudalis = claustrum. The diencephalon and the cerebellum were, in general terms, poor in these binding sites. While 5-HT displaced in all regions more than 90% of ^{3}H-sumatriptan binding, the level of binding insensitive to 5-CT (5-HT$_{1F}$ receptors) was relevant, around 50% of total binding, in several areas such as hippocampus (CA$_1$ field), neocortex (internal layers) and n. trigeminalis caudalis, among others. Most 5-HT$_{1D}$ receptors labelled by ^{3}H-sumatriptan belonged to the 5-HT$_{1D\beta}$ class. 5-HT$_{1D\alpha}$ receptors represented only 15% of ^{3}H-sumatriptan binding sites and only over the locus niger.

These results confirm that ^{3}H-sumatriptan binds to 5-HT$_{1F}$ receptors in several areas of the human brain, though the labelling to 5-HT$_{1D\beta}$ receptor is still predominant. To our knowledge, this is the first report analysing the autoradiographic distribution of 5-HT$_{1F}$ receptors in the whole human brain.

Supported by FIS (94/1382) and FAES grants

528.6

HIGH AND LOW AFFINITY SEROTONIN 5-HT$_{1D}$ RECEPTOR SITES IN BRAIN: CHARACTERIZATION USING A NEW RADIOLABELED ANTAGONIST.

G. Mengod[1], A. Baurich[1], J.M. Palacios[2]*, and R. Cortés[1].
[1]Dept. Neurochem., IIBB/CSIC, 08034 Barcelona, Spain.

Serotonin 1D receptor (5-HT$_{1D}$R), a G-coupled receptor, is one of the most abundant 5-HT receptors in the mammalian brain and the target of anti-migraine drugs. Until now, only radiolabeled agonist compounds were available for direct labeling of these receptors. Agonist ligands, however, recognize only the high affinity state of receptors and provide only a partial view of the density and localization of these sites. The compound GR 125743 is a selective 5-HT$_{1D}$R antagonist. We have used ^{3}H-GR 125743 to characterize and visualize (using quantitative autoradiographic techniques) 5-HT$_{1D}$R in the brain of rat, guinea pig, monkey and man. The binding of ^{3}H-GR 125743 was saturable, with comparable nanomolar affinity and with low non-specific binding in all brain regions examined in the four species. ^{3}H-GR 125743 binding was competitively blocked by co-incubation with both unlabeled agonists and antagonists of the 5-HT$_{1D}$R. When compared with agonists ligands the density of sites labeled by the radiolabeled antagonist was considerable higher in all the regions examined. The ratio between antagonist and agonist binding site densities was not the same in every brain region studied. The results show that ^{3}H-GR 125743 is a new useful tool for the study of 5-HT$_{1D}$ receptors. This ligand appears to label both high and low affinity states of these receptors, and can be used to map these sites differentially and study the alterations after physiological or pharmacological modifications of these receptors.

[2] Permanent address: Research Institute, Laboratorios Almirall, Barcelona.

Supported by FIS grant number 94/0864

528.7

CLONING AND CHARACTERIZATION OF RECOMBINANT GUINEA PIG 5-HT$_{1D\alpha}$ AND 5-HT$_{1D\beta}$ RECEPTORS.

J.M. Zgombick*, J.A. Bard, S.A. Kucharewicz, D.A. Urquhart, R.L. Weinshank and T.A. Branchek. Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652.

The 5-HT$_{1D}$ receptor has been implicated as a molecular target for the treatment of acute migraine based upon the pharmacological actions and clinical efficacy of sumatriptan, a selective 5-HT$_{1D}$ receptor agonist. The guinea pig has served as an animal model of 5-HT$_{1D}$ receptor function, most recently in evaluating 5-HT$_{1D}$ receptor agonists as potential anti-migraine agents. Since the 5-HT$_{1D}$ receptor subfamily is comprised of two distinct, but closely-related subtypes, the genes encoding these receptors were cloned from a guinea pig liver genomic DNA library using oligonucleotide probes targeted to nonconserved regions of recombinant human 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors. Sequence analysis indicates that guinea pig 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors are comprised of 378 and 390 amino acids, respectively. Comparison of the deduced amino acid sequences of guinea pig 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptor subtypes indicate that they display overall and transmembrane (TM) identities of 63% and 77%, respectively. Both clones contain a conserved threonine residue in TM 7, a structural feature imparting 5-HT$_{1D}$ receptor pharmacology. The 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptor genes were transiently expressed in Cos-7 cells and their binding properties were evaluated using [^{3}H]5-HT. Membranes expressing either of the cloned guinea pig 5-HT$_{1D}$ receptor subtypes displayed classical 5-HT$_{1D}$ receptor pharmacology with the following rank order of binding affinities: 5-CT > 5-HT > sumatriptan > 8-OH-DPAT > (-)-pindolol. Ketanserin displayed modest (7-fold) 5-HT$_{1D\alpha}$ receptor selectivity while methiothepin exhibited a similar selectivity for the 5-HT$_{1D\beta}$ subtype. Although a few compounds displayed modest subtype selectivity, the pharmacological profiles of recombinant guinea pig 5-HT$_{1D}$ subtypes were nearly indistinguishable. Intraspecies and interspecies comparisons of mammalian 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors reveal high similarities in amino acid sequences and binding properties. High correlations were observed between the binding affinities of compounds for 5-HT$_{1D}$ binding sites measured in guinea pig cortical membranes and both recombinant guinea pig 5-HT$_{1D\alpha}$ (r^2=0.80) and 5-HT$_{1D\beta}$ (r^2=0.87) receptors. The determination of the potencies and intrinsic activities of the compounds tested at cloned guinea pig 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors awaits evaluation in second messenger assays.

This work was supported by Eli Lilly and Company and Synaptic Pharmaceutical Corporation.

528.8

CLONING AND CHARACTERIZATION OF RABBIT AND DOG 5-HT$_{1F}$ RECEPTOR SUBTYPES: A COMPARISON OF THE PHARMACOLOGICAL PROFILE TO THE HUMAN SPECIES HOMOLOGUE.

J.A. Bard*, S.A. Kucharewicz, J.M. Zgombick, R.L. Weinshank and T.A. Branchek. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

The pharmacology of 5-HT$_1$-like receptors which mediate vasoconstriction and are implicated in the treatment of migraine has been studied in a variety of tissues from several species, including man, rabbit and dog. Since species differences in the pharmacology of 5-HT$_{1D}$ binding sites have long been observed, we further explored this possibility for the 5-HT$_{1F}$ receptor subtype. We cloned the rabbit and dog 5-HT$_{1F}$ genes, transiently transfected them into Cos-7 cells and compared their binding profiles in membrane preparations with that of the previously cloned human 5-HT$_{1F}$ receptor. The 5-HT$_{1F}$ genes from rabbit and dog show a high degree of homology to each other and to the previously published human gene, both at the nucleotide and amino acid levels, as predicted for species homologues. The K_d of [^{3}H]-5-HT determined from saturation experiments for the rabbit and dog 5-HT$_{1F}$ receptors was 10 and 13nM, respectively. The B_{max} for rabbit and dog 5-HT$_{1F}$ was determined to be 3.2 ± 0.3 and 5.1 ± 0.2 pmol/mg protein, respectively. The rabbit, canine and human 5-HT$_{1F}$ receptors all displayed similar pharmacological profiles. However, sumatriptan exhibited a 10-fold lower affinity at the canine receptor when compared to the human or rabbit 5-HT$_{1F}$ homologues. Finally, we have used reverse transcription-PCR (RT-PCR) to demonstrate the presence of mRNA in both rabbit and dog saphenous veins, which have been used as models for vasoconstriction as related to therapeutics for migraine.

This work was supported by Eli Lilly and Company and Synaptic Pharmaceutical Corporation.

528.9

CLONING AND CHARACTERIZATION OF A RECOMBINANT GUINEA PIG 5-HT$_{1F}$ RECEPTOR.

N. Adham*, J.A. Bard, J.M. Zgombick, M.M. Durkin, R.L. Weinshank and T.A. Branchek. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

The human, mouse and rat serotonin 5-HT$_{1F}$ receptors have been cloned previously by several groups. The antimigraine compound sumatriptan has been shown to have substantial affinity for the cloned human 5-HT$_{1F}$ receptor, suggesting that in addition to 5-HT$_{1D}$ receptor subtypes the 5-HT$_{1F}$ receptor may be a therapeutic target for the treatment of migraine. The guinea pig plasma extravation model has been used to evaluate potential antimigraine drugs. Since species differences in the pharmacology of serotonin receptors are well known, we compared the pharmacological profiles of the cloned human and guinea pig 5-HT$_{1F}$ receptors in order to evaluate the usefulness of the *in vivo* model in predicting activity of compounds targeted for humans. The guinea pig 5-HT$_{1F}$ receptor was cloned by homology to the human 5-HT$_{1F}$ receptor and transiently transfected into Cos-7 cells to evaluate its binding properties using [^{3}H]5-HT. High affinity (K_d ~ 10 nM) [^{3}H]5-HT binding was detected to membranes obtained from transfected cells. The cloned guinea pig receptor displayed typical 5-HT$_{1F}$ receptor pharmacology with the following rank order of binding affinities: 5-HT > sumatriptan > 1-NP > α-Methyl 5-HT > metergoline > methiothepin > 5-CT. The pharmacological profiles of the cloned guinea pig and human 5-HT$_{1F}$ receptors were almost identical. *In situ* hybridization studies in guinea pig tissue revealed 5-HT$_{1F}$ receptor mRNA expression in the nuclei of the trigeminal ganglion suggesting that the 5-HT$_{1F}$ receptor may play a role in the presynaptic inhibition of neuropeptide release at the level of the intracranial vasculature, thereby blocking the development of neurogenic inflammation. These findings indicate that a selective 5-HT$_{1F}$ agonist may provide a novel approach to treat migraine.

This work was supported by Eli Lilly and Company and Synaptic Pharmaceutical Corporation.

528.10

INHIBITION OF NEUROGENIC PROTEIN EXTRAVASATION IN THE DURA VIA 5-HT$_{1F}$ RECEPTOR ACTIVATION - IMPLICATIONS TO MIGRAINE THERAPY,

K.W. Johnson*, J.M. Schaus, M.L. Cohen, J.E. Audia, S.W. Kaldor, M.E. Flaugh, J.H. Krushinski, K.W. Schenck, A.D. Kiefer Jr., J.S. Nissen, B.A. Dressman, J.M. Zgombick, T.A. Branchek, N. Adham and L.A. Phebus, Eli Lilly and Company, Indianapolis, IN 46285 and Synaptic Pharmaceutical Corp., Paramus, NJ 07652.

Neurogenic inflammation of the dura is thought to be an important component of the pathophysiology of migraine pain. Electrical stimulation (1 mA, 5 Hz, 4 msec) of the guinea pig trigeminal ganglion for 3 min. produced dural extravasation, a component of neurogenic inflammation, which was quantified by an *in situ* fluorescence measurement using Evans Blue. 5-HT$_{1D\beta}$ and 5-HT$_{1D\alpha}$ receptor activation was originally thought to be associated with the blockade of dural extravasation, in addition to contraction of the rabbit saphenous vein *in vitro*. We determined the potency of several 5-HT agonists (sumatriptan, DHE, L-694247, LY306258, LY334370, LY302148, and MK-462) 15 minutes after intravenous administration in the dural extravasation model. We correlated the potency in this model with the compounds' binding affinity at the cloned human 5-HT$_{1A}$, 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ receptors transfected in a mammalian cell line. We also correlated the potency in the extravasation model to each compound's functional activity to inhibit forskolin-stimulated adenylate cyclase in cell lines expressing the same human receptors. The 5-HT$_{1F}$ receptor had the highest correlation coefficient in both analyses (R^2 = 0.89 and 0.73 respectively). In contrast, no correlation existed between potency to inhibit dural extravasation and rabbit saphenous vein contraction *in vitro* for these same compounds. Thus, the beneficial effects of serotonin agonists and partial agonists to inhibit dural extravasation most likely occur via activation of the 5-HT$_{1F}$ receptor without requiring vasoconstriction. Such a mechanism may be relevant to the efficacy of other serotonergic anti-migraine agents that have high 5-HT$_{1F}$ receptor affinity. In addition, a selective 5-HT$_{1F}$ receptor agonist may be beneficial in migraine pain without the liability of coronary vasoconstriction. (Funded by Eli Lilly and Co.)

528.11

CHARACTERIZATION OF LY334370, A POTENT AND SELECTIVE 5-HT$_{1F}$ RECEPTOR AGONIST, IN THE NEUROGENIC DURAL INFLAMMATION MODEL OF MIGRAINE PAIN. L.A. Phebus*, K.W. Johnson, J.E. Audia, M.L. Cohen, B.A. Dressman, J.E. Fritz, S.W. Kaldor, J.H. Krushinski, K.W. Schenck, J.M. Zgombick T.A. Branchek, N. Adham, and J.M. Schaus, Eli Lilly & Co., Indianapolis, IN 46285 and Synaptic Pharmaceutical Corp., Paramus, NJ 07652.

Inhibition of trigeminal stimulation-induced neurogenic dural inflammation by serotonergic agents in guinea pigs is best correlated with affinity and agonist activity at the 5-HT$_{1F}$ receptor (see K.W. Johnson et al). LY334370 is a selective, high affinity agonist at 5-HT$_{1F}$ receptors with a K_i of 1.6 nM. It is devoid of in vitro vasoconstrictor properties at the rabbit saphenous vein in concentrations up to 10-5 M. LY334370 is a potent inhibitor of neurogenic dural inflammation in both the rat and guinea pig models (see table), which may be predictive of efficacy in acute migraine. It was active in this model when administered to animals via the intravenous, subcutaneous, oral or sublingual routes. When guinea pigs were pretreated (p.o.) with LY334370 at 200 pg/kg, a dose which approximates its oral ID_{100}, neurogenic dural inflammation was inhibited for at least 16 hours. Inhibition of trigeminal neurotransmitter release through 5-HT$_{1F}$ receptor agonist activity, as demonstrated for LY334370 in the dural inflammation model, may provide a treatment for migraine pain without vasoconstriction and its associated side effects.

Species	Route	Pretreatment Time	Estimated ID50
Rat	i.v.	10 min.	20 pg/kg
Rat	p.o.	60 min.	30 pg/kg
Guinea Pig	i.v.	10 min.	30 pg/kg
Guinea Pig	p.o.	60 min.	45 pg/kg

(Funded by Eli Lilly & Co.)

528.12

LY334370 IS SELECTIVE FOR THE CLONED 5-HT$_{1F}$ RECEPTOR. C.D. Overshiner, N. Adham, J.M. Zgombick, T.A. Branchek, D.O. Calligaro, L.A. Phebus, M.E. Roush, K.W. Johnson, S.K. Hemrick-Luecke, R.W. Fuller, V.L. Lucaites, D.B. Wainscott, D.L. Nelson, M.C. Wolff, M.J. Benvenga*, J.E. Audia, J.M. Schaus, J.H. Krushinski, S.W. Kaldor, B.A. Dressman and J.D. Leander, Eli Lilly & Company, Indianapolis, IN 46285 and Synaptic Pharmaceutical Co., Paramus, NJ 07652.

LY334370 fumarate is a high affinity 5-HT$_{1F}$ receptor agonist. LY334370's binding affinity was determined at other serotonin receptors; 5-HT$_{1A}$, 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$, 5-HT$_{1E}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_3$, and 5-HT$_7$. The K_i values were greater than 1 μM at numerous other neurotransmitter receptors, ion channels and uptake sites. Among these binding sites significant cross reactivity was found only at the 5-HT$_{1A}$ receptor. However, in functional assays measuring the inhibition of forskolin-stimulated cAMP production LY334370 acted as a full agonist at both receptors but was 1000-fold more selective for the 5-HT$_{1F}$ receptor than the 5-HT$_{1A}$ receptor. The compound was evaluated in several animal models sensitive to *in vivo* 5-HT$_{1A}$ receptor activity. Rats dosed with LY334370 (10-30 mg/kg, s.c.) did not exhibit serotonin syndrome, hypothermia or antagonize the increased temperature produced by 8-OH-DPAT (0.1 mg/kg, s.c.). The compound (1-10 mg/kg, s.c.) only modestly increased rat plasma corticosterone, and did not block increased corticosterone produced by 8-OH-DPAT (0.3 mg/kg, s.c.). LY334370 fumarate (0.03-10 mg/kg, s.c.) did not decrease exploratory behavior in rats , although 8-OH-DPAT did at a low dose (0.1 mg/kg, s.c.). The compound (1-10 mg/kg, i.m.) also did not mimic 5-HT$_{1A}$ agonists in the pigeon punished responding test. Pigeons trained to discriminate small doses of 8-OH-DPAT (0.16 mg/kg, i.m.) from distilled water could not discriminate LY334370 fumarate (5-20 mg/kg, i.m.) as a 5-HT$_{1A}$ agonist. LY334370 fumarate (1-10 mg/kg, s.c.) failed to lower brain 5-HIAA levels in rats. LY334370 fumarate (0.1 ng/kg, s.c.) was fully active in the rat dural extravasation model illustrating drug exposure following s.c. dosing. Thus, LY334370 fumarate is a potent and selective 5-HT$_{1F}$ receptor agonist. (funded by Eli Lilly and Company)

528.13

[^{3}H]LY334370, A SELECTIVE RADIOLIGAND FOR LABELING THE SEROTONIN$_{1F}$ (5-HT$_{1F}$) RECEPTOR. D.B. Wainscott, J.H. Krushinski, J.M. Schaus*, S.W. Kaldor, B.A. Dressman, J.E. Audia, N. Adham‡, and D.L. Nelson, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 and ‡Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

The compound LY334370 fumarate has been identified as a high affinity, selective agonist at the 5-HT$_{1F}$ receptor. Based on these properties the tritiated version of this molecule was examined as a potential radioligand for the study of this receptor subtype. Initial studies focused on characterization at the cloned human 5-HT$_{1F}$ receptor where [^{3}H]LY334370 exhibited a K_d value of 0.451 ± 0.025 nM and a B_{max} of 7608 ± 143 fmol / mg protein. Specific binding was > 80 % over a concentration range of 0.031 to 4.56 nM. Examination of possible effects of cations showed that $CaCl_2$ or $MgCl_2$ did not enhance, and in fact concentrations above 1 mM $CaCl_2$ or $MgCl_2$ inhibited [^{3}H]LY334370 binding to the human 5-HT$_{1F}$ receptor. In addition, NaCl and KCl did not enhance [^{3}H]LY334370 binding to the human 5-HT$_{1F}$ receptor, with concentrations above 1 mM NaCl inhibiting the binding. At 37°C association of the ligand-receptor complex was relatively slow, reaching equilibrium only by 120 minutes in the presence of 10 mM $MgCl_2$. In the absence of $MgCl_2$, equilibrium was reached within 60 minutes. Studies investigating the distribution of [^{3}H]LY334370 binding in rat brain homogenates showed highest labeling in cortical areas, corpus striatum, hippocampus and olfactory bulb. In guinea pig, a similar distribution was seen with the exception that more labeling was detected in caudate homogenates and less labeling in homogenates of olfactory bulb. These data demonstrate that [^{3}H]LY334370 can be used as a high affinity, selective radioligand for the study of the 5-HT$_{1F}$ receptor. (Research funded by Eli Lilly and Company).

528.14

AUTORADIOGRAPHIC LOCALIZATION OF THE SEROTONIN$_{1F}$ (5-HT$_{1F}$) RECEPTOR IN RAT BRAIN USING [^{3}H]LY334370, A SELECTIVE 5-HT$_{1F}$ RECEPTOR RADIOLIGAND . V.L. Lucaites, J. Krushinski, J.M. Schaus, J.E. Audia, D.R. Gehlert* and D.L. Nelson, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

LY334370 is a high affinity, selective agonist at the 5-HT$_{1F}$ receptor. On this basis, the tritiated compound was examined for its utility in autoradiography to localize the 5-HT$_{1F}$ receptor in rat brain regions. Maximum specific binding in 12 micron brain sections occurred at 0.5 nM [^{3}H]LY334370 in buffer containing 50 mM tris-HCL, 0.5 mM EDTA and 104 mM NaCl, pH 7.4. The binding reached equilibrium in 3 hours at room temperature. Nonspecific binding was determined in the presence of 1 μM 5-HT. The overall level of binding sites was low. This, coupled with the low specific activity of [^{3}H]LY334370 (31 Ci/mmol), resulted in requiring 12 week film exposure. A band of specific 5-HT$_{1F}$ receptor binding was found in intermediate layers of all cortical regions examined. [^{3}H]LY334370 bound in a stippled pattern in caudate putamen. 5-HT$_{1F}$ receptor binding was also prominent in hippocampus, particularly the CA3 region and was highly expressed in olfactory tubercle. No detectable specific binding was seen in cerebellum and very little was present in most brain stem regions. These findings suggest a discrete localization of the 5-HT$_{1F}$ receptor in the rat brain, and confirm the utility of [^{3}H]LY334370 as a potential tool to explore further the localization and possible functions of the 5-HT$_{1F}$ receptor.
(Research funded by Eli Lilly and Company.)

528.15

INVESTIGATION OF THE AGONISTIC ACTION OF ANTI-MIGRAINE COMPOUNDS ON HUMAN 5HT$_{1D\alpha}$- AND 5HT$_{1D\beta}$-RECEPTORS EXPRESSED IN VARIOUS MAMMALIAN CELLS. A.S. Lesage§*, R. Wouters§, P. Van Gompel§, W.H.M.L. Luyten§, P. Vanhoenacker°, G. Haegeman° and J.E. Leysen§. §Janssen Research Foundation, Beerse, Belgium; °University of Gent, Belgium.

Alniditan is a novel migraine abortive agent with nM affinity for 5HT$_{1D}$-receptor subtypes. We investigated the agonistic properties of alniditan, sumatriptan, dihydroergotamine and 5-hydroxytryptamine (5HT) on cloned human 5HT$_{1D\alpha}$-receptors expressed in C6 glioma cells and on cloned human 5HT$_{1D\beta}$-receptors expressed in HEK293, C6 glioma and L929 cells. Receptor expression levels were 700 to 2000 fmoles/mg protein, after induction of the latter with Na-butyrate and interferon β, respectively. Without induction, receptor expression was only about 50 and 200 fmoles/mg protein. 5HT$_{1D}$-Receptors are negatively coupled to adenylate cyclase. For measuring cAMP formation cells were grown in MW96 plates. In all the receptor transfected cells, alniditan and the reference compounds produced, similarly as 5HT, a concentration dependent reduction of forskolin stimulated cAMP formation. Hence all compounds were full agonists at both 5HT$_{1D}$-receptors and for the 5HT$_{1D\beta}$-receptors in different cells and at high and low receptor expression levels (but pIC_{50}-values tended to decrease in the latter); preliminary pIC_{50}-values (-log M) are presented in the table.

	5HT$_{1D\alpha}$ C6 glioma	5HT$_{1D\beta}$ C6 glioma	HEK293	L929
alniditan	8.9	8.1	8.8	9.0
sumatriptan	8.4	7.3	-	-
dihydroergotamine	8.7	7.9	-	-
5HT	8.6	7.5	8.0	8.3

Stimulation of 5HT$_{1D\beta}$-receptors, predominantly present in cranial blood vessels and mediating vasoconstriction and stimulation of 5HT$_{1D\alpha}$-receptors, predominantly present in trigeminal nerves and probably causing inhibition of inflammatory peptide release, may have a role in the migraine abortive properties of the compounds.

528.16

DISCRIMINATIVE STIMULUS PROPERTIES OF RU 24969: ANTAGONISM BY THE PUTATIVE 5-HT$_{1B/1D}$ RECEPTOR ANTAGONIST GR 127935.

*P.M. Callahan, M.G. Bankson and K.A. Cunningham**
Dept. of Pharmacology and Toxicology, Univ. of Texas Medical Branch, Galveston, TX 77550-1031.

The neuropharmacological effects of the 5-HT$_{1A/1B}$ receptor agonist RU 24969 were investigated in rats (N=16) trained to discriminate RU 24969 (0.5 mg/kg) from saline using a standard two-lever, water-reinforced FR 20 drug discrimination task. In substitution tests, the 5-HT$_{1B}$ agonists CGS 12066B and CP 94,253 (1.25-20 mg/kg) and the 5-HT$_{1B/2C}$ agonist TFMPP (0.125-1 mg/kg) substituted completely for the RU 24969 stimulus. The 5-HT$_{1A}$ agonists 8-OH-DPAT and ipsapirone, the 5-HT$_{1B/2C}$ agonist mCPP, the 5-HT$_{2C}$ agonist MK 212, the 5-HT$_{2A}$ agonists DOI and LSD and the 5-HT$_3$ agonist m-chlorophenylbiguanide as well as the 5-HT releaser fenfluramine and the 5-HT reuptake inhibitor fluoxetine engendered ≤50% drug-lever responding. In combination (antagonism) tests, the 5-HT$_{1B/1D}$ antagonist GR 127935 (0.125-4 mg/kg) produced a dose-dependent blockade of the RU 24969 (0.5 mg/kg) cue. Likewise, the substitution of CGS 12066B and TFMPP was also completely blocked by GR 127935. Administration of the 5-HT$_{1A}$ antagonist WAY 100135, the 5-HT$_{2A/2C}$ antagonist ketanserin and the 5-HT$_3$ antagonist zacopride failed to alter the RU 24969 stimulus. These results suggests that GR 127935 is an efficacious antagonist of RU 24969 and that the discriminative stimulus properties of RU 24969 are mediated primarily by 5-HT$_{1B/(1D?)}$ receptors.

Supported by NIDA 06511

528.17

EFFECTS OF GR 127935 ON TERMINAL 5-HT1D AUTORECEPTORS AND 5-HT SYNTHESIS IN GUINEA PIG BRAIN. C. Moret* and M. Briley. Pierre Fabre Research Center, 17 Av. J. Moulin, 81100 Castres, France.

Guinea pig brain was used to study the effect of the putative 5-HT1D antagonist, GR 127935, on the terminal 5-HT autoreceptor modulating 5-HT release in vitro and in vivo, and on ex vivo 5-HT synthesis. GR 127935 only partially antagonized the inhibitory effect of the non-selective 5-HT agonist, 5-carboxamidotryptamine, on electrically evoked release of [3H]5-HT from hypothalamic slices, whereas the non-selective 5-HT antagonist, methiothepin, blocked this effect totally. In vivo the terminal 5-HT1D autoreceptor was studied by measuring the extracellular levels of 5-HT by microdialysis in hypothalamus of freely moving guinea pigs. The 5-HT1D agonist, naratriptan, infused through the microdialysis probe, reduced in a concentration-dependent manner (1-10 μM) the extracellular levels of 5-HT. Naratriptan-induced inhibition (1 μM) was not modified by GR 127935 whereas it was reduced by methiothepin (1 μM), a concentration which had no effect by itself. Neither local nor systemic administration of GR 127935 (10-100 μM or 2.5-10 mg/kg ip) modified the in vivo output of 5-HT while methiothepin increased it (10-100 μM and slightly at 10-40 mg/kg ip). The synthesis of 5-HT was determined by measuring the accumulation of 5-HTP after blockade of the decarboxylase. GR 127935 (0.63-10 mg/kg ip) dose-dependently increased 5-HT synthesis by 50-60 % in hypothalamus, hippocampus and substantia nigra. These results suggest that in vitro GR 127935 does not act as a full antagonist at the 5-HT1D autoreceptor in the hypothalamus of guinea pig. In vivo its lack of effect locally or after systemic administration could also be explained by mixed agonist-antagonist properties. The moderate enhancing effect of GR 127935 on 5-HTP formation suggests that tonically activated 5-HT1D receptors modulate directly or indirectly 5-HT synthesis.

TRANSMITTERS IN INVERTEBRATES

529.1

INHIBITORY AMINO-ACID RECEPTORS FROM THE PARASITIC NEMATODE, *HAEMONCHUS CONTORTUS*. N.S. Delany*, T.M. Skinner, D.L. Laughton & A.J. Wolstenholme. School of Biology & Biochemistry, Univ. of Bath, Bath BA2 7AY, U.K.

GABA and glutamate act as inhibitory transmitters at nematode neuromuscular junctions. GABA is the major inhibitory transmitter on body and head muscles, whereas glutamate inhibits pharyngeal pumping. The receptors for these transmitters show interesting pharmacological properties and are major targets for existing and future anthelminthics.

We have isolated 5 cDNA clones (designated HG1-5) encoding putative inhibitory amino acid receptor subunits from *Haemonchus contortus*, a parasitic nematode of sheep and goats. All the clones contain the sequence motifs characteristic of GABA$_A$ and related receptors, but show only a low level (~30%) identity to vertebrate GABA and glycine receptor subunits. Expression of HG1, which responds weakly to glycine when expressed as a homomer in *Xenopus* oocytes, has been localised to the nematode nerve cord using an antibody raised against a synthetic peptide corresponding to a unique region of the extracellular domain. It is thus a good candidate for a component of the inhibitory GABA receptor found at the nematode neuromuscular junction. HG2 & 3 are homologues of two putative GABA receptor subunits isolated from *C. elegans* and are alternatively spliced products of a single gene. They share a common N-terminal extracellular domain, but have different C-terminal channel-forming domains. HG4 and 5 show a high level of sequence identity to the β- and α-subunits, respectively, of the *C. elegans* glutamate-gated chloride channel (the avermectin receptor) and may form part of the analagous receptor in *H. contortus*.

Funded by the Wellcome Trust, a BBSRC studentship to NSD and a BBSRC-CASE award with Merck, Sharp & Dohme to TMS.

529.2

TWO NOVEL INHIBITORY AMINO ACID RECEPTORS FROM *C. ELEGANS* FORMED BY ALTERNATIVE SPLICING POSSESS IDENTICAL EXTRACELLULAR DOMAINS. D. L. Laughton, G. G. Lunt and A. J. Wolstenholme *. Department of Biology and Biochemistry, University of Bath, Bath, BA7 2AJ. U.K.

Inhibitory amino-acid receptors, including those for GABA and glutamate, play an important role in the functioning of the nematode nervous system, involved in such activities as locomotion and pharyngeal pumping. We have isolated two novel inhibitory receptor cDNAs from the nematode *Caenorhabditis elegans* which are alternatively spliced products of a single gene. Both receptors are identical within the first 237 amino acids of the N-terminus, but differ in their C-terminal transmembrane domains, which are 83% identical. Northern blot analysis has confirmed the presence of both transcripts in a developmentally mixed population of *C. elegans*. Analysis of the genomic sequence has also confirmed that the junction between the shared extracellular domain and the alternative transmembrane domains corresponds to exon/intron boundaries within the gene. These results suggest that in nematodes alternative splicing may be employed to create receptor subunits with identical ligand binding domains but different channel forming domains and thus potentially different biophysical channel properties. A similar genomic organisation is found for a related gene, *unc-49*, discovered during the *C. elegans* genome sequencing project. To date, this phenomenon appears to be unique to nematode ligand-gated ion channel receptors.

This work is supported by the Wellcome Trust

529.3

DISTRIBUTION OF GABA IMMUNOREACTIVE NEURONS IN THE LEECH, *HIRUDO MEDICINALIS*. D.J. Bailey and J.L. Witten*. Department of Biological Sciences, U. Wisconsin-Milwaukee, Milwaukee, WI 53201.

We have used wholemount immunohistochemical methods to begin mapping the distribution of GABAergic neurons in the central nervous system of the medicinal leech, *Hirudo medicinalis*. The anti-GABA serum was generously supplied by Dr. Tim Kingan (USDA). Our preliminary results demonstrate a reproducible but sparse distribution of GABA-like immunoreactivity (GLI). Only 1 to 2 pairs of dorsal, anterior neurons per ganglion express GLI. These neurons send processes toward the midline and then travel ventrally towards the Retzius cells. Our findings are in marked contrast to the large number of neurons that exhibit ^{3}H-GABA uptake (Cline, 1983). To clarify this discrepancy and produce a complete map of GABA-like immunoreactive neurons, we are trying to improve penetration of the antisera in the wholemount preparations and are processing sectioned CNS material for GLI. Furthermore, we are trying to localize GLI to terminals on longitudinal body wall muscles that are known to be innervated by inhibitory motoneurons that take up GABA (Cline et al., 1985).

This work was begun in a Neurobiology Laboratory course taught by J.L.W. and has been supported by the College of Letters and Sciences at the U. of Wisconsin-Milwaukee.

529.4

GLUTAMATE IMMUNOSTAINING IN THE MUSHROOM BODIES AND CENTRAL BODY OF CRICKETS H. W. Honegger*, B. Lauer and M. Bartos. Institute for Zoology, TU Munich, Germany, and Dept. Biology, Vanderbilt Univ., Nashville, TN 27235

Glutamate is the transmitter at the neuromuscular junction of insects, but it also acts at central synapses. We used an anti-glutamate antiserum from O.P. Otterson (antiserum 607) to double label proctolinergic antennal motoneurons in crickets. In parallel, other central regions of the cricket brain were investigated. The antiserum was routinely preincubated with glutaraldehyde conjugates of aspartate, alanine and to supress the possible detection of amino acids similar to glutamate. In control it was preincubated additionally with glutaraldehyde-glutamate. Immunostaining was done on frozen or paraplast-embedded sections using the avidin-biotin-HRP method.

Most brains showed appreciable background staining, probably due to the function of glutamate in metabolism. However, the mushroom bodies including the calyx, pedunculus, α- and ß-lobes, the central body and 50-60 large neurons (20-40 μm diameter) in the median pars intercerebralis showed intense labeling. In some preparations only these regions were stained. In these it became evident that many of smaller glutamatergic neurons in the median pars intercerebralis project into the central body via the protocerebral bridge. Sections of the 4 brains exposed to the anti-antiserum, preincubated with glutaraldehyde-glutamate showed no staining indicating that glutamate was specifically detected. Incubation of brains with an anti-GABA antiserum stained only GABAergic input into the calyx and large extrinsic neurons projecting into the α lobes and pedunculi. Our data thus show that glutamate may be the one transmitter in restricted neuropiles of the insect brain which are important for olfactory learning and memory (mushroom body), and act as interface between sensory and motor activity (central body).

Supported by the Deutsche Forschungsgemeinschaft and Vanderbilt University.

529.5

A PUTATIVE NICOTINIC ACETYLCHOLINE RECEPTOR ALPHA SUBUNIT MESSENGER RNA IS PRESENT IN BOTH NERVE CELLS AND MUSCLE CELLS IN *APLYSIA CALIFORNICA*. R.E. McCaman*[1], D. Tamura[2], F. Loera[2] T. Chang[1], and J. K. Ono[2]. [1]Div. of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte, CA. 91010, [2]Dept. of Biological Science, California State University, Fullerton, CA. 92634.

The accessory retractor muscle (ARC, or the I5 muscle) in *Aplysia californica* has been extensively studied and shown to be innervated by identified cholinergic motorneurons which release a number of neuropeptides. Electrophysiological recordings from these muscles indicate that they have two types of nicotinic acetylcholine receptors (nAChR), one that mediates an excitatory response blocked by hexamethonium as well as an inhibitory response due to gating of chloride channels and blocked by alpha bungarotoxin (Kozak, J.A., K. R. Weiss, & V. Brezina, 1996, *J. Neurophysiol., 75:* 660-677). These studies suggested that the excitatory and inhibitory receptors in the ARC had identical characteristics to those found in central neurons. A putative alpha subunit for a nAChR was cloned from the *Aplysia* central nervous system and neurons known to express the excitatory nicotinic AChR were found to contain mRNA with this cloned sequence (McCaman, R.E. and Ono, J.K., 1993, *Soc. Neurosci. Abstracts, 19*: 1339). The present study suggests that the ARC muscle and isolated ARC muscle cells contain the same alpha subunit, based on the polymerase chain reaction of cDNA from reverse transcribed mRNA, restriction analysis of the PCR products, hybridization with the cloned sequence, and cloning and sequencing of the amplified sequence from the muscle. In contrast to other systems, it appears that both neuronal and smooth muscle nAChRs may be composed of the same alpha subunit.

Supported by the Minority Biomedical Research Support Program at CSUF (NIH grant S06GM08258), California State University State Mini-Grants and by Beckman Research Institute of the City of Hope Endowment funds.

529.6

PURIFICATION OF CHOLINE ACETYLTRANSFERASE FROM THE BRAIN OF THE LOBSTER, *HOMARUS AMERICANUS*. R. Guo and C. Brandon*. Department of Cell Biology and Anatomy, The Chicago Medical School, North Chicago, IL 60064.

Choline acetyltransferase (ChAT) has not heretofore been purified from crustacean brain. Therefore, as a prelude to immunocytochemical studies in crustacean brain, we have purified ChAT from the brain of the lobster, *Homarus americanus*.

Neural tissue (cerebral, sub-esophageal, and thoracic ganglia, plus connectives) was dissected from 140 lobsters, yielding about 75 grams of tissue. Purification of ChAT involved the following steps: 1) Homogenization and centrifugation at 39,000 x g (Specific Activity (SA)=0.0245 Units/mg, Total Activity (TA)=31.319U); 2) 0-60% ammonium sulfate precipitation; 3) gel filtration (SA=0.26U/mg, TA=12.48U); 4) gradient elution from DEAE-cellulose (SA=1.056U/mg, TA=8.96U); 5) gradient elution from Cibachron-F3GA dextran (TA=2.35U); 6) gradient elution from hydroxyapatite (SA=12.73U/mg, TA=0.76U).

The final product had a specific activity of 12.73U/mg, representing a purification of about 520-fold, with a yield of about 2.5%. Lobster ChAT has a molecular weight of 86,000 by SDS-PAGE, and a pH optimum of 7.0. ChAT concentration was highest in the cerebral and thoracic ganglia (0.0245U/mg), intermediate in optic lobe (0.0187U/mg), and lowest in abdominal ganglia and connectives (0.0113U/mg).

Supported by FUHS/CMS.

529.7

DEVELOPMENTAL AND SPATIAL EXPRESSION OF THE MUSCARINIC ACETYLCHOLINE RECEPTOR IN THE TOBACCO HORNWORM, *Manduca sexta*. A. Wang* and B. A Trimmer, Dept. of Biology, Tufts University, Medford, MA 02155.

Electrophysiological, pharmacological and biochemical approaches have been used to characterize putative muscarinic acetylcholine receptors (mAChR) in the central nervous system of *Manduca sexta*. However, little was known about the molecular properties and the temporal and spatial distribution of mAChRs in *Manduca*. Using degenerate primers based on the *Drosophila* mAChR clone in the polymerase chain reaction (PCR), two fragments, MSF 1 and MSF 2, were isolated from genomic DNA and identified as regions of the mAChR gene in *Manduca*. MSF 1, a 239 nucleotide fragment, corresponds to transmembranes (TM) 3-5 of a mAChR gene while MSF 2, 180 basepairs in length, represents TM domains 6-7. We have used these fragments to characterize the developmental expression and tissue specific distribution in the fifth instar larva by reverse transcriptase (RT)-PCR. Poly A RNA extracted from whole animals at various developmental stages, including the egg, larva, wanderer, pupa, and adult were used with specific primer pairs for MSF 1 and MSF 2. Both fragments were detected in all developmental stages tested with one exception: MSF 1 was not found to be expressed in the pupal stage. Further tissue specific distribution of MSF 2 was investigated using total RNA isolated from selected tissues of the fifth larval instar in RT-PCR. MSF 2 was identified in the central nervous system and the fat body. However, other tissues such as the salivary glands and intersegmental muscles lacked MSF 2 expression. These studies describe the differential expression of MSF 1 and MSF 2 in the developmental stages and larval tissues evaluated. Furthermore, because MSF 1 and MSF 2 represent different regions of the mAChR gene, the absence of MSF 1 in the pupa introduces the possibility of mAChR heterogeneity in *Manduca sexta*.

Supported by a Sloan Foundation Fellowship, Whitehall Grant and NIH Grant 30566 to BAT.

529.8

CHARACTERIZATION OF INOSITOL (1,4,5) TRISPHOSPHATE-5-PHOSPHATASE IN THE CNS OF *Manduca sexta*. S. Qazi* and B. A. Trimmer. Dept. of Biology, Tufts University, Medford, MA 02155.

Stimulation of muscarinic receptors in purified CNS membrane preparations of *M. sexta* with oxotremorine-M results in the generation of inositol (1,4,5) trisphosphate (IP_3). We are studying the metabolism of this important signaling molecule by cystolic enzymes (in nerve cord homogenates) and by membrane associated enzymes. The primary products formed in homogenates have been identified by high pressure liquid chromatography analysis to be inositol 1,4 bisphosphate and inositol monophosphate, suggesting the presence of cytosolic IP_3-5-phosphatases. Enzyme rate kinetics of this IP_3 metabolism indicates a low affinity for the substrate (*Km* 25 - 100μM). However, IP_3 breakdown by purified membranes appears to result from an enzyme with an even lower affinity for IP_3 (*Km* greater than 100μM) suggesting that IP_3 is metabolized by multiple phosphatases. In many systems, inositol 1,3,4,5-tetrakisphosphate (IP_4) is also a substrate for IP_3-5-phosphatase. Measurements of the rate kinetics of [^{3}H]-IP_4 conversion to inositol trisphosphate in purified membrane preparations (*Km* = 1.00 μM) and homogenates (*Km* = 0.93 μM) are indistinguishable and reveal the presence of a phosphatase with high affinity for IP_4. This metabolism of [^{3}H]-IP_4 (1μM) can be completely inhibited by 100μM IP_3 suggesting that IP_4 can be used as a high affinity substrate for IP_3-5-phosphatase. Using this method we will compare the inhibitory rate constants (*Ki*) of IP_3 and other analogs of inositol trisphosphate to identify potent inhibitors of 5-phosphatase for use in single cell studies.

Supported by a Whitehall Foundation grant and NIH Grant NS 30566 to BAT.

529.9

AMINO ACID TRANSMISSION IN HYDRA. A. Pannaccione, P. Pierobon and G. Kass-Simon.* Dept. Biological Sciences, Univ. of Rhode Island, Kingston, R.I. 02881.

Evidence for the existence of classical neurotransmitters in hydra is based on electrophysiological, biochemical and histochemical studies. Evidence exists for ACH, 5-HT, and dopamine (Kass-Simon and Passano, '78, Venturini, '92.) Binding studies have provided evidence for glutamate (kainate and quisqualate) and GABAa receptors (Bellis, et al., '91 and Pierobon, et al., '95.) The present study provides electrophysiological evidence for glutamate and GABA involvement in hydra's pacemaker coordinating systems.

Hydra has 3 main pacemaker systems controlling its behavior: 1) the ectodermal Contraction Burst Pulse (CBP) system controlling longitudinal body contractions; 2) the endodermal Rhythmic Potential (RP) system causing elongation, and the Tentacle Pulse (TP) system producing individual tentacle contractions.

Hydra were recorded from with standard extracellular suction electrodes and treated with GABA, and GABAa and GABAb agonists & antagonists, glutamate and kainate , AMPA & NMDA receptor agonists & antagonists. Dose response curves were made for all substances and the data analyzed by ANOVA's.

GABA and GABAa agonists significantly reduced CBPs and RPs, but increased TPs; glutamate and kainate significantly increased RPs and reduce CBPs,and caused the body to elongate.

Supported in part by a post-doctoral fellowship to A.P. from the University of Naples, Federico II.

529.10

CHARACTERIZATION OF NEMATODE GLUTAMATE RECEPTORS E. Bush, H. Brooks, D.J. Brownlee, L. Holden-Dye*, R.C. Foreman, and R.J. Walker. Dept. of Physiol. & Pharmacol., Univ. Southampton, UK, SO16 7PX.

Two cDNAs isolated from *C.elegans*, GluClα and GluClβ, expressed in *Xenopus* oocytes form a glutamate-gated chloride channel which is exquisitely sensitive to the anthelmintic ivermectin. Here we have undertaken to i) identify blockers of the *C.elegans* glutamate-gated chloride channel ii) identify responses to glutamate in the parasitic nematode *Ascaris* and assess the action of ivermectin and chloride channel blockers on this response and iii) isolate cDNAs encoding for glutamate receptors from *Ascaris*

C.elegans GluClα and GluClβ cDNAs were expressed in *Xenopus* oocytes for voltage clamp experiments. Glutamate elicited an inward current (at -80mV; EC_{50} 948μM; maximum current 195 nA). The reversal potential was -20 mV. The response to 1mM glutamate was blocked by 100μM picrotoxin (55%), 4,4-dinitrostilbene-2,2-disulphonic acid (DNS; 36%) and 5-nitro-2-(3-phenylpropylamino)benzoic acid (38%). In *Ascaris*, glutamate (1mM) had no effect on somatic muscle but inhibited pharyngeal muscle (EC_{50} 490μM). This inhibition was potentiated by 1pM ivermectin. The response to glutamate was blocked by picrotoxin (500μM) but not by 100μM DNS. This suggests the presence of an ivermectin sensitive glutamate receptor regulating the pharyngeal muscle of *Ascaris*. We are screening an Ascaris L2 cDNA library with *C.elegans* GluClα and GluClβ to isolate equivalent clones from *Ascaris*. This will enable a comparison of the pharmacological properties of *Ascaris* glutamate receptor subunits with the *C.elegans* hetero-oligomer GluClα-GluClβ, and with the native glutamate response in Ascaris pharyngeal muscle.

Thanks to Dr J.Arena and Dr D.Cully (Merck, Sharp and Dohme) for the cDNAs for GluClα and GluClβ and to the BBSRC and MRC for funding.

529.11

MELATONIN IN A PRIMITIVE METAZOAN: RHYTHMICITY AND NEURONAL LOCALIZATION. N. Mechawar and M. Anctil*. Dept. Sci. Biol., Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

Although melatonin has long been considered a vertebrate hormone acting as a photoperiodic messenger, recent findings indicate that it is also present in some invertebrates in which it may be secreted rhythmically. We now report evidence that melatonin is present in neurons of a representative of the lowest invertebrates possessing a nervous system and that melatonin contents exhibit both diel and annual rhythmic patterns. Colonies of the cnidarian *Renilla koellikeri* were sampled over a 24h period once monthly and extracts were assayed for melatonin by RIA. Melatonin levels were highest in June at the peak of the reproductive season, and lowest in December. There was also a biphasic diel rhythmicity only during the latter part of the reproductive season (June to August). In addition, melatonin and its precursors serotonin (5-HT) and N-acetyl-5-HT were detected by HPLC. Melatonin-like immunoreactivity was localized in ectodermal neurons resembling previously identified 5-HT-immunoreactive neurons, but also in endodermal neurons associated with the gametes. The results suggest that (1) melatonin is synthesized from 5-HT in *Renilla* neurons, (2) it is an evolutionarily conserved neurochemical transmitter, and (3) it plays a role in the seasonal reproductive process of this primitive metazoan. (Support: NSERC, FCAR)

529.12

NEUROHORMONAL MODULATION OF CARDIAC PERFORMANCE AND CULTURED CARDIAC GANGLION NEURONS IN THE SHORE CRAB. M.A. Saver[1], J.L. Wilkens[1*], and N.I. Syed[2], Departments of Biological Sciences[1] and of Medical Physiology[2], University of Calgary, Calgary, Alberta, Canada T2N 1N4.

The modulatory effects of crustacean neurohormones on neurogenic heart performance were studied using perfused *in situ* hearts of *Carcinus maenas*. Heart rate, extracellular EMGs, myocyte membrane potential, and contractile force were measured in response to proctolin, crustacean cardioactive peptide (CCAP), serotonin, octopamine, and dopamine. The amines and CCAP induced strong positive chronotropic effects, and moderate positive inotropic effects consistent with simple facilitation. Proctolin caused moderate positive chronotropic and large positive inotropic effects.

To understand the actions of neurohormones at the neuronal level, we have developed an enzymatic dissociation technique to isolate the cardiac ganglion (CG) and to culture the isolated ganglionic neurons. Sharp electrode intracellular recordings from large cells in intact ganglia revealed spontaneous pacemaker and bursting potentials, and showed responses to neurohormones consistent with those shown by intact hearts. The responses of cultured neurons during exposure to neurohormones were similar to those exhibited by intact ganglia. Our data demonstrate that isolated cardiac ganglion neurons maintain their intrinsic membrane properties in cell culture and remain responsive for pharmacological and biophysical analyses. These cells provide an opportunity to examine the neuronal mechanisms modulating excitability in pacemaker neurons. Supported by NSERC Canada.

529.13

THE ROLE OF BUCCAL NEURON B1 IN THE OSMOTICALLY-INDUCED ALTERATIONS IN HEART ACTIVITY IN THE TERRESTRIAL GASTROPOD, *Limax maximus*. I.G. Welsford* and C. Rhine Dept. of Biology, Keene State College, Keene, NH 03435.

Terrestrial gastropods, such as *Limax maximus,* can experience dramatic alterations in hemolymph osmolality due to evaporative water loss across the moist integument. Previous work has implicated the small cardioactive peptides, SCP_A and SCP_B, in osmotically-induced alterations in heart activity. We investigated the potential role of buccal neuron, B1, which contains SCP-like immunoreactive material (SLM), in these alterations. Exposure of the buccal ganglia alone to normal slug saline made hyperosmotic (190 mOsm/kg H_2O) by addition of mannitol (normal level is 145 mOsm/kg H_2O), caused a decrease in heart atrial contraction force of 48± 18.3% ($\bar{X} \pm$ SEM, n=10). Exposure of the buccal ganglia alone to hyposomotic saline (110 mOsm/kg H_2O) caused an increase in these parameters (110 ± 8.6%). B1 firing frequency increased (38± 5.6%) in response to application of hyposmotic saline and decreased(-29 ± 7.9%) in response to hyperosmotic saline. When B1 was hyper-polarized during exposure of the buccal ganglia to hyposmotic saline, the increase in heart activity was significantly reduced (44 ± 16.2%; $p < 0.001$). Video-microscopic examination of the buccal ganglia during osmotic shock revealed osmotically-induced alterations in apparent cell volume of B1 which were blocked by both $HgCl_2$ (0.1 mM) and pCMBS (0.01 mM). These results are consistent with the hypothesis that B1 is involved in osmotically-induced alterations in heart activity and that this process involves aquaporin-like water channels (AQPs) in B1.
Supported by the Whitehall Foundation and NSF DUE-9352732

BEHAVIORAL PHARMACOLOGY

530.1

CONTINUOUS ACCESS TO SUCROSE INDUCES DEPRESSIVE-LIKE BEHAVIOR IN THE RAT V.J Djurić, D.H. Overstreet[1], D. Crosthwaite, E. Dunn and M. Steiner*. Father Sean O'Sullivan Rsrch. Cntr., Dept. of Psychiatry, St. Joseph's Hospital, McMaster Univ., Hamilton, Ontario, Canada L8N 4A6 and [1]Skipper Bowles Cntr. for Alcohol Studies, Univ. of North Carolina, Chapel Hill, NC 27599-7175, USA

Ingestion of carbohydrate-rich meal enhances serotonergic transmission, and carbohydrate craving has been observed in many depressed patients. The objective of this study was to investigate whether continuous access to sucrose affects indices of depressive behavior in the rat. In two independent experiments, cholinergic- and serotonergic hypersensitive Flinders Sensitive Line rats (a genetic animal model of depression) and their control counterparts Flinders Resistant Line rats were allowed *ad lib* access to 16% sucrose solution supplementing their regular lab diet for 30 days. Subsequent behavioral tests revealed striking effects of sucrose consumption on depressive-like behavior. Rats of both lines that were exposed to sucrose had longer periods of immobility in the forced swim test relative to the control animals of both lines whose diet was not supplemented with sucrose ($F_{1,26} = 47.14$; $p < .001$ and $F_{1,12} = 9.26$; $p = .01$; for the first and the second experiment, respectively). This finding was also paralleled with results from an open field test: rats that have been exposed to sucrose showed hypoactivity and thigmotaxis. Our data may help to explain serotonergic mechanisms that have been implicated in both eating and mood disorders in view of recent reports which indicate that swim test immobility is related to both cholinergic and serotonergic sensitivity.

(Supported by the St. Joseph's Health Care Foundation)

530.2

PREFRONTAL COGNITIVE IMPAIRMENT IN THE PATHOPHYSIOLOGY OF THOUGHT DISORDER: EFFECTS OF NMDA ANTAGONISM C.M. Adler[1], T.Goldberg[2], A.K. Malhotra[1], L. Elman[1], T. Yoshikawa*[3], D. Pickar[1], A. Breier[1]. [1]Experimental Therapeutics Branch, National Institute of Mental Health (NIMH), Bethesda, MD 20892. [2]Clinical Brain Disorders Branch, NIMH, Washington, DC 20032. [3]Clinical Neurogenetics Branch, NIMH, Bethesda, MD 20892.

Objective: The N-methyl-d-aspartate (NMDA) receptor has been implicated in the pathophysiology of thought disorder. We have hypothesized that dysfunction in prefrontal cortex cognitive processes may cause thought disorders. Ketamine is a general anesthetic that antagonizes the NMDA receptor at the PCP site and produces cognitive abnormalities in healthy controls (Malhotra et al, 1996). In this study, we sought to determine if ketamine produces thought disorder and if there is a relationship between thought disorder and prefrontal cognitive dysfunction. **Method:** A double blind placebo controlled infusion of ketamine was performed in 10 normal volunteers (0.12 mg./kg. bolus followed by a 0.65 mg./kg./hour infusion). Thought disorder, working memory, and verbal fluency were assessed during each infusion. Thought disorder was rated with the Scale for the Assessment of Thought, Language, and Communication (TLC). **Results:** Ketamine induced thought disorder and impairment in working memory and verbal fluency. **Conclusions:** These data suggest that NMDA receptor antagonism is involved with the pathophysiology of both thought disorder and cognitive impairment. The relationship between these two NMDA receptor antagonist effects may provide informative data on the role of cognition in thought disorder. A relationship between a subset of thought disorder and cognitive dysfunction was noted.

This study was funded through the intramural program of the National Institute of Mental Health.

530.3

ANTI-IMMOBILITY EFFECTS OF A MELATONIN RECEPTOR AGONIST, BUT NOT ANTAGONIST, IN A GENETIC ANIMAL MODEL OF DEPRESSION. D.H. Overstreet*, O. Pucilowski, M.-C. Rettori# and P. Delagrange#. Skipper Bowles Center for Alcohol Studies, Univ. North Carolina, Chapel Hill, NC 27599-7178 and #IRIS, 92415 Courbevoie, France.

The Flinders Sensitive Line (FSL) rats have been proposed as a genetic animal model of depression because they exhibit increased REM sleep and their innately high immobility in the forced swim test is counteracted by the tricyclic antidepressants, imipramine and desipramine. This model was used to test the acute and chronic effects of two novel compounds which have either an agonist or antagonist-like effect at melatonin receptors. In the acute study FSL and control Flinders Resistant Line (FRL) rats received either vehicle (DMSO) or the agonist (S 20304, 1 or 20 mg/kg), or desipramine (DMI, 10 mg/kg) at 08:00 or vehicle or the antagonist (S 20928, 1 or 10 mg/kg) or DMI at 10:00. A single 5-min swim test was performed by a blind observer 1 hr later. Only DMI in the FSL rats slightly reduced immobility; no other treatment was effective. In the chronic study FSL and FRL rats received vehicle or the agonist (1, 5, 10 mg/kg) chronically at 08:00 for 14 days or vehicle or the antagonist (1, 5, 20 mg/kg) chronically at 10:00 for 14 days. The swim test was performed by a blind observer 25 hr later. One-way ANOVAs indicated significant inter-group differences in both the agonist and antagonist treatments. However, the differences were primarily due to the large differences between the FSL and FRL rats. The only significant drug effect was a reduction in immobility in the FSL rats at the highest dose of the agonist. These findings suggest that the melatonin receptor agonist S 20304 may have antidepressant potential.
Supported by IRIS.

530.4

DISCRIMINATIVE STIMULUS CONTROL WITH OLANZAPINE: GENERALIZATION TO THE ATYPICAL ANTIPSYCHOTIC CLOZAPINE. **S. E. Strong* and J. H. Porter. Psychology Dept., Virginia Commonwealth University, Richmond, VA 23184.**

Olanzapine (Lilly & Co.) is a putative atypical antipsychotic that is currently being tested in clinical trials. Preclinical behavioral and pharmacological testing have revealed a profile very similar to the atypical antipsychotic clozapine. For example, in rats trained to discriminate clozapine from vehicle, olanzapine (1.25 mg/kg) substituted for clozapine, suggesting that olanzapine has discriminative stimulus properties similar to that of clozapine (Moore et al., 1992). The purpose of the present study was to determine if rats could be trained to discriminate olanzapine from vehicle in a two-lever discrimination procedure and, if so, if olanzapine would generalize to clozapine.

Initially, discrimination training with 22 rats was attempted with a training dose for olanzapine of 1.0 mg/kg (i.p.). Because of significant suppression of response rates, the training dose was reduced to 0.5 mg/kg and then to 0.25 mg/kg for 8 rats. For the 0.5 group, 11 of 14 rats acquired the two-lever discrimination (mean of 41.0 training sessions) and generalization testing (0.3125 to 2.0 mg/kg) yielded an ED_{50} of 0.18 mg/kg (95% C.I. = 0.13 to 0.24 mg/kg). For the 0.25 group, 4 of 8 rats acquired the discrimination (mean of 48.0 sessions). No ED_{50} was calculated as % drug lever responding was not below 50%. Clozapine fully substituted for olanzapine in all rats at one or more of the doses tested (0.156 to 10.0 mg/kg). These results support the suggestion that olanzapine has discriminative stimulus properties similar to clozapine, demonstrate that there is symmetrical generalization between olanzapine and clozapine, and support the conclusion that olanzapine has an atypical antipsychotic profile similar to that of clozapine in preclinical tests.

530.5

THE EFFECTS OF THE D_1 DOPAMINE RECEPTOR AGONIST SKF-81297 ON SOCIAL-EMOTIONAL REACTIVITY AND ACOUSTIC STARTLE RESPONSE IN MICE. P. L. Gendreau[1,2], J. M. Petitto[1], J.-L. Gariépy[2], and M. H. Lewis[1*]. [1]Department of Psychiatry, University of Florida, Gainesville, FL, 32610; [2]Department of Psychology, University of North Carolina, Chapel Hill, NC, 27599.

Our previous studies suggested an important role for D_1 dopamine receptors in mediating emotional behavior. These studies examined emotional reactivity in a social context and used dihydrexidine, a dopamine agonist which is only 10-fold selective for D_1 over D_2 receptors. Thus, the present study examined the effects of the highly selective, full-efficacy D_1 agonist SKF-81297 on emotional reactivity in isolated C57BL/6 mice. Moreover, drug effects were examined in tests of both social and nonsocial reactivity. First, the effects of SKF-81297 (0, 1, 3, 10 mg/kg) were assessed in response to mild social contact provided by a nonaggressive conspecific. SKF-81297 increased escape/jump behavior and markedly reduced the frequency of social approach initiated by the test animal. These effects were fully blocked by the D_1 antagonist SCH-23390 (0.1 mg/kg) but only partially by the D_2 antagonist sulpiride (20 mg/kg). In a second experiment, the effects of SKF-81297 (3 mg/kg) alone or in combination with SCH-23390 or sulpiride were examined in an acoustic startle experiment. SKF-81297 increased startle response and this effect was blocked by SCH-23390 but not by sulpiride. These results provide evidence for an important role for D_1-like dopamine receptors in the mediation of emotional reactivity to social and nonsocial stimuli. Supported by NSERC of Canada and MH45371.

530.6

EFFECTS OF REPEATED ADMINISTRATION OF A LOW DOSE OF THE D2 AGONIST QUINPIROLE ON THE STIMULUS PROPERTIES OF D1 AND D2 DOPAMINE AGONISTS. M.J. Pokora,* and D.A. Cory-Slechta. Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

In the absence of tolerance, repeated administration of a presynaptic dose of the D2 agonist quinpirole (QUIN) might be expected to result in a sustained decrease in dopamine (DA) release and eventual DA receptor supersensitivity. If so, it could enhance the stimulus properties of D1 and D2 DA agonists. Rats were trained to discriminate the stimulus properties of either 0.05 mg/kg of the D2 agonist QUIN, or 6.0 mg/kg of the D1 agonist SKF38393 from saline using standard operant drug discrimination procedures. Following the determination of dose-effect curves for the training drug, drug discrimination was suspended and repeated twice daily injections of either saline or 0.05 mg/kg QUIN separated by 12 hr were initiated for 4 weeks. During the final 2 weeks of repeated QUIN, the first dose was replaced by a drug discrimination substitution test every other day to re-establish the training drug dose-effect curves. QUIN sensitivity was increased and SKF38393 sensitivity marginally increased by chronic saline administration, as indicated by average decreases in the ED_{50} values derived from the dose-effect curves of 28% and 19%, respectively. In contrast, QUIN sensitivity decreased and SKF38393 sensitivity marginally decreased in response to chronic QUIN administration, as indicated by average increases of 27% and 28%, respectively, in dose-effect curve ED_{50} values. Sustained D2 DA autoreceptor agonism thus appears to attenuate both D1 and D2 DA agonist stimulus properties. These data could indicate that DA tolerance develops with repeated low dose QUIN treatment with recovery occurring following its cessation, as indicated by increased sensitivity following chronic saline administration. ES05017, ES05903 and ES01247.

530.7

THE EFFECTS OF PREFEEDING AND DOPAMINE AGONISTS ON WITHIN-SESSION MATCHING CURVE PARAMETERS OBTAINED USING A CONCURRENT VI 30 VI-INCREMENTING PROCEDURE. B.J. Brockel* and D.A. Cory-Slechta. University of Rochester, Rochester. NY 14642.

Twelve rats were trained on a 2-lever, concurrent variable interval (VI) schedule in which a complete matching curve could be obtained within a single 30-min session. One lever was associated with a "base" VI schedule (VI 30 s) while the other lever (incrementing lever) was associated with a percentage of the base VI. The percentage of the incrementing lever increased after every 6 reinforcers earned on this lever (i.e., 12%, 25%, 50%, 100%, 200%, 400%). Sensitivity and bias estimates were calculated using the generalized matching equation and were similar to parameters previously reported with matching curves generated using across-session, concurrent VI procedures. Changing the base VI from 30 s to 60 s had no effect on overall response rates or bias and sensitivity parameters. In probe sessions in which reinforcement densities were altered, response ratios were modified and obtained reinforcement ratios were similar to the programmed values. A prefeeding procedure produced decreases in overall response rates, increases in sensitivity, and alterations in bias estimates. Similar to prefeeding, the D2-D3 dopamine agonist quinpirole (0.0-3.0 mg/kg) and the D1-specific dopamine agonist SKF 82958 (0.0-0.4 mg/kg) decreased overall response rates. Low doses of the indirect-dopamine agonist d-amphetamine (0.25-1.0 mg/kg) increased response rates; whereas high doses (2.0-3.0 mg/kg) decreased rates. Of the 3 agonists, only quinpirole caused changes in sensitivity by producing a biphasic function of decreased sensitivities at low doses. Both d-amphetamine and quinpirole produced bias. These data indicate that a matching curve for concurrent VI schedules can be obtained within a single session using this procedure. In addition, this paradigm can be used to quantify the effects of alterations to the dopamine system on sensitivity to changes in reinforcement density and bias estimates. Supported by T32 ES07026, ES05017, ES05903, ES01247.

530.8

GRADUAL SLOWING IN RATS' RHYTHM OF LICKING BEHAVIOR AS CHRONIC HALOPERIDOL DOSING ENSUES: IMPLICATIONS FOR RODENT MODELS OF NEUROLEPTIC-INDUCED DYSTONIA AND PARKINSONISM. S.C. Fowler* and G. Wang. Dept. of Human Development, Univ. of Kansas, Lawrence, KS 66045.

Earlier reports indicated that acute haloperidol treatment (0-0.24 mg/kg) dose-relatedly decreased force of rats' tongue contact with a force-sensing disk, but rhythm of licking, as quantified by power spectrum analysis, was little affected at these acute doses. We report here that chronic daily doses of 0.24 mg/kg haloperidol produced a gradual slowing of the lick rhythm that first became detectable after about 10 days and continued to slow through the 21-day dosing period. This effect was not observed for lower haloperidol doses (0.06 and 0.12 mg/kg, in separate groups of 12 rats each). Over the same time period, all three doses produced an enduring and dose-related tongue-force deficit that remained stable compared to the vehicle control group. Early tongue-force deficits induced by haloperidol may be related to acute dystonia, whereas the later developing rhythm slowing maybe reflective of concurrent dystonia and parkinsonism. Supported by MH43429.

530.9

CHOLECYSTOKININ ATTENUATION OF SKF 38393-INDUCED BEHAVIOURS DOES NOT INVOLVE REMOVAL OF DOPAMINE D2 RECEPTOR ACTIVITY. J.M. Van Kampen* and A. Jon Stoessl. Clin. Neurol. Sci., University of Western Ontario, CANADA

The neuropeptide cholecystokinin (CCK) is known to interact with dopamine in various ways. We have demonstrated that CCK-8S effectively attenuates dopamine D_1 receptor mediated vacuous chewing movements (VCMs) and grooming. This ability of CCK to attenuate behavioural responses to a direct-acting agonist, suggests a **postsynaptic** action. However, for some dopamine-mediated behaviours dopamine D_1 and D_2 receptors demonstrate a synergistic relationship, requiring activation of both receptor types for their expression. In this context, attenuation of **presynaptic** dopamine release could alter the effects of a dopamine D_2 receptor agonist. In these experiments we sought to determine the role of dopamine D_2 receptors in the attenuation of SKF 38393-induced VCMs and grooming by CCK. Acute dopamine depletion by reserpine and α-methyl-p-tyrosine in male Sprague Dawley rats blocked SKF 38393-induced VCMs and grooming (5 mg/kg s.c.) suggesting that tonic dopamine D_2 receptor activation may play an important role in the expression of these behaviours. However, this effect was not reversed by concurrent administration of the dopamine D_2 receptor agonist quinpirole (10, 30, 50 µg/kg i.p.). As well, in non-depleted animals, quinpirole **potentiated** the effects of CCK on both SKF 38393-induced VCMs and grooming. These data suggest that while CCK attenuation of presynaptic dopamine release remains a possible means of action, removal of tonic dopamine D_2 receptor activity does not appear to be involved.

Supported by the Medical Research Council of Canada and the Ontario Mental Health Foundation.

530.10

AGING AND RESPONSES TO PROPOFOL IN RATS - R. Dirksen*, C.M. VanRijn, J. VanEgmond, E.L.J.M. VanLuijtelaar, T. Vree. Inst. Anesthesiology, Psychology NICI, Nijmegen University, Postbus 9101, 6500 HB Nijmegen, The Netherlands.

Aging may result in changes in GABA transmission and this transmission is essential to effects of various anesthetics including propofol [1]. This study compares effects of propofol between elderly and young adult WISW rats. No age-dependent differences were found in the incidence of narcosis (4 -20 $mg.kg^{-1}$) or in the potency of propofol to inhibit withdrawal reflexes to noxious stimuli (ED_{50}: 1.2 $mg.kg^{-1}$) [2]. The brain concentrations at given doses were similar for the two groups. The time course of these effects was however different, the narcosis lasted longer in the elderly than in the young adult animals (20 $mg.kg^{-1}$: 25 min v. 17 min) and the reflex inhibition had a slower onset ($t_{½}$: 0.7 min v. 0.3 min) and a slower decay ($t_{½}$: 16 min v. 7 min) in the elderly than in the young adult rats. Noteworthy, plasma concentrations at given doses were higher in elderly than in young adult rats, and the $t_{½}$ of plasma propofol was 8 min for the two groups. The elderly rats showed a greater reduction in heart rate and a higher incidence of apnea than the young adult rats. Moreover, propofol elicited a number of behavioral elements. The incidences of tail flicks, head and body shakes, and walking difficulties were lower in the elderly rats than in the young adult ones, whereas vacuous chewing occurred more often in the elderly than in the young adult animals. The observed shakes and walking difficulties are proposed to express abruptness of disruption of signal transfer in the central nervous system and consequently the transfer of faulty information to parts of the receiving neuronal networks during the transit between the awake and narcotic state [3]. The elderly rats showed a lower incidence of both items and recovered more slowly from narcosis. A higher incidence of vacuous chewing in elderly rats may reflect a low GABA activity in the substantia nigra, pars reticulata [1]. [1] Dirksen, R. et al.: Abstr. Soc. Neurosci. 20: 1360; 1994. [2] Dirksen, R. et al.: Eur. J. Anaesth. S3: 41-49; 1990. [3] Cools, A.R. et al.: Ann. NY Acad. Sciences Vol. 525, 1988: 338.

We acknowledge financial support by EUREKA grant EU51of the European Society.

530.11

ANXIOLYTIC EFFECT OF POLYAMINES MICROINJECTED INTO THE DORSAL PERIAQUEDUCTAL GRAY MATTER. A.P. Carobrez* and J.G. Pereira-da-Cruz. Depto. de Farmacologia, CCB, UFSC, Florianópolis, SC, 88040-900 Brazil

The relationship of the dorsal periaqueductal gray matter (DPAG) and anxioselective effects of drugs acting at the N-methyl-D-aspartate receptor (NMDAR) have been examined recently by our group. In brief, compounds applied within DPAG, that depress NMDAR/glycine-receptors, showed anxiolytic effects, whereas agonists of the glycine-site at the NMDAR exhibit a anxiogenic profile. NMDAR complex also supports binding sites for the polyamines and a dose-dependent effect, potentiation or inhibition of the NMDAR channel currents can be obtained. In the current studies the behavioral effects of spermidine (SD) or spermine (SP) into DPAG were analysed using male Wistar rats, with a chronically implanted cannula aimed to DPAG, placed in the elevated plus-maze test (EPM). An increase ($p<0.05$) in the open arms exploration, was observed after the microinjection (0.4 µl) of 0.62 nmol of SD [Rao $R(10,116)=2.33$; $p<0.05$] or of 2.5 nmol of SP [Rao $R(8,80)=2.49$; $p<0.05$] into DPAG. The anxiolytic effect of 2.5 nmol SP (0.2 µl) was blocked in rats previously (5 min) microinjected (0.2 µl) with ifenprodil (5 nmol) or arcaine (4 nmol) into DPAG. In addition, SP (2.5 nmol, 0.2 µl) failed to show an increase in open arms exploration of rats pre-treated (5 min) with glycine (80 nmol, 0.2 µl). However, previous (5 min) treatment with ±HA-966 (50 nmol, 0.2 µl) revealed an increase in open arms exploration in an ineffective dose of SP (1.25 nmol, 0.2 µl). The data obtained show that the anxiolytic action of the polyamines microinjected into DPAG is due to its action at the polyamine site of the NMDAR, sensitive to the specific antagonists, ifenprodil and arcaine. The data obtained also shows an interference on the anxiolytic action of polyamines applied to DPAG with compounds binding at the glycine sites of the NMDAR channel complex.

Supported by: Brazilian Government: CNPq and CAPES

530.12

THE EFFECTS OF TROPICAMIDE ON MYDRIASIS IN YOUNG RATS EXHIBITING A NATURAL DEFICIT IN PASSIVE-AVOIDANCE RESPONDING RD Smith,* CA Morgan & VL Coffin. *Schering-Plough Research Institute, Kenilworth NJ 07033.*

The young rat at post-natal day 18-22 exhibits a natural deficit in passive-avoidance responding that can be corrected with the acute systemic administration of different cholinomimetic drugs, such as tacrine (Smith et al., 707 pp13-21, 1996). In order to evaluate the generality of this apparent cholinergic hypofunction, different doses of the anticholinergic agent, tropicamide, were administered either systemically or dropped directly into the eye of young or adult rats. Tropicamide produced mydriasis in a dose-dependent manner. The ED50 for tropicamide dropped into the eye was 0.12% for adult rats and 0.25% for young rats. When doses between 0.3 and 100 mg/kg were delivered systemically, the mean time course for recovery to baseline pupil size was accelerated in young rats. The average time to recovery across all doses was 112 ± 27 min (mean ± SE) for young rats and 274 ± 70 min for adults. When subcutaneous tacrine was given immediately to young rats after training in a passive-avoidance response (PAR) task, retention was enhanced at testing 24 hours later in a dose-dependent manner. The response latencies were statistically different from saline-treated controls at doses of 0.003 and 0.01 mg/kg. This was not observed in adult rats. Taken together, these results suggest that the PAR, in addition to the mydriacyl response of the young rat to tropicamide, may be regulated by a system of subsensitive cholinergic receptors.

530.13

COMPARISON OF THE BEHAVIORAL EFFECTS OF SELECTIVE INHIBITORS OF PHOSPHODIESTERASE TYPES (PDE2, PDE3, PDE4, AND PDE5). S. Frith, R.E. Maloney*, and J. M. O'Donnell. LSU Medical School, Shreveport, LA 71130.

Selective inhibitors of PDE4, such as rolipram, have been shown to produce antidepressant-like effects on a number of behaviors, including behavior maintained under a differential-reinforcement-of-low-rate (DRL) schedule. In order to begin to determine whether similar effects are produced by selective inhibitors of other PDE types, the effects of the PDE2 inhibitor trequinsin, the PDE3 inhibitor milrinone, the PDE4 inhibitors rolipram and Ro 20-1724, and the PDE5 inhibitor zaprinast on the behavior of rats maintained under variable interval (VI) 30-sec and DRL 72-sec schedules were determined. For comparative purposes, the effects of the tricyclic antidepressant desipramine also were determined. At the higher dose ranges tested, all drugs reduced response rate under the VI schedule, except for milrinone; at lower doses, trequinsin (3, 5.6 mg/kg), milrinone (3 mg/kg), rolipram (0.003 mg/kg), and desipramine (0.03 mg/kg) increased response rate. Only desipramine and rolipram produced clear antidepressant-like effects on DRL behavior, reducing response rate and increasing reinforcement rate; Ro 20-1724, however, did tend to increase average reinforcement rate. Selective inhibitors of PDE2, PDE3, and PDE5 did not produce antidepressant-like effects on DRL behavior. The pattern of behavioral activity of the selective PDE4 inhibitors most closely resembles that of the proven antidepressant desipramine. (Supported by grants from NIMH.)

530.14

EFFECTS OF SCOPOLAMINE ON DRL RESPONSE RATE IN RATS GIVEN TRIMETHYLTIN. J. N. Freeman, III, R. H. Baisden and M. L. Woodruff*. Dept. of Anat. & Cell Biol., Quillen Coll. Med., E. Tenn. St. Univ., Johnson City, TN 37614.

Trimethyltin (TMT) produces obvious gross damage to the hippocampus. Many of the behavioral changes that follow TMT exposure can be attributed to hippocampal damage. TMT also significantly reduces the number of cholinergic muscarinic receptors in hippocampus (Earley et al., Neurochem. Int., 21, 351-366, 1992; Cannon et al., Mol. Chem. Neuropath. 23, 47-62, 1994). Because of the general damage caused to the hippocampus by TMT it is difficult to attribute specific behavioral changes to alterations in muscarinic receptors produced by the toxin. However, it could be that the TMT-induced reduction in muscarinic receptors might alter behavioral response to anti-cholinergic agents. This possibility was examined in the present experiment. Twenty-two male Long-Evans rats were trained to respond for a food reinforcement on DRL schedules with gradually increasing intervals until the interval reached 15 s. Twelve of the rats were then given 6 mg/kg TMT (p.o.). Testing on the DRL schedule was resumed 6 wks later with a 3 s interval. The interval was increased by 2 sec every 5 days until it reached 15 s. After 10 wks of training the rate of response did not differ statistically between the groups and daily saline injections were begun. These were continued for 2 wks and then injections of 0.1, 0.2, 1, 1.5, 2 and 3 mg/kg of scopolamine were given in a random order with 5 days separating each injection. Saline injections were given on the days that scopolamine was not given and injections of methscopolamine that were equimolar to the 1, 2 and 3 mg/kg doses of scopolamine were randomly interspersed. The 0.1 mg/kg dose increased responding in both groups and increased response rate in the TMT-treated rats more than controls. The 0.2 dose increased response rate in the control rats but not the TMT-treated rats. The higher doses decreased response rate in both groups, but significantly more in the TMT-treated rats. The 3 mg/kg methscopolamine injection reduced responding equally in both groups. These data indicate that pharmacologic challenge can reveal a behavioral effect of the reduction muscarinic receptors produced by TMT. (Supported by NIH grant ES 0407003-08.)

530.15

ROLE OF NITRIC OXIDE IN THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS: MAINTENANCE OF NORMAL SLEEP. S. Datta*, E. Patterson, Z. Xie and D. F. Siwek. Dept. of Psychiatry, Harvard Med. School, Boston, MA 02115 & VA Hosp., Bedford, MA.

Mesopontine cholinergic cells in the pedunculopontine tegmental (PPT) nuclei modulate the control of behavioral states by releasing acetylcholine to their target structures (Datta, Neurosci. Biobehav. Rev. 19:67-84, 1995). These cells also synthesize nitric oxide (NO). This NO diffuses into the extracellular space and acts as a neuronal messenger. The present study examines the function of NO synthesis and its presence in the extracellular space in the PPT in the behavioral states of waking and sleep. Cats (n=5) were implanted with standard electrodes to measure states of waking, Slow-wave sleep (SWS), and REM sleep and with bilateral guide tubes for PPT microinjection. Five hr. free-moving polygraphic recordings were made following each of 3 discrete PPT microinjections (n_i) of: control saline (n_i=10, 0.25 µl); NO donor, S-Nitroso-N-acetylpenicillamine (SNAP, n_i=10, 3mM); competitive inhibitor of NO synthase enz., N^G-Nitro-L-arginine methylester hydrochloride (L-NAME, n_i=5, 10 mM). Following PPT microinjection of SNAP, SWS and REM sleep was increased by 50% and 67% respectively compared to the control microinjection. Microinjection of L-NAME reduced SWS and REM sleep by 32% and 58% respectively compared to control microinjection. The present result demonstrates that endogenous NO synthesized within the PPT cells functions as a paracrine signal in the control of behavioral states of sleep and waking by modulating local cholinergic cells. [Supported by grants from NIH, NS 34004-01A1 NEUA and William F. Milton award from Harvard Medical School to S. Datta].

530.16

DETECTION OF, AND SCOPOLAMINE REVERSAL OF, LOW-DOSE PHYSOSTIGMINE-INDUCED INCREASES IN FORELIMB TREMOR IN THE RAT. J.A. Stanford* and S.C. Fowler. Dept. of Human Development, Univ. of Kansas, Lawrence, KS 66045.

The effects of the cholinesterase inhibitor physostigmine (<= 0.1 mg/kg) and the antimuscarinic scopolamine (<=0.2 mg/kg) were assessed separately and in combination in rats trained to use a single forelimb to exert 5-s bouts of continuous pressure on a force-sensing operandum. Power spectrum analysis of the resulting force-time recordings indicated that 0.1 mg/kg physostigmine substantially increased power in the 10-25 Hz frequency band (i.e., increased tremor), and this effect was completely reversed by 0.1 mg/kg of scopolamine HCl. Neither drug nor their combination affected power in the lower 5-9.9 Hz frequency band. The peak force observed at the beginning of each 5-s "response" was increased by physostigmine, decreased by scopolamine, and returned to vehicle levels when the drug treatments were combined. At doses low enough to permit the expression of operant behavior, cholinergic mechanisms adequately account for physostigmine's tremorogenic effects. Supported by MH43429.

530.17

UNIQUE BEHAVIORIAL EFFECTS OF A METABOLITE OF FELBAMATE; GLYCINE-LIKE? VL Coffin,* M Cohen-Williams, ME Grzelak & RD Smith. *Schering-Plough Research Institute. Kenilworth NJ 07033.*

Felbamate (2-phenyl-1,3-propanediol dicarbamate) is a novel antiepileptic with a unique structure and mechanism of action, possibly involving glycine sites at the N-methyl-d-aspartate (NMDA) receptor complex. A monocarbamate metabolite of felbamate (SCH 54388), was compared to felbamate in tests of pharmacological activity. The anticonvulsive effect of these two compounds was studied in three rodent models (CF-1 mice): NMDA-induced, electroshock (ECS)-induced, and pentylenetetrazol (PTZ)-induced convulsions. SCH 54388 weakly prevented NMDA-induced convulsions (0.5nmol/mouse) and ECS-induced convulsions (50 mA, 60 HZ, 0.2s through corneal electrodes) at 74 and 173 mg/kg-sc, respectively. These doses were 4 to 5-fold larger than effective doses of felbamate in the same models. In contrast, SCH 54388 potently prevented tonic convulsions produced by 100 mg/kg of PTZ, with an ED50 of 1.0 mg/kg-sc. Felbamate was 100-fold less potent in this test. Glycine did not block the effects of SCH 54388 in any of the models and was itself active in the PTZ-induced seizure model. Furthermore, SCH 54388 (0.01-10 mg/kg-sc, po) was able to prevent cognitive deficits produced with either scopolamine or MK-801, a non-competitive NMDA channel-blocker, on the mouse passive-avoidance response.

530.18

THE CRITICAL ROLE OF NUCLEUS ACCUMBENS IN THE MEDIATION OF FIXED-INTERVAL SCHEDULE-CONTROLLED BEHAVIOR. D.A. Cory-Slechta*, R. Pazmino, M.J. Pokora and C. Bare. Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Low-level lead exposure selectively alters nucleus accumbens (NAC) dopamine (DA) binding and reliably modifies fixed-interval (FI) schedule-controlled behavior. To determine whether any relationship existed between these behavioral and binding effects of Pb, the role of NAC DA systems in mediating FI performance was assessed in noraml rats during daily 30 min sessions. Rats were first trained on a FI 1 min schedule of food reinforcement until behavior stabilized. At that point, the comparative effects of the irreversible DA antagonist EEDQ (90-150 ug total dose in a volume of 0.25 ul/side) microinjected bilaterally into either dorsal striatum (DS) or NAC on FI performance was examined in groups of 9 rats each. EEDQ microinjections into NAC produced dose-related decreases in FI response rates to levels of 40-70% of control over the first 1-2 subsequent sessions. Rates gradually recovered over 4-6 sessions. EEDQ microinjections into DS had no impact on FI performance. Sparing either D1 or D2 receptors by treatment with either the D1 antagonist SCH23390 (0.15 mg/kg) or the D2 antagonist eticlopride (0.1 mg/kg), respectively, prior to EEDQ microinjection into NAC restored FI response rates, and activation of D1 receptors alone (SCH23390 co-treatment with EEDQ) actually increased FI response rates above control levels. These data demonstrate that NAC DA function is critical for the mediation of normal FI performance. The increased FI rates when only D1 NAC receptors are activated could reflect loss of D2 presynaptic control over DA synthesis and release. ES05017, ES05903 and ES01247.

530.19

NOREPINEPHRINE ALTERS THE CENTRALLY CONTROLLED RATE AND PERIPHERALLY CONTROLLED WAVEFORM OF THE ELECTRIC SIGNAL IN A WEAKLY DISCHARGING ELECTRIC FISH. C. Lin, S. G. Gupta, L. J. Perrotti and R. E. Landsman*. Academy for the Advancement of Science and Technology, Lab. of Behavioral Ichthyology, 200 Hackensack Ave., Hackensack, NJ 07601.

This study investigated the effects of norepinephrine (NE) on the electric organ discharge (EOD) in the weakly discharging electric fish, *Brienomyrus brachyistius*. Characteristics of this EOD are both centrally- and peripherally-controlled. Fish received either a NE or a vehicle control injection, or served as nonhandled controls. For each fish, both EOD rate data, taken every 5 seconds for 3 minutes, and one EOD were collected pre-treatment, post-treatment, 15 minutes post-treatment, and 30 minutes post-treatment. Pre- and post-treatment comparisons were made for both mean EOD rates and phase durations (expressed as % of pre-treatment). Two-way ANOVAs with repeated measures on one factor indicated significant condition x treatment period interactions for EOD rate [$F(6,81) = 9.54$, $p < 0.0000005$], durations of phases 2 [$F(6,81) = 9.07$, $p < 0.000005$] and 3 [$F(6,81) = 2.79$, $p = 0.016$], and for total EOD duration [$F(6,81) = 3.48$, $p = 0.004$]. Tests for simple main effects followed by multiple comparisons indicated that EOD rate decreased directly following the NE injection, differing from both controls, and remained at this level through 30 minutes post-treatment. The durations of phases 2 and 3 increased 15 minutes after the NE injection, differing from both controls, where they remained through 30 minutes post-treatment. Total EOD duration significantly increased by 30 minutes post-NE injection, differing from the vehicle control. No significant NE effects were found on the durations of phases 1 or 4. Neither EOD rate, nor EOD phase or total durations changed in the vehicle or nonhandled control fish over the study period. NE-induced changes in the centrally controlled EOD rate and the peripherally controlled EOD shape suggest that the EOD could serve as a model for the study of the simultaneous effects of neurotransmitters on peripherally- and centrally-mediated behavior.

Funded by the Bergen County Technical Schools' Board of Education.

530.20

FLUORIMETRY AND UNIFORM ROBOT-MADE CORTICAL PYRAMIDAL CULTURES MONITOR NEUROTRANSMITTER RECEPTOR MODULATION BY PHARMACEUTICAL AGENTS, L.A. Riblet*, B.D.Stein, R.M. Villalba and A.H. Cornell-Bell. Viatech Imaging, Ivoryton, CT 06442.

Primary cortical cultures (>85% neurons) are constructed in 96 well Primaria plates (Falcon) using a fluid-handling robot (Tomtec Quadra 96). Neurofilament, GFAP and myelin basic protein and total DNA levels (SYTO-17, Molecular Probes) are measured on a CytoFluorII fluorimeter (PerSeptive Biosystems) to determine % of neurons, astrocyte and oligodendrocytes. DNA levels vary little from well to well (mean= 3.9% difference with S.E.= 0.89). Cell viability is monitored (Live Cell/Dead Cell). All dye loading and agonist or antagonist additions are made by robot. The Ca^{2+} dye Fluo3AM has been used to monitor receptor activation following robot addition of glutamate, GABA, ACh, norepinephrine, dopamine, 5HT, neuropeptides (VIP, NPY, and Substance P) and growth factors (IGF-1, NGF and EGF) . Fluorescence is read pre and post agonist addition. $Ca^{2+}{}_{in}$ measurements are determined by a macro (Excel5.0) which determines %change in fluorescence (deltaF/F). This technology is sensitive enough to monitor effects of antagonists (MK801 and CNQX) on glutamate receptor subtypes (t-ACPD, kainate, AMPA and NMDA; all 100uM) at time courses extending from 15 sec to 3.0 min. $Ca^{2+}{}_{in}$ following KCl (1-50mM KCl) has been inhibited using Nimodipine (Bayer Pharmaceuticals) demonstrating the sensitivity of this system. Membrane potential changes (di-8 Anepps) following KCl depolarization and pH changes (BCECF) in response to extracellular matrix interactions have also been determined. Pharmaceutical effects on receptor activity in **primary** cortical cultures can be screened. These methods are sensitive enough to monitor second messenger signalling.

Viatech Imaging, LLC

531.1

DOWN REGULATION OF α_2-ADRENERGIC RECEPTORS BY HYPOXIA.

N.R.Prabhakar*, D.Kline, P.Ernsberger S.Bibevski, and J.R.Romaniuk, Dept. of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH 44106.

Chronic hypoxia has long term physiological consequences and adaptations. In the present study, we examined the effects of prolonged hypoxia on α_2-adrenergic receptors. Experiments were performed on rats exposed either to normoxia (n = 12) or to 24 hr. of hypobaric hypoxia (0.4 ATM; n = 12). Following which they were anaesthetized, artificially ventilated and bilaterally vagotomized. Arterial blood pressure, efferent phrenic and cervical sympathetic nerve activities were recorded. Systemic administration of guanabenz, an α_2-adrenergic receptor agonist inhibited respiratory rate in a dose-dependent manner with maximal responses seen at 60 and 100 µg (37 ± 8.4 % of control) without affecting the amplitude of the tidal phrenic nerve activity. Sympathetic nerve activity was augmented by about 40% at doses of guanabenz that depressed breathing. The effects of guanabenz appear to be of central nervous origin as they were unaffected by denervation of the carotid bodies. In animals exposed to 24 hr of hypobaric hypoxia, guanabenz-induced respiratory inhibition and sympathetic excitation were either markedly attenuated or abolished. [^{125} I] p - iodoclonidine binding analysis of rat brain membranes showed significant decreases (50 %) in α_2-adrenergic receptor density in the dorsal but not in ventral pons-medulla of animals exposed to hypoxia. These results demonstrate that hypobaric hypoxia down regulates α_2-adrenergic receptors in the brain stem in a regional specific manner and attenuates or abolishes the physiological responses to α_2-adrenergic receptor agonist. Supported by NIH HL-45780 and HL-02599.

531.2

REGULATION OF α_{2A}-ADRENERGIC RECEPTOR MRNA GENE EXPRESSION BY FORSKOLIN AND PHORBOL ESTER.

M. A. Reutter*, E. M. Richards, and C. Sumners. Dept. of Physiology, College of Medicine, Univ. of Florida, Gainesville, FL 32610.

Treatment of rat astroglial cultures with an adenylyl cyclase activator forskolin (FSK) or a PKC agonist phorbol 12-myristate 13-acetate (PMA) resulted in time- and concentration-dependent decreases in steady state levels of α_{2A}-adrenergic receptor (α_{2A}-AR) mRNA. Membrane permeable analogues of cAMP induced decreases in α_{2A}-AR mRNA to levels similar to those seen with FSK treatment (to 10-20% of control levels), indicating that the FSK-mediated decrease was due to increases in intracellular cAMP concentration. Analysis of cAMP content confirmed that FSK increased the cAMP content in these cultures of rat astroglia. Activation of PKC with phorbol 12, 13-dibutyrate or mezerein produced decreases in α_{2A}-AR mRNA levels similar to those seen with PMA, indicating that PKC also regulates this message. The degradation rate of the α_{2A}-AR mRNA ($t_{1/2} \approx 2$ hr) did not change with FSK or PMA treatment, suggesting that the decreased steady-state levels observed were due to decreases in transcription of the α_{2A}-AR gene. (Supported by NIH grant NS-19441 to CS and an AHA-FL Affiliate graduate student fellowship to MAR).

531.3

CHRONIC IMIPRAMINE TREATMENT MODULATES IMIDAZOLINE RECEPTORS IN RAT BRAINSTEM.

H. Zhu, I. Paul, A. Halaris, J. E. Piletz*. Dept. of Psychiatry and Human Behavior, Univ. of Mississippi Medical Center, Jackson, MS 39216

Previous studies have suggested that α_2-adrenoceptors as well as nonadrenergic imidazoline receptors (subtypes IR_1 and IR_2) are linked to depression. In this study we have measured α_2 and I_1-imidazoline binding sites with [^{125}I]*p*-iodoclonidine and I_2-imidazoline binding sites with [^{3}H]-idazoxan in rat brainstem and assessed the effects of chronic imipramine treatment in vivo on these sites. The nonadrenergic IR_1 and IR_2 binding sites were assessed under a 10 µM NE mask of α_2-adrenoceptors. Compared with saline-treated controls, imipramine treatment (i.p.,20mg/kg/day for 25 days) had no effect on the density (Bmax) or affinity (K_D) of α_2-adrenoceptors in brainstem membranes. However, the treatment decreased significantly the density of brainstem I_1-binding sites with no change in radioligand affinity in a dose dependent manner (minimum effective dose, i.p., 10mg/kg/day). Furthermore, it was found that 25 days of imipramine treatment (i.p.,20mg/kg/day) could also lead to a significant enhancement in the density of IR_2-binding sites with no influence on the affinity. These results suggest that brainstem I_1-imidazoline receptors as well as I_2-imidazoline receptors might be targets for antidepressants. Supported by NIH grants MH49248 and MH42859.

531.4

ADRENALECTOMY DOES NOT PREVENT FLUOXETINE-INDUCED DESENSITIZATION OF 5-HT$_{1A}$ AUTORECEPTORS IN THE RAT DORSAL RAPHE NUCLEUS.

E. Le Poul, N. Laaris, M. Hamon and L. Lanfumey*. INSERM U288, CHU Pitié-Salpêtrière, 91 Bld de l'Hôpital, 75634 Paris cedex 13, France.

Electrophysiological studies have shown that somatodendritic serotonin 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus (DRN) desensitize upon their chronic stimulation by exogenous agonists or 5-HT (through the blockade of its reuptake by selective inhibitors such as fluoxetine) in rats. This functional desensitization might be due to an alteration of transduction mechanisms which involve Gi/Go proteins. In rats with elevated plasma corticosterone levels, expression of Gi has been found to be reduced as a result of chronic stimulation of glucocorticoid receptors (GR). Since 5-HT$_{1A}$ receptor activation leads to an increased secretion of corticosterone, we investigated whether this hormone, through its negative influence on Gi synthesis, could be responsible for the 5-HT$_{1A}$ autoreceptor desensitization in rats treated chronically with the 5-HT transporter blocker fluoxetine.

To this end, experiments were performed on mesencephalic slices from adrenalectomized (ADX) or sham-operated Sprague-Dawley male rats which had been treated with fluoxetine (5 mg/kg/day i.p.) or saline for 3 or 7 days. A single barrel micropipette (15 MΩ) filled with 2M NaCl was used for extracellular recording of 5-HT neurons in the DRN.

The concentration-dependent inhibition of the firing of 5-HT neurons by the direct 5-HT$_{1A}$ agonist ipsapirone was similar in ADX (IC_{50}=74 nM) and in sham-operated (IC_{50}=67 nM) rats which had been treated with saline for 3 or 7 days. Administration of fluoxetine for 7 days resulted in a shift to the right of the concentration-response curve of ipsapirone which was as pronounced in ADX (IC_{50}=226 nM, +205% as compared to saline, $P<0.05$) as in sham-operated rats (IC_{50}=196 nM, +193%, $P<0.05$). A significant desensitization of 5-HT$_{1A}$ autoreceptors was also found in both groups after only a 3-day treatment with fluoxetine. Combined treatment with the specific GR antagonist RU 38486 (25 mg/kg/day; s.c.) did not affect the fluoxetine-induced shift to the right of the inhibition curve of ipsapirone in sham-operated as well as in ADX rats.

Because the potency of ipsapirone to inhibit the firing of DRN 5-HT neurons was reduced by fluoxetine treatment to the same extent in the absence (ADX) or the presence (sham-operated rats) of corticosterone, it can be concluded that this hormone does not participate in the functional desensitization of 5-HT$_{1A}$ autoreceptors in rats chronically treated with 5-HT uptake blockers.

531.5

PERIPHERAL TARGETS INFLUENCE EFFICACY OF SYNAPTIC TRANSMISSISION BETWEEN PREGANGLIONIC AND SYMPATHETIC NEURONS

P Devay*, D S McGehee and L W Role. Dept of Anat and Cell Biol, Ctr for Neurobiol & Behav P&S, 722 W 168th St NY, NY 10032;

Sympathetic neurons innervate a variety of targets in vivo. Earlier studies showed that target contact influences the ACh responsiveness and the expession of nAChR subunit genes in embryonic chick autonomic neurons (Levey et al., Neuron 14: 153 1995; Devay et al., NS Abstr. 528.20,1995). Furthermore, distinct targets differentially alter the profile of nAChR subunit genes expressed by sympathetic neurons. Thus co-culture of sympathetic neurons with embryonic kidney increases the magnitude of ACh-evoked currents, while heart co-culture decreases the responses. The profile of nAChRs channel subtypes are also differentially regulated by these targets (Devay et al., NS Abstr. 528.20,1995). In view of these findings we examined the impact of these target on synaptic transmisson.

Naive sympathetic neurons were co-cultured with heart or kidney and innervated by preganglionic spinal cord explants. In control cultures, neurons were innervated by preganglionic neurons but no target was present. Spontaneous miniature EPSCs were recorded from sympathetic neurons and the expression level of nAChR subunits were examined in these neurons. While innervation alone increases the levels of α3, α5 and β4 mRNAs, input plus target greatly augments the levels of α5 mRNA relative to α3 and β4. Examination of sympathetic neurons innervated in the presence of target also reveals significant increases in the amplitude of the synaptic currents compared with neurons innervated without heart or kidney. These studies suggest that target contact influences the expression profile and/or distribution of nAChRs at sites of synaptic input on sympathetic neurons (supported by NS29071).

531.6

UPREGULATION OF α7 NICOTINIC ACETYLCHOLINE RECEPTORS IN RAT HIPPOCAMPAL CULTURES: PHARMACOLOGICAL CHARACTERISATION.

S.Wonnacott* and A.T.Rogers. Sch Biol Biochem, Univ Bath, Bath, BA2 7AY, U.K.

The α7 nicotinic acetylcholine receptor (nAChR) subunit, which has been correlated with [^{125}I]αBgt binding, is particularly abundant in the mammalian hippocampus. Chronic nicotine treatment *in vivo*, and exposure of hippocampal cultures *in vitro* to 10µM nicotine for 7 days leads to an upregulation of surface [^{125}I]αBgt binding sites (Barrantes *et al.*, Brain Res. **672**: 228-236, 1995).

To provide a comprehensive pharmacological characterisation of this response, high density primary cultures of E18 rat hippocampal neurons were treated with nicotinic agonists and antagonists for 7 days prior to [^{125}I]αBgt (10nM) binding assays (Kd ≈ 1nM). In the absence of drugs, cultures expressed 59.5±6.3(n=17) fmol [^{125}I]αBgt binding sites / mg protein. The agonists cytisine (20µM), nicotine (10µM), DMPP (10µM) and anatoxin-a (10µM) significantly increased the number of binding sites by 28±9%, 34±4%, 38±12%, and 61±8% respectively above control levels, whereas the muscarinic agonist muscarine had no significant effect. The antagonists methyllycaconitine (MLA;100nM) and d-tubocurarine (10µM) gave increases of 23±7% and 26±8% respectively, but the non-competitive antagonist mecamylamine (10µM) was without effect. Co-application of nicotine (10µM) and MLA (100nM) resulted in an increase of 23±4%, close to that seen with each drug alone. Depolarisation of the neurons with KCl (20mM) elevated [^{125}I]αBgt sites by 52±12%, while veratridine (1µM) had no effect.

These results suggest that occupancy of the binding site may trigger the upregulation response.The ability of KCl depolarisation to produce a similar response may signify the operation of Ca^{2+}-mediated mechanisms, as proposed by De Koninck & Cooper (J.Neurosci. **15**: 7966-7978, 1995) in cultured rat sympathetic neurons.

This study was supported financially by BAT Co. Ltd.

531.7

DISTRIBUTION OF GLYCINE RECEPTORS ON ABDUCENS MOTONEURONS AFTER BOTULINUM TOXIN BLOCKADE OF NEUROMUSCULAR TRANSMISSION. B. Moreno-López[§], R R de la Cruz*, A.M. Pastor, J. M. Delgado-García and F. J. Alvarez[§]. Lab. de Neurociencia, Facultad Biologia, 41012-Sevilla, Spain and (§) Dept. of Anatomy, Wright State University, Dayton, OH.

High doses of botulinum neurotoxin type A (BoNT) alter firing and synaptic properties of abducens motoneurons. Inhibitory synaptic potentials virtually disappear for at least 2 months (Soc. Neurosci. 21:749, 1995). We have analyzed the distribution of glycine receptors in cat abducens motoneurons after BoNT injection (3 ng/Kg) in the lateral rectus muscle. Gephyrin, a protein of the glycine receptor complex, was detected using immunofluorescence and electron microscopy immunohistochemistry in abducens motoneurons at 5, 19 and 35 days post-treatment. Motoneurons were identified by their immunoreactivity to calcitonin gene-related peptide or by the retrograde transport of horseradish peroxidase.

By immunofluorescence we found a significant reduction in the density of glycine receptor clusters by 19 and 35 days post-injection as compared to control values whereas by 5 days no significant change was observed. These data correlated well with the ultrastructural analysis of gephyrin-immunopositive postsynaptic densities, which also revealed a parallel reduction in the density of glycinergic presynaptic boutons. The distribution of glycine receptors over the somatic membrane surface was homogeneous in both control and treated motoneurons. Previous physiological results indicated that inhibitory synaptic transmission is abolished as soon as 3 days after BoNT injection. Therefore, physiological alterations were more pronounced and preceded structural changes affecting the number of postsynaptic receptors. The drop in the number of glycine receptors following BoNT blockade can be explained as a down-regulation related to the lack of trophic influence from the target or by a direct action of the toxin itself on the motoneuron cell body and/or its presynaptic inputs. (Supported by DGICYT PB-1175, NIH 33555 and NATO CRG 960221).

531.8

INCREASED DENSITY OF RENAL PERIPHERAL BENZODIAZEPINE RECEPTORS IN AUTOIMMUNE MICE. R. Drugan*[1], L. Schrott[2], D. Healy[1] and L. Crnic[3] 1) Dept. of Psychology, Univ. of New Hampshire, 2) Dept. of Pharmacology Univ. of Minnesota Medical School and 3) Depts. of Pediatrics and Psychiatry University of Colorado Medical School.

The female NZB X NZW F1 hybrid (B/W) mouse spontaneously develops a lupus-like autoimmune disorder. In comparison to non-autoimmune controls from the paternal progenitor NZW strain, 12 week old B/W female mice show increased anxiety-related behaviors in the elevated plus maze and decreased exploratory drive in the novel object task. The Peripheral Benzodiazepine Receptor (PBR) is altered in both acute and chronic states of anxiety or stress. We examined the PBR in several peripheral tissues in both B/W and NZW mice 8-12 days following behavioral testing. Importantly, previous work has demonstrated that stress-induced PBR changes return to baseline levels within 24 hours. All subjects were sacrificed by rapid decapitation and peripheral tissues were dissected on ice, fast-frozen and stored at -80°C until assay. In vitro radioligand binding of [3H]Ro5-4864 to PBR was conducted in lung, heart liver and kidney for both groups. We observed a significant and consistent increase in renal PBR in B/W mice in comparison to the NZW controls. This effect was confirmed by Scatchard analysis indicating an increase in density (Bmax) and not affinity (Kd). No other differences were observed. Research supported by NIMH grants 45475,10643 and 49043.

531.9

$GABA_A$ RECEPTOR SUBUNIT CHANGES IN THE INFERIOR COLLICULUS MAY RESULT IN ENHANCED RECEPTOR FUNCTION IN AGED RATS. J.C. Milbrandt*, T. Blomquist, and D.M. Caspary. Dept. of Pharmacology, SIU School of Medicine, Springfield, IL 62702.

γ-aminobutyric acid (GABA) acting through $GABA_A$ receptors is critically involved in processing acoustic information in the inferior colliculus (IC). The $GABA_A$ receptor is thought to exist as a pentameric complex composed of an unknown combination of at least 13 possible subunits. The IC contains high concentrations of GABA and high levels of $GABA_A$ receptor binding, mRNA, and subunit protein. It appears that presynaptic markers for GABA neurotransmission are reduced in the IC of aged Fischer 344 (F344) rats (Caspary et. al., 1995). The present study attempts to compare $GABA_A$ receptor subunit mRNA levels to subunit protein levels in the IC of F344 rats: 3-, 18-, and 26-months of age.

Using *in situ* hybridization, no significant age-related changes were observed in the mRNA hybridization levels of the most common (α_1, β_2, γ_2) $GABA_A$ receptor subunits in the IC. However, significant age-related increases in the α_2 and γ_1 receptor subunit hybridization levels were detected in the IC. Follow-up Western blots indicated no significant changes in the α_1, β_2, and γ_2 subunit proteins, but a *significant* age-related increase in the γ_1 subunit protein. Western blot results were similar in two rat strains, the F344 and F344/Brown Norway F1 hybrid.

Previous studies suggest that the α_2 and γ_1 subunits, when assembled into a $GABA_A$ complex, can produce receptors with greater sensitivity to GABA (Levitan et al., 1988; Puia et al., 1991; Ducic et. al., 1995). These findings are consistent with an age-related increase in the ability of GABA to modulate binding at the picrotoxin site (Milbrandt et. al., 1996). In addition, GABA evoked Cl^- flux in age rats maybe higher than in young adults (unpublished). Taken together, an age-related increase in the α_2 and γ_1 subunits may represent a compensatory upregulation in order to increase the sensitivity of IC $GABA_A$ receptors to GABA, and therefore maintain functional levels of inhibition in aged rats.(Supported by NIH DC 00151)

531.10

VARIATIONS IN ENVIRONMENTAL LIGHT/DARK CYCLE INDUCED CHANGES IN GLUTAMATE RECEPTOR EXPRESSION IN THE RAT HIPPOCAMPUS. P. Nagappan, T. Morcol, J.W. Patrickson, G. Small, K.H. Thomas, and J.A. Whittaker. Department of Anatomy, Morehouse school of Medicine, Atlanta, GA 30310.

In this study, we investigated the effects of environmental light variations on the expression of glutamate GluR1-4 receptor subtypes in the rat hippocampus. Glutamate is known to play key roles in learning and memory, behavioral functions, and in the entrainment of the hypothalamic suprachiasmatic nucleus to environmental light/dark cycles. In addition, the loss of hippocampal neurons in Alzheimer's disease and in CNS ischemic insults have been correlated with excessive glutamate release, resulting in cell death or altered excitatory amino acid (EAA) receptor sensitivities. The mechanisms underlying changes in EAA receptor functions remain unclear however, and it is not known whether EAA regulation follows a circadian pattern. Adult male Sprague-Dawley rats were entrained to constant light and constant dark conditions for 24 and 48 hour periods. Rats housed under 12 h light/12 h dark conditions were used as controls. Soluble membrane proteins were prepared from hippocampal tissue extracts and SDS-PAGE and Western blot analysis were performed to evaluate changes in total protein and glutamate receptor subtype expression. Although no significant change in total protein concentration was observed at constant dark conditions, exposure to constant light resulted in 1.5 to 2 fold increases in hippocampal total protein expression within 48 hours. Exposure to constant light (24 h and 48 h) induced a noticeable decrease in GluR1 receptor subtype expression, whereas an approximate 2-fold increase was observed in 48- hours of constant dark exposure. No detectable signal could be obtained for the GluR4 or GluR2/3 receptor subtypes. Immunohistochemical assays confirmed data obtained from Western blot analysis. All results will be discussed relative to hippocampal function. Support: NIH Grants GM08248, RR03034 and NASA Grant NCCW0083.

531.11

EFFECTS OF THE LIGHT/DARK VARIATIONS ON GLUTAMATE RECEPTOR UP-REGULATION IN THE RAT CEREBELLUM. T. Morcol, J.W. Patrickson, J. Springfield, K.H. Thomas, and J.A. Whittaker*. Department of Anatomy, Morehouse school of Medicine, Atlanta, GA 30310.

We have previously shown that certain receptor subtypes in the rat basal ganglia varies with environmental light/dark cycles or circadian rhythms. In this study, we examined the effects of variations in light/dark cycle on total protein expression and on the expression levels of glutamate receptor subtypes in the rat cerebellum. Functionally, the cerebellum is import in the coordination of movement, posture, and balance. This study was designed to determine a possible role for light induced glutamate receptor modulation of cerebellar output activity. Adult male Sprague-Dawley rats were entrained to constant light and constant dark conditions for 24 and 48 hour periods. Cerebellar tissue extracts were prepared and integral membrane proteins were solubilized for total protein determination. Digitonin solubilized receptors were fractionated to evaluate changes in glutamate receptor subtype expression using SDS-PAGE and Western blotting techniques. Our findings indicate that exposure to 48 hour constant light significantly increased total protein expression (approximately 2.5 fold) in soluble cerebellar membrane preparations. Glutamate receptor subtypes GluR1 and GluR4 were also found to be noticeably increased following 48 hours of constant light exposures. Whether these AMPA type glutamate receptors are expressed as individual subtypes or co-expressed combination of GluR1-GluR4 subunits is still undetermined. No significant changes in GluR1 or GluR4 expression levels were observed in the cerebellum under constant dark conditions and no detectable signal was observed for the GluR2/3 subtype. Immunohistochemical analysis of cerebellar tissues exposed to varying light/dark conditions demonstrated results consistent with the Western blot data and may suggest possible light-induced receptor modulation of cerebellar functions.. Support: NIH Grants GM08248, RR03034, and NASA Grant NCCW0083.

531.12

PROTON POTENTIATION OF ATP-ACTIVATED INWARD CURRENT IN SENSORY NEURONS. Chaoying Li*, Robert W. Peoples and Forrest F. Weight. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse & Alcoholism, National Institutes of Health, Bethesda, MD 20892-8205.

We studied the modulation of ATP-gated ion channels by protons in rat nodose and bullfrog dorsal root ganglion (DGR) neurons using the whole-cell patch-clamp technique. Both in nodose and DRG neurons, reduced external pH enhanced, whereas elevated external pH suppressed, the ATP-activated current. The pH producing the half-maximal effect (EC_{50}) was 7.1 - 7.2. Proton potentiation of ATP-activated current was voltage-insensitive, and did not involve a shift in reversal potential. Lowering pH shifted the ATP concentration-response curve to the left, decreasing the EC_{50} for ATP, but did not alter the maximal response to ATP. Protons decreased the rate of deactivation without affecting the rate of activation of ATP-gated ion channels, which is consistent with an increase in the affinity of ATP for its receptor rather than an increase in the concentration of one or more active forms of ATP. The time constant for desensitization of ATP-activated current was unchanged by acidic pH. Proton potentiation of ATP-activated current appears to be independent of intracellular pH. Treatment of neurons with diethylpyrocarbonate, dithiothreitol, 5,5'-dithio-bis-(2-nitro-benzoic acid), or *N*-ethylmaleimide did not affect proton potentiation of ATP-activated current. Our results suggest that extracellular protons, at physiological concentrations, could regulate the function of P_{2X} purinoceptors by modulating the affinity of the binding site for ATP on these ATP-gated receptor-channels.

This work was supported by NIAAA, NIH.

532.1

THE ASTROGLIAL RESPONSE TO PSYCHOLOGICAL AND INFLAMMATORY STRESSORS IN TRANSGENIC ANIMALS EXPRESSING TYPE II GLUCOCORTICOID RECEPTOR (GR) ANTI-SENSE RNA. [1,2]B. Marchetti*, [1]F. Gallo, [3]R. Avola, [4]G.P. Leanza, [1]Z. Farinella, [2]C. Tirolo, [2]N. Testa, and [5]N. Barden. Depts.[1]Pharmacol. [3]Biochem. and [4]Physiol., Catania Univ., [2]OASI Institute (IRCCS), Troina (EN), Italy, and [5]Mol. Psychogen., CHUL, Quebec, Canada.

Transgenic animals with impaired type II glucocorticoid receptor (GR) function display a number of neuroendocrine, immune and behavioral abnormalities including a. increased hypothalamus-pituitary-adrenal (HPA) axis activity; b. hyper-responsiveness of the T-helper cell compartment; and c. deficits in tests of anxiety and cognitive performance. Neurons of the hippocampal formation are known to be selectively vulnerable to certain stresses, and this may be the cause of impaired cognitive behavior. Astrocytes have been found to express mRNA and protein for a number of defined neurotrophic factors such as members of the neurotrophin family, pleiotropic factors such as bFGF, as well as a number of cytokines, suggesting their potential role in neuronal survival/protection from cellular insults. In the present study we have attempted to assess the astroglial response to a number of stressful situations in transgenic mice expressing GR anti-sense RNA. For this aim, we have monitored alterations in glial fibrillary acidic protein (GFAP) and proliferative state, as markers of astroglia reactivity, basic fibroblast growth factor (bFGF)-like immunoreactivity and astroglial cytokine expression in response to different stresses, lipopolysaccharide (LPS), or dexamethasone (DEX) administration. Proliferation of the astroglial compartment was significantly stimulated in transgenic mice. While resistant to the effects of DEX, astroglial cells responded to LPS with a further stimulation of astroglial proliferative activity. On the other hand, the glucocorticoid feedback on astroglia cytokine expression and bFGF-like immunoreactivity was inefficient in transgenic mice. Further studies on alterations in different neurotrophins and their receptors are in progress to clarify whether a defective glucocorticoid feedback might alter the astroglial neurotrophic factor response to neuronal insults resulting in increased neuronal vulnerability.

532.2

ENDOGENOUS CORTICOSTERONE DOES NOT INHIBIT NOCICEPTIVE RESPONSES IN THE FORMALIN TEST. B.K.Taylor, S.F.Akana*, M.A.Peterson, and A.I.Basbaum. Departments of Anatomy and Physiology and the W.M. Keck Foundation Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143-0452.

Hindpaw injection of dilute formalin produces acute and persistent nociceptive responses. Since physiological stress can significantly influence pain, we evaluated formalin-evoked nociceptive behaviors, cardiovascular responses, inflammation, and increases in plasma ACTH and corticosterone concentrations in adult, male Sprague-Dawley rats. After overnight acclimation to the test environment, we collected multiple 140µl blood samples via chronic carotid arterial catheters both before and after the injection of saline, 1.5% formalin, or 5.0% formalin. In a concentration-dependent manner, formalin produced flinching and licking behavior, increased paw thickness, and increased ACTH and corticosterone. Although we did not detect distinct acute and persistent phases of HPA activity, these results indicate that nociceptive input activates the HPA axis.

We next evaluated the contribution of formalin-evoked endogenous corticosterone release to the magnitude of nociceptive responses. All animals were instrumented with chronic femoral arterial catheters and then either sham-operated or bilaterally-adrenalectomized (ADX). Corticosterone (25 ug/ml) was supplied in the drinking water of the ADX group. Although ADX produced subtle decreases in licking behavior and formalin-evoked heart rate responses, it did not change flinching behavior, blood pressure responses, or the increase in paw thickness. We conclude that corticosterone released during the formalin test does not produce a strong feedback inhibition of nociceptive responses. Supported by NS21445, NS 14627 and DK 28172, and thanks to Mary Dallman for helpful comments.

532.3

SPATIAL AND TEMPORAL STUDIES OF IMMOBILIZATION-INDUCED CELLULAR ACTIVATION IN STRESS-RELATED NEURONS: ANOTHER NEUROGENIC STRESS MODEL. H.-Y. Li* and C.-W. W. Department of Anatomy, Chang Gung College of Medicine and Technology, Tao-Yuan, Taiwan, R.O.C.

Previous findings suggest that the responses of hypothalamic neurosecretory neurons to neurogenic stress and systemic stress are mediated by differential driving mechanisms (Li, et al., PNAS, 93:2359-2364, 1996). To test the commonality among neurogenic stress models, acute immobilization stress was used to identify the pattern of cellular activation. Animals were immobilized by fixing rats on their backs against a board and securing each limb with tape for 2 hours, and were sacrificed at different time points after stress. Maximal Fos expression was detected at 1-2 hr and disappeared by 4 hr after stress. Cell groups displaying Fos-ir included the parvocellular division of paraventricular nucleus of hypothalamus (PVH), which is rich in CRF-containing neurons. Double immunohistochemical labeling confirmed that the scattered Fos positive neurons in the surrounding of magnocellular PVH and dorsal edge of the supraoptic nucleus were oxytocinergic. Widespread Fos expression was also found in the limbic areas, including ventral lateral septum, bed nucleus of stria terminalis, and medial nucleus of amygdala. It is of interest to note the prominent Fos staining in the horizontal limb of diagonal band, which is suggested to be associated with serotonin-containing neurons in the raphe nuclei, where nociceptive information is relayed. In the brainstem, Fos expression was located in areas involved in processing somatosensory/nociceptive information, such as periaqueductal gray, raphe nuclei, and the dorsal column nuclei. Similar to footshock and restraint stresses, immobilization elicits Fos expression in the catecholaminergic neurons in the nucleus of the solitary tract and ventrolateral medulla. These findings indicate a similar pattern of cellular activation among immobilization and other neurogenic stress models. It remains to be determined the role of activated extrahypothalamic regions in conveying immobilization stress information to trigger neuroendocrine responses. (Supported by CMRP 587)

532.4

DIFFERENTIAL TEMPORAL AND SPATIAL ACTIVATION OF VISCERO-MOTOR CELL TYPES OF THE PARAVENTRICULAR NUCLEUS IN RESPONSE TO ACUTE AND REPEATED RESTRAINT. V. Viau* and P.E. Sawchenko. The Salk Institute, La Jolla CA 92037.

Habituation of HPA responses with repeated exposure to homotypic stress is well-documented. To explore the central bases for this effect, we compared the time courses of cellular activation seen in visceromotor compartments of the paraventricular hypothalamic nucleus (PVH) of rats subjected to a single 30 min restraint session or 2 weeks of repeated daily exposure. Acute stress-induced Fos-ir in the CRF-rich part of the hypophysiotropic zone, autonomic-related subdivisions, and in oxytocinergic aspects of the magnocellular system were all prominent in animals sacrificed at the termination of restraint (0 hr), peaked at 2 hr after stress, and abated to control levels by 3-4 hr. An additional cell group occupying the most medial aspects of the PVH and extending into the anterior periventricular nucleus displayed a Fos response that peaked at 0.5 hr post-stress and was diminished by 1 hr. The most marked departures from this pattern in repeatedly stressed rats were highly preferential attenuations of Fos-ir in the CRF-rich and periventricular aspects of the PVH. The repeated stress group also displayed a paradoxical recruitment of supraoptic vasopressin neurons to Fos expression at 1-2 hr post-stress. Finally, we also tracked the time course of CRF and AVP hnRNA within the parvocellular and magnocellular divisions of the PVH, respectively. In response to acute restraint, both primary transcripts peaked at 0 hr and reattained control levels by 0.5 hr post-stress. While the magnocellular AVP hnRNA response was blunted following repeated restraint, the elevation of the parvocellular CRF intronic response was enhanced and prolonged. Visceromotor compartments of the PVH are activated with distinct time courses following acute stress, and these compartments are differentially affected by repeated exposure. Early Fos-ir and hnRNA responses indicate that such recruitment is initiated during stress.
(supported by NS-21182)

532.5

ELEVATED FAT INTAKE DURING LACTATION INCREASES STRESS RESPONSIVENESS OF PREPUBERTAL RAT OFFSPRING. G. Trottier*[1], K. Koski[2], and C.-D. Walker[1]. Douglas Hospital Research Center, Depts of Psychiatry[1] and Nutrition[2], McGill University, Montreal, PQ H4H 1R3, Canada.

High fat diet and elevated plasma levels of free fatty acids are known to increase ACTH response to stress in adult rats. We investigated whether changes in fat intake during nursing could have prolonged effects on stress responsiveness in the offspring. Lactating females were fed either a control (C: 5% fat, 60% CHO), high fat (HF: 20% fat, 53% CHO) or low carbohydrate (LC: 30% fat, 24% CHO) diet during the last week of gestation and throughout lactation. Lipid, protein and CHO content of the milk was measured at different stages of lactation. After weaning, pups from the various maternal diets were kept on the C diet for 2 weeks and tested for their ACTH and corticosterone (B) response to 15 min swim stress on day 35 of age. Maternal energy intake and pup weight gain were not altered by the diets during the first 21 days of nursing although total milk lipid content was significantly increased in the HF and LC compared to C group. On day 35, after 2 weeks of normal diet, the magnitude and duration of the ACTH response to stress were significantly higher in pups from the HF compared to the C diet. Basal or stress-induced B secretion was similar between diet groups. Fat pad weight was higher in 21 day-old pups from HF and LC than C diet, but these differences were abolished by day 35. These results show that changes in maternal diet during the nursing period could have profound and prolonged effects on stress responsiveness in the offspring. These effects persist at least 2 weeks after weaning and return to a normal diet. Alterations in fat supply in the milk might produce long-term changes in energy distribution, liver function and food preference in pups, thereby influencing adrenocortical regulation. (Supported by NSERC, Canada).

532.6

REDUCTION IN NORADRENERGIC POTENTIATION OF THE STRESS RESPONSE DURING LACTATION IN THE RAT IS ASSOCIATED WITH CHANGES IN ADRENERGIC α-2 RECEPTOR DENSITY IN THE PARAVENTRICULAR NUCLEUS (PVN). D.J.Toufexis* and C-D Walker. Douglas Hospital Research center, Dept of Neurology and Neurosurgery, McGill University, Montreal, PQ H4H 1R3, Canada.

During lactation, tonically elevated glucocorticoid secretion and a blunted ACTH response to stressors are observed in the rat. The mechanisms underlying lactation-induced stress hyporesponsiveness remain largely unknown. In these studies we first tested the role of noradrenergic (NA) afferents to the PVN in mediating stress hyporesponsiveness. Virgin and lactating females on day 2 of lactation, received either sham- or 6-hydroxydopamine (6OH-DA) lesions over the PVN. Specific dopamine and serotonin reuptake blockers were given prior to lesioning to spare non-NA inputs. Nine days later plasma ACTH and corticosterone (B) responses to swim stress were significantly attenuated in lesioned compared to sham virgin females ($p<0.05$). In lactating females 6OH-DA lesions did not affect ACTH or B responses to stress. Next changes in α-1 and α-2 adrenergic receptor (AR) density were measured in virgins and throughout lactation (Days 3, 12, 21) by autoradiography using 3H-prasozin or 3H-rauwolscine as specific ligands. The density of α-2 AR binding declined in the the PVN and in the CA1 and dentate gyrus (DG) of the hippocampus over the course of lactation, while the density of the α-1 AR binding decreased only in the DG. These results indicate that 1) brainstem NA inputs to the PVN act to potentiate ACTH stress response in virgin females, while in lactating rats this potentiation is absent and 2) changes in the level of α-2 adrenergic receptors might be involved in modulating the HPA axis during lactation. (Supported by MRC Canada).

532.7

PRENATAL PSYCHOLOGICAL STRESS IN THE CONGENITAL LEARNED HELPLESS RAT(cLH): BEHAVIORAL AND NEUROENDOCRINE CORRELATES S. Shukla and E. Edwards. Department of Pharmaceutical Sciences, University of Maryland, Baltimore MD 21201

This study examined the effects produced by **maternal restraint stress during pregnancy** on behavioral indices of emotionality and on measures of hypothalamic-pituitary-adrenal axis (HPA) physiology. The congenital learned helpless rat (cLH), a genetic model of depression, allowed the assessment of interaction between environmental influences aand genetic susceptibility. In an open-field paradigm, cLH rats stressed in utero exhibited significant increases in activity at day 14, 21, 45 and 90 (day 14, 55 %↑; day 21, 45 & 90, 37%↑), as compared to the cLH no stress group. In a hot plate test, the latency to hindpaw withdrawal was significantly increased in the cLH rats stressed in utero (day 14 & 21, 50 %↑; day 45 and 90, 30 %↑). At weaning, cLH rats tested in the spontaneous alternation paradigm displayed an increase in vacillatory behavior (decision making choice) but they also exhibited higher alternation patterns than the no stress control. However, these differences were not evident at day 45 and day 90. Adrenocorticotropin hormone (ACTH) and Corticosterone (CORT) plasma levels were monitored at day 7, 14, 21, 45 and 90 in both cLH stressed in utero and no stress cLH controls. In response to prenatal stress, baseline levels of ACTH and CORT were elevated 60% and 100 % respectively early in development (day 7 and day 14 only). At day 21, 45 and 90, there was no difference in basal levels of ACTH and CORT in the stress in utero group and the no stress control. Our results suggest that exposure to prenatal stress in the cLH rat has long term consequences such as a dampened emotionality profile. The altered functionality of the HPA axis was only evident in the early stages of development and the hypersensitivity of the system may contribute to the demonstrated blunted response to various stressors in the adult cLH rat. Supported by School of Pharmacy DRIF Grant

532.8

POSTNATAL HANDLING/MATERNAL SEPARATION ALTERS RESPONSES TO NOVELTY STRESS, OPEN FIELD EXPLORATION AND CENTRAL BENZODIAZEPINE RECEPTOR LEVELS IN ADULT RATS. C. Caldji[1]*, S. Sharma[1], P.M. Plotsky[2] and M.J. Meaney[1]. [1]McGill Univ., Douglas Hosp. Res. Ctr., Montreal, Canada, H4H 1R3. [2]Stress Neurobiology Lab., Dept. Of Psychiatry, Emory Univ., Atlanta, GA 30322.

Postnatal handling (H) results in reduced novelty induced fearfulness and increased forebrain 3H flunitrazepam binding compared to non-handled (NH) rats (Bodnoff et al., 1987, European J. Pharmacol. 144: 105-107). In the current study, H, NH and rats maternally separated for 180 minutes per day for the first 3 weeks of life (MS) were food deprived for 24 hours and placed in a novel environment with food. H rats showed a significantly reduced novelty induced suppression to feeding as compared to NH rats. In addition, MS rats made significantly less visits to the food as compared to H and NH rats . In a novel open field environment, H rats spent significantly greater time exploring this environment as compared to both MS and NH rats . 3H flunitrazepam binding autoradiography revealed that H rats had significantly greater levels of central benzodiazepine (CBZ) receptors in the lateral nucleus of the amygdala , the locus coeruleus (LC) and both C1-C2 adrenergic cell bodies as compared to MS rats. In addition, H rats also had significantly greater CBZ levels in the central nucleus of the amygdala and LC as compared to NH rats. These results suggest that extrahypothalamic regions such as the amygdala and the locus coeruleus may play an important role in controling the central events leading to the H rats enhanced inhibition of anxiogenic noradrenergic responses to novelty stress. (Supported by NIMH grant to PMP and MJM)

532.9

EFFECT OF NEONATAL HIPPOCAMPAL AND AMYGDALA LESIONS ON SYMPATHO-ADRENAL SYSTEM AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS RESPONSIVENESS TO IMMOBILIZATION STRESS IN RHESUS MONKEYS. K. T. Kalogeras*, L. Malkova, K. Pacak, R. Kvetnansky, J. Bachevalier, P. W. Gold, M. Mishkin, and R. L. Wilder. Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS 39216; NIMH, NINDS and NIAMS, National Institutes of Health, Bethesda, MD 20892.

The amygdala has been shown to play a stimulatory role in the activation of both the sympatho-adrenal system (SAS) and the hypothalamic-pituitary-adrenal (HPA) axis during stress in rats. By contrast, the hippocampus is thought to either have no effect or play an inhibitory role. To examine the role of these two structures in the activation of the SAS and the HPA axis, we studied stress responses in adult rhesus monkeys that had received bilateral neonatal lesions of either the amygdala (A), the hippocampus (H), or both (AH) structures. The completeness of the lesions was verified by magnetic resonance imaging. A total of 3 A, 4 H and 3 AH lesioned animals (originally lesioned for behavioral studies) and 5 normal controls were exposed to 20 min immobilization stress. Blood samples were obtained through an indwelling iv cannula before, during, and after the stress. The SAS activation during stress was reflected by peak values of 2372±975 pg/mL for epinephrine (EPI), and 1748±607 pg/mL for norepinephrine (NE) in normal controls, with all lesioned monkeys exhibiting a blunted response of plasma EPI (715±268, 708±291 and 567±219 pg/mL for A, H, and AH, respectively) and NE (870±103, 533±178 and 740±465 pg/mL, respectively). Plasma cortisol reached a peak of 17.7±2.4 µg/dL 5 min after termination of the stress in normal controls and gradually declined to baseline values (6.2±1.2 µg/dL) 120 min later. All lesioned primates showed a blunted cortisol response as well, reaching peak values of 11.4±5.5, 12.1±1.8 and 8.0±2.9 µg/dL for A, H, and AH lesions, respectively. Arginine vasopressin and neuropeptide Y showed a trend of an attenuated response in lesioned primates. In conclusion, neonatal lesions of the amygdala as well as the hippocampus appear to decrease the responsiveness of the sympatho-adrenal system and the HPA axis to stress. Combined lesions of both structures do not result in an additive or synergistic effect. (Intramural NIH support).

532.10

MATERNAL DEPRIVATION IN THE BROWN NORWAY RAT RESULTS IN LONG-TERM ALTERATIONS IN THE HYPOTHALAMUS-PITUITARY-ADRENAL AXIS AND IN BEHAVIOR. J.O. Workel, E.R. de Kloet, M.S. Oitzl*. Div. of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research, (LACDR), University of Leiden, P.O. Box 9503, 2300 RA Leiden, The Netherlands.

Glucocorticoids play an important role in developmental processes. In the rat, corticosteroid levels are low from postnatal day (pnd) 4 until 14, even after stress. During this period, the stress hyporesponsive period (SHRP), maternal factors have an inhibitory influence on the hypothalamus-pituitary-adrenal (HPA) axis of the neonate, since maternal deprivation of the pup for 24 hours (DEP) results in an immediate increase in HPA reactivity. We hypothesized that a single 24-hour maternal deprivation at pnd 3 results in long-term alterations in HPA reactivity and behavior. We used male Brown Norway (BN) rats, a rat strain known for its long healthy life span. As indices for HPA activity we measured basal CRF mRNA in the hypothalamus, and both basal as well as stress-induced ACTH and CORT levels in plasma. We found that BN rats display a SHRP which resembles the one reported for albino rat strains. DEP at pnd 3 did not alter basal and stress induced HPA reactivity at pnd 10, but resulted in an enhanced ACTH response after stress at pnd 18. Comparing DEP rats and their controls (NDEP) at the age of 3 months, exposure to a novel environment resulted in a decreased CORT response, while ACTH levels were similar. At all ages tested, CRF mRNA expression was not different. Behaviorally, DEP rats showed an increased reactivity (exploration of an object in an open field) and an enhanced locomoter activity. Furthermore, DEP rats displayed a time delay in the acquisition of spatial learning in the water maze. Taken together, these data suggest that a single 24-hour maternal deprivation at pnd 3, results in long-term alterations in HPA reactivity and in behavior of young adult BN rats. We are currently investigating 12-month old animals. Supported by the Netherlands Organization for Scientific Research (NWO), grant # 554-545.

532.11

RESTRAINT STRESS PRODUCES REGION SPECIFIC CHANGES IN RAT BRAIN PREPRO-TRH 178-199, A PROPOSED NOVEL CORTICOTROPIN RELEASE INHIBITING FACTOR. P.A. Rittenhouse*, E.P. Zorrilla, R.F. McGivern#, and E. Redei. Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia PA 19104 and #Dept. of Psychology, San Diego State Univ., San Diego, CA 92120.

We have described that prepro-TRH 178-199 shows corticotropin release inhibiting activity *in vitro* and *in vivo*. As this peptide is encoded within the precursor for TRH, which is widely distributed in brain, the concentration of prepro-TRH 178-199 may change region specifically in response to stress. A specific RIA was developed with a sensitivity of 19 pg/tube using a polyclonal antibody and ^{125}I-Tyr^{178}-prepro-TRH 178-199 as tracer. Brain regions were dissected from adult male rats (n=6/group) at 9:00 am or 9:00 pm, and 30, 90 and 180 min after 5 min of restraint stress. The rank order of prepro-TRH 178-199 content (ng/mg protein) was: hypothalamus 9.2; septum 6.3; caudate 2.9; amygdala 0.82; prefrontal cortex 0.75; periaquaductal gray (PAG) 0.6. The highest amount was found in the median eminence (ME) at 3.6/tissue with negligible amounts found in other brain areas. There was a trend for a diurnal morning decrease in the ME, pituitary and septum with an increase in morning PAG. Following stress there was a regionally specific change in prepro-TRH 178-199 content ($F_{[5,16]}$=1.98, $p<0.05$). A small increase at 30 min was seen in the hypothalamus, septum, caudate and amygdala followed by a decrease in the hypothalamus, caudate and amygdala at 90 or 180 min post-stress. Prepro-TRH 178-199 decreased in response to stress without an initial increase in the prefrontal cortex and PAG. In contrast, prepro-TRH 178-199 increased in the ME and pituitary at all time points post stress. These results show that this RIA is suitable for detection of prepro-TRH 178-199 concentrations, and suggest that brain prepro-TRH 178-199 is differentially sensitive to stressful stimuli. *Supported by the Berman Foundation.*

532.12

CORTICOSTERONE TONICALLY INHIBITS THE HPA AXIS RESPONSE TO AIRPUFF STARTLE IN CONSCIOUS RATS. K.V. Thrivikraman*, Y. Su, and P.M. Plotsky. Stress Neurobiology Laboratory, Dept. of Psychiatry & Behavioral Sci., Emory Univ., Atlanta, GA 30322.

We previously demonstrated that suppression of hypothalamic-pituitary-adrenal (HPA) axis activity by glucocorticoid-induced feedback was stressor-specific. Furthermore, it was speculated that this apparent specificity was related to differences in the neuronal pathways conveying stimulus-specific information to the neuroendocrine cells in the hypothalamic paraventricular nucleus (PVN). Thus a physical stressor, represented by a controlled hemorrhage (H) which elicits activation of hemodynamically sensitive brainstem catecholaminergic cell groups, was not responsive to feedback inhibition, whereas an emotional stressor, represented by airpuff-startle (APS) which does not activate these cell groups, was susceptible to feedback suppression. We have now extended these studies in adrenalectomized (ADX) rats with or without exogenous corticosterone (B) replacement to evaluate the contribution of stable circulating B within the basal range to this phenomenon. Four treatment groups of male Sprague-Dawley rats were used in this study: (1) sham-ADX (sADX), (2) ADX, (3) ADX +40%B subcutaneous pellet (pADX), (4) ADX+water containing 25µg/ml B in 0.5% saline (wADX). The pADX treatment maintained a body weight between 248-277g and achieved basal plasma ACTH and B levels of 30±3 pg/ml and 46±3 ng/ml (mean±sem), respectively. Basal ACTH and B levels of wADX rats within the same weight range were 19±7 pg/ml and 16±6 ng/ml, respectively. Groups of rats from each treatment were exposed to either 20% H over 3 min or APS. The ACTH and B responses of sADX rats after either H or APS were similar to those of intact rats. Relative to sADX, the integrated ACTH response to APS increased 5.9-fold in ADX and 2.2-fold in wADX; in pADX the response to APS was only 16% of sADX. In contrast, both pADX and wADX groups responded with robust H-induced increases in ACTH relative to sADX (2.1- and 1.8-fold, respectively). These results suggest that circulating B within the normal basal range is sufficient to attenuate the pituitary response to emotional stressors at a site proximal to the PVN. Supported by NIMH grant MH/OD51745.

532.13

Antagonism of release of ACTH in response to hypoxemia in fetal sheep by the CRH antagonist Astressin. P.W. Nathanielsz*, L.F. Buchwalder, W. Vale[2], J. Rivier[2], A. Yen, and T.J.McDonald. Laboratory for Pregnancy and Newborn Research, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 and [2]The Salk Institute for Biological Studies, The Clayton Laboratories for Peptide Biology, La Jolla, CA 92037

The fetal hypothalamo-pituitary axis plays a critical role responding to various physiological challenges (e.g. hypoxemia) and in promoting the birth process. CRH and AVP have been implicated in ACTH regulation in late gestation. The present study evaluated Astressin-cyclo(30-33)[D-Phem^{12},Nle^{21,38},Glu^{30},Lys^{33}]- hCRF(12-41)inhibition of the ACTH response to hypoxemia in fetal sheep.

METHODS: Five fetal sheep were studied at 135-145 d (term 148 d in sheep). Hypoxemia was induced via N_2 infusion to a maternal tracheal catheter for 1h. Fetal arterial blood was sampled at -30, -15 and 0 min before injection of either 0.8-1 mg Astressin or vehicle and at +15 and +30 min when hypoxemia started. Fetal PaO_2 fell ~7.5 mmHg (Fig. 1). Further samples were taken at 70, 90, 105, & 150 min. ACTH was measured by RIA.

RESULTS: Astressin reduced the rise in fetal ACTH peak by 70% (Fig. 1).

CONCLUSIONS: This is the first demonstration that the fetal ACTH rise in response to hypoxemia in late gestation results predominantly from CRH stimulation. (HD 21350).

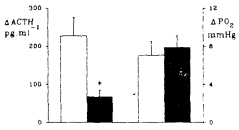


Fig. 1 Fetal plasma ACTH rise and PO2 fall during fetal hypoxemia with Astressin treatment (■) or vehicle (□), n=5 (mean±SEM). *p<0.05.

532.14

RESPONSE TO COLD STRESS FOLLOWING PRENATAL ETHANOL EXPOSURE. **C. K. Kim*, P. K. Giberson, W. Yu and J. Weinberg. Department of Anatomy, University of British Columbia, Vancouver, B.C., Canada, V6T-1Z3.**

Prenatal ethanol exposure (PEE) produces hyperresponsiveness to stressors in offspring. This study tested the hypothesis that PEE will result in increased physiological responses to chronic cold stress (4 °C for 0, 1 or 3 days), and increased sensitization to an acute stressor (15 min following IP stick) imposed during the chronic cold stress. The subjects were adult male and female offspring from prenatal ethanol exposed (E, 36% EDC), pair-fed (PF) and ad libitum-fed control (C) rats.

Adrenocorticotropic hormone (ACTH) levels in males were not affected by cold stress, and although ACTH levels were significantly elevated following acute stress there was no sensitization. Females showed significantly elevated basal ACTH levels in response to cold stress, and E rats displayed significantly higher ACTH levels than PF rats following acute stress. Furthermore, overall ACTH levels were significantly higher in E compared to PF and marginally higher than in C females. Basal corticosterone (CORT) levels in males were significantly elevated following cold stress only in E rats; significant sensitization was evident in all groups. Females showed significantly elevated basal CORT levels following cold stress, and although CORT levels significantly increased with acute stress there was no sensitization. Corticosterone binding globulin levels were not affected by any of the experimental manipulations in either males or females. Overall body weights of both males and females significantly decreased with cold stress; this decrease was most pronounced in E males. Adrenal weight and adrenal/body weight ratios of males significantly increased with cold stress. Thus, PEE produced an elevated CORT response to chronic cold stress in males, and an elevated ACTH response to acute stress in females. (Supported by a grant from NIAAA to JW)

532.15

MIDLINE THALAMIC LESIONS ALTER HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) ACTIVITY IN CHRONICALLY STRESSED ANIMALS. S. Bhatnagar*, C.J. Horsley & M.F. Dallman, Dept. of Physiology, UCSF, San Francisco, CA, USA, 94143-0444.

We have previously shown that animals exposed to chronic, intermittent cold exhibit elevated circulating levels of ACTH and corticosterone in response to a novel, heterotypic stressor, restraint. In earlier studies using fos immunoreactivity, we identified, amongst other areas, the paraventricular nucleus of the thalamus (PVTh) as a potential site mediating these elevated responses. In the present study, we examined the role of the PVTh in HPA responses to a novel stressor in chronically stressed animals. Adult, male, Sprague-Dawley rats were stereotaxically injected with either ibotenic acid or vehicle into the midline thalamic area which encompasses the PVTh. 48 hours following surgery, animals were either exposed to 7 days of chronic, intermittent cold (4h a day at 4°C; CHR) or left undisturbed (CTL). On the eighth day, all animals were sampled via the tail vein prior to restraint,at 15 and 30 min during a 30 min period of restraint, and at 30 min following return to the home cage. We found that lesioned CHR animals exhibited elevated basal levels of ACTH relative to all other groups. Furthermore, midline thalamic lesions increased ACTH responses to restraint in both CTL and CHR animals, but these two groups did not differ from each other. As a result, the differences in HPA responses to restraint seen between non-lesioned CTL and CHR animals were eliminated in the lesioned groups. These data provide direct evidence for a role of the midline thalamic nuclei in regulation of HPA responses to acute stress and in regulation of chronic stress-induced hyperresponsiveness to novel stressors. *Supported by the Medical Research Council of Canada and DK28172.*

532.16

FOOTSHOCK INDUCED C-FOS PROTEIN IN THE BRAIN: EFFECTS OF ADRENALECTOMY. L. A. Wetmore* and D. M. Nance. Depts. Physiology & Pathology, Univ. Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3.

Footshock invokes neurochemical alterations in specific brain regions, activates the hypothalamic-pituitary-adrenal (HPA) axis and affects both endocrine and immune system function. Corticosterone mediates inhibitory feedback on the HPA axis however, the interaction of corticosterone with footshock induced neurobiological changes is not entirely known. Using c-fos immunodetection, the functional neuroanatomy activated by footshock has been reported. In the present study, the role of the adrenal gland in footshock induced brain c-fos protein expression was examined. Rats were adrenalectomized (ADX) or SHAM operated and later exposed to footshock (+FS) or tone (-FS). A subgroup of ADX and SHAM rats were pretreated with dexamethasone (DEX) or saline (SAL). SHAM/+FS rats had identical brain c-fos protein immunostaining, as previously reported for unoperated rats. ADX alone did not alter brain c-fos immunostaining however, footshock induced c-fos protein in the hypothalamic para-ventricular nucleus (PVN) was reduced. C-fos immunostaining in other brain regions was not altered by ADX. PVN c-fos protein in ADX/+FS rats was not reinstated by DEX. However, DEX pretreatment decreased footshock induced c-fos immunostaining in the PVN of SHAM rats, relative to SAL controls. SAL and DEX had no effect on brain c-fos protein in ADX/-FS or SHAM/-FS groups. The regionally selective effect of ADX on footshock induced c-fos protein highlights the importance of this region in stress processing. The data suggest a functional interaction between the adrenal gland and footshock regulated c-fos protein expression in the PVN, although the exact mechanisms involved remain to be determined. Supported by the Medical Research Council of Canada.

532.17

COMBINED TYPE I AND TYPE II CORTICOSTEROID RECEPTOR ANTAGONISM ENHANCES BASAL AND ACUTE-STRESS INDUCED HPA AXIS ACTIVITY IN BOTH THE MORNING AND EVENING. P.J. Kim*, B.A. Kalman, M.A. Cole, M.S. Chi, E.S. Schuiling, R.L. Spencer. Dept. of Psychology, Univ. of Colorado., Boulder, CO 80309.

This study examined the relative roles of the corticosterone (CORT) receptor subtypes in mediation of CORT regulation of the basal and acute stress-induced activity of the HPA axis during the trough (AM) and the peak (PM) of the HPA axis circadian rhythm. One hour prior to one hour restraint stress, male Sprague-Dawley rats were injected sc with the putative selective CORT receptor antagonists, RU28318 (50mg/kg; Type I receptor antagonist), or RU40555 (30mg/kg; Type II receptor antagonist), or the combination of both. RU28318 increased basal CORT in the AM, but not the PM, and had no effect on the HPA axis stress response at either time of day. RU40555 had no effect on basal or stress-induced CORT levels at either time of day. Treatment with the combination of RU28318 and RU40555 increased basal CORT, increased peak stress CORT levels and delayed the return of post-stress CORT levels to basal levels in both the AM and PM. Measurement of available corticosteroid receptor levels after antagonist treatment indicated that RU28318 occupied over 90% of Type I receptors and 30% of Type II receptors, whereas RU40555 selectively occupied 70% of Type II receptors. Thus, combined antagonist treatment occupied a greater percent of Type II receptors than RU40555 treatment alone, and this combined antagonist treatment was effective in increasing basal and stress-induced HPA axis activity in both the AM and PM. [Supported by UROP program at the Univ. of CO, and USPHS grants DK49143 and MH54742]

532.18

BLOCKING CORTICOSTEROID NEGATIVE FEEDBACK IMPAIRS THE EXPRESSION OF HABITUATION TO A REPEATED DISCRETE STRESSOR IN RATS. M.A. Cole*, B.A. Kalman, P.J. Kim, M.S. Chi, R.L. Spencer. Dept. of Psychology, University of Colorado, Boulder, CO 80309.

The role of corticosterone (CORT) negative feedback in the expression of habituation of the HPA axis response to a discrete repeated stressor was investigated. Male, Sprague-Dawley rats were restrained for one hour a day in cylindrical, Plexiglas tubes for six consecutive days. On day six, one hour prior to restraint stress, rats were injected s.c. with either the combination of the putative selective Type I CORT receptor antagonist RU28318 (50mg/kg) and the selective Type II receptor antagonist RU40555 (30mg/kg) (n=6) or vehicle (n=6). Blood samples were collected for CORT measurement (days 1, 5, and 6) just prior to stress, 30 and 60 minutes into stress, and one hour after stress termination. All rats significantly habituated to the stress-induced CORT secretion by day five. The antagonist pretreatment on day 6 completely blocked the expression of the habituation. The day 6 peak CORT stress response in the antagonist group was significantly greater than their day 1 prehabituation levels in addition to being greater than the day 6 control (vehicle group) levels. Similar increases in peak stress response CORT levels were seen when these antagonists were administered one hour prior to stress in nonhabituated rats. These results indicate that expression of the habituated HPA axis stress response is dependent on CORT negative feedback. A possible implication is that an impairment in CORT negative feedback would interfere with HPA axis stress adaptation. [Supported by USPHS grants DK49143 and MH54742]

532.19

EFFECTS OF ACUTE CORTICOSTEROID RECEPTOR ANTAGONISM ON BASAL AND STRESS-INDUCED C-FOS-LIKE IMMUNOREACTIVITY (C-FOS-LI) IN SPECIFIC BRAIN REGIONS. M.S. Chi, B.A. Kalman, M.A. Cole, P.J. Kim, R.L. Spencer*. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

The nature of the relationship between stress-induced c-fos protein induction in various brain regions and HPA axis activity remains to be established. Acute blockade of corticosteroid receptors with the combined sc injection of the Type I receptor antagonist, RU28318 (50 mg/kg) and of the Type II receptor antagonist, RU40555 (30 mg/kg) increases both basal and stress-induced corticosterone (CORT) secretion in the rat. We have examined the concurrent effect of this corticosteroid receptor antagonist treatment on c-fos protein induction in brain regions involved in HPA axis function (PVN of hypothalamus and hippocampus). Four groups of male Sprague Dawley rats were tested: 1) Vehicle + No Restraint, 2) Antagonist + No Restraint, 3) Vehicle + Restraint, and 4) Antagonist + Restraint. All rats were anesthetized and perfused transcardially with 4% paraformaldehyde in phosphate buffer 3 h after injection. Restraint rats were placed in Plexiglas tubes for the final 2 h prior to perfusion. C-fos-LI was visualized on brain sections using immunohistochemistry. Cell counts indicate that antagonist pretreatment of non-restrained rats resulted in an increase in c-fos-LI in PVN and pyramidal cells in hippocampus, however, the increase in PVN did not reach statistical significance. Stress produced a large increase in c-fos-LI positive cells in the parvocellular region of the PVN, which was comparable for both vehicle and antagonist pretreatment, perhaps due to ceiling levels of c-fos-LI resulting from the stress of restraint. [Supported by UROP program at the Univ. of CO, and USPHS grants DK49143 and MH54742]

532.20

ULTRADIAN RHYTHMS OF CORTICOSTERONE RELEASE: EFFECT ON DIFFERENTIAL RESPONSE TO STRESS IN SPRAGUE-DAWLEY, FISCHER-344 AND LEWIS STRAINS OF RAT. C.D. Ingram*, R.J. Windle[1,2] and S.L. Lightman[1]. [1]Dept. Medicine and [2]Dept. Anat., Univ. Bristol BS8 1TD, Bristol, U.K.

An automated system which allows frequent and remote blood sampling was used to study patterns of corticosterone (CORT) release in the female rat. In Sprague-Dawley (SD) rats, CORT release occurred in discrete pulses (26 ± 3 pulses/24 h) each of which decayed rapidly, consistent with burst-like release of the hormone, given the short half life (<10 min) of exogenous CORT that we observe in adrenalectomised rats. Pulse frequency did not vary over the day, although an increase in pulse magnitude generated a circadian pattern of circulating glucocorticoid. The ultradian pattern of CORT release had a profound effect on the response to a mild psychological stress (white noise, 10 min, 114 dB). When the stress fell on the rising phase of a pulse, there was a large increase in plasma CORT, reaching 800 ± 152% of the mean pre-stress value at 20 min, before falling rapidly to baseline. However, when the stress occurred on the falling phase of the pulse no significant increase in plasma CORT was seen. This relationship between pulse pattern and responsiveness to stress was further studied in the Lewis (LEW) and Fischer-344 (F344) strains of rat which show differential sensitivity to stress. Both strains showed pulsatile release of CORT similar to the SD rat (F344: 32±2; LEW: 31±1 pulses/24 h). LEW rats also showed a relationship between pulse phase and stress-induced CORT levels comparable to SD rats. However, the more reactive F344 rats displayed a prolonged elevation of CORT (60 min) following noise stress, and the phase of the pulse on which the stress fell did not affect the magnitude or duration of the response. Therefore, the HPA axis appears to alternate between periods of hormone release, during which it can respond to stress, and periods of quiescence, when it is unresponsive. The absence of quiescent periods in the F344 rat may account for its greater reactivity to stress and may provide a model for other conditions of stress hypersensitivity.

Supported by the Wellcome Trust

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION III

533.1

EFFECTS OF UNILATERAL MECHANICAL STIMULATION OF THE OVARIAN PEDICLE PERFORMED ON EACH DAY OF THE ESTROUS CYCLE ON OVULATION AND NEURAL MONOAMINE-ACTIVITY IN THE POA-AHA. A. Domínguez-González*, M.E. Cruz and N.Cruz. UIBR, Fes-Zaragoza, UNAM, México

We have previously shown that hemiovariectomy performed at 13.00 h on each day of the estrous cycle affects ovulation and serotonin concentration of POA-AHA in different way (S.Nsc. 21;743.11;1995). Because there is evidence that Unilateral Mechanical Stimulation of the Ovarian Pedicle (UMSOP) performed at 07.00 h affects ovulation depending on the day of the cycle when it is performed (Med.Sci.Res. 18;819;1990), in present study we compared the effects of UMSOP performed at 13.00 on each day of the estrous cycle on ovulation rate and monoamine-activity in POA-AHA. Adult cyclic females rats were ether-anaesthetized and laparotomized. Right (R-UMSOP) or Left (L-UMSOP) ovary was exteriorized, manipulated and returned to the abdominal cavity. A group of intact cyclic animals were used as control. All animals were killed on the next estrus day.

L-UMSOP blocked ovulation when was done on D2 (0/4 vs.7/8); the activity of the serotoninergic-neuron of the left side of POA-AHA was significant lower than control (0.18±0.1 vs. 1.44±0.4, $p<0.05$), while changes in the right side of POA-AHA were not observed (1.39±0.8 vs.1.68±0.5). The R-UMSOP did not affect ovulation (4/6 vs 7/8) nor the serotoninergic neuronal activity. UMSOP performed on others days of the estrous cycle did not modify ovulation, nor neuronal activity.

Present results suggest that the stimulation of the ovarian pedicle affects ovulation and serotoninergic neuron in POA-AHA in an opposite way than hemiovariectomy, and both present laterality.

Supported by Grant DGAPA:IN204693; IN210843; PADEP 500307

533.2

BEHAVIORAL INTERACTIONS CAUSE A DECLINE IN GONADOTROPIN-RELEASING HORMONE IMMUNOREACTIVE CELL NUMBERS. V.C. Tai, L. E. Muske* and E. F. Rissman. Biology Department, University of Virginia, Charlottesville, VA 22903.

Previously we reported that in the female musk shrew, interactions which accompany the mating bout promote rapid increases in the numbers of gonadotropin-releasing hormone immunoreactive (GnRH-ir) cells in olfactory-related forebrain regions (Dellovade et. al., Neuroendo. 62:385-395, 1995). In this study we asked whether the changes are caused by male-related olfactory stimuli. Females were housed in one of 4 conditions. Either they resided in a split cage in male-soiled bedding with an active adult male on the other side of a barrier, or under identical conditions, with an anesthetized male, or in a clean cage with a male on the other side of a transparent solid barrier. Control animals resided in a clean cage without a male present. After one hour females were perfused, brains removed, processed and stained for GnRH with a commercial antibody (SMI-41). A "blind" observer counted GnRH-ir neurons in the forebrain. Behavioral interactions with an awake male resulted in a decrease in the numbers of GnRH-ir cells located in the ventral forebrain ($P<0.05$). This effect was caused by changes in the GnRH cells located in the ventral forebrain ($P<0.05$). The data suggest that behavioral interactions, not pheromones, promote GnRH release from cell bodies and thus the appearance of fewer GnRH-ir cells in the hypothalamus. This work is supported by NSF IBN 94-12605 (EFR).

533.3

EFFECT OF ESTRADIOL (E) ON LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) AND LH RELEASE IN MALE SHEEP. Lubbers LS, Hileman SM, Kuehl DE, Ferreira SA and Jackson GL*. Department of Veterinary Biosciences, University of Illinois, Urbana-Champaign, Il 61801.

In the male, the inhibitory effects of testosterone on LH release may be mediated in part by E. To determine the sites at which E acts, we tested the hypothesis that E acts first on the pituitary, to reduce responsiveness to LHRH, and then on the hypothalamus, to reduce LHRH release. Hypophyseal-portal cannulated, castrated male sheep were infused with E (15 ng/kg/h) or vehicle (V). Portal and jugular blood samples were collected at 10 min intervals for 4 h prior to, and for either 12 h (E, n=4; V, n=4) or 24 h (E, n=8; V, n=3) following the start of infusion. In animals sampled for 16 h, temporal changes of both LHRH and LH were assessed. In animals sampled for 28 h, only LH data were analyzed. Prior to the start of either the 12 h or 24 h infusion, LHRH and/or LH mean concentrations, pulse amplitude and interpulse interval (IPI) did not differ ($p>0.05$) between E- and V-infused animals. In animals sampled for 16 h, mean LHRH and LHRH pulse amplitude was greater ($p<0.01$) in the V-infused than E-infused males, however, there was no significant ($p>0.05$) effect of time or steroid x time interaction. LHRH IPI was unaffected ($p>0.05$) by infusion. In contrast, both mean LH and LH pulse amplitude declined within 4-8 h ($p<0.01$) after the start of E infusion while mean LH IPI was unaffected ($p>0.05$). In animals sampled for 28 h, both mean LH and LH pulse amplitude declined within 4-8 h ($p<0.01$) and remained suppressed for the remainder of the 24 h infusion period. Mean LH IPI was unaffected ($p>0.05$). In conclusion, in male sheep the acute inhibitory effects of E on LH release are achieved by reducing pituitary responsiveness to LHRH and not by decreasing LHRH release. (Supported by USPH HD-27453)

533.4

NEUROENDOCRINE REGULATION OF THE EFFECTS OF PMSG ADMINISTRATION ABOUT PREOVULATORY PEAK OF ESTRADIOL AND PROGESTERONE IN PREPUBERTAL RATS. **J. Villavicencio*, M.C. Peñasco, R. Chávez and Díaz. Vicente. UIBR-FES Zaragoza, UNAM., and INNS-Zubirán , México, AP 9-020, CP 15 000.**

Gonadotropin administration in prepubertal rats induce an increase in plasma progesterone levels and advanced ovulation, effects mediated by the central catecholaminergic system. In this work we study the participation of the cholinergic and catecholaminergic systems on preovulatory peak of progesterone and estradiol in 27 days old rats treated with: a) PMSG (8 i.u). b) PMSG, 48 h after Reserpine (RSP: 2.5 mg/kg bw) or Atropine (ATR: 400 mg/Kg bw) and c) PMSG+RSP or ATR and 6 h later with hCG (10 i.u.) Animals were sacrified 54 or 72 h after PMSG treatment. The results are shown in the next table:

Group	Ovulation rate	Ova shed	Estradiol (pg/ml)	Progesterone (ng/ml)
54 h-PMSG	0/10	0 ± 0	118 ± 16	23 ± 2
PMSG+RSP	0/5	0 ± 0	145 ± 25	5 ± 1 a
PMSG+ATR	0/5	0 ± 0	584 ± 17 a	9 ± 1 a
72 h-PMSG	14/14	16 ± 2	40 ± 9	17 ± 2
PMSG+RSP	2/9 a	0 ± 0 a	211 ± 30 a	10 ± 1 a
PMSG+ATR	0/9 a	0 ± 0 a	144 ± 22 a	11 ± 2

a. $p < 0.05$ vs group PMSG same hour

All animals treated with hCG ovulated (PMSG: 6/6, 28±2 ova shed; PMSG+ATR+hCG: 6/6, 10±2 ova shed and PMSG+RSP+hCG: 6/6, 17±1 ova shed). Our results shown that both steroid production and the ovulation are regulated by cholinergic and catecholaminergic systems.

Supported by DGAPA, PUIS and CONACyT

533.5

CHANGES IN CELLULAR GONADOTROPHIN-RELEASING HORMONE (GnRH) mRNA CONTENT IN THE ROSTRAL PREOPTIC AREA (rPOA) PRIOR TO THE ESTRADIOL-INDUCED LUTEINISING HORMONE (LH) SURGE IN THE EWE.

T.G. Harris, N.P. Evans*, J.E. Robinson, D.C. Skinner and A.E. Herbison. Lab. of Neuroendocrinology, The Babraham Institute, Cambridge, CB2 4AT, U.K.

During the ovine estrous cycle, the follicular phase rise in estradiol (E) stimulates the preovulatory LH surge by initiating a massive and sustained rise in GnRH. The objective of this study was to determine whether E-induced changes in GnRH secretion were associated with temporal changes in GnRH mRNA expression. 48 Clun Forest ewes were ovariectomised (OVX) and ovarian steroids replaced to mimic the follicular phase rise of E. Hourly blood samples for LH were taken through the follicular phase. Animals in group 1 were killed at five specific time points for analysis of GnRH mRNA expression using in situ hybridisation (ISH): *A*, Prior to E administration (n=6); *B*, 8 hrs after E (n=5); *C*, on the ascending limb of the LH surge (18-23h following E;n=5); *D*, at the peak of the LH surge (n=7); and *E*, 40 hrs after E (n=5). A separate group of animals (group 2) were killed at identical time points to assess changes in GnRH mRNA when E was not increased (n=5, each group). Animals were killed, and the rPOA rapidly dissected and frozen. Every tenth 12 μm coronal section through the rPOA was processed for ISH using a ^{35}S-labelled oligonucleotide probe directed against ovine GnRH. Cellular analysis revealed no temporal changes in the number of GnRH mRNA expressing neurons in either group. Analysis of cellular silver grain density in E-treated ewes revealed a non-significant increase in GnRH mRNA content between points *A* (0.56±0.03 silver grains/μm^2) and *B* (0.64±0.06) followed by a significant ($P<0.05$) decline to point *C* (0.47±0.04). Levels remained at this significantly depressed level through points *D* (0.47±0.04) and *E* (0.47±0.03). No significant temporal changes were detected in group 2. These data indicate that there is an E-induced decrease in GnRH mRNA expression prior to the GnRH surge. This suggests that changes in GnRH mRNA expression may precede the increased secretory activity of GnRH neurons at the time of the GnRH surge. TGH was supported by an MRC studentship.

533.6

MECHANISMS OF LHRH RELEASE REGULATED BY GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF).

M. Kimura*, W. H. Yu, A. Walczewska, V. Rettori and S. M. McCann. Pennington Biomedical Research Center, Louisiana State Univ., Baton Rouge, LA 70808-4124, and CEFYBO-CONICET, 1414 Buenos Aires, Argentina.

Granulocyte-macrophage colony stimulating factor (GM-CSF), a hemapoietic cytokine, has a dose-related inhibitory effect on luteinizing-hormone releasing hormone (LHRH) release as we have shown recently. It is known that γ-aminobutyric acid (GABA) also inhibits LHRH release. However, glutamate and norepinephrine stimulate LHRH release resulting from the activation of nitric oxide (NO) neurons. In the present study, we examined *in vitro* how GM-CSF was involved in the regulation of LHRH release. Medial basal hypothalami were dissected from male rat brains. After 1-h preincubation, each individual tissue was incubated with recombinant murine GM-CSF (10^{-11} M) combined with either sodium nitroprusside (NP, NO releaser, 300 μM) or bicuculline ($GABA_A$ receptor antagonist, 10^{-5} M) for 0.5 h in Krebs-Ringer bicarbonate glucose media in a Dubnof metabolic shaker under 95 % O_2/5 % CO_2. LHRH release into media and NO synthase (NOS) activity in the tissues were determined by RIA and conversion of ^{14}C-arginine to citrulline, respectively. GM-CSF completely suppressed NP-induced LHRH release ($p < 0.01$) and lowered NOS activity in the hypothalamus ($p < 0.001$). Bicuculline partially reversed the inhibitory effects of GM-CSF on LHRH release ($p < 0.05$) at a dose that reversed completely GABA-suppressed LHRH release. The present results suggest that the action of GM-CSF on LHRH release uses a dual pathway: blocking stimulatory NO-induced release but signaling GABAergic neurons with an inhibition of the hypothalamic release of LHRH. (Supported by MH51853 & DK43900)

533.7

DECREASE IN PEPOA FOS ACTIVATION DURING THE LH SURGE IN AGED RATS

W-W. Le,* J.M. Lloyd, P.M. Wise, and G.E. Hoffman. Dept. Neurobiology, Univ. Pittsburgh, Pittsburgh, PA, 15261, Dept Anatomy Univ Oklahoma, Oklahoma City, OK 73190 and Dept. Physiology, Univ. Kentucky, Lexington, KY 40536.

Neurons in a narrow strip of the periventricular preoptic area (pePOA) regulate the activation of LHRH neurons in young female rats during the afternoon of proestrus. PePOA neurons are coordinately activated with LHRH neurons at the time of LH surge and possess receptors for both estrogen (ER) and progesterone (PR). Tract-tracing experiments determined that these neurons provide afferent input to LHRH neurons. In cycling middle-aged rats, the observed reductions in LHRH Fos activation and LH secretion during the LH surge raises the question of whether the decline the surge mechanism involves a diminution in pePOA neuronal activation. To test this hypothesis, we examined cFos staining in pePOA neurons in young (3-4 month old) and middle-aged (10-12 month old) animals. Double labeling of cFos/LHRH in LHRH neurons determined the activational status of the surge mechanism. All animals were perfused during the afternoon of proestrus at the time of the LH surge. A blood sample for plasma LH was taken at the time of perfusion. In the middle aged group, as expected, LH values and the degree of Fos activation in LHRH neurons were reduced by 60% and 58% respectively at the time of the surge and there was a similar reduction (57%) in cFos activation in pePOA as compared to the younger group ($p<0.05$). On an animal-by-animal basis, the level of pePOA activation reflected well the level of LHRH cFos activation. These data provide further support linking the pePOA to LHRH activation during an LH surge. (Supported by NS 28730 and AG 02224).

533.8

THE GnRH SYSTEM AND PITUITARY GLAND RESPONSE DURING AGING.

M.A. Ottinger*, A. Walz, Q. Li, and S. Ishii+. Dept. of Poultry Science and Cytoimmune Sci., Univ. of Maryland, College Park, MD 20742, USA and +Dept. of Biology, Waseda University, Tokyo 169-50, Japan.

Age-related changes in the GnRH system of the male Japanese quail include a significant ($p<0.05$) reduction in both preoptic-septal region and the median eminence concentrations of cGnRH-I. Similarly, in vitro perifusion of longitudinal hypothalamic slices from young reproductive males compared to senescent males also showed a diminution of cGnRH-I release and reduced response to norepinephrine challenge. Surprisingly, plasma and pituitary gland LH levels were not found to decline significantly (collaboration with John Proudman, USDA, ARS). In a separate study, pituitary gland LH was studied immunocytochemically during aging. Male Japanese quail were grouped according to chronological age and sexual activity. Experimental groups included young, middle-aged and old males, categorized as sexually active or inactive. In addition, there were groups of young photoregressed and old senescent testosterone implanted males. Sections were analyzed using cell counts and image analysis (Opelco Image Analysis System). Results showed an age-related decline in the number of cells staining, size of the cells, and total area stained. Testosterone treatment increased immunostaining in old senescent males. These results provide evidence for age-related alterations in pituitary gonadotropin cell response that appear to be more distinct than age-related effects on the plasma or pituitary LH. Supported in part by USDA/NRI 92-37023-7742 (MAO).

533.9

NON-ISOTOPIC MEASUREMENT OF LHRH FROM POSTNATAL HYPOTHALAMIC SLICE EXPLANT CULTURES.

J.A. Maurer* and S.Wray. Lab. of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

Luteinizing hormone releasing hormone (LHRH) cells control gonadal function by regulating gonadotropin release from the pituitary. Studies on immortalized and embryonic LHRH neurons indicate that they express an array of membrane receptors and possess a variety of intracellular signalling pathways. However, study of intra- and extracellular signalling pathways in postnatal LHRH neurons is hampered by their dispersed location in the CNS. To examine regulation of LHRH release from primary LHRH neurons in an organotypic environment, a competitive enzyme-linked immunosorbant assay (EIA) was designed to measure LHRH from long-term, roller-tube slice explant cultures of PN5 rat hypothalami. The EIA, which uses affinity-purified antibodies against mammalian LHRH, has a detection limit of 10 pg/ml, a range of 10-1000 pg/ml, an intraassay coefficient of variation (cv) of 2.2% (n=7), and an interassay cv of 2.0-6.8% (n=4); the ED_{50} of the standard curve is 68.0 [50.4-91.8] pg/ml (n=4). LHRH extracted from slices prior to culturing measured 43.6±7.7 pg per 4 slices/animal (n=4). No sex differences were noted. The majority of LHRH was found in the slice containing the OVLT (21.5±3.1 pg), followed by the most caudal slice at the level of the SCN (14.9±6.9 pg). Slices containing the DBB and mPOA contained 8.9±3.2, and 8.7±0.5 pg LHRH, respectively. LHRH extracted from slices maintained in culture for 18-21 days was 5-fold greater than that extracted from freshly-cut slices. Preliminary studies show that after 21 days in culture, 56 mM KCl stimulates a 6-fold increase in LHRH release over basal.

This work was supported by the NINDS, NIH Intramural Program.

533.10

NEURONS CULTURED FROM ADULT HYPOTHALAMUS EXPRESS LHRH, LHRH mRNA, AND RELEASE LHRH.

P.W. Coates*, L. L. Murphy†, S. Thode§, R.L. Norman and F.H. Gage§. Dept. Cell Biol./Biochem., TTUHSC, Lubbock, TX 79430, †Dept. Physiol., SIU-SM, Carbondale, IL 62901 and §Lab Genetics Salk Inst., San Diego, CA 92186.

We recently reported on a novel system for long and short-term culture of neurons from normal adult (90 d. old) female rat hypothalamus (Coates et al., SN Abs., 1995) that was patterned after a system used to successfully culture adult hippocampal cells (Gage et al., PNAS, 1995). Adult-derived hypothalamic cells grown in serum-free, defined medium + FGF-2 proliferate abundantly and subpopulations differentiate into neuronal and glial-like cells. Such cells have been grown for over a year in continuous culture, can be passaged, cryopreserved and re-initiated into culture. To determine whether the cells can express a regionally specific hypothalamic neurohormone and its message, we examined the cultures with immunocytochemistry using the LR1 and EL-14 antibodies for luteinizing hormone releasing hormone (LHRH). Approximately 40% of the cells were modestly immunopositive for LHRH. RT-PCR was used to analyze message for LHRH. mRNA for LHRH was detected unambiguously at 40 cycles from cells harvested at different culture times. A sensitive radioimmunoassay (RIA) using the CRR11B73 antibody to LHRH was used to assess LHRH in media obtained from cultures after varying periods of time. RIA revealed that LHRH was released into the medium. Thus data from three independent analyses suggest that at least some cells in adult-derived hypothalamic cultures differentiate into neurons that display properties similar to typical mature hypothalamus *in vivo*. Supported by HD22806.

533.11

IMMUNOCYTOCHEMICAL LOCALIZATION OF GLUTAMATE, GLUTAMATE RECEPTORS OR NITRIC OXIDE SYNTHASE IN THE MEDIAN EMINENCE OF FEMALE RATS. S-I. Kawakami[1], H. Tsukamura[1], K-I. Maeda*[1] and M. Ichikawa[2]. [1]School of Agricultural Sciences, Nagoya Univ., Nagoya 464-01, Japan. [2]Department of Anatomy and Embryology, Tokyo Metropolitan Institute for Neuroscience, 2-6 Musashidai, Fuchu-shi, Tokyo 183, Japan.

We have previously found that glutamate (Glu) or its agonists stimulate *in vitro* LHRH release from median eminence (ME) fragments of ovariectomized (OVX) rats. In the present study, we examined immunocytochemical localization of Glu, Glu receptors or nitric oxide synthase (NOS) in the external layer of the ME of OVX rats. Animals were perfused with 4% paraformaldehyde-0.1% glutaraldehyde in 0.1 M PB and 50 µm coronal sections were cut with a microslicer. After blocking, sections were incubated with polyclonal antibody raised against Glu, NR1, GluR1, GluR2/3, GluR6/7, mGluR2/3, mGluR5 or NOS. Immunoreactivity was visualized using ABC method and diaminobenzidine. Stained sections were embedded in Ouetol 812, cut on an ultramicrotome and observed with an electron microscope. Glu or Glu receptors immunoreactivity was observed in the nerve terminals of the ME. These nerve terminals contained clear and/or dense-core vesicles, and most of these formed nonsynaptic contacts. A few Glu-, GluR1-, GluR2/3- or mGluR5-immunopositive nerve terminals formed synaptic contacts in the ME. GluR1-, GluR2/3- or mGluR5-immunoreactivity was only postsynaptically observed. Glu-immunoreactivity was observed in both pre- and postsynaptic areas. NOS-immunoreactivity was found in glial processes and nerve terminals which contained dense-core vesicles. A few NOS-immunopositive synapses were observed, strong immunoreactivity was found on the postsynaptic density and mitochondria membrane. These results suggest that Glu and NO play a role in regulating LHRH release at the nerve terminals of the ME.

533.12

ESTROGEN INCREASES NADPH-DIAPHORASE REACTIVITY IN THE HYPOTHALAMUS AND BASAL FOREBRAIN OF FEMALE RATS. T. J. Hebert*, Z. Zhao, J. M. Pace, C. S. Menard, and G. P. Dohanich. Department of Psychology, Tulane University, New Orleans, LA 70118 and Department of Psychology, University of Southwestern Louisiana, Lafayette, LA 70503.

NADPH-diaphorase histochemistry provides a useful method for identifying and characterizing neurons that produce nitric oxide (Hope et al.; PNAS, 88, 2811-2814; 1991). A previous study demonstrated that treatment with high doses of estrogen (10µg/day of estradiol benzoate for 2 days) resulted in an increase in the number of NADPH-diaphorase reactive neurons in the ventromedial nucleus of the hypothalamus (VMH) of female rats (Okamura, et al.; Endocrinology, 135, 1705; 1994). In an initial experiment, we determined the effect of natural cyclicity on diaphorase reactivity in the hypothalamus and basal forebrain. Gonadectomy significantly reduced the number of diaphorase reactive neurons in the VMH and horizontal limb, but not in the vertical limb, of female rats. In the VMH, diaphorase reactivity varied significantly across the estrous cycle, with the greatest number of diaphorase neurons detected at proestrus. An additional experiment indicated that estrogen treatment (10µg/day of estradiol benzoate for 3 days), similar to that previously reported to increase the number of diaphorase reactive neurons in the VMH, also increased the number of diaphorase reactive neurons in the horizontal limb, but not the vertical limb. These results indicate that the gonadal steroid, estrogen, can increase the activity of the nitric oxide synthase, NADPH-diaphorase, in areas of the hypothalamus and basal forebrain.

Supported by NSF award BNS-9021447 to GPD.

533.13

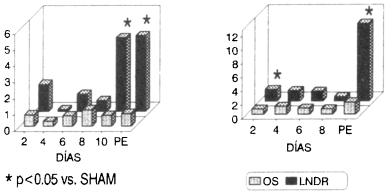
EVIDENCE THAT HYPOTHALAMIC NITRIC OXIDE SYNTHASE (NOS) mRNA LEVELS INCREASE BEFORE THE PREOVULATORY LH SURGE IN RATS: EFFECTS OF AGE. A. Sahu*. Dept. Cell Biol. & Physiol., Univ. Pittsburgh, Pittsburgh, PA 15261.

Recent evidence suggests an important role of nitric oxide (NO), a free radical gas, in regulation of GnRH and luteinizing hormone (LH) secretion. In the present study, we tested the hypothesis that gene expression of hypothalamic NOS, the enzyme that catalyzes NO production, is increased before the LH surge and that alteration in NOS neuronal activity may be one of the causal factors for the delayed and attenuated LH surge in middle-aged (MA) rats. Young (2-3 mo old) and MA (8-10 mo old) rats showing regular 4-day estrous cycles were killed at 1000, 1200, 1400, 1600, 1800, 2000 or 2200 h on proestrus. Trunk blood was collected for LH determination by RIA. Medial basal hypothalamus (MBH) was dissected out and frozen on dry ice. NOS mRNA levels in the MBH were measured by solution hybridization/RNAse protection assay using a cRNA probe for brain NOS and expressed in relation to cyclophilin mRNA. In young rats, the LH surge occurred at 1600 h with peak values at 1800 h. As compared to 1000 h, NOS mRNA levels increased significantly at 1200 h when LH levels were low basal, remained elevated during 1600 to 1800 h and returned to low levels by 2200 h. In MA rats, NOS mRNA levels remained unaltered between morning and afternoon associated with a delayed and attenuated LH surge. Also, NOS mRNA levels were significantly decreased at 1200, 1600 and 1800 h in MA rats as compared to those in young rats. These results show for the first time that hypothalamic NOS mRNA levels increase long before the LH surge in young rats and that this antecedent increase in NOS mRNA levels does not occur in MA regularly cycling rats. Overall, our results suggest an enhanced NOS neuronal activity, and thereby an increased NO production, before the generation of the LH surge. This early increase in NO production could play an important role in stimulating other excitatory neural signals that are obligatory for the preovulatory LH surge. Thus, absence of antecedent increase in NO production could, partially, be responsible for delayed and attenuated LH surge in middle-aged rats. (Supported by NIH AG 10868).

533.14

TEMPORAL CHANGES IN THE HYPOTHALAMIC SEROTONINERGIC-NEURONAL ACTIVITY AFTER THE LESION OF THE DORSAL RAPHE NUCLEUS (DRN). M.E. Ayala, J. Monroy, L. Morales and R. Dominguez*. UIBIR. FES Zaragoza, UNAM. México AP 9-020 CP 15,000.

The lesion of the DRN of 30-day old rat blocked the ovulation, while the same treatment performed on 27-day old animals resulted in an increase on the number of ova shed by ovulating animals. To analize if the changes in ovulation depend on the hypothalamic serotoninergic-neuronal activity, the activity of the neuron was calculated as [5-hydroxyindole 3-acetic acid]/ [serotonin] measured by HPLC, in control, sham operated and rats with a lesion on the DRN performed on days 27 or 30 and autopsied every two days until first vaginal estrus. The figures 1 and 2, show the changes observed in 27-day and 30-day old rats.



Present results suggest that there the differences in the first ovulation induced by a lesion of the DRN on 27-day and 30-day old rats, are not related with the hypothalamic serotoninergic neural activity.

Supported by DGPA IN 210893, CONACyT N1719 and PUIS.

533.15

GNRH AXONS TRAVEL WITH NEURONAL, NOT GLIAL, ELEMENTS WHEN EXITING PREOPTIC AREA (POA) EXPLANTS. M-C. Rogers, A-J. Silverman[1], M.J. Gibson. Dept.Med. & Fishberg Ctr. Neurobiol., Mount Sinai Sch. Med.NY 10029. [1]Dept.Cell Biol.&Anat, Columbia Coll.Phys.& Surg, NY 10032.

GnRH fibers follow defined routes to project to the median eminence (ME) during development. Similarly, GnRH axons grow from intraventricular grafts in hypogonadal mice, to innervate the host ME. Using organotypic co-cultures of embryonic (E15) tissue in insert chambers, we showed that GnRH terminals grow from the POA explant preferentially towards a mediobasal hypothalamus (MBH) explant that includes the ME (Soc.Neurosc.Abstr.20:664; 21:1898). These findings suggest the existence of a diffusable factor chemotropic for GnRH axons. The purpose of the present study was to evaluate associations of outgrowing GnRH axons with other elements on the membrane. Postnatal day 1 tissue was used, and as with E15, GnRH outgrowth was higher in the region facing the MBH ($p<0.001$). Double labeling for GnRH and GFAP, vimentin, or S100 was performed to see if GnRH terminals are using a glial substrate to grow on the membrane. Only erratic associations were seen between GnRH and glial processes. Staining for growth associated protein 43 (GAP-43) labels a general population of neurons elongating their axons. Double staining with GAP-43 and GnRH showed that, in contrast to its effect on GnRH axons, the MBH did not induce a differential outgrowth of those axons labeled with GAP-43. GAP-43 axonal outgrowth extended from all borders of the explants and always grew further from the POA explant than the GnRH fibers. Moreover, GnRH fibers traveled in the company of GAP-43 axons. In some cases, the GnRH axons followed a bundle of GAP-43-reactive fibers, and in other instances, GnRH axons appeared to be crossing on a dense network of GAP-43-reactive fibers. Some GnRH axons growing on the membrane were also double-labeled with GAP-43. Whether there is a sub-population of neurons that provide guidance to GnRH axons remains to be determined. *Supported by NS20335.*

533.16

INHIBITION OF PROTEIN KINASE A (PKA) STIMULATES GnRH RELEASE IN GT1 CELLS AND DOES NOT BLOCK cAMP-INDUCED STIMULATION OF GnRH. E.A. Vitalis, A.L. Choi and R.I. Weiner*. Reproductive Endocrinology Center, UCSF, San Francisco, CA 94143.

Pharmacological and receptor-mediated activation of adenylyl cyclase (AC) and elevation of cAMP stimulate GnRH secretion from GT1 cells. To determine if PKA mediates the stimulatory action of cAMP, GT1 cells were treated with H-89, a selective, permeable inhibitor of PKA. *In vitro* PKA assays on GT1 lysates verified that the cells express PKA activity which is inhibited by H-89 in a dose-dependent fashion. Unexpectantly, H-89 treatment for 30 minutes caused a statistically significant, dose-dependent, stimulation of basal GnRH secretion from GT1 cells measured by RIA. Furthermore, it did not block GnRH release induced by dopamine, forskolin, or 8-Br-cAMP, agents which increase cAMP levels. The stimulatory effect of inhibition of PKA could be explained by removal of a negative feedback action upon AC. A family of AC isoforms have been described of which two, V and VI, are inhibited by PKA. By RT-PCR and restriction map analysis, we showed that the cells express AC types V and VI. In conclusion, elevation of cAMP in GT1 cells results in a stimulation of GnRH release which is not dependent upon PKA activation. We hypothesize that activation of PKA inhibits GnRH release via inhibition of AC resulting in a decrease in cAMP levels.

This work was supported by NIH grant HD0894.

533.17

The Neurosteroid, 3α-Hydroxy-4-pregnen-20-one (3αHP), Suppresses GnRH-Induced FSH Release by Rapid Interaction With the Gonadotrope Membrane

J.P. Wiebe*, L. Murphy, and M. Wolfe. Hormonal Regulatory Mechanisms, B&G Bldg, Univ. Western Ontario, London, Canada N6A 5B7.

The gonadal- and neurosteroid, 3αHP, has been shown to selectively suppress GnRH-induced pituitary FSH release *in vivo* and *in vitro* by means of the cell signaling pathway involving protein kinase C and Ca^{2+} mobilization. To determine if additionally 3αHP action on the gonadotrope is at the level of the cell membrane, three approaches were used. [A]. 3αHP was conjugated to BSA (26 moles 3αHP/mole BSA) to prevent entry into cells. Treatment with 3αHP-BSA resulted in a significant ($p<0.01$) suppression of, while progesterone(P)-BSA or 3βHP-BSA showed no effect on FSH release from female rat pituitary cells. [B]. Pituitary cell membrane fractions showed specific binding to [1,2,6,7-^{3}H]3αHP which was not displaced by 1200-fold P, 3βHP or testosterone. [C]. The time course of suppressive action of 3αHP in GnRH-induced FSH release was examined in a perifusion system employing pituitary cells attached to cytodex beads. Cells received a 5 min pulse of GnRH (10^{-7}M) with/without 3αHP or other steroids. GnRH pulses resulted in a short (10-15 min) 4-30-fold peak (above baseline) of FSH release. When a 5-min pulse of 3αHP (10^{-9}M) was applied simultaneously with the GnRH pulse, the GnRH-induced FSH release was completely suppressed while pulsing with P or estradiol resulted in a significantly greater ($p<0.05$) release of FSH than the control (GnRH only). When a 3αHP pulse was applied 5 min after the start of GnRH pulse, the GnRH-induced FSH release was about 85% of the control peak. The pituitary levels of 3αHP (measured by specific RIA) on the day of proestrus oscillate between a low of 6 ng and a high of 64 ng and show an inverse oscillatory relationship to the FSH levels. The results suggest that the 3αHP regulated suppression of GnRH-induced FSH release involves a rapid interaction of this neurosteroid with the gonadotrope membrane. (Supported by NSERC of Canada).

533.18

ACUTE ETHANOL INGESTION AND EXPRESSION OF SYNCHRONIZED LH PREOVULATORY SURGES USING A FOLLICULAR MODEL IN EWES.

S. Goebbert, J. Klein, J. Rabii*, and JP Advis. Depts of Animal Sciences and Biological Sciences, Rutgers University, New Brunswick, NJ 08903.

We developed an animal model for studying potential effects of ethanol (EtOH) ingestion on in vivo release of multiple neuropeptides, from discrete brain areas, during the genesis of a synchronized LH preovulatory surge. This synchronized follicular model used sequential P4 removal (P4 out, day 18) and E2 implantation of silastic packets (E2 in, 16h later) into cycling ewes (20 day synchronized cycle, P4 in on day 10, and E2 out on day 20). Gastric EtOH infusion (0.25 g/kg in a 25% saline solution) was provided at the beginning of a synchronized follicular phase (at E2 in) through a chronic abomasal cannula. For the next 18h, blood EtOH level was maintained between 0.15 - 0.20 % (w/v) by further intragastric EtOH administration and hourly monitoring of blood EtOH (Sigma EtOH Diagnostic Kit). Plasma LH also determined at hourly samples was continued for up to 36h after E2 in. In this model, each ewe served as both control (saline abomasal infusion, LH surge onset: 18±2h after E2 in, mean ± sem, n=6) and experimental ewe (EtOH abomasal infusion for 18h from E2 in, during another synchronized estrous cycle of the same ewe, LH surge onset: 26± 2h after E2 in, n=6). Each ewe underwent a control synchronized cycle, followed by an EtOH experimental cycle, and then back to a control synchronized cycle. In control cycles, all surges began between 17-20h after E2 in, each lasted approx. 5h and reached peak LH values between 19 - 22h after E2 in. In experimental EtOH cycles, all surges began between 25 - 27h after E2 in, each lasted approx.7h and reached peak LH values between 28 - 29h after E2 in. Thus, our model allows study of neuroendocrine effects of EtOH in well-defined reproductive conditions (e.g. median eminence in vivo release of multiple neuropeptides after EtOH ingestion). Support NJAES-Hatch 06108 & USDA 94-37203-0721 to JP Advis

NEUROENDOCRINE REGULATION: GROWTH HORMONE AND SOMATOSTATIN

534.1

EFFECT OF GONADAL STEROIDS ON GROWTH HORMONE (GH) RELEASE IN OVARIECTOMIZED SHEEP IN RESPONSE TO L-692,585, A NOVEL NON-PEPTIDYL GH SECRETAGOGUE.

G. E. Dahl*[1], E. Russek[1], L. J. Craig[1], C. H. Chang[2], G. J. Hickey[2], [1]University of Maryland, College Park, 20742 and [2]Merck Research Laboratories, Rahway, NJ, 07065.

L-692,585 (585) is a novel GH secretagogue that stimulates GH release in guinea rigs, pigs and dogs. Gonadal steroids influence endogenous GH secretion, but their impact on responses to 585 are unknown. Objectives of this study were to evaluate 585 stimulated release of GH in ovariectomized (O) sheep and to determine if steroidal environment (i.e. androgenic or estrogenic) influenced these responses. Also, the influence of steroidal environment on the GH response to 585, GHRH and 585+GHRH was determined. In a preliminary experiment, 585 at doses of .033 and .1 mg/kg BW stimulated GH release relative to saline injection in a dose dependent manner. Ewes (n=6) were ovariectomized and received estradiol (E; ~2-3 pg/ml), testosterone (T; ~2.5ng/ml) or no replacement (O) in random succession with a 2-3 wk equilibration period between each steroid. In Experiment 1, GH was quantitated in serum samples collected at 10-min intervals for 2 hr before and during a 6 hr period when 585 (.1 mg/kg) was administered i.v. at 1 hr intervals. Responses to 585 were analyzed using a pulse detection algorithm; increases in GH were detected to 106 of 108 injections. Responses, as assessed by area under the GH curve, were greatest with the first injection and declined with time ($P<.01$). Responses were not affected by steroidal treatment. In Experiment 2, responses to a single injection of 585 (.1 mg/kg), GHRH (.75 μg/kg), or the combination were assessed. Regardless of steroid, GH AUC for GHRH was greater than that for 585 ($P<.05$). GH AUC for 585+GHRH was not different from GHRH. Steroid treatment altered GH AUC responses, with O≥T≥E (O vs. E, $P<.05$), but this effect of E was specific to GHRH. In summary, 585 stimulates GH secretion in sheep. Repetitive 585 stimulation is associated with a decline in GH output over a 6 hr period. Steroids had no effect on responses to 585 but E decreased responses to GHRH. Support from MD-AES & Merck Res. Lab.

534.2

REDUCTION OF INTRINSIC NEGATIVE FEEDBACK REGULATION OF NEUROENDOCRINE AXES INCREASES THE APPROXIMATE ENTROPY (SERIAL IRREGULARITY) OF THE PITUITARY HORMONE RELEASE PROCESS FOR TSH, GH, AND LH.

J.D. Veldhuis*, A. Iranmanesh, S. Pincus Univ. of Virginia Hlth Sci Ctr, Charlottesville, VA, VA Med Ctr, Salem, VA , 990 Moose Hill Rd. Guilford, CT.

To evaluate feedback regulation of hypothalamo-pituitary-target organ axes, we investigated 3 paradigms of decreased negative feedback signaling. First, we sampled blood at 10 min intervals for 24 h in 6 men with primary hypothyroidism before and after L-thyroxine replacement. Secondly, men and women were subjected to a 5 day fast consisting of water only, which reduced plasma IGF-I concentrations and increased GH release in an "open loop" model. Thirdly, we administered the steroidogenesis inhibitor, ketoconazole, to lower testosterone secretion and reduce androgen negative feedback. To estimate network complexity, we used a model-independent and scale-invariant statistical measure of quantifiable regularity in the hormone profiles, approximate entropy (ApEn, PNAS 88:2297, 1991), with increased disorderliness or greater irregularity quantified by larger ApEn values. In primary hypothyroidism, ApEn for TSH increased significantly from 1.073 ± 0.081 (normal) to 1.514 ± 0.066 (hypoT4) ($P=0.0086$), and this increase was reversed by the administration of L-thyroxine (ApEn = 1.146 ± 0.1012, $P=0.013$). Similarly, 24-hour GH profiles in fasting men and women with low IGF-I concentrations showed a rise in ApEn from 0.331 ± 0.133 to 0.592 ± 0.227 ($P = 0.0072$) (JCI 94:1277, 1994). Lastly, when testosterone concentrations fell, the ApEn of LH secretory time series rose from 0.638 ± 0.032 to 0.893 ± 0.022 ($P<0.01$). Collectively, these observations provide evidence for feedback-dependent modulation of hypothalamo-pituitary regulatory mechanisms that control the temporal secretory patterns of TSH, GH, and LH. Thus, we infer a general principle that for many endocrine networks reduction of intrinsic negative feedback is manifested in more irregular secretory dynamics at the target node.

NIH

534.3

BEDREST PREVENTS RELEASE OF TIBIAL GROWTH FACTOR BUT NOT GROWTH HORMONE IN RESPONSE TO MUSCLE ACTIVITY.

G.E. McCall*, C. Goulet, J.A. Hodgson, R.E. Grindeland†, and V.R. Edgerton. Dept. of Physiological Sci., Univ. of California, Los Angeles, CA 90066 and NASA Ames Research Center, Moffet Field, CA 94035†.

Hormonal responses of 8 men to a muscle fatigue test before (-12 and -7 days), during (days 2/3, 8/9, and 13/14) and following (+2/3 and +10/11 days) 17 days of bedrest were studied. The fatigue test involved a series of unilateral isometric plantar flexions and included 4 maximal voluntary contractions (MVC), 48 contractions at 30% MVC, and 12 contractions at 80% MVC all performed at a 4:1 s work:rest ratio. Blood was collected prior to and immediately following the fatigue test to measure plasma growth hormone (GH) by radioimmunoassay and a tibial growth factor (TiGF) by bioassay of tibia epiphyseal cartilage growth in hypophysectomized rats. Plasma GH was unchanged by the fatigue test during all testing periods. Prior to bedrest plasma TiGF increased ($p<0.05$) following the fatigue test (-12 d 2146 ± 192 to 3565 ± 197; -7 d 2162 ± 159 to 4161 ± 204; μg • L^{-1}). During bedrest the TiGF response was absent at 2/3 d, and decreased ($p<0.05$) by 8/9 (2433 ± 185 to 2105 ± 106) and 10/11 d (2594 ± 211 to 2085 ± 109). Following bedrest the TiGF response at +2/3 d was not significant. The TiGF response had normalized by +10/11 d (1881 ± 75 to 4160 ± 315; $p<0.05$). Although significant ($p<0.05$) increases in thyroid hormone (T3 and T4) and decreases in cortisol and testosterone plasma concentrations occurred in response to the fatigue test, these changes were independent of bedrest. In conclusion, the release of TiGF in response to a fatigue test was inhibited by bedrest, but returned to normal 10/11 days following bedrest.

Supported by NASA #199-26-12-09, and NIH, NRSA(DE07212) from NIDR.

534.4

D_2 DOPAMINE RECEPTOR-MEDIATED REGULATION OF FOS PROTEIN EXPRESSION IN SOMATOSTATIN-CONTAINING NEURONS IN THE HYPOTHALAMIC PERIVENTRICULAR NUCLEUS IN MALE RATS.

K.J.Lookingland*, S. Cheung, J.D.Johnson and K.E.Moore. Dept. of Pharm. & Toxicol., Michigan State University, East Lansing, MI 48824

Somatostatin (SS)-containing neurons located in the hypothalamic periventricular nucleus (PeVN) project to the median eminence. SS released from these neurons into the hypophysial portal blood inhibits growth hormone secretion from the anterior pituitary. The close proximity of SS perikayra to A_{14} dopaminergic neuronal axons in the PeVN suggests that these peptidergic neurons may be regulated by dopamine receptor-mediated mechanisms. To test this, the effects of agonists selective for either D_1 or D_2 receptors were examined for their ability to alter FOS immunoreactivity (IR) in SS-IR neurons in the PeVN. Frontal sections (30 μm) through the middle PeVN were chosen macroscopically and the number of SS-IR and Fos-IR + SS-IR cells were counted bilaterally. Ninety min after systemic injection of the D_1 agonist SKF 38393 (20 mg/kg; i.p.) there was no difference in the number of Fos-IR + SS-IR double labeled cells as compared with vehicle-treated controls. However, 90 min after systemic injection of the D_2 agonist quinelorane (100 μg/kg; i.p.) the number of SS-IR neurons containing FOS-IR decreased from 13.60 ± 3.43 to 5.00 ± 1.38 in the PeVN. Prior administration of the D_2 antagonist raclopride (3 mg/kg; i.p.) had no effect *per se*, but prevented the quinelorane induced decrease in the number of SS-IR neurons with FOS-IR. These results reveal that selective activation of D_2 receptors inhibits expression of FOS protein in SS-containing neurons in the PeVN. (Supported by NIH grant NS15911)

534.5

COMPARISON OF SPONTANEOUS ELECTRICAL ACTIVITY IN RAT SOMATOTROPHS AND LACTOTROPHS. S. Sankaranarayanan* and S. M. Simasko. Dept. of VCAPP, College of Veterinary Medicine, Washington State Univ., Pullman, WA 99164-6520.

The perforated patch variation of whole cell patch-clamp technique was used to determine the characteristics of the spontaneous action potentials exhibited by primary cultures of rat somatotrophs and lactotrophs. This spontaneous activity is hypothesized to be responsible for spontaneous secretion from these cells. Pituitary cells were obtained from random-cycling female Sprague-Dawley rats. Lactotrophs were isolated using percoll-density gradient and identified using quinpirole-induced hyperpolarization. Somatotrophs were identified using the reverse-hemolytic plaque assay. Spontaneous action potentials were exhibited by approximately 75% of somatotrophs and 65% of the lactotrophs. The following characteristics of spontaneous action potentials in somatotrophs (n = 19, 1st value listed) and lactotrophs (n = 28, 2nd value listed) were significantly different (*t*-test, $P < 0.002$): threshold for spike generation: -38 ± 1 mV, -34 ± 0.8 mV; duration: 238 ± 20 ms, 500 ± 48 ms; peak amplitude: -12 ± 2 mV, -17 ± 0.7 mV; slope of upstroke: 0.8 ± 0.1 mV/ms, 0.4 ± 0.06 mV/ms; and afterhyperpolarization: -51 ± 1.0 mV, -43 ± 1.0 mV. The underlying ionic basis for these differences and the significance in terms of regulation of secretion remains to be determined.

In both cell types removal of bath sodium caused a hyperpolarization that resulted in cessation of spontaneous action potentials and was associated with an increase in input resistance. However, the magnitude of the hyperpolarization was larger in lactotrophs (ΔV_M = -22 ± 2.5 mV, n = 25) than somatotrophs (ΔV_M = -13 ± 1.4 mV, n = 14). On the other hand, somatostatin-induced hyperpolarization in somatotrophs (ΔV_M = -18.5 ± 1.9 mV, n = 18) was larger than quinpirole-induced hyperpolarization in lactotrophs (ΔV_M = -13 ± 1.0 mV, n = 11). This suggests a difference in the relative proportion of background sodium current versus agonist-modulated inward-rectifier current expressed in these cells.

This work was supported by NSF grant No. IBN-9319536.

534.6

CELL-TO-CELL PROPAGATION OF CALCIUM SIGNALS DUE TO ELECTRICAL ACTIVITY IN ACUTE ANTERIOR PITUITARY SLICES. N. C. Guérineau*, L. Stoeckel, F. Mercier and P. Mollard.
INSERM U401, Rue de la Cardonille, 34094 Montpellier Cedex, France.

Individual endocrine pituitary cells, like neurons, generate action potential-linked [Ca2+]i transients leading to exocytosis. However, the exact role of membrane excitability in the entire gland is still unknown. We therefore studied calcium signals and associated electrical events in acute slices from guinea-pig anterior pituitary. Cells within cell cords are surrounded by basal membrane and conjonctive tissues. Spontaneous short-lived [Ca2+]i transients were detected in many cells using real-time (30 to 480 frames / sec) confocal Ca2+ imaging (fluo-3 as fluorescent Ca2+-sensitive probe). These transients were presumably due to electrical activity, since action potential firing was found in ~ 80% of patch-clamped cells. In parts of the slices, neighboring cells (up to 4) showed spontaneous [Ca2+]i transients that occurred synchronously. Cells behaved like somatotrophs since somatostatin (100 nM) was effective in blocking synchronized [Ca2+]i transients. The involvement of gap junctions was investigated using octanol, a gap junction blocker. In some cases, octanol (1 mM) blocked cell-to-cell propagation of [Ca2+]i transients. No direct effect of octanol on [Ca2+]i spiking was noticed. Dye-coupling studies using neurobiotin (1% in the patch-pipette solution) showed that ~ 40% of cells were coupled to neighboring partners belonging to the same cell cord. Taken together, these data show that excitable endocrine (GH) cells synchronize their spontaneous [Ca2+]i transients *via* gap junctions within the anterior pituitary gland.

This work was supported by grants (to P.M.) from INSERM, FRM, ARC, Région Languedoc-Roussillon, and French Ministry of Education and Science (ACC-SV11).

534.7

DIFFERENTIAL G PROTEIN-MEDIATED COUPLING OF SOMATOSTATIN RECEPTORS TO POTASSIUM AND CALCIUM CURRENTS IN PRIMARY CULTURED OVINE SOMATOTROPHS. C. Chen*. Prince Henry's Institute of Medical Research, P. O. Box 5152, Clayton, Victoria 3168, Australia.

We have shown that Go2 protein mediates the reduction in Ca^{2+} currents by somatostatin in primary cultured ovine somatotrophs (Chen & Clarke. J. Physiol. 491:21-29, 1996). To investigate the role of G proteins in the augmentation of voltage-gated K^+ currents by somatostatin, classical whole-cell recordings were made on ovine somatotrophs identified by post-recording immunocytochemistry. Using Ca^{2+}-free, TTX-containing (1 μM) bath solution and K^+-containing pipette solution, outward transient (I_A) and delayed rectifying (I_K) K^+ currents were recorded. Both I_A and I_K were reversibly increased by local application of somatostatin (10 nM) onto the recorded cells. After intracellular dialysis of GTP-γ-S (0.2 mM) via whole-cell patch pipette, the increase in K^+ currents by somatostatin became irreversible. However, dialysis of GDP-β-S (0.2 mM) significantly reduced the increase in K^+ current by somatostatin. These data indicate an involvement of G proteins in the alteration of K^+ current by somatostatin. In order to study the role of different subtypes of G protein, specific antibodies to various α subunits of G proteins were dialysed into the cells. Effective dialysis was confirmed by immunofluorescence staining of the antibodies in recorded cells. Intracellular dialysis of the antibodies to αi, but not that to αo, significantly attenuated the increase in K^+ currents by somatostatin. The blocking effect on the response to somatostatin was more obvious with αi3 antibodies than αi1-2 antibodies. These results suggest that Gi proteins (probably Gi3) mediate the effect of somatostatin on K^+ currents in ovine somatotrophs in primary culture whereas Go2 protein mediates the effect on Ca^{2+} currents. (Supported by Australian NH & MRC)

534.8

EXPRESSION OF GALANIN RECEPTOR mRNA BY SOMATOSTATIN NEURONS IN THE HYPOTHALAMUS OF THE MALE RAT. E. Grafstein-Dunn, R.A. Steiner, and D.K. Clifton*. Departments of Obstetrics & Gynecology and Physiology & Biophysics, University of Washington, Seattle, WA 98195

Galanin (GAL) is expressed in growth hormone-releasing hormone (GHRH) neurons in the arcuate nucleus (ARC) and has been implicated in the control of growth hormone (GH) secretion; however, the target cells for GAL's action in the hypothalamus are unknown. Because GHRH neurons make synaptic contact with the somatostatin (SS) neurons that regulate GH—those with cell bodies in the periventricular nucleus (PeN)—we postulated that GAL acts at these synapses to influence GH secretion. To examine this possibility, we sought expression of GAL receptor (GAL-R1) mRNA in hypothalamic SS neurons, using double-label *in situ* hybridization. We used a digoxigenin-labeled cRNA probe to identify SS mRNA-expressing neurons. A ^{33}P-labeled GAL-R1 cRNA probe was used to estimate cellular GAL-R1 mRNA levels by counting silver grains over SS neuronal cell bodies in the PeN, ARC, and cortex. GAL-R1 mRNA signal levels (grains/cell) over SS neurons in the PeN were significantly greater than background ($p<0.0001$), whereas signal levels over SS neurons in the cortex and ARC could not be distinguished from background. *Conclusion*: GAL released from GHRH neurons in the ARC can act directly on SS neurons whose cell bodies reside in the PeN. Based on GAL's putative cellular mechanisms of action, we infer that GAL inhibits SS secretion and thereby reduces SS's inhibition of GH secretion. *(Supported by NIH grants R01 HD29039 and R01 HD27142)*

NEUROENDOCRINE REGULATION: PROLACTIN

535.1

EFFECT OF CONTINUOUS AND DISCONTINUOUS ESTROGEN TREATMENT ON PROLACTIN (PRL) mRNA IN THE RAT PITUITARY AND BRAIN.
A. Dutt*, H. Bergen and D. W. Pfaff. Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA 19104; Dept. of Anatomy, Univ. of Manitoba, Winnipeg, Canada and Dept. of Neurobiology and Behavior, The Rockefeller University, New York, NY 10021.

Besides the expected expression of the PRL gene by lactotrophs, surprisingly robust demonstrations of immunoreactive PRL have come from work with rat brain (Emanuele et al.,Brain Res.,421:255-262, '87; Harlan et al., Neuroendocrinology, 49:7-22, '89), where PRL can promote female reproductive behavior (Harlan et al., Science 219:1451-1453, '83). To investigate estrogenic influence on PRL mRNA in pituitary and brain, we have used in situ hybridization with PRL P-32 labelled riboprobes, and experiments designed with concepts derived from Harris and Gorski, Endocrinology 103:240-245, '78. Discontinuous estradiol-17β treatments (2 i.p. injections of 5 μg at hours 0 and 8) were compared with continuous treatment (10 μg estradiol benzoate s.c. in oil), sacrificed at hour 24. Control rats were treated with vehicle alone. In female rat anterior pituitary, both estrogen treatments raised PRL mRNA levels significantly, with continuous treatment yielding a larger effect. Examining female and male rat brain, small numbers of positive PRL mRNA expressing cells were detected. They were in medial hypothalamus (matching previous immunocytochemistry) and surprisingly, the amygdala. The magnitude of changes observed in pituitary PRL mRNA, suggest a difference in PRL responsiveness to discontinuous and continuous estrogen treatment.

Supported by The Rockefeller Foundation Fellowship to A. Dutt.

535.2

EFFECT OF ADENOSINE ON THE RELEASE OF PROLACTIN FROM THE ANTERIOR PITUITARY *IN VITRO*. W.H. Yu*, M. Kimura, A. Walczewska, J.C. Porter and S.M. McCann. Pennington Biomedical Research Center, LSU, Baton Rouge, LA 70808 and Department of OB/Gyn, University of Texas Southwestern Medical Center., Dallas, TX 75235.

Adenosine was identified as a pituitary cytotropic factor for hypothalamic dopaminergic neurons (Porter et al, 1995). It was reported that intraventricular injection of adenosine elevated plasma levels of prolactin (PRL) (Ondo et al, 1989). However, adenosine inhibited prolactin release in cultures of anterior pituitary cells (Schettini et al, 1993). To clarify the effect of adenosine on the release of PRL, we incubated hemi-anterior pituitaries with adenosine in the presence or absence of its receptor antagonists. After 1 h preincubation, hemipituitaries from adult male rats were incubated in fresh Krebs-Ringer bicarbonate (KRB) buffer containing adenosine, its receptor antagonist or both compounds. Incubation was continued for 3 h in a Dubnoff Incubator (50 cycles/min) in an atmosphere of 95% O_2-5% CO_2 at 37^0 C. The concentration of PRL in the medium was measured by RIA. Incubation with adenosine (10^{-3} to 10^{-10} M) significantly stimulated PRL release. A selective A1 adenosine receptor antagonist, 8-phenyltheophylline (8-PT), had no effect on PRL release by itself, but significantly inhibited the adenosine-induced PRL release at doses of 0.1, 1 or 10 μM. A more potent A1 receptor antagonist, 8-cyclopentyltheophylline (8-CPT), also suppressed PRL release when it was added to the medium together with adenosine. However, 8-(3-chlorotyryl) caffeine (CSC), an A2a receptor antagonist, had little or no effect on PRL release by itself or in the presence of adenosine. In conclusion, our results indicate that adenosine stimulates PRL release through the action of A1 adenosine receptors. (This work was supported by NIH grants DK43900 and MH51853.)

535.3

MELATONIN AND RHYTHMIC CHANGES OF TUBERO-INFUNDIBULAR DOPAMINERGIC NEURON ACTIVITY AND PROLACTIN SECRETION. Y.S. Chu, S.H.H. Chan*, and J.T. Pan. Inst. Physiol., Natl. Yang-Ming Univ., Taipei, Taiwan R.O.C.

A diurnal rhythm of tuberoinfundibular dopaminergic (TIDA) neuron activity correlated with prolactin (PRL) secretion was recently reported. Whether melatonin plays a role in its regulation was the focus of this study. Under regular lighting environment (LD=14:10), melatonin given in the morning dose- (0.01, 0.1, 1 mg/kg, ip) and time- (15, 30, 60 min) dependently stimulated TIDA neuron activity as determined by DOPAC or DOPA levels in the median eminence. Serum PRL levels were concurrently inhibited. Melatonin given in the late subjective day (1600 h) seemed to be more effective on stimulating the lowered TIDA neuron activity and on inhibiting the increased PRL level than that given in early subjective day (1000 h). Under constant lighting (LL), basal TIDA neuron activity was increased and no diurnal variation could be observed. The stimulatory effect of melatonin still persisted, though. Given 4 interspersed injections of melatonin between 1800 and 0400 h for 3 days could restore the TIDA rhythm in rats under LL. To further test if melatonin indeed possesses an entraining effect on TIDA neuron activity, daily injections of melatonin (1 mg/kg at 1800 h) were conducted in rats kept under regular lighting for 10 days. The results showed that the treatment significantly decreased the afternoon PRL surge and induced a phase advance of the diurnal change in TIDA neuron activity. In all, melatonin may indeed play a role in regulating the rhythmic activity of TIDA neurons, and in turn, the PRL secretion. (Supported in part by grant NSC 85-2331-B010-071).

535.4

ATRIAL NATRIURETIC PEPTIDE EXHIBITS A NEGATIVE MODULATION ON ANGIOTENSIN II'S STIMULATORY EFFECT ON TIDA NEURON ACTIVITY: NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES. J.T. Pan* and S.H. Yen. Inst. Physiol., Natl. Yang-Ming Univ., Taipei, Taiwan R.O.C.

Both atrial natriuretic peptide (ANP) and angiotensin II (AII) are located in the mediobasal hypothalamus and have been shown to affect the secretion of prolactin. Their effects on tuberoinfundibular dopaminergic (TIDA) neuron activity have not been reported and were the focus of this study. Intracerebroventricular injection of AII (0.01, 0.1 and 1 μg) dose-dependently stimulated TIDA neuron activity in long-term ovariectomized, estrogen-treated rats as determined by 3,4-dihydroxyphenylacetic acid (DOPAC) concentration in the median eminence. The stimulatory effect of AII (1 μg/rat, icv) was significantly inhibited by co-administered ANP in a dose-dependent manner (0.1, 1 and 10 μg/rat, icv). In study using single-unit recording of dorsomedial arcuate (DM-ARC) neurons in brain slices, AII stimulated 67.2% of 64 units recorded. Few units were inhibited (6.3%) by it and the rest were not responsive. ANP alone was mostly ineffective on DM-ARC neurons (60% of 50 units), and it stimulated or inhibited only a few DM-ARC neurons: 24% and 16%, respectively. When ANP was co-administered with AII to AII-responsive units, however, it significantly attenuated the effect of AII in 75.8% of 33 units. The rest were not effective. Results from both in vivo and in vitro studies indicate that ANP can have a negative modulation on the stimulatory effect of AII on hypothalamic TIDA neurons. (Supported in part by grant NSC 85-2331-B010-051).

535.5

A NOVEL INTRAPITUITARY ENDOCRINE AXIS; MELATONIN AND PROLACTIN.

Morgan PJ*, P, Barrett, Webster CA JG Mercer, A Ross, DG Hazlerigg. Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, AB21 9SB

It has been shown that melatonin may regulate seasonal cycles in plasma prolactin independently of the brain (Lincoln and Clarke 1994 J Neuroend 6:251-260). These results suggest regulation of prolactin secretion by melatonin may occur via the pituitary gland. In adult sheep melatonin receptors, detected through 2-[^{125}I]-iodomelatonin binding, are not expressed on the pars distalis (PD) cells, of the pituitary gland, but are present on the cells of the pars tuberalis (PT). This suggests indirect regulation of prolactin secretion and lactotroph activity by melatonin through the PT. In these studies, two end-points have been used to detect the release of biologically active factors secreted from ovine PT cells cultures. The first is the expression of c-fos mRNA in, and the second is the release of prolactin by, PD cell cultures. Using Northern blotting for the c-fos mRNA, and dual *in situ* hybridization labelling for c-fos and prolactin mRNA it was found that conditioned medium from PT cells increased c-fos expression in ovine lactotrophs relative to controls. By RIA, the release of prolactin was also increased by culture medium conditioned by PT cells relative to controls. Similar experiments performed using medium conditioned by PD cells had no effect relative to controls. These experiments suggest therefore that ovine PT cells release a factor which enhances both c-fos gene expression and the secretion of prolactin by PD cells. This represents a new endocrine axis within the pituitary gland. We have called the factor released by the pars tuberalis, 'tuberalin'. Chromatographic isolation of this factor is in progress.
This work was supported by research funding from SOAEFD.

535.6

CENTRAL EFFECTS OF PROLACTIN ARE INHIBITORY FOR MILK EJECTION IN CONSCIOUS LACTATING RATS. **Morales, MT*, Shapiro, E and Mena, F. Centro de Neurobiología, Universidad Nacional Autónoma de México, 04510 México D.F., México.**

Previous work showed that in urethane-anesthetized lactating rats centrally-administered PRL, i.e., intracerebroventricular (ICV) or intrathecally (IT), facilitated mammary contractility in response to exogenous oxytocin. These effects were exerted through inhibition of β-adrenergic neural elements regulating ductal tone within the mammary glands. The present experiments were designed to determine whether similar effects by PRL could be obtained in conscious than in anesthetized lactating rats upon normal milk ejection. The rate of milk ejection was determined by applying a 15 min period of suckling by the litter, following a 6h period of isolation. The results showed that centrally-administered PRL in these rats provoked a dose-related, long-lasting inhibition of milk ejection. Thus, opposite effects of the hormone could be obtained upon milk ejection in anesthetized and concious rats. Further experiments measuring ductal tone as a mean to determine the mechanisms involved, showed that in conscious rats this effect of PRL was associated with increased ductal constriction within the mammary glands. Also, this effect was mimicked by ICV administration of the β-adrenergic agonist isoproterenol, and could be prevented, by prior systemic or central administration to the mother of the ß-adrenergic blocker propranolol. On the other hand, when administered to anesthetized rats, ICV-PRL counteracted the effect of centrally-administered isoproterenol upon ductal tone. These results indicate that centrally-administered PRL may modulate adrenergic mechanisms regulating milk ejection in conscious rats. Such influence, however, may be reversed through an anesthesia-mediated action.

535.7

SOURCE OF DOPAMINERGIC CONTRIBUTIONS TO THE NEURO-ENDOCRINE REGULATION OF PROLACTIN SECRETION. J. Gelineau-van Waes, M. Cassens and C. A. Johnston*. School of Pharmacy, Dept. of Pharm. Sci., Univ. of Montana, Missoula, MT 59812.

Dopamine (DA) from both the tuberoinfundibular (TIDA) and tuberohypophyseal neuronal systems has been implicated in the tonic inhibitory control of prolactin (PRL) secretion. The relative contributions of these DA systems on PRL secretion were studied using an in vivo rat model in which the neurointermediate pituitary (NIL) was exposed (SHAM) or surgically removed (NILX), leaving the anterior pituitary (AP) and its vascular connections to the hypothalamus (TIDA neuronal control) intact. Two weeks following NIL surgery animals were treated with alpha-methylparatyrosine (AMPT, 250 mg/kg BW, ip) to inhibit DA synthesis, and blood samples were obtained at 5 min before, and throughout the next 4 hr. In other animals the DA agonist, bromocriptine, was administered 2 hr after the AMPT injection to determine the effects of DA on PRL secretion. DA content and metabolism were analyzed in the median eminence and AP by HPLC with ED. DA content, metabolism, basal PRL, and the sensitivity of the AP PRL response to DA inhibition were not altered. However, the removal of DA inhibition produced a much greater increase in plasma PRL in SHAM animals than in NILX animals. The data suggest that the NIL contains a PRL-releasing factor which is tonically inhibited by DA, or that another PRL inhibitory factor plays a greater role in NILX rats. This work was supported by NIH grant, MH48228 (CAJ).

535.8

EFFECTS OF CHRONIC BROMOCRIPTINE TREATMENT ON HYPOTHALAMIC DOPAMINE FUNCTIONS DURING AGING. P. S. MohanKumar*, S. M. J. MohanKumar, S. K. Quadri, and J. L. Voogt. Department of Physiology, University of Kansas Medical Center, Kansas City, KS, Department of Anatomy & Physiology, Kansas State University, Manhattan, KS 66506.

Tuberoinfundibular dopaminergic (TIDA) neurons constitute a major hypothalamic dopaminergic system involved in the regulation of prolactin (PRL) through negative feedback mechanism. DA from the TIDA neurons inhibits PRL, which in turn, results in decreased activity of TIDA neurons. This study was done to investigate the effects of prolonged hypoprolactinemia produced by bromocriptine (BC), a dopamine agonist, on TIDA neuronal activity in the median eminence (ME) of middle-aged rats. BC was dissolved in alcohol, mixed with drinking water and given to 10-12 months old female Sprague-Dawley rats. The final concentration of BC was 25 μg/ml of water containing 1.7% alcohol. Control rats received water-alcohol mixture only. After 6 months of treatment, TIDA activity was estimated by determining tyrosine hydroxylase (TH) activity in the stalk ME on the basis of accumulation of l-dopa after administration of m-hydroxybenzylhydrazine (NSD 1015), a dopa decarboxylase inhibitor. TH activity (l-dopa, pg/μg protein) in BC-treated rats (11.9±1.4) was significantly lower than that in the control rats (19.6±1.8; $p<0.01$). The incidence of spontaneous mammary tumors in the BC-treated rats (5%) was markedly lower than that in the control rats (37%), strongly suggesting that BC-treated rats were hypoprolactinemic. These results indicate that no compensatory mechanisms are initiated by TIDA neurons to overcome the chronic loss of stimulatory effects of PRL. Supported by NIH grant AG 11561.

535.9

NALOXONE INCREASES HYPOTHALAMIC TYROSINE HYDROXYLASE ACTIVITY AND mRNA DURING LACTATION, L.A. Arbogast* and J.L. Voogt, Dept. of Physiology, University of Kansas Medical Center, Kansas City, KS 66160

Naloxone (NAL), an opioid antagonist, blocks the suckling-induced PRL surge, indicating the endogenous opioid peptides (EOP) contribute to the control of PRL release during lactation. Yet, it is not clear if the EOP influence PRL secretion by reducing dopaminergic input. The aims of this study were: 1) to assess the effect of NAL on suckling-dependent PRL release and 2) to determine if NAL infusion alters TH activity in the stalk-median eminence (SME) and TH mRNA levels in the arcuate nucleus. Exp 1: Lactating rats 7 days postpartum were constantly exposed to pups during the experiment. NAL (60 mg/kg/h) or saline was infused for 12h. Blood was collected before and at 2h intervals during the infusion. TH activity was assessed by the accumulation of 3,4-dihydroxyphenylalanine (DOPA) after m-hydroxybenzylhydrazine (25 mg/kg, iv). TH mRNA levels were evaluated by *in situ* hybridization. NAL lowered plasma PRL to <36% of control values at 4, 6, 8 and 12h. TH activity in the SME increased 2.5-fold after 12h NAL treatment. TH mRNA content in the arcuate nucleus of NAL-treated rats was greater than control rats. Litter (8 pups) weight in the NAL-treated group did not change during the 12h infusion, but control litters gained 5g. Both groups of dams spent equal time with the pups. Exp. 2: Pups were removed from the dams before the 12h NAL infusion and were returned after 11h. Blood was collected before the infusion, at 3h intervals during pup separation and at 15, 30, 45 and 60 min after reunion with the pups. Plasma PRL in control and NAL-treated rats was low (1-15 ng/ml) during the separation period. The suckling-induced PRL surge in NAL-treated rats was attenuated to 9-25% of control levels (350-650 ng/ml). After a 1h suckling episode, TH activity in the SME of NAL-treated rats was 4.5-fold greater than controls. Litter weight gains were significantly less in NAL-treated rats. These data indicate that the EOP are an integral component for increasing PRL in response to suckling and act to decrease tubero-infundibular dopaminergic neuronal activity during lactation, in part by suppressing TH gene expression. Supported by HD24190 and KUMC Research Institute.

535.10

CIRCADIAN CHANGES OF CENTRAL DOPAMINERGIC NEURON ACTIVITY AND ITS DEVELOPMENTAL ASPECT K.R.Shieh, L.M. Mai*, and J.T. Pan. Inst. Physiol., Natl. Yang-Ming Univ., Taipei, Taiwan ROC

We recently reported that a diurnal change of the tuberoinfundibular dopaminergic (TIDA) neuron activity exists in both intact and ovariectomized (OVX) female rats with or without estrogen replacement. However, a complete 24-h profile of TIDA and other central DA neurons' activities were not determined. Long-term OVX rats plus estrogen treatment for 6 days were decapitated every 4 h from 0100 to 2100 h. Either DOPAC or DOPA levels in the median eminence (ME), striatum (ST) and nucleus accumbens (NA) were determined in two groups of rats. Significant decreases of both ME DOPAC and DOPA levels were observed at 1700 and 1900 h. The dramatic change occurred at two times of the day, i.e., between 1300 and 1700 h and between 2100 and 0100 h. The activities of DA neurons in the ST and NA were significantly higher during the dark (2100-0500 h) compared with those during the light (0900-1700 h) phase. The rhythm of TIDA neuron activity has been correlated with the initiation of estrogen-induced PRL surge; while the rhythms observed in the ST and NA may be related to the animal's locomotor activity. We further determined the diurnal variations of TIDA neuron activity from pre- (28-, 35-, 39-day old), peri- (42-day) to post- (49-day) pubertal female rats. There was a gradual increase in basal TIDA neuron activity during the development and the diurnal rhythm occurred only after puberty. Thus, this pre-determined, sex-specific rhythm of TIDA neuron activity appears to develop peripubertally.

(This study was supported in part by grant NSC 85-2331-B010-071).

535.11

RT-PCR ANALYSIS OF D1 DOPAMINE RECEPTOR SUBTYPES IN TURKEY BRAIN. S.A. Schnell,* S. You, D.N. Foster and M.E. El Halawani. Dept. of Animal Science, Univ. of Minnesota, St. Paul, MN 55108.

VIP is a prolactin-releasing factor in birds. Recently, our laboratory has shown that D1-specific dopamine receptor agonists stimulated vasoactive intestinal peptide (VIP) release from turkey hypothalamus. The genes encoding chicken D1A, D1B,and D1D receptor subtypes have been cloned and pharmacologically characterized. We have used the reverse transcription-polymerase chain reaction (RT-PCR) technique to determine the mRNA expression of D1 subtypes in various turkey brain regions.

Two μg total RNA from turkey cortex (CTX), cerebellum (Cb), hypothalamus (hypo), diencephalon (die), medulla (med), brainstem (BS), spinal cord (SC), pituitary (pit), pineal (pin), olfactory bulb (OB) and optic chiasm (OC) were reverse transcribed. Oligonucleotide primers specific for the carboxy terminus of chicken D1A, D1B or D1D dopamine receptors were used to generate a single turkey PCR product corresponding to the size predicted for each of the chicken D1 cDNAs. All of the central nervous system regions examined revealed the presence of each of the D1 receptor subtyes. The products from the D1A and D1B subtypes were subcloned, sequenced and shown to be >90% homologous to their chicken counterparts and were used as probes to determine the relative mRNA amounts in the different brain regions. The rank order for D1A mRNA: CTX>hypo>BS>SC>die>Cb>pin>med>OC>OB>pit; D1B mRNA: CTX>hypo>die>SC>OC>BS>Cb> pin>med>OB>pit.

Dopamine inhibits the release of PRL birds, mainly through tonic inhibition via D2 dopamine receptors. The present results indicate a possible modulatory role for dopamine on the release of prolactin in birds by D1 receptors as well. Supported by USDA grant 94-37203-0897.

535.12

REPRODUCTIVE EXPERIENCE INCREASES STRIATAL AND HYPOTHALAMIC DOPAMINE LEVELS IN PREGNANT RATS. [1]L.F.Felicio*, [1]J.C.Florio, [1]L.H.Sider, [1]P.E.Cruz-Casallas and [2]R.S.Bridges. [1]Department of Pathology, School of Veterinary Medicine, University of São Paulo, Brazil; [2]Department of Comparative Medicine, Tufts University, School of Veterinary Medicine, North Grafton, MA, USA.

Effects of parity on dopaminergic function of rats were studied. Striatal and hypothalamic levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine (NE), serotonin (5HT) and 5-hydroxyindoleacetic acid (5HIAA) as well as serum prolactin (PRL) levels of 7 days primi- and multigravid rats were compared. Brains and trunk blood were collected from 1200 to 1400 h on day 7 of pregnancy and assayed for monoamines and their metabolites, and prolactin, respectively. Multigravid rats showed a significant increase in striatal and hypothalamic dopamine levels ($p<0.05$). A tendency to increase in striatal DOPAC ($p=0.06$) levels was also observed in multigravid rats. Levels of other neurotransmitters and metabolites were not statistically different. Haloperidol (1 mg/kg) treatment induced a significant increase in multigravid 5HT striatal levels. There was no statistical difference among primi- and multigravid serum PRL levels after either saline or haloperidol treatment. These data suggest that prior parity produces a shift in dopaminergic activity in multigravid rats.

Supported by NIH, FAPESP and CNPq

535.13

FUNCTION OF GALANIN IN THE ANTERIOR PITUITARY OF ESTROGEN-TREATED FISCHER 344 RATS: AUTOCRINE REGULATION OF PROLACTIN SECRETION. A. Cai* and J.F. Hyde, Dept. Anatomy and Neurobiology, Univ. Kentucky College of Medicine, Lexington, KY 40536.

Galanin peptide has been localized in lactotrophs, somatotrophs and thyrotrophs in the anterior pituitary of female rats. In order to obtain precise information of the specific population of galanin-expressing cells and to quantify the estrogen-induced changes in galanin cell phenotypes, we performed dual *in situ* hybridization to detect the colocalization of galanin mRNA with PRL and GH mRNAs in the anterior pituitary. Female Fischer 344 rats (n=6) were ovariectomized and half of the animals were implanted with an estradiol capsule s.c. After two weeks, animals were euthanized and dispersed anterior pituitary cells were prepared. Dual *in situ* hybridization was performed using ^{33}P-UTP (galanin) and digoxigenin-UTP (PRL or GH) labeled cRNA probes. In ovariectomized rats galanin mRNA signals were not detectable. In contrast, in estrogen-treated animals galanin mRNA was localized in 31% of lactotrophs and 2.3% of somatotrophs. Galanin-expressing cells, lactotrophs and somatotrophs were 16%, 51%, and 13.5% of the total cells in the anterior pituitary, respectively. Within those detectable galanin-expressing cells, more than 90% of cells coexpressed PRL mRNA and less than 5% of the cells coexpressed GH mRNA. Our data confirm that estrogen treatment dramatically increases the number of galanin-expressing cells in the anterior pituitary and the level of galanin gene expression in the individual cells. Galanin-expressing cells are mainly lactotrophs suggesting that galanin may contribute to the heterogeneous secretion of prolactin from lactotrophs, and galanin may regulate prolactin secretion in an autocrine manner. To support this hypothesis we are performing the reverse hemolytic plaque assay (prolactin secretion) combined with *in situ* hybridization (galanin mRNA) to measure the correlation of prolactin secretion and galanin gene expression in the same cell. Supported by NIH grant DK-45981 (J.F.H).

535.14

ELEVATED LEVELS OF CIRCULATING GROWTH HORMONE-RELEASING HORMONE ALTERS LACTOTROPH FUNCTION IN THE HUMAN GHRH TRANSGENIC MOUSE. J.P. Moore, Jr.*, A. Cai, and J.F. Hyde, Dept. Anatomy and Neurobiology, Univ. Kentucky College of Medicine, Lexington, KY 40536.

Human growth hormone-releasing hormone (hGHRH) transgenic mice sustain a twenty-fold increase in circulating hGHRH, a sixteen-fold increase in plasma growth hormone (GH), and no difference in plasma prolactin levels when compared to non-transgenic siblings. After ten months of age an adenoma begins to develop within the anterior pituitary (AP) of hGHRH mice which is described as consisting primarily of somatotrophs. We have recently reported that GH as well as galanin secretion is significantly increased from individual pituitary cells of the hGHRH mouse as measured by the cell immunoblot assay (CIBA). This study focuses on the regulation of prolactin production and secretion within the AP of the 5 month old, male hGHRH mouse. We first performed the CIBA and discovered no significant differences in the amount of prolactin secreted from individual pituitary cells from transgenic and control mice. Preliminary studies utilizing dual *in situ* hybridization have revealed a significant increase in the number of lactotrophs and an increase in the number of mammosomatotrophs within the AP of the hGHRH mouse, yet plasma prolactin levels remained unchanged. To further investigate this discrepancy, we administered NSD-1015 (100 mg/kg, i.p.), a DOPA decarboxylase inhibitor, to both transgenic and control mice and observed a significant 2-fold increase in DOPA accumulation within the median eminence of the hGHRH mice, suggesting an elevation in dopamine production and secretion from the hypothalamus of transgenic mice. Taken together, these data suggest that elevated levels of hGHRH or GH can expand the population of prolactin-producing cells and can affect hypothalamic dopamine neurons regulating prolactin secretion from the AP of the hGHRH transgenic mouse. (Supported by DK-45981 and HD-07436.)

536.1

ESTRUS MODULATES STRESS EFFECTS ON BEHAVIORAL DESPAIR AND IMMUNE FUNCTION IN FEMALE RATS S.E. Hendricks*, J.Wieseler, A. Cyr, M.Frank, M. Nakamura, M. Menolascino, D. Johnson. Dept. of Psychol., Univ. Neb. at Omaha, and Dept. of Path. & Microbiol. and Dept. of Psychiatry, Univ. Neb. Med. Cntr., Omaha, NE 68198.

The forced swim test (FST) has been demonstrated to induce changes in behavior taken to reflect despair or depression that are sensitive to antidepressants. Specifically, the increased immobility observed in the second swim trial is reduced by prior exposure to antidepressants. Previous research has demonstrated that female rats in estrus are more resistant to some of the consequences of stress compared to anestrous rats. Among responses often observed to stress are reductions in immune competency particularly as measured by natural killer cell cytolytic activity (NKCA). Previous research in our laboratory demonstrated reduced NKCA in rats exposed to the FST. In the present study we evaluated effects on behavior in the FST and on NKCA in estrous and anestrous female rats. All rats were ovariectomized. Estrus was induced by 5 μg estradiol benzoate followed 40 hrs later by 500 μg progesterone. Rats were exposed to the first swim trial of the FST 6 hrs after the progesterone injection. Rats exposed to the first swim trial while in estrus, when exposed to the second trial 24 hrs later, exhibited less immobility than rats exposed to the first swim trial while in an anestrous state. Also, rats exposed to the first swim trial while in estrus exhibited higher levels of NKCA after the first swim trial compared to rats exposed to the first swim trial while anestrus. (Supported by: Nebraska Research Initiative and UNMC Dept. of Psychiatry)

536.2

CYTOKINE LEVELS IN MAJOR DEPRESSSIVE DISORDER. A. B. Negrão, J. Licinio*, M.-L. Wong, P. Bongiorno, T. D. Geracioti, P. W. Gold, M. Kling. Clinical Neuroendocrinology Branch, NIMH. NIH. Bethesda, MD 20892-1284.

Dysregulation of hypothalamic-pituitary-adrenal (HPA) function is an element in the pathophysiology of major depressive disorder. The cytokine IL-1ß can potently stimulate the HPA axis, and functions as a communication signal between the peripheral immune system and the central nervous system. In order to determine whether neuro-immune interactions might have a role in the pathophysiology of major depression we examined cytokine levels in patients meeting DSM-III-R criteria for major depressive disorder (MDD). Using a high sensitivity enzyme-linked immunoassay we measured circulating IL-1ß levels in 16 healthy controls, and 14 depressed patients, 7 of whom were re-studied during the course of antidepressant treatment. We found that IL-1ß levels were 0.33±0.13 pg/ml in patients with MDD and 0.60±0.17 pg/ml in healthy controls (NS). During antidepressant treatment, there was a trend for a 20% increase in the basal plasma levels of IL-1ß. Further studies are being conducted to ascertain cytokine levels longitudinally in MDD.

536.3

MODERATE STRESS ENHANCES, AND CHRONIC STRESS SUPPRESSES, CELL-MEDIATED IMMUNITY *IN VIVO*. F. S. Dhabhar* & B. S. McEwen. Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

Delayed type hypersensitvity (DTH) reactions are antigen-specific, cell-mediated immune responses which mediate beneficial (resistance to viruses, bacteria, and tumors) and harmful (allergic dermatitis, autoimmunity) aspects of immune function. Contrary to the popular notion that stress suppresses immune function, we have demonstrated that moderate stress significantly enhances a cutaneous DTH response. We have also shown that a stress-induced trafficking of leukocytes to the skin may mediate this enhancement.

Here we investigate the effects of varying the duration, intensity, and chronicity of stress on the DTH response. DTH was induced by challenging the pinnae of previously sensitized rats with the chemical antigen 2,4-dinitro-1-fluorobenzene (DNFB). Animals moderately stressed (2h restraint) immediately before challenge showed a significant enhancement of DTH as measured by an increase in pinna thickness relative to the pre-challenge baseline. Increasing the duration of stress to 5h produced the same magnitude of enhancement as 2h restraint. Increasing the severity of stress (immobilization + mild shaking) produced an even greater enhancement of the DTH response. In contrast, increasing the chronicity of stress (6 h restraint/day, 6 days) significantly suppressed the DTH response. These results demonstrate a biphasic relationship between stress and immune function, such that moderate stress enhances, while chronic stress suppresses, an important class of immune responses in vivo. *Supported by: The MacArthur Foundation, The Arthur Vining Davis Foundation, and The DeWitt Wallace Foundation.*

536.4

IMMUNE AND BEHAVIORAL CORRELATES OF TYPICAL AND ATYPICAL DEPRESSION. J. Griffiths*, A.V. Ravindran, Z. Merali and H. Anisman. Dept of Psychiatry, Royal Ottawa Hospital, and School of Psychology, University of Ottawa, Ottawa, Ontario, Canada.

Although depressive illness has been associated with immune alterations, inconsistent and even contradictory data have been reported in this respect. It has been suggested that the appearance of immune variations may be related to the severity of depression. As well, the immune alterations may vary across subtypes of depression, given the marked differences of neurovegetative symptoms. In the present investigation we demonstrate that both typical major depressive (i.e., showing reduced eating, reduced sleep) and atypical patients (increased neurovegetative signs) show increased perception of day-to-day stresses, and the use of inadequate coping styles. Moreover, relative to controls, mitogen-induced cell proliferation was reduced in typical major depression, but not in atypical patients. Interestingly, in the atypical patients serum interleukin-1β levels were significantly higher than in controls or patients with typical depressive features. Following a 12 week course of antidepressant treatment (sertraline), IL-1 levels returned to control values in those patients that showed a positive therapeutic response. It is suggested that the immune alterations associated with depression are independent of stress/coping factors. However, the immune variations may be secondary to the symptoms characteristic of the illness, or common mechanisms may support the immune alterations and the neurovegetative features of major depressive illness.

Supported by the Medical Research Council of Canada and by Pfizer Canada Inc.

536.5

IMMUNE CORRELATES IN SUBTYPES OF DEPRESSIVE DISORDERS. A.V. Ravindran*, J. Griffiths, Z. Merali and H. Anisman. Dept of Psychiatry, Royal Ottawa Hospital, and School of Psychology, University of Ottawa, Ottawa, Ontario, Canada.

Major depressive disorder has been associated with immune alterations, including reduced mitogen-induced cell proliferation, diminished natural killer (NK) cell activity, increased interleukin-1β (IL-1), as well as variations of several lymphocyte subsets. While these immune alterations may be related to the mood disturbances, it is possible that the symptoms that characterize the disorder (e.g., anorexia, sleep disturbances, increased stress perception) contribute to the immune alterations. In the present investigation we assessed endocrine and immune alterations in major depressive and dysthymic (chronic, low grade depression) patients that exhibited either the typical (reduced sleep, reduced food consumption) or atypical symptom profile (increased sleep, increased eating). While plasma cortisol did not differ between groups, ACTH levels were modestly increased in major atypical depressive patients. The number of circulating NK cells was increased in all depressive groups, but this effect was greater in the major depressive than the dysthymic patients, and greater in typical than in atypical patients. Levels of IL-1 in supernatants of mitogen stimulated blood cells were also found to differ in the depressive subgroups. In particular, while IL-1 was reduced in typical major depressive patients, a pronounced elevation of IL-1 was evident in the atypical patients. It appears that both mood disturbances and neurovegetative symptoms contribute to the immune alterations associated with depression.

Supported by the Medical Research Council of Canada and by Pfizer Canada Inc.

536.6

SYSTEMIC ADMINISTRATION OF BACTERIAL LIPOPOLYSACCHARIDE (LPS) ENDOTOXIN INDUCES SYNTHESIS OF PITUITARY ADENYLATE CYCLASE ACTIVATING PEPTIDE (PACAP) IN CENTRAL NEURONES OF THE HYPOTHALAMO-PITUITARY-ADRENOCORTICAL (HPA)-AXIS OF THE RAT. P.J. Larsen*, D.S. Jessop†, J. Fahrenkrug‡, and J. Hannibal‡, Dept. Anatomy, Univ. Copenhagen, Denmark; †Dept. Medicine, Univ. Bristol, U.K., ‡Dept. Clinical Biochemistry, Bispebjerg Hospital, Copenhagen, Denmark.

The neurosecretory neurones of the HPA-axis constitutively express corticotrophin-releasing hormone (CRH), but when challenged by various stressors they respond with de novo synthesis of neuropeptides specifying the type of stress. Among numerous stressors, icv infusion of colchicine is the only one capable of eliciting PACAP mRNA expression in the neurones of the HPA-axis of the rat. Presently, we demonstrate that an ip injection of LPS (500 μg/animal), partly mimicking the immunological stress associated with bacterial infections, induces PACAP mRNA expression in medial parvocellular region of the paraventricular nucleus (PVN) which harbours the neurones of the HPA-axis. LPS induced expression of PACAP and CRH mRNAs in the PVN peaked 24 hours after the infusion. After the LPS challenge, PACAP-immunoreactive cells became visible in the PVN and a dense plexus of PACAP-immunoreactive nerve fibres emerged in the external layer of the median eminence, reflecting increased PACAP synthesis in the neurones of the HPA-axis. To further identify the signal(s) which mediate the LPS induced expression of PACAP synthesis in the HPA-axis, animals were injected intraperitoneally with either interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α). However, neither of these cytokines induced PACAP mRNA synthesis in the HPA-axis, irrespective of the fact that the employed doses (1μg) caused rises in plasma corticosterone similar to that obtained by LPS. In addition, both IL-1β and TNF-α robustly induced CRH mRNA expression in the PVN. In conclusion, the LPS-induced PACAP mRNA expression in the central neurones of the HPA-axis is independent of the cytokines believed to be released from macrophages exposed to LPS.

536.7

INCREASED TUMORIGENESIS IN A TRANSGENIC MOUSE MODEL OF THE ENDOCRINE CHANGES SEEN IN DEPRESSION CAN BE REVERSED BY ANTIDEPRESSANT THERAPY. J.M.C. Blom[a]*, N.Barden[b], D. Viglienghi[a], and G. Racagni[a]. [a]Center of Neuropharmacology, Univ. of Milan, 20133 Milan, Italy, and [b]Molecular Psychogenetics, CHUL, Québec, Canada.

Episodes of severe depression affect up to 5% of the general population and occur with a threefold higher frequency in patients with cancer. Given recent serious concerns regarding the carcinogeneity and the tumor promoting activity of antidepressants and the fact that antidepressants are prescribed for extensive periods of time, a transgenic mouse model of the endocrine changes observed in 60% of depressive patients characterized by partial knockout of glucocorticoid receptor gene expression, resulting in defective glucocorticoid feedback inhibition and HPA-Immune axis dysfunction, allowed to test the tumor promoting effects of this particular class of drugs in an animal that displays a pathophysiology similar to that observed in depression. Adult transgenic mice and intact controls were injected s.c. either with the chemical carcinogen 9,10 dimethylbenzanthracene (DMBA) or C-3 fibrosarcoma cells. Tumor incidence was 4 times higher in DMBA treated transgenics than in control animals (86% vs 23%). More importantly tumor incidence could be reversed by treatment with the antidepressant Desimipramine (33%). Similarly C-3 induced tumorigenesis was enhanced in transgenic mice as compared to control mice (60% vs 15%). Treatment of transgenics with DMI reduced tumor incidence by 50 % to only 30%. Furthermore, tumor incidence correlated with altered immune responsiveness, as measured by lymphocyte cell number and lymphoproliferative assays. These results indicate that in this animal model of depression where general defense mechanisms appear to be compromised, treatment with an antidepressant such as desimipramine, seemed to normalize and restore both neuroendocrine functioning as well as general defense mechanisms, resulting in decreased risk of tumor development.

536.8

CENTRAL α-MSH$_{(1-13)}$ BLOCKS STRESS-INDUCED ALTERATIONS IN TEMPERATURE, CORTICOSTERONE AND CBG. E.D. Milligan*, K. T. Nguyen, T. Deak, M. Fleshner, L. R. Watkins, and S. F. Maier. Dept of Psych. Univ. of Colorado, Boulder, CO., 80309.

Both intracerebroventricular (i.c.v.) IL-1β and exposure to acute stressors such as inescapable tail shock (IS) activate acute phase responses (APRs) such as fever and decreases in carrier proteins including corticosterone binding globulin (CBG). These and other data have led to the suggestion that stressors may produce acute phase as well as other responses by inducing brain IL-1β. The pro-opiomelanocortin derivative, alpha-melanocyte-stimulating hormone (α-MSH$_{(1-13)}$) when injected i.c.v., has been shown to antagonize a variety of IL-1β effects. We therefore sought to determine whether i.c.v. α-MSH$_{(1-13)}$ would block the effects of IS thought to be mediated by brain IL-1β. Male Sprague-Dawley rats received either 0.5μg in 2.5μl α-MSH$_{(1-13)}$ or vehicle prior to a single session of 100, 5 sec., 1.6mA, with a 1 min. ITI ISs delivered to the tail via fixed electrodes, or control treatment. Core body temperature (CBT), from probe implanted i.p., and plasma corticosterone (CORT), assessed by RIA, were measured at times of IS-pre-exposure, the 25th and 100th tail shock, and 2, 24, and 48 hours after IS. CBG levels from plasma at pre-IS exposure and 24-hour post-IS exposure were measured by RIA. α-MSH$_{(1-13)}$ had no effect on CBT or the rise in CORT during IS. However, α-MSH$_{(1-13)}$ blocked the elevated CBT and basal CORT levels, and blocked the reduced levels of CBG and food intake, observed 24 hours after IS. These data provide further indirect support for a role of brain IL-1β in mediating these outcomes of IS.
Supported in part by grants, NIMH MH 45045 and NIH MH 55283.

536.9

EVIDENCE FOR ACUTE PHASE ACTIVATION FOLLOWING EXPOSURE TO AN ACUTE STRESSOR. T. Deak*, J.L. Meriwether, M. Fleshner, R.L. Spencer, A. Abouhamze[1], L.L. Moldawer[1], L.R. Watkins, & S.F. Maier. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309, [1] Dept. of Surgery, Univ. of Florida, Gainesville, FL 32610.

We recently reported that exposure to a single session of inescapable shock (IS) produces a reduction in corticosteroid binding globulin (CBG) 24 hrs later (Fleshner et al., 1995). Subsequent studies have shown that this effect is independent of glucocorticoid regulation. Since CBG is known to be a negative reactant of the acute phase response (i.e., circulating CBG is *decreased* by at least 25% during acute phase activation), we investigated the possibility that exposure to IS leads to activation of the acute phase response. After a baseline blood sample was taken from a lateral tail vein (300 µl in <2 min), rats were exposed to 100 inescapable tail shocks (5 sec, 1.6 mA, 1 min ITI), and blood samples were taken 6 hrs and 24 hrs following IS. Analysis of samples demonstrated that haptoglobin and seromucoid protein levels were significantly increased, CBG levels were significantly decreased, and there were no changes in serum albumin or total serum protein levels 24 hrs after IS. Since fever is also used as an index for acute phase activation, we investigated whether IS exposure leads to elevated core body temperature (CBT). Rats were implanted with thermisters under halothane anesthesia and permitted to recover for 3 days prior to experimentation. Baseline CBT was recorded, and rats either remained in their home cages (HCC) or received IS. Subsequent readings of CBT were taken during IS, for 10 hrs following IS, and in the morning after IS. CBT was significantly increased during the shock session and for 1 hr immediately following IS treament. CBT was also significantly elevated in IS treated rats during the AM of the next day (p's<.05). All of these data support the hypothesis that IS exposure leads to activation of the acute phase response. Supported by NIH Grant MH45045.

536.10

STRESS-INDUCED NITRIC OXIDE SUPPRESSES SPECIFIC IMMUNITY IN KLH IMMUNIZED RATS. KT Nguyen*, M Fleshner, LR Watkins, & SF Maier. Department of Psychology, University of Colorado at Boulder, Boulder, CO, 80309

Nitric oxide (NO) has been implicated as an important mediator in a variety of immune functions. NO is suppressive to T cell, but not B cell, proliferation in response to ConA or alloantigen [Fleshner et. al. 1995, J. Neuroimmunology (56):45-52]. Previous findings from our laboratory have shown that exposure to inescapable tail shock (IS) suppresses T cell proliferation and expansion to antigen. We, therefore, investigated whether IS could increase NO production by lymphocyte/monocytes and in turn, result in a suppression of T cell expansion and T cell associated cytokines. Male Harlan Sprague Dawley rats were immunized with 200 µg IP of KLH and exposed to either IS (n=6) or returned to their home cages (HCC, n=6). Cells were collected from the peritoneal cavity, mesenteric lymph nodes, and spleen at 0, 48, & 96 hours after IS. Total cell numbers were assessed in each compartment. HCC animals showed an increase in cell number in response to KLH at 24 & 48 hours after immunization. In contrast, IS animals failed to show an increase in cell number in response to KLH. After 48 hours of cell culture, supernatants were harvested and assayed for nitrite & IFNγ. NO production was enhanced by IS compared to HCC. In contrast, HCC-IFNγ levels showed an increase in response to KLH, while IS-IFNγ levels failed to show this increase at both 48 and 96 hours. Given the recent finding from our laboratory showing that IS can suppress the expansion of Th1-like cells in response to an antigen, these findings suggest that IS-induced production of NO may play a role in the mechanism of stress-induced immunosuppression. Supported by NIH Grant MH45045.

536.11

CENTRAL GLUCOCORTICOID RECEPTOR DYSFUNCTION: PROFOUND CHANGES IN CIRCADIAN RHYTHM OF BODY TEMPERATURE AND LOCOMOTION, AND ALTERED NEUROENDOCRINE AND AUTONOMIC RESPONSES TO ENDOTOXIC STIMULATION. A.C.E. Linthorst*, S. Karanth, N. Barden[1], F. Holsboer and J.M.H.M. Reul. Max Planck Inst. of Psychiatry, Clinical Inst., Munich, Germany; [1]Molecular Psychogenetics Lab., Laval Univ., Quebec, Canada.

Recently, a transgenic mouse has been created bearing a transgene expressing antisense RNA directed against the glucocorticoid receptor (GR). In view of the intimate involvement of glucocorticoids in the cascade of responses following activation of the immune system, we investigated the consequences of impaired GR function for the physiological responses observed after an endotoxic challenge. First, plasma levels of ACTH, corticosterone and interleukin-6 (IL-6) were measured 1, 3, 6 and 24 hr after i.p. injection of endotoxin. We found that the transgenic mice show an amplified and prolonged endotoxin-induced increase in plasma ACTH, but not in corticosterone and IL-6. In a second set of experiments, mice were implanted with a biotelemetric transmitter for continuous recording of body temperature and locomotion. Transgenic mice showed markedly lower body temperature and locomotor activity levels during the dark phase of the diurnal cycle. I.p. administration of endotoxin caused a biphasic increase in body temperature in normal mice, returning to baseline levels ~15 hr after injection. In contrast, in transgenic mice endotoxin and saline produced similar increases in body temperature during the first 6 hr after injection. However, body temperature of endotoxin-injected transgenic mice started to rise after 6 hr and was still elevated 24 hr post-injection. In both strains the endotoxin produced an inhibition of locomotor activity and other signs of sickness. Our data demonstrate that central GR plays a pivotal role in the diurnal cycle of body temperature and behavioral activity and in the neuroendocrine and pyrogenic responsiveness to an endotoxic challenge.
Supported by the VW-Foundation (I/70 543)

536.12

TUMOR NECROSIS FACTOR-α, INTERLEUKIN-1ß AND INTERLEUKIN-2 PRODUCE DIFFERENTIAL EFFECTS ON HIPPOCAMPAL SEROTONERGIC NEUROTRANSMISSON, FREE CORTICOSTERONE LEVELS, BODY TEMPERATURE AND BEHAVIOR. S. Pauli*, A.C.E. Linthorst and J.M.H.M. Reul. Max Planck Inst. of Psychiatry, Clinical Inst., Munich, Germany.

Recently, we have shown that i.p. injection of endotoxin results in an activation of distinct central neurotransmitter pathways, rises in free corticosterone levels, fever and behavioral depression (sickness behavior). Brain interleukin (IL)-1 was found to participate in the endotoxin-induced increase in hippocampal serotonergic neurotransmission. Moreover, the endotoxin effects were largely mimicked by i.c.v. injected human IL-1ß. Because endotoxin induces apart from IL-1 also other proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-6, we included these cytokines in our studies. In addition, we investigated the effects of the non-inflammatory cytokine IL-2. *In vivo* microdialysis in freely moving rats was used to continuously monitor hippocampal extracellular levels of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), and free corticosterone. Biotelemetry was used to assess body temperature and locomotion. I.c.v. administration of mouse TNF-α produced a dose-dependent increase in free corticosterone levels and body temperature, but had no effect on hippocampal 5-HT and 5-HIAA. In contrast, i.c.v. injection of human IL-2 dose-dependently increased free corticosterone levels, body temperature and hippocampal concentrations of 5-HT and 5-HIAA. IL-2 produced behavioral depression, whereas TNF-α did not. Moreover, the effects of TNF-α and IL-2 on body temperature and free corticosterone followed different time courses. It may be concluded that cytokines produced as a result of immune and inflammatory challenges play differential but overlapping roles in the brain subserving homeostatic responses to cope with the challenge.
Supported by the VW-Foundation (I/70 543)

536.13

CILIARY NEUROTROPIC FACTOR IS A POTENT ANORECTIC CYTOKINE ACTING THROUGH HYPOTHALAMIC NEUROPEPTIDE Y
S.P. Kalra*, B. Xu, M. Dube, A. Kaibara, D. Martin and L.L. Moldawer, Depts. Neuroscience, Physiology and Surgery, Univ. of Fla. Col. Med., Gainesville, FL 32610 and Amgen, Inc., Boulder, CO 80301

Anorexia is one of the most common neurological manifestation observed during acute and chronic disease. Neither the identity nor the mode of action of cytokine(s) that induce chronic anorexia is known. We evaluated the effects and mode of action of the cytokine, ciliary neurotropic factor (CNTF), on daily food intake and body weight gain in male rats. Daily intracerebroventricular (icv) injection of CNTF (0.5, 2.0 or 5 μg/rat) produced a steady dose-dependent decrease in food intake and body weight over a 4-day treatment period. Food intake was suppressed by 80% with a concomitant loss of body weight by 20%. PreproNPY mRNA in the hypothalami of CNTF-treated rats was significantly less than in fasted or pair-fed controls ($p < 0.05$), and the characteristic fasting-induced increase in hypothalamic NPY gene expression was also blocked by CNTF. Further, acute, as well as chronic CNTF treatment markedly suppressed NPY-induced stimulation of feeding. Cumulatively, these results show that CNTF produces severe and persistent anorexia by decreasing both the synthesis as well as the post-synaptic stimulatory effects of hypothalamic NPY, the endogenous orexigenic signal. (Supported by NIH DK37273).

536.14

EPINEPHRINE STIMULATES IL-6 RELEASE IN MAJOR DEPRESSION AND HEALTHY CONTROLS
G. H. Pelton*, L. H. Price, R.T. Malison, G. R. Heninger, Clin. Neurosci. Res. Unit, Dept. of Psychiatry, Yale Univ. School of Med., 34 Park St., New Haven, CT. 06519.

In order to evaluate possible abnormalities in stress induced alterations in immune function in depressed patients, we have examined the interleukin-6 (IL-6) and cortisol (C) response to infused epinephrine (E) in six patients with Major Depression (MD) and four healthy controls (HC). **METHODS**: MD patients had HAM-D scores $\geq$18 and were drug-free for at least 3 weeks. All subjects underwent randomized double-blind testing with a 60-min continuous i.v. infusion of E 0.2 ug/kg/min or saline placebo (P). Plasma levels of E and norepinephrine, C, and IL-6 were measured, in addition to blood pressure (BP), heart rate (HR), and emotional state. **RESULTS**: In HCs, we previously reported a clear dose-response curve for effects of E on IL-6 levels (at E doses of 0.1, 0.15 and 0.2 ug/kg/min). At the 0.2 ug/kg/min dose, base-to-peak IL-6 change was significantly less in MDs than in HCs ($p<.05$), 5.5$\pm$3.4 pg/ml and 10.7 $\pm$3.4 pg/ml, respectively. Baseline IL-6 levels were not significantly different. E produced a mean base-to-peak increase in systolic BP of 12$\pm$ 15 mm HG and15$\pm$12 bpm and 15$\pm$15 mm Hg and HR 20$\pm$10 bpm in MD and HCs respectively, that were not significantly different. IL-6 plasma levels peaked at 90 min and between 90 min and 180 min, C level tended to increase ($p=0.061$) during E vs P. Positive correlations between peak IL-6 and peak HR or change in IL-6 and change in C were seen in HC but not in MD. There was no change in emotional state in either group during E. **DISCUSSION**: This is the first demonstration that peripheral E causes an increase in plasma IL-6 levels in both HC and MD human subjects. The results support the hypothesis that E directly affects the immune system (probalbly via Beta receptors) in HCs, which may be dysregulated in MD. E may also indirectly effect the HPA axis via increasing plasma levels of IL-6. These results suggest a novel mechanism which could explain immune and HPA axis dysregulation during stress and depression. **(Supported by NARSAD and the Stanley Foundation)**

536.15

MODULATORY EFFECTS OF GLUCOCORTICOIDS AND CATECHOLAMINES ON HUMAN INTERLEUKIN-12 AND INTERLEUKIN-10 PRODUCTION: POTENTIAL RELEVANCE FOR CHANGES OF THE TH1/TH2 BALANCE IN STRESS AND STATES ASSOCIATED WITH DYSREGULATION OF THE STRESS SYSTEM.
L.J. Elenkov, D.A. Papanicolaou, R.L. Wilder, and G.P. Chrousos*, DEB, NICHD and ARB, NIAMS, NIH, Bethesda, MD 20892.

To examine the potential effects of stress on the Th1/Th2 balance, we studied the ability of 3 prototype stress hormones, dexamethasone, norepinephrine and epinephrine, to alter the production of interleukin (IL)-12 (p70) and IL-10 induced by bacterial lipopolysaccharide (LPS) in human whole blood. Dexamethasone inhibited LPS-induced bioactive IL-12 production in a dose-dependent fashion and at physiologically relevant concentrations; it had no effect on IL-10 secretion. The glucocorticoid-induced reduction of IL-12 production was antagonized by RU 486, a glucocorticoid-receptor antagonist, suggesting that it was mediated by the glucocorticoid receptor. Norepinephrine and epinephrine also suppressed IL-12 production in a dose-dependent fashion and at physiologic concentrations: both catecholamines, however, increased, dose-dependently, the production of IL-10. The effects of either catecholamine on IL-12 or IL-10 secretion were completely blocked by propranolol, a b-adrenoreceptor antagonist, indicating that they were mediated by the b-adrenergic receptor. These findings suggest that the central nervous system may regulate IL-12 and IL-10 secretion, and, hence, the Th1/Th2 balance via the peripheral end-effectors of the stress system. Stress may, thus, cause a shift towards Th2, rather than generalized Th suppression. This shift may be responsible for the stress-induced increase of susceptibility of the organism to certain infections, and may influence their course. In addition, through this mechanism, states associated with chronic hyper- or hypo-activity of the stress system may influence the susceptibility of an individual to certain autoimmune, allergic, infectious or neoplastic diseases.
Source of funding: NICHD and NIAMS intramural.

536.16

CENTRAL AMYGDALA LESIONS REDUCE BEHAVIORAL SUPPRESSION AFTER ENDOTOXIN.
N.C. Tkacs*. Depts. of Psychiatry and Neurosciences. UMDNJ New Jersey Medical School and Grad. School of Biomedical Sciences, Newark, NJ 07103.

Administration of bacterial endotoxin (ETX) to rats induces centrally-mediated responses including fever, stress hormone release, and behavioral suppression. This experiment tested the hypothesis that the central nucleus of the amygdala (CeA) mediates the behavioral response to ETX.

Behavioral and febrile responses to IV E. coli ETX 10 μg/kg were recorded from rats with electrolytic or sham CeA lesions. Movements between two halves of a plexiglass chamber were recorded for 5 minutes, two hours after ETX injection. The rats were divided into three groups, based on activity data: SHAM (n=4), MISS (activity after ETX=shams, n=3), and LESION (activity after ETX>shams, n=5). Results (activity): SHAM=6.2$\pm$0.9 counts; MISS=5.7$\pm$1.3; LESION=15.8$\pm$1.7. ANOVA revealed a significant effect of lesion on activity ($F=17.02$, $p=.0009$). Fever was slightly but not significantly higher in SHAM rats, while MISS and LESION rats had identical fever profiles. Histological analysis showed that LESION rats had bilateral ablation of the mid-region of the CeA, 2.3 - 2.8 mm behind bregma) with sparing of the rostral and caudal poles of CeA. In MISS rats, bilateral damage only included the caudal CeA with unilateral sparing of the mid-region. These results support the hypothesis that the CeA mediates behavioral responses to systemic ETX treatment, and demonstrates a possible dissociation between fever and behavioral responses to ETX.
(Support: NR03801)

536.17

EFFECTS OF INTERFERONS ON CATECHOLAMINE SECRETION FROM BOVINE ADRENAL CHROMAFFIN CELLS.
E. Tachikawa*, Y. Kondo, K. Kudo and T. Kashimoto. Dep. of Pharmacol., Sch. of Med. Iwate Med. Univ., Morioka 020, Japan.

When bovine adrenal chromaffin cells were previously exposed to human recombinant interferon (IFN) -α (1,500 units/ml) for 72 hrs, the secretion of catecholamines from the cells stimulated by acetylcholine (ACh) (25 μM) was greatly reduced. On the other hand, human fibroblast IFN-β and recombinant human IFN-γ had no effect on the secretion. The IFN-α inhibition was concentration (30-1,500 units/ml) - and time (18-96 hrs) -dependent manners. IFN-α also inhibited ACh-induced Ca^{2+} influx into the cells, whereas it inhibited neither Ca^{2+} influx into nor secretion from the cells induced by high K^+. The inhibitory effect of IFN-α on the secretion was completely overcome by the equivalent human anti-IFN-α antibody and by the increase in ACh concentration (200 μM) but not by the increase in the external Ca^{2+} concentration.

These results indicate that IFN-α affects nicotinic ACh receptors, and reduces the Ca^{2+} influx and the consequent secretion of catecholamines in bovine adrenal chromaffin cells stimulated by ACh. The results further suggest that immune systems may regulate the function of autonomic nervous systems via the immunotransmitter, IFN-α, and that the side effects occurring during IFN-α therapy (depression, neurasthenica, somnolence, lethargy, anorexia, etc.) may be related to the IFN-α action on the chromaffin cells.

536.18

ATTENUATION OF BEHAVIORAL ABNORMALITIES IN AUTOIMMUNE MICE BY SOLUBLE IFN-γ RECEPTOR TREATMENT.
L.M. Schrott* and L.S. Crnic. Dept. of Pharmacology, Univ. of MN Med. School, Minneapolis MN 55455; Depts. Pediatrics and Psychiatry, Univ. of CO Med. School, Denver CO 80220.

Autoimmune NZB x NZW F1 hybrid (B/W) mice develop reduced exploratory drive and increased anxiety behavior compared to non-autoimmune NZW mice as their lupus-like disease progresses. The present study examined if treatment with murine soluble IFNγ receptor (sIFNγR), a blocker of an early step in the cytokine cascade which retards disease progression, would attenuate behavioral alterations of B/W mice. Female B/W (n=19) and NZW (n=18) mice were injected 3 times a week from 6 to 13 weeks of age with either sIFNγR (3.33 μg/g; gift of L. Ozmen, Hoffmann-LaRoche) or saline vehicle and tested in the elevated plus maze (EPM) at 12 weeks and the novel object (NO) task at 13 weeks of age. Although B/W mice were less active and exploratory and more anxious than NZW mice, as seen in prior studies, sIFNγR treatment significantly attenuated these effects. Compared to B/W saline controls, sIFNγR treated B/W mice were more active in EPM ($p<.02$) and NO task ($p<.006$) and showed decreased EPM anxiety behaviors with greater % open arm time ($p<.0004$), shorter closed arm visits ($p<.02$), and less difference between closed and open arm visit ($p<.008$). In the NO task sIFNγR treated B/W mice explored more, as they crossed more % center squares ($p<.0001$), reared more ($p<.02$), and had shorter contact latency ($p<.002$), more contacts ($p<.02$), and more contact time with the NO ($p<.009$) than B/W saline controls. NZW mice were unaffected by sIFNγR treatment. Thus, down-regulation of autoimmunity by sIFNγR treatment was effective in attenuating behavioral abnormalities of B/W mice and suggest that prior differences seen between B/W and NZW mice in anxiety and exploratory behaviors are the result of autoimmune disease processes rather than genetic differences. These findings may have implications for lupus patients suffering from psychiatric symptoms. Supported by NIH grants MH 10643, MH 49043, and HD 04024.

536.19

HYDROLYSIS OF LEUCINE ENKEPHALIN IN HUMAN PLASMA AFTER SEVERE TRAUMA. L. Bongiorno-Borbone*, A. Urbani, M. Marini, E. Trani, L. G. Roda. Dept. Medicina Sperimentale, Universita' "Tor Vergata", 00133 Roma, Italy.

Plasma hydrolysis of leucine enkephalin, and several cellular immunity parameters, have been evaluated in an homogeneous group of human subjects undergone severe trauma (proximal femur fracture). The possible relationship between the stressful event and the degradation of opioid peptides has been evaluated by chromatographic separation of the hydrolysis by-products formed in the presence of plasma obtained immediately after the trauma, and in controls obtained during a successive follow-up. By results obtained, trauma induces a net reduction in the amount of substrate hydrolyzed, as well as significantly modifying the pattern of the hydrolysis by-products formed. The analysis of these data indicates that the reduction of substrate hydrolysis is attributable in part to decreased activity of the enkephalin-degrading enzymes, in part to increased inhibition by the low molecular weight plasma inhibitors. In the same subjects, several of the immunological indexes measured underwent to modifications that can be considered related to stress. The lack of quantitative relationship between reduction of hydrolysis and modifications of immune parameters does not however allow to hypothesize the existence of direct dependence between these two sets of data.

536.20

IMPAIRED FIGHTING BEHAVIOR IN AUTOIMMUNE MRL-lpr MALES. Boris Šakić*, Leonard Gurunlian, Judah A. Denburg, and Henry Szechtman. Departments of Biomedical Sciences and Medicine, McMaster University, 1200 Main St. West, Hamilton, CANADA L8N 3Z5.

We have reported previously that lupus-prone MRL-lpr mice float excessively in the forced swim test (an animal model of "behavioral despair") and show blunted concentration-intake response in the sucrose test (an animal model of "anhedonia"). The results are consistent with the hypothesis that development of systemic autoimmune disease produces depressive-like behavior. The present study is a further test of the above hypothesis. Fighting behavior and social encounters in autoimmune MRL-lpr males and congenic MRL +/+ controls were measured. It was expected that MRL-lpr males will show reduced fighting behavior as autoimmune symptoms develop. Twelve-week old males (n=10/ substrain) were housed singly for 4 weeks. Isolation-induced aggressiveness was tested in a resident-intruder paradigm where each mouse was either the resident or the intruder across nine sessions (one session/day). Frequency and duration of social contacts (sniffing, mounting, touching) and aggressive encounters (wrestling, biting, chasing) were measured during 10-min session by custom-made computer software. Social contact did not differ between the MRL-lpr and MRL +/+ males. However, fighting was reduced in autoimmune MRL-lpr males, as evidenced by rare fighting episodes and short aggressive encounters. These results strengthen the hypothesis that autoimmune disease produces depressive-like behavior and suggest that central mechanisms which mediate aggressiveness are targeted by immune factor(s).

(Supported by NSERC. HS is a Research Associate of the Ontario Mental Health Foundation).

NEURAL-IMMUNE INTERACTIONS: CYTOKINES II

537.1

TRANSDUCTION PATHWAYS INVOLVED IN THE DOWNREGULATION OF IL-2 EXPRESSION BY VIP. Z. Xin*, L. Sun, and D. Ganea. CMBN and Biological Sciences, Rutgers Univ., Newark, NJ 07102.

Neuropeptides have been reported to modulate the immune response *in vivo* and *in vitro*. Previous studies from our laboratory indicated that VIP inhibits IL-2 production by murine T lymphocytes and thymocytes activated through T cell receptors. In EL-4.IL-2 cells, a murine thymoma cell line expressing VIP-R2, VIP does not inhibit IL-2 production following stimulation with PMA and calcium ionophores. These results suggest that VIP interferes with T cell receptor mediated signal transduction pathways. Here we first investigated whether VIP affects cellular tyrosine phosphorylation, and/or the activity of TCR-associated tyrosine kinases (lck^{56}, fyn^{59} and ZAP-70), which represent early events in T cell activation. Western blots using anti-phosphotyrosine antibodies showed that VIP does not alter tyrosine phosphorylation at three different time points. VIP does not influence the activity of lck^{56} as demonstrated by both immunoblot and immune complex kinase assays in the absence or presence of enolase. In contrast, VIP induces fyn^{59} activity in unstimulated cells. We also examined the effect of VIP on IL-2 transcriptional factors. The binding activity of NF-AT, but not NF-kB, AP-1, and the TGGGC-binding factor, is inhibited by VIP in electrophoretic mobility shift assays (EMSA). Since translocation and dephosphorylation of NF-AT is mediated by calcineurin, a calcium-calmodulin dependent phosphatase, we investigated the effect of VIP on calcineurin activity, and compared it to forskolin, another cAMP inducer, and cyclosporin A, a known inhibitor of IL-2 expression. NIMH grant MH49079

537.2

MOLECULAR CLONING OF THE cDNA CODING SEQUENCE OF IL-2 RECEPTOR-γ (γ_c) FROM MURINE FOREBRAIN AND HUMAN HIPPOCAMPUS: EXPRESSION BY ASTROCYTES AND NEUROBLASTOMA CELLS. J.M. Petitto[1], Z. Huang[1], C.M. Rinker[1], M.K. Raizada[2], D.L. Evans[1]* and D.B. McCarthy[1]. Departments of Psychiatry[1] and Physiology[2], University of Florida, Gainesville, FL, 32610.

IL-2 has been implicated in various neurobiological processes of the mammalian CNS. To understand how IL-2 acts in the brain, our lab has sought to determine the molecular pharmacological characteristics of brain IL-2 receptors (IL-2R). The lymphocyte IL-2Rγ, an essential subunit for IL-2 signaling, is also a common subunit (γ_c) for multiple immune cytokines (e.g., IL-4, IL-7, IL-9, IL-15). Having previously cloned the α and β subunits of the IL-2R heterotrimer complex from normal murine forebrain, we examined the hypothesis that the brain IL-2Rγ is derived from the same or a closely related gene coding sequence as that expressed by lymphocytes. In this study, we cloned and sequenced the full length IL-2Rγ coding region from saline-perfused mouse forebrain and from a human hippocampal library. The cDNA sequences of IL-2Rγ from murine and human brain were 100% homologous to their lymphocyte sequences. Northern analysis showed that the predominant mRNA transcript in murine brain was the expected size, but was expressed at very low levels compared to spleen. IL-2Rγ was constitutively expressed in SCID mouse forebrain, primary astrocyte cultures, and by murine neuroblastoma and AtT-20 pituitary cells using RT-PCR. These data suggest the possibility that IL-2Rγ could be an essential common subunit (γ_c) for multiple cytokine receptors which may be operative in the mammalian CNS. (Supported by NIH grant NS343623).

537.3

CORTICOTROPIN-RELEASING FACTOR (CRF) EXPRESSION IN THE IMMUNE SYSTEM FOLLOWING IL-2 ADMINISTRATION S.M. Brouxhon, D.L. Bellinger, D.L. Felten*. Dept. Neurobiol. & Anat. Univ. of Roch. Sch. of Med., Rochester, NY 14642, U.S.A.

A growing body of evidence has established the importance of cytokines in maintaining homeostatic conditions via a bidirectional communication between the neuroendocrine and immune systems. Recently, Raber et al (J.P.E.T, 1995) using an in vitro slice preparation, demonstrated a calcium- and dose-dependent release of CRF from the hypothalamus and amygdala following incubation with IL-2. In addition, IL-2 has been shown to regulate pro-opiomelanocortin (POMC) mRNA expression in pituitary cells (Brown et al., J. Immunol., 1987) as well as inducing a plasma increase in the POMC-derived products corticotropin (ACTH) and ß-endorphin (Denicoff et al., J. Clin. Endocr. Metab, 1989).

In this study, we examined CRF immunoreactivity in primary and secondary lymphoid organs following IL-2 administration in male Fischer 344 rats. Rats were administered IL-2 (50, 100, or 200ng/rat, i.p.) and sacrificed at varying time points (4, 12, and 24 hours) after treatment. The primary and secondary lymphoid organs were then removed and processed for immunocytochemistry (ICC). In vehicle-treated and non-treated rats we were unabled to demonstrate CRF-ir fibers in the thymus, spleen, or mesenteric lymph nodes. IL-2 administration induced the expression of CRF-ir nerves in the interlobular septa and capsular region of the thymus, and in the hilar region in the spleen.

IL-2 and monoclonal antibodies directed against IL-2 receptors have been used to treat a variety of hematologic malignancies, autoimmune diseases, and to prevent rejection following transplantation. Our findings suggest that IL-2 can interact with peptidergic neurons in the periphery, and indicate that more research needs to be done to determine the effect of IL-2 on nonlymphoid targets, including the central and peripheral nervous system.

This work was supported by MH42076 and a grant from the Markey Charitable Trust.

537.4

IL-2 INCREASES IL-2R EXPRESSION IN SUPERIOR MESENTERIC-CELIAC GANGLION AND ALTERS SPLENIC NOREPINEPHRINE CONCENTRATION. D.L. Bellinger*, S. Brouxhon, D. Henderson, D.L. Felten, and S.Y. Felten. Dept. Neurobiol. & Anat. Univ. of Rochester Sch. of Med., Rochester, NY 14642, U.S.A.

The autonomic nervous system through its innervation of lymphoid organs, provides a direct route for communication between the nervous and immune systems. Noradrenergic (NA) sympathetic innervation of both primary and secondary lymphoid organs has been well described with a variety of staining methods. NA innervation of the spleen is regional and specific, and distributes along the vasculature and in the parenchyma among cells of the immune system. Electron microscopic studies have demonstrated direct appositions between NA nerve terminals and T lymphocytes and macrophages. This spatial relationship provides an anatomical substrate for neural signaling of cells of the immune system. Additionally, we have hypothesized that cells of the immune system may modulate sympathetic outflow through the release of signal molecules such as IL-2.

To explore this possibility, immunocytochemistry (ICC) for IL-2 receptors (IL-2R) was performed in superior mesenteric-celiac ganglion (SMCG) from male Fischer 344 rats following the administration of IL-2 (range of 50, 100, or 200 ng/rat, i.p.). IL-2R-immunoreactivity was greatly increased in the SMCG from IL-2-treated animals compared with vehicle-treated controls. Enhanced IL-2R immunoreactivity in SMCG neurons from IL-2-treated rats was observed at 4, 12, and 24 h after IL-2 treatment at each dose administered. Since the spleen is a target of SMCG neurons, we also examined splenic norepinephrine content in vehicle- and IL-2-treated rats. Norepinephrine concentration in spleens was significantly decreased at 4 h after treatment with 50 ng of IL-2. Splenic norepinephrine content was not significantly different from vehicle controls at any other dose or time point. This study provides evidence for the presence of IL-2R on sympathetic neurons, and suggests that IL-2 can alter sympathetic outflow to the spleen through its interaction with IL-2R on SMCG neurons. Supported by R29 MH47783.

537.5

DIFFERENTIAL EXPRESSION AND POSSIBLE FUNCTION OF VIP-R1 AND VIP-R2 IN T LYMPHOCYTES. C. Coke, W. Sun, Z. Xin, and D. Ganea*. CMBN and Biological Sciences, Rutgers Univ., Newark, NJ 07102.

Immune cells express specific receptors for immunomodulatory neuropeptides. Previous studies from our laboratory indicated that murine T cells express both VIP-R1 and VIP-R2. The two receptors are differentially expressed, with unstimulated cells expressing only VIP-R1, recent antigen-stimulated cultures expressing both VIP-R1 and VIP-R2, and fully differentiated T cell lines expressing only VIP-R2. The differential expression of the two VIP-Rs in T lymphocytes may reflect a different function in mediating the immunomodulatory effects of VIP. Our laboratory reported previously that VIP inhibits both IL-2 and IL-10 expression in antigen-stimulated normal T cells. Here we first studied the effect of VIP on IL-2 and IL-10 production in mature TH1 and TH2 cell lines, which express VIP-R2. VIP inhibits IL-2 production in TH1 cell lines, but does not affect IL-10 production in TH2 cell lines, suggesting that inhibition of IL-2 is mediated by VIP-R2, and inhibition of IL-10 by VIP-R1. This hypothesis was investigated next in normal splenocytes treated with various stimuli which induce IL-2 and/orIL-10 production, by studying the effects of VIP, the related neuropeptide PACAP, and different agonists and antagonists, in conjunction with the expression of the two VIP-Rs. The differential expression of the two VIP-Rs in T lymphocytes may reflect different functions in mediating the effects of VIP on cytokine production, and therefore, acquire an important physiological, significance.

NIMH grant MH49079

537.6

REGULATION OF IL-6 RELEASE FROM CORTICAL ASTROCYTES BY LPS: ROLE OF LIPOOXYGENASE PRODUCTS AND NITRIC OXIDE. Grimaldi M., #Navarra P., #Preziosi P, ^Favit A*,and $G. Schettini Dip. Neuroscienze e della Comunicazione Interumana, Sez. di Farmacologia, Univ. Napoli "Federico II", Via S. Pansini 5, 80131 Napoli, #Ist. Farmacologia, Univ. Cattolica del Sacro Cuore, Largo Francesco Vito 1, Roma; $Ist. di Oncologia Clinica e Sperimentale, Un. Neurobiologia C.B.A., Univ. Genova, Largo R. Benzi 10, I-16132 Genova; ITALY; ^ LAS/NINDS/NIH, 36 Convent Dr. Bethesda MD-20892, USA;

Among neuroactive substances released by astrocytes, interleukin 6 (IL-6), a pleiotropic cytokine that regulates immune responses and inflammatory processes, has been demonstrated to play a role in neuronal differentiation and survival. The mechanisms whereby Bacterial lipopolysaccharide (LPS), a component of gram negative bacteria wall, induce IL-6 release has not yet been clarified. Here we studied, by means of a bioassay, the modulation of IL-6 release from rat cortical astrocytes, induced by LPS, by anti inflammatory agents.

LPS stimulated concentration-dependently the release of IL-6 from rat cortical type I astrocytes. This LPS effect was not blocked by non steroidal anti inflammatory agents belonging to the class of cyclooxygenase inhibitors, while it was partially inhibited by lipooxygenase inhibitors. Dexamethasone (DEX) completely blunted the stimulatory effect of LPS upon IL-6 release. These data suggest that other mechanisms could be activated by LPS in order to fully achieve the stimulation of IL-6 release. Since LPS increased, and DEX inhibited, the expression of inducible nitric oxide synthase, we investigated the role of nitric oxide synthase inhibitors upon the stimulatory action of LPS. We found that L-NO-ARG, an inhibitor of nitric oxide production, inhibited the release of IL-6 induced by LPS in astrocytes.

We conclude that the stimulatory effect of LPS is achieved by the activation of either the metabolism of arachidonic acid with the production of lipooxygenase metabolites and by the activation of nitric oxide production, and that the high effectiveness of DEX could be due to the blockade of both of these pathways.

537.7

THE ROLE OF INTERLEUKIN-6 IN THE ACTIVATION OF THE HYPOTHALAMO-PITUITARY-ADRENOCORTICAL AXIS INDUCED BY ENDOTOXIN AND IL-1

Jianping Wang and Adrian J. Dunn*
Dept. of Pharmacology, LSU Medical Center, Shreveport, LA 71130.

Endotoxin (LPS) and interleukin-1 (IL-1) have been shown to be potent activators of the hypothalamo-pituitary-adrenocortical (HPA) axis, and both induce secretion of interleukin-6 (IL-6). LPS and IL-1 administration also increases brain 3-methoxy,4-hydroxy-phenylethyleneglycol (MHPG), as well as tryptophan and 5-hydroxyindoleacetic acid (5-HIAA), with peak responses at 2h and 4h following injection, respectively. Other studies have shown that IL-1 and IL-6 exhibit similar pharmacokinetics after i.p. or i.v. administration. However, the peak HPA axis responses to LPS and mIL-1β are around 2h after i.p. injection, whereas the response to IL-6 is significantly earlier (30-60 min). Moreover, the tryptophan and 5-HIAA responses to IL-6 appear earlier (2h), than those to LPS (6-8h) and IL-1 (4h).

LPS is known to induce IL-6 production and secretion starting around 1-2h after administration. Mouse IL-1β administration (100 ng i.p.) was shown to elicit a substantial, but short-lived increase of plasma IL-6 concentrations with a peak at 2h. Mice pretreated with a monoclonal antibody against mIL-6 showed attenuated corticosterone responses to IL-1 and LPS. However, anti-IL-6 treatment did not significantly alter the increases of MHPG, free tryptophan or 5-HIAA in either hypothalamus or brain stem. These results suggest that IL-6 may play a significant role in the LPS- and IL-1-induced stimulations of the HPA axis.

Supported by NIMH and NINDS (MH46261 and NS25370).

537.8

INTERLEUKIN-6-INDUCED POTENTIATION OF THE LOCOMOTOR RESPONSE TO AMPHETAMINE. S. Zalcman*, I. Savina, and R. A. Wise. Center for Studies in Behav. Neurobio. Concordia U. Montreal Canada H3G 1M8.

IL-6 is a macrophage and Th2 lymphocyte-derived glycoprotein that been detected in various central structures. It has been implicated in playing a role in various CNS pathologies, including ones associated with alterations of mesolimbic and mesostriatal activity. Zalcman et al. (1995) reported that IL-6 induced behavioral activating effects. In the present study, we examined locomotor responses to acute and repeated IL-6 administration. Long-Evans rats were administered IL-6 (0.0, 0.5, 1.0 or 2.0 μg, ip) and immediately thereafter placed in a locomotor box for two hours. Locomotor counts of rats receiving IL-6 (1 μg) were initially increased compared with those of controls. Although counts declined over the two hour period in all animals, a slower rate of decline was evident in IL-6-treated animals, whose counts significantly exceeded those of controls during the final 30 minutes of the session. The latter effect was also evident in rats treated with a 2 μg dose. Locomotion was reduced in rats treated with 0.5 μg of IL-6. Locomotor responses of rats receiving repeated intermittent administration of IL-6 were consistently greater than responses of rats that received repeated saline injections; however, responses did not increase with repeated injection. Interestingly, repeated IL-6 treatment appeared to sensitize animals to the locomotor-activating effects of amphetamine. In an initial treatment phase, rats received five daily injections of IL-6 (1.0 μg, ip) or saline and immediately thereafter were exposed to the test box for two hours. Daily counts of all animals declined over this five day treatment phase, although those of IL-6-treated rats were consistently higher (20%) than those of controls. Seven days after the last IL-6 injection, rats were administered amphetamine (1.0 mg/kg, ip) and again five days thereafter (0.5 mg/kg, ip). Locomotor counts of rats previously treated with IL-6 were about 45% higher than those of saline-treated animals for the 2 hour test session (and about 75% greater 40-80 minutes post-amphetamine injection). At the end of the session, IL-6-treated animals were observed to be in a slightly prone position, staring in one direction, with eyelids widely opened (whereas saline-treated rats were simply moving around the test box). It is suggested that IL-6 might proactively influence behavioral responses that are associated with psychopathological states (e.g., schizophrenia). Supported by NIDA.

537.9

ENTERIC ENDOTOXIN MAY TRIGGER THE IMMOBILIZATION-INDUCED PLASMA IL-6 INCREASE. A.Takaki*, S. Shioda, A. Arimura, T. Hori. Dept of Physiol, Fac of Medicine, Kyushu Univ, Fukuoka 812-82, Japan; U.S.-Japan Biomed Res Labs, Tulane Univ, Belle Chasse, LA 70037, USA.

Interleukin (IL)-6 has many non-immune functions including the production of acute phase proteins in the hepatic cells, the stimulation of the hypothalamic-pituitary-adrenal axis, the cytoprotection against lethal irradiation, etc. These pleiotropic activities of IL-6 must be important for the host defense against stress. We have recently demonstrated that immobilization (IM) stress increases plasma IL-6 levels; and that the partial hepatectomy or the hepatic sympathectomy attenuated IM-induced plasma IL-6 increase. Furthermore, the kupffer (K) and endothelial (E) cells in the liver were stained by anti-IL-6 antibody in the immobilized rats (Takaki et al., 1995). Since it is well known that physical stresses, such as burn injury, gut ischemia, cold exposure, etc., induce leakage of the intestinal flora and/or the bacterial endotoxin (LPS) into the circulation, we hypothesized that enteric LPS was released into the portal vein during IM stress, and then triggered the IL-6 production in the K and E cells in the liver. To confirm this possibility, LPS concentration in the portal plasma was measured in immobilized (1h) rats. Results showed that the portal LPS level certeinly increased in response to IM. The peak LPS level was about 3 times higher than that of the resting level (about 2pg/ml). The peak time was 30 min after the initiation of the restraint, whereas the restraint continued for 60 min. Since plasma IL-6 concentration began to increase at 30 min and reached to the peak level at 90-120 min, the portal LPS was likely to activate the K cell functions resulting in IL-6 release. In addition, the LPS stimulated IL-6 production in the K cells in a dose-dependent manner *in vitro*; and norepinephrine significantly enhanced this response. These data suggest that enteric LPS triggers the IM-induced plasma IL-6 increase, and that sympathetic innervation in the liver enhances this response.

537.10

CENTRAL OVEREXPRESSION OF INTERLEUKIN-6 MODULATES HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) FUNCTION. R. D. O' Shea*¶, F. E. Bloom¶, I. L. Campbell¶ and J. Raber¶#. ¶Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037; #New affiliations: Gladstone Molecular Neurobiology Program and Department of Neurology, University of California, San Francisco, CA 94141.

Interleukin-6 (IL-6) and IL-6-receptor mRNA and protein have been detected in different brain regions under normal and pathophysiological conditions. Although previous studies reported hypothalamic-pituitary-adrenal (HPA) axis stimulation after acute IL-6 administration, little is known about the chronic effects of IL-6 on the modulation of the HPA axis. Recently, a transgenic model was established in which CNS overexpression of IL-6, under the control of the glial fibrillary acidic protein promoter, produced severe neurologic disease with profound neuropathological manifestations (Campbell et al., 1993; Chiang et al., 1994). The function of the HPA axis in these IL-6 transgenic mice was investigated. IL-6 transgenic mice of the low expressor line G167 showed normal basal plasma corticosterone levels, but abnormally increased levels after restraint stress, as compared to non-transgenic littermate controls. The high expressor line G369 had elevated basal plasma corticosterone levels. Increased plasma corticosterone levels in G167, but not in G369 mice, were associated with increased adrenal corticosterone content and hyperplasia of both adrenal cortex and medulla. Notably, plasma ACTH levels and pituitary ACTH content were either not changed or decreased in these mice. G167 mice showed increased plasma levels of arginine vasopressin (AVP). The decreased ACTH response in the IL-6 transgenic mice and the adrenal hyperplasia in the G167 line support a direct activation at the level of the adrenal gland. The adrenal gland may be directly activated by AVP or be sensitized to ACTH. Thus CNS overexpression of IL-6 modulates the HPA axis in a complex, expression level-dependent fashion. These data further support a role for IL-6 in interactions between the neuroendocrine and immune systems. (supported by NIMH AIDS Center Grant MH47680 (FEB), MH50426 (ILC), NHMRC (RDO) and SKF (JR)).

537.11

LIPOPOLYSACCHARIDE-INDUCED PROINFLAMMATORY CYTOKINE mRNA EXPRESSION IN MIXED GLIA CULTURES: SUPPRESSION BY PROTEIN TYROSINE KINASE INHIBITORS. G.-H. Jeohn, L.-Y. Kong, and J.-S. Hong*. Neuropharmacology Section, Systems Toxicology Branch, NIEHS/NIH, Research Triangle Park, NC 27709.

Protein tyrosine kinases are involved in signaling events for proinflammatory cytokine production in glial cells. However, the mechanisms by which protein tyrosine kinases influence the production of proinflammatory cytokines have not been well understood. This study examined the effects of two potent protein tyrosine kinase inhibitors, genistein and tyrphostin A25, on lipopolysaccharide (LPS)-induced mRNA expression for tumor necrosis factor-α (TNFα), interleukin-1α (IL-1α) or interleukin-6 (IL-6) in mouse primary mixed glia cultures. LPS dose-dependently increased the expression of the mRNA for TNFα, IL-1α, or IL-6 in the mixed glia cultures. Genistein or tyrphostin A25 significantly inhibited the LPS-induced expression of the mRNA for these cytokines. These results demonstrate that protein tyrosine kinases are involved in the LPS-induced production of TNFα, IL-1α, or IL-6 in glial cells by modulating the transcription of the mRNA for these cytokines.

537.12

INHIBITION OF LIPOPOLYSACCHARIDE-INDUCED NITRIC OXIDE AND CYTOKINE PRODUCTION BY ULTRALOW CONCENTRATIONS OF DYNORPHINS IN MIXED GLIA CULTURES. L.-Y. Kong*, M. K. McMillian, B. C. Wilson, P. M. Hudson, L. Jin, G. Bing, and J.-S. Hong. Neuropharmacology Section, Systems Toxicology Branch, NIEHS/NIH, Research Triangle Park, NC 27709.

Dynorphins are a major class of endogenous opioid peptides that modulate functions of immune cells. However, the effects of dynorphins on the immune functions of glial cells in the CNS have not been well characterized. As nitric oxide (NO) and the proinflammatory cytokine tumor necrosis factor-α (TNFα) produced by glial cells influence various physiopathological conditions in the CNS, this study examined the effects of dynorphins on the production of NO and TNFα from mouse glial cells treated with lipopolysaccharide (LPS). LPS induced a concentration-dependent increase in the production of NO or TNFα from the mouse primary mixed glia cultures. Ultralow concentrations (10^{-16}-10^{-12} M) of dynorphin (dyn) A-(1-8) significantly inhibited the LPS-induced production of NO or TNFα. The inhibitory effects of dyn A-(1-8) were not blocked by nor-binaltorphimine, a selective *kappa* (κ) opioid receptor antagonist. Ultralow concentrations (10^{-16}-10^{-12} M) of des-[Tyr¹]-dyn A-(2-17), a non-opioid analog which does not bind to κ opioid receptors, exhibited the same inhibitory effects as dyn A-(1-17). The production of NO and TNFα in the LPS-stimulated microglia-enriched cultures was significantly higher than that in the identically stimulated astrocyte-enriched cultures, indicating that microglia are more responsive to the stimuli which cause NO and TNFα production. These results suggest that dynorphins modulate immune functions of microglia and/or astrocytes in the brain and these modulatory effects of dynorphins are not mediated by classical κ opioid receptors.

537.13

POTENTIAL ROLES FOR THE 75 KD TNF RECEPTOR AND INTERFERON-α IN SENSITIZING NEURONS TO TNF-α INDUCED CELL DEATH K.J. Sipe[1], K.W. Kelley[2], R. Dantzer[3] and J.A. Weyhenmeyer[1]*. Departments of [1]Cell and Structural Biology and [2]Animal Sciences, University of Illinois, Urbana, IL 61801 and [3]INRA-INSERM, Unité 394, Bordeaux, France.

Using the N1E-115 neuroblastoma line (N1E's) as a model system, we are examining the direct effects of the inflammatory cytokine, tumor necrosis factor-α (TNF), on neurons. We previously reported that the N1E's express only the 55kD TNF receptor (TNFR) which mediates TNF induced apoptotic cell death in the N1E's in the presence of the transcriptional inhibitor, ActD (ActD). A physiological agent that might sensitize neurons to TNF cytotoxicity is the antiviral cytokine, interferon α/β (IFN). Initial studies indicate that IFN is less potent than ActD in inhibiting N1E proliferation and in sensitizing the N1E's to TNF cytotoxicity. It is likely that viral infection is also required to obtain the full antiviral effect, including inhibition of RNA synthesis and proliferation, of IFN. If this is the case, concurrent viral infection, TNF and IFN production may represent one mode of neuronal destruction in viral infections of the CNS. Another mode of neuronal sensitization to TNF cytotoxicity may be the upregulation of the 75kD TNFR. While upregulation of the 75kD TNFR has been reported in TNF treated cells (Kalthoff et a., *J. Biol. Chem.* 268, 2762-2766), we have not observed this in N1E's. We are currently transfecting N1E's with an expression construct for the 75kD TNFR to examine this issue. Although we anticipate that expression of the 75kD TNFR will increase N1E sensitivity to TNF cytotoxicity, based on the ligand passing model proposed by Tartaglia et al.(*J. Biol. Chem.* 268, 18542-8) and results in 75kD TNFR knock-out mice (Erickson et al., *Nature* 372,560-563), it is conceivable that that N1E's may express endogenous TNF that, in a membrane bound form, engages expressed 75kD TNFR, leading to cytoxicity in the absence of exogenous TNF. This work was supported by NIH Grant DK49311 and NSF Grant IBN 9320158.

537.14

ENCEPHALOPATHY INDUCED BY THE ASTROCYTE-TARGETED EXPRESSION OF INTERFERON-α IN TRANSGENIC MICE. Y. Akwa*[1], M. L. Eloranta[2], K. Sandberg[3], H. Powell[4], F. E. Bloom[1] and I. L. Campbell[1]. [1] Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037 [2] Univ of Uppsula, Sweden, [3] Astra Arcus Sodertälje, Sweden, [4] Dept. of Pathology, Univ of San Diego, CA.

Interferon-α (IFN-α) is an immunoregulatory cytokine pivotal in the anti-viral response that may be both protective or injurous to the host in viral infections of the central nervous system (CNS). In order to assess the possible neuropathogenic properties of IFN-α, we used a transgenic (Tg) approach to constitutively express IFN-α in the CNS of mice. Expression of IFN-α was targeted to astrocytes by placing the IFN-α gene under the regulatory control of a glial fibrillary acid protein (GFAP) genomic expression vector. Two GFAP- IFN-α Tg lines, termed GIFN-39 and GIFN-12, were developed with moderate and low levels of IFN-α mRNA expression in the brain, respectively. Significant production of IFN-α protein was demonstrated from astrocytes from GIFN-39 but not wild type mice. GIFN-39 mice which were runted and moribund, died at 5-10 months of age. A similar but much less severe phenotype was observed in some older (8-12 mo) GIFN-12 mice. In the brain of Tg mice a spectrum of alterations, correlated with the levels and distribution of transgene expression, was observed. At the cellular level, gliosis, angiopathy with mononuclear cell cuffing and progressive calcification affecting basal ganglia and cerebellum were prominent. Spongiform changes were evident in regions affected by calcification. At the molecular level, the expression of two IFN-α inducible genes, 2',5'-oligoadenylate synthetase and major histocompatibility complex class 1, were markedly increased. In conclusion, these findings demonstrate that the chronic cerebral expression of IFN-α is sufficient to produce specific structural and functional changes in the CNS. This model therefore offers great potential in further dissecting the mechanism of IFN-α in neuropathogenesis and developing therapeutic approaches to abolish the harmful actions of IFN-α. This work was supported by USPHS grant MH47680 (I.L.C.), a WHITTIER Foundation grant (F.E.B.) and a PHILIPPE Foundation grant (Y.A.).

537.15

INTRACEREBROVENTRICULAR INJECTION OF ANTI-TNF-α BLUNTS THE ONSET OF THE ACTH RESPONSE TO ENDOTOXIN: IS THIS EFFECT DUE TO NEUTRALIZATION OF TNF-α WITHIN THE BRAIN OR IN THE PERIPHERY? A.V. Turnbull* and C. Rivier. Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037

Tumor necrosis factor-α (TNF-α) undoubtedly plays an important role in regulating neuroendocrine responses to inflammatory/disease processes. However, whether TNF-α acts within the brain or whether it influences the neuroendocrine hypothalamus via intermediates released in the periphery, remains unclear. In the present study, we found that intracerebroventricular (icv) injection of a specific, neutralizing rabbit anti-TNF-α antisera (5 μl) 20 h earlier, significantly blunted the onset of the ACTH response to intravenous (iv) lipopolysaccharide (LPS, 5μg/kg). However, in control experiments the same dose of antisera injected iv produced quantitatively similar effects. Surprisingly, the increase in plasma TNF-α biological activity (assessed by cytotoxicity to L929 cells) produced by LPS was similarly reduced by iv and icv anti-TNF-α treatment. Furthermore in normal animals injected with anti-TNF-α icv, recovery of TNF-α biological activity from "spiked" plasma samples was reduced. This inhibitory factor could be removed by pre-precipitation with a sheep anti-rabbit IgG, suggesting the presence of antisera in blood after icv injection. In separate experiments, we found that icv injection of a number of antisera, raised in different host species and against different peptides, produced rapid and sustained elevations in the plasma titer of the antisera as assessed by specific binding of the appropriate I^{125}-labeled ligand. Indeed after icv injection, measurable plasma titers were apparent within 30 min, and by 8 h of injection, plasma titers were equivalent to those observed after iv injection. Collectively, these data indicate that icv injection of antisera produces plasma titers sufficient to exert significant actions within the periphery, and suggest that TNF-α acts within the periphery to stimulate ACTH secretion in response to LPS.

Supported by NIH DK-26741.

537.16

ANANDAMIDE EFFECTS ON NITRIC OXIDE AND CYTOKINES PRODUCTION BY ASTROCYTES STIMULATED WITH THEILER'S MURINE ENCEPHALOMYELITIS VIRUS OR LIPOPOLYSACCHARIDE.

F. Molina-Holgado*, A. Lledó and C. Guaza. Instituto Cajal, CSIC, 28002 Madrid, Spain.

Astrocytes are an important cell population in the central nervous system (CNS) cytokine network. The astroglial response to diverse neurological disorders, such as multiple sclerosis (MS) or experimental allergic encephalomyelitis (EAE), include the production of nitric oxide (NO) as well as several cytokines. In the present study we showed that primary cultures of neonatal mouse cortical astrocytes directly exposed to lipopolysaccharide (LPS:1μg/ml, 18 hours) or to the Theiler's murine encephalomyelitis virus (TMEV), increased the release of nitrites (NO_2^-), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). The presence of anandamide (10, 50 and 100 μM) in the astrocyte (Balb/c mice strain) culture medium, an endogenous ligand for cannabinoid receptor, blocked dose-relatedly the LPS-induced release of NO_2^- and TNF-α. Exposure of cultured astrocytes (SJL/J mice strain) to TMEV (10^5 PFU, 24 hours) together with anandamide (10, 25 and 50 μM) suppressed the stimulatory effects of TMEV infection on NO and TNF-α in a dose-related manner. By contrast stimulated IL-6 release from astrocytes exposed to TMEV or LPS treatment was enhanced by the presence of anandamide in a concentration dependent manner.These results strongly suggest that in glial cells the cannabinoid endogenous ligand, anandamide, exert a modulatory role which may have protective effects. These results could have important implications in the modulation of immunological and inflammatory processes in the CNS.

Suported by DGICYT (PB94-0098) and Fundacion Salud 2000

537.17

Th2-DERIVED CYTOKINES ACT AS IMMUNOSUPPRESSIVE FACTORS AND PROVIDE NEUROTROPHIC SUPPORT IN THE CNS. Chaya Brodie* and Nurit Goldreich Department of Life-Sciences, Bar-Ilan University, Ramat-Gan, Israel 52900.

Astrocytes function as immunocompetent cells in the central nervous system and play an important role in immune-related processes by their interactions with various cytokines. The development of the immune response in the brain has been shown to be mediated by proinflammatory cytokines such as IL-1, IL-6, TNFα and IFNγ, whereas, the Th2-derived cytokines, IL-4 and IL-10 have been implicated as negative regulators of immune responses in the CNS. In this study we characterized the expression of IL-4R and IL-10R in astrocytes derived from the cortex and the cerebellum and explored the effects of IL-4 and IL-10 on immune-related properties of these cells. We found that astrocytes express the mRNA for both the membranal and the soluble forms of the IL-4R, whereas, they do not secrete IL-4. In contrast, mRNA for both IL-10R and IL-10 are expressed by these cells. Pre-treatment of the cells with IL-4 and IL-10 inhibited both NO production and iNOS expression induced by LPS treatment as well as the secretion of TNF-α. In contrast, neither cytokine blocked NO production by astrocytes stimulated with exogenous TNF-α. Both IL-10 and IL-4 induced the secretion of NGF by astrocytes and synergized with TNF-α in this effect. These results suggest an important role for IL-4 and IL-10 as immunosuppressive and neurotrophic factors in the CNS during instances of CNS trauma and inflammation.

This study was supported in part by grant No. 3980 from the Chief Scientist Office of Ministry of the Health, Israel. Supported by Ministry of Health, Israel

537.18

INHIBITION OF KAINIC ACID-INDUCED ELEVATED TNF α LEVELS IN BRAIN BY ICV GI 129471. A.L. Nikkel*, K.E. Asin, P.S. Puttfarcken, E.D. Çadman, A.M. Manelli and K. Shiosaki. Neuroscience Res. Div., Pharmaceutical Discovery, D-47W, Bldg. AP9A, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-3500.

Tumor necrosis factor alpha (TNFα) has been implicated as a possible mediator of the brain's response to diverse forms of injury. The neurotoxin, kainic acid (KA), when administered systemically at doses that cause convulsions, produces widespread damage and cytokine induction, including an increase in TNFα mRNA expression. Other investigators have demonstrated that the metalloproteinase inhibitor, GI 129471 (Glaxo, Inc.), can block TNFα production both *in vitro*, from peripheral blood monocytes, and *in vivo*, in plasma (McGeehan et al., 1994). GI 129471 can also inhibit TNFα release from CNS-derived astrocytes (see Puttfarcken et al. this meeting).

We investigated the ability of ICV-administered GI 129471 to inhibit elevated TNFα in the brain, following SC-administered KA (10 mg/kg) to rats with indwelling cannulae terminating in the left lateral ventricle. TNFα levels from brain homogenates were quantitated by ELISA. In unoperated rats, TNFα levels were elevated within 3 hours, reached maximum within 9 to 12 hours, and declined within 18 to 24 hours post-KA treatment. Cannulated rats were subsequently pretreated with 30 μg GI 129471 or vehicle ICV just prior to KA or saline injection and again at 4 hours after KA or saline injection prior to sacrifice at 9 hours. ICV vehicle-treated rats that received KA and displayed convulsions exhibited elevated TNFα when compared to SC vehicle-treated controls. When compared to ICV vehicle controls, ICV GI 129471 significantly attenuated TNFα production in KA-treated, convulsing rats (45-63% reduction). ICV GI 129471 did not alter TNFα in SC vehicle-treated rats and did not affect the proportion of KA-treated rats that displayed convulsions.

These data demonstrate that GI 129471 can inhibit TNFα production in brain in the face of excitotoxic damage and suggest that TNFα inhibition may provide a productive area of investigation for CNS disorders that involve elevated TNFα production.

This research was supported by Abbott Laboratories.

537.19

GI 129471 INHIBITS LPS-INDUCED TNF-α RELEASE FROM PRIMARY CULTURES OF RAT ASTROCYTES P.S. Puttfarcken*, A.M. Manelli, E.D. Cadman, and K. Shiosaki. Neuroscience Research, D-47W, Abbott Laboratories, 100 Abbott Park, IL 60064-3500.

Elevated levels of TNF-α have been reported in various CNS infections, including HIV-1, meningitis, and following brain injury. TNF-α is synthesized as a precursor, then cleaved by a protease to yield the active form. Although a specific TNF-α processing enzyme(s) has not been identified, the effectiveness of selected hydroxamate-based inhibitors indicates that this enzyme resembles a matrix metalloproteinase (McGeehan et al., 1994; Gearing et al, 1994). Previous studies have demonstrated inhibition of lipopolysaccharide (LPS)-induced TNF-α release by the metalloproteinase inhibitor GI 129471 in the periphery (McGeehan et al., 1994). To investigate the ability of GI 129471 to inhibit TNF-α release in the CNS, rat astrocyte cultures were treated with 10ng/ml LPS. Elevated TNF-α levels appeared within 1.5 to 3 hours, reached a maximum at 8 hours, and declined by 24 hours. This time course correlated with *in vivo* findings (see Nikkel et al., this meeting). Treatment with GI 129471 inhibited LPS-induced TNF-α release in a concentration-dependent manner with an estimated IC_{50} of 50 nM, a value approximately 2- to 3-fold more potent than that previously reported in the periphery (McGeehan et al., 1994]. In contrast, pentoxifylline, a methylxanthine derivative previously determined to inhibit TNF-α production through a undefined mechanism, was less potent with an estimated IC_{50} of 100 μM. These data suggest that the enzyme(s) responsible for processing TNF-α may be similar in the CNS and periphery and that inhibition of this process may be a suitable target to investigate the pathophysiology of CNS disorders associated with elevated TNF-α levels.

This work was supported by Abbott Laboratories.

537.20

INDUCTION OF TNF-α EXPRESSION BY PNEUMOCOCCAL CELL WALL COMPONENTS (PCW) IS INDEPENDENT FROM NFκB ACTIVATION IN CEREBRAL ENDOTHELIAL CELLS (EC). D.Freyer, A.Meisel, M.Weih*, K.Angstwurm, J.R.Weber, R.Manz, A.Ziegenhorn, W.Bürger, U.Dirnagl. Depts. of Neurology and Microbiology, Humboldt University Berlin, FRG.

Cell wall components of gram-positive Streptococcus pneumoniae, as they result from bacteriolysis, have experimentally been shown to cause meningeal inflammation, similarly to LPS of gram-negative bacteria. The cellular mechanisms during inflammation are well established for LPS and a central role of of the NFκB in regulation cascades has been demonstrated. However, little is known about the PCW-induced transduction cascade. In this study we investigated iNOS and TNFα-expression in EC upon stimulation with PCW. EC were prepared from rat brain and cultivated by standard procedures. The activation by PCW was altered by unspecific and specific blocking of NFκB. For unspecific inhibition, EC were simultaneously incubated with 0.1 mM pyrrolidine-dithiocarbamic acid (PDTC) and $5*10^5$ cfu/ml PCW. According to the decoy approach, oligonucleotide duplexes (20μM) containig the cognate NFκB-binding site were used for specific NFκB inhibition. By using the unspecific approach the TNFα as well as the iNOS expression were reduced to baseline levels. In contrast, specific inhibition of NFκB produces only a 50-60% reduction of iNOS expression whilst the TNFα expression was increased by incubation with PCW and oligonucleotides to 140% of the value from incubation with PCW alone. These results suggest a different transcriptional activation of iNOS and TNFα. Since the antioxidant PDTC is known to have unspecific effects, it is more likely that in addition to NFκB there is a second inducible transcription factor for the transactivation of the iNOS gene and the TNFα expression upon stimulation with PCW seems to be independent from NFκB activation. (supported by the DFG)

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS VII

538.1

INTERLEUKIN 6 (IL-6) SUPPRESSES AFFERENT SENSORY TRANSMISSION IN THE PRIAMRY SOMATOSENSORY (SI) CORTEX. K.H. Lee*, C.K. Won, H.C. Shin Dept. of Neurosurgery*, Dept. of Physiol., Coll. Med., Hallym Univ., Chunchon, Korea

Although IL-6 and IL-6 receptors are present in the adult cerebral cortex in the noraml rat, the functional role of IL-6 has not been characterized. In the present study, we have examined the effects of topical application of human recombinant IL-6 to the cortical surface on the afferent sensory transmission in the SI cortex of anaesthetized rats. Quantitative determination of the effect of IL-6 on the afferent sensory transmission to the neurons in the SI cortex was carried out by generating poststimulus time histograms of unit responses to the stimulation of receptive field located in the whisker area of the face. Application of 0.1 U (n=8) IL-6 caused a slight reduction (-9.30±4.7%, $p < 0.01$, 15-20 min post-drug) in afferent sensory transmission 15-25 min after drug administration. Administration of 1.0 U (n=8) IL-6 exerted much stronger (-15.13±3.4%, $p < 0.01$, 20-25 min post-drug) and long-lasting, but reversible, inhibitory influences on the afferent sensory transmission. IL-6-induced suppression fully recovered by 60 min after drug. Lowest dose of IL-6 (0.01 U, n=8) as well as saline solution (n=9) containing 0.2% bovine serum albumin, used as a vehicle, did not affect afferent sensory transmission. The results of this study provide evidence that IL-6 may be involved in the processing of afferent sensory information in the SI cortex of rats (supported by KOSEF grant, 961-0701-1).

538.2

TIME COURSE OF EFFECTS OF LOCAL SUPERFUSION OF BICUCULLINE ON RECEPTIVE FIELD PROPERTIES AND TEMPORAL INTEGRATION OF RAT SOMATOSENSORY CORTICAL NEURONS. A. Benali, F. Spengler, R. Leonhardt*, H.R. Dinse. Institut f. Neuroinformatik, Ruhr-Univ. Bochum, Germany

We investigated the role of $GABA_A$-receptor mediated inhibition on a number of parameters reflecting cortical information processing properties such as receptive field (RF) organization, topographic maps, response latencies, temporal integration properties (repetition rate coding) and spontaneous and stimulus-induced activity. Neurons were recorded in layer IV of the fore- and hindpaw representation of somatosensory cortex of urethane anaesthetized rats. $GABA_A$-receptor antagonist (-) bicuculline-methiodide (BMI) was locally superfused (single application of 1μl of 1 mM, washed out after 1 h) onto the cortical surface restricted to about 1 mm^2 with the remaining cortex covered with paraffin film. In view of the highly interconnected cortical network properties, a local superfusion method was chosen in order to affect a large population of neurons.

Mapping RFs around the application site revealed that an area of 0.25-1 mm^2 was affected by the superfusion method used. Three different episodes of BMI effects could be distinguished: 1. Complete suppression of tactile-driven activity following BMI superfusion for about 10 to 20 min paralleled by high spontaneous, bursting activity. 2. Restoration of tactile responsiveness paralleled by severalfold RF enlargement and strong suppression of repetition rate responsiveness. 3. After wash-out, we observed recovery after 1 to 2 h. At the begin of this period, RF size was still highly enlarged and temporal integration was shifted towards lower cut-off frequencies due to a removal of suppression typical for short ISIs (35 ms).

The results indicate that BMI-mediated disinhibition affects all spatial and temporal aspects of cortical information processing tested. However, the time course of the effects includes transient episodes of complete suppression of sensory responsiveness. The differential results are compared to plastic reorganizational changes to elucidate the role of inhibition in cortical plasticity.

Supported by DFG and the Institut für Neuroinformatik, RUB, Germany

538.3

DIFFERENTIAL EFFECTS OF THE Ca^{2+} INFLUXBLOCKER NIMODIPINE ON RECEPTIVE FIELD SIZE AND RESPONSE LATENCIES OF SOMATOSENSORY CORTICAL NEURONS OF AGED RATS. M. Jürgens* and H.R. Dinse. Institut für Neuroinformatik, Theoretische Biologie. Ruhr-Univ. Bochum RUB, Bochum, Germany

During aging the regulation of Ca^{2+} homeostasis can be disturbed, among others by an excessive influx through voltage gated calcium channels of the L-type. The dihydropyridine Ca^{2+} antagonist nimodipine is able to block this type of channels, thus protecting the cell from intracellular Ca^{2+}-overload (Freund & Redding 1989, Brain Res 654: 257).

We have previously shown that rats older 24 months display a number of age related impairments in sensorimotor state as well as in cortical organization (Spengler, Godde, Dinse 1995, Neuroreport 6: 469). In this investigation we studied the effects of long-term application of nimodipine on the cortical organization of RFs and response latencies of somatosensory neurons recorded in the fore and hindpaw representations of urethane anesthetized aged rats (23-30,5 months). In addition to electrophysiological recordings, the sensorimotor state of each animal was assessed.

In parallel to the beneficial effect on the overall state and walking pattern of the hindextremity, we found a significant influence on RF-size of the hindpaw compared to untreated age-matched animals. The positive effects resulted in a reversal of the normally observed age-related enlargement of RFs that came to match the values found in normal adult animals. This reversal was restricted to a period of 4 to 5 months. RFs on the forepaw that showed no age-related increase even in untreated animals, were not affected by nimodipine treatment. Response latencies of neurons from the hindpaw as well as the forepaw were not altered by nimodipine too, but showed the characteristic lengthening with chronological age. Taken together we were able to demonstrate an effect of nimodipine on cortical RF-organization, whereas response latencies remained unaffected. Accordingly, the results indicate a selective involvement of nimodipine in the treatment of age-related changes.

Supported by Tropon, Bayer Leverkusen, Germany

538.4

OPTICAL IMAGING OF AGE-RELATED CHANGES OF RAT SOMATOSENSORY CORTICAL REPRESENTATIONS AND THEIR SENSITIVITY TO THE Ca^{2+} INFLUXBLOCKER NIMODIPINE. T. Berkefeld, B. Godde and H.R. Dinse (SPON: Europ. Brain Behav. Soc). Institut für Neuroinformatik, Theoretische Biologie. Ruhr-Univ. Bochum RUB, Bochum, Germany

Impairment of the Ca2+ homeostasis during aging is assumed to be causally related to many age-related neuronal changes. To address the question of age-related changes of cortical representational properties [Spengler, Godde, Dinse 1995 Neuroreport 6: 469] and their sensitivity to the Ca2+ antagonist nimodipine, we studied the functional organization of somatosensory cortex (SI) of aged nimodipine- and aged non-treated animals by means of optical imaging of reflectance changes of intrinsic signals and by recording local field potential (LFP) maps in supragranular and granular layers.

The size of the hindpaw representations of aged animals was significantly reduced compared to normal adults as measured both optically and electrophysiologically. We found a striking correspondence of areas of activation for different activity levels between optical imaging and LFP data recorded in layer IV, but not for layer II/III. Several months of nimodipine treatment resulted in a substantial increase of the hindpaw representational areas which became even larger than in normal adult animals. This restaurative effect was more prominent for the optical imaging data, but also present in the LFP maps recorded in cortical layers II/III and IV, suggesting an additional effect of nimodipine related to cerebral blood flow.

In contrast to the hindpaw with its age-related locomotion impairment, the forepaw maintains a fairly unimpaired behavior even in old animals. We observed that the forepaw representational areas were indeed comparable in adult and old, untreated animals, indicating a lack of age-related cortical degradations. After nimodipine treatment, the forepaw representations were not significantly increased. Combined, the results indicate a differential effect of nimodipine for the differentially affected fore- and hindpaws, thereby suggesting a specific involvement of nimodipine in plastic and restaurative processes.

Supported by Tropon, Bayer Leverkusen, GERMANY

538.5

PERSISTENT REDUCTION IN WHISKER BARREL AREAS FOLLOWING SYSTEMIC AND LOCAL PARACHLOROAMPHETAMINE-INDUCED SEROTONIN DEPLETION IN NEONATAL RATS. F. Keller*[1], A.M. Persico[1], E. Calia[1], and S. Puglisi-Allegra[2]. [1]Laboratory of Neuroscience, Libero Istituto Universitario Campus Bio-Medico, Rome, Italy, and [2]Dept. of Psychology, Univ. "La Sapienza", Rome, Italy.

Increasing evidence supports neurodevelopmental roles for serotonin in several species. Whisker barrels located in the posteromedial barrel subfield (PMBSF) of the rodent somatosensory cortex allow reliable assessment of serotoninergic modulation of thalamocortical pathway development and plasticity. Single (P0) or repeated (P0 and P2) injections of parachloroamphetamine (PCA: 3.5 or 14 mg/kg s.c.) reduced left hemisphere PMBSF 5-HT and 5-HIAA content, assessed using HPLC on P7, by 32-60% ($p<.001$) and 10-45% ($p<.01$), respectively, compared with saline-injected rats. The right hemispheres of the same animals assessed using acetylcholinesterase histochemistry displayed 18.7% ($p<.0001$) and 14.2% ($p<.01$) reductions in mean whisker barrel and total PMBSF areas, respectively. Decreases in mean whisker barrel area appear to follow a gradient, ranging from 23.6% reductions in row-A ($p<.0001$) down to 14.9% decreases in row-E ($p<.01$). Systemic PCA also blunted body weight gain (126% increase with saline vs 30-57% increase with PCA between P0 and P7) and reduced mean brain weight by 12.5% on P7. We therefore employed elvax and gelfoam implants (see abs. by Persico & Keller) providing slow-release of PCA or saline over the somatosensory cortex to verify the specificity of systemic PCA effects. Although saline-containing implants frequently produce non-specific reductions in whisker barrel and PMBSF areas, PCA-containing implants consistently produce much more sustained decreases, providing further support to intracortical 5-HT-mediated neurodevelopmental effects on thalamocortical pathways. *This work was supported by the Margherita Lama Caputo Memorial Fund and by EC Biomed grant PL950730.*

538.6

ELVAX vs GELFOAM IMPLANTS FOR SUSTAINED DRUG RELEASE OVER THE WHISKER BARREL FIELD: THE PCA-INDUCED SEROTONIN DEPLETION PARADIGM. A.M. Persico*, and F. Keller. Laboratory of Neuroscience, Libero Istituto Universitario Campus Bio-Medico, Rome, Italy.

Systemic drug treatments frequently produce neurodevelopmental effects with questionable specificity. Systemic parachloroamphetamine (PCA) treatment, for example, may reduce whisker barrel areas by specifically depleting cortical 5-HT, or by non-specifically reducing body weight in neonatal rats (see abs. by Keller et al.). To circumvent these limitations, implants providing sustained drug release over specific cortical areas have been employed. We have initially defined the stereotaxic coordinates for correct implant targeting by injecting India ink at P0 and verifying injection location relative to the posteromedial barrel subfield (PMBSF) at P7. We then compared PCA- and saline-containing implants made with elvax chips or with gelfoam. Both acetylcholinesterase histochemistry and 5-HT immunocytochemistry were performed to assess implant effects on thalamo-cortical and raphe-cortical pathways. Elvax chips of 2 x 2 mm size and approximate thickness of 200 μ or more, produced extensive tissue damage (no interpretable data out of 18 animals). Chips of approximately 100 μ yielded 1/11 informative brains when slid under the skull and 2/10 when the bone was removed and the chip covered with a sterile 7 x 7 mm Saran Wrap sheet. Gelfoam implants yielded 6 informative brains out of 7 animals. Rats receiving saline gelfoam implants, however, displayed 20-30% reductions in mean whisker barrel areas, which were instead unmodified in saline elvax-treated animals. In conclusion, elvax chips have the advantage of providing more interpretable results, while gelfoam implants provide much better yields. Given the limitations inherent to both these approaches, we are actively searching for new polymers to test for drug treatments localized over specific cortical regions.

This work was supported by the Margherita Lama Caputo Memorial Fund and by EC Biomed grant PL950730.

538.7

GAP CROSSING TRAINING ENHANCES WHISKER PAIRING BIAS IN ACETYLCHOLINE DEPLETED RATS. R. N. S. Sachdev*, M. Stonecypher, M. Egli, R. G. Wiley[†], S.-M. Lu, F. F. Ebner. Institute for Developmental Neuroscience, Kennedy Center, Vanderbilt University, [†]& Dept. of Neurology, VAMC Nashville, TN 37203

Neurons in the D2 barrel column develop a bias in their responses after trimming all but two adjacent whiskers on one side of the adult rats face, such that these neurons respond more to whiskers that are spared or "paired". We have shown that whisker pairing plasticity can be prevented by depletion of acetylcholine (ACh) from the barrel cortex. In this study we have examined whether the whisker pairing bias can be restored when a naive ACh depleted animal is trained to use the "paired" whiskers in a gap crossing task. For each experiment, two adult male litter-mates were depleted by making a single 1 μl injection of IgG 192-saporin (0.62 mg / ml) medial to the barrel field. A week after the injections, both animals were weighed and a food deprivation regimen was initiated. One week later, all whiskers -- except D2 and either D3 or D1 on the right side of the face -- were trimmed. One rat was then trained everyday on a gap crossing task, while the other animal was handled daily but not trained on the task. In the gap-crossing task, the rat is placed on one of two platforms 60 cm above the desktop and is required to make a crossing over a variable gap between the two platforms by using its whiskers. One week after either pairing the whiskers or pairing and training on gap-crossing, the response properties in the D2 barrel column were assayed with extracellular single-unit recording. Following the recording session animals were perfused transcardially, their brains were fixed, sectioned and stained for cytochrome-C oxidase (CO) and acetylcholinesterase (AChE). Electrode tracks were confirmed in CO stained sections, and the depletion was confirmed on AChE stained sections. In the gap-crossing trained ACh depleted animals, a bias toward the paired whisker is apparent while in the untrained animals no significant bias develops, suggesting that the active use of whiskers can reinstate a whisker pairing bias even in the ACh depleted animal. (Supported by NS 25907 to FFE and NS 09929 to RNSS)

538.8

EFFECTS OF NOREPINEPHRINE (NE) DEPLETION IN THE RAT BARREL CORTEX S.-M. Lu*, R. Sachdev, M. Picklo, D. Robertson, and F. F. Ebner, Inst. for Dev. Neurosci., Kennedy Center and Dept. of Pharmacol., Vanderbilt Univ. Nashville, TN 37203

Previous studies have shown that trimming all but 2 whiskers (whisker-pairing) in an adult rat produces robust changes in cortical receptive field (RF) properties that can be used as an assay for the status of cortical activity-dependent plasticity (called whisker pairing plasticity). NE has been proposed as an important regulator of activity-dependent plasticity. The present experiments examine whether NE is a necessary requirement for whisker pairing plasticity to occur in adult rat barrel cortex.

NE innervation of barrel cortex was depleted using a dopamine-ß-hydroxylase (DBH) immunotoxin, saporin conjugated anti-DBH antibody, which binds specifically to NE containing terminals when DBH is exposed extracellularly during NE release. The bound DBH immunotoxin is internalized and transported back to cell bodies in the locus coeruleus where the saporin kills the host cells. Fewer than 5% of the NE containing axons remain detectable in barrel cortex 10-14 days after a single 1 μl DBH immunotoxin (0.2 mg/ml) intracortical injection.

Cortical RF properties were recorded under urethane anesthesia from adult rats who had a unilateral depletion of cortical NE with or without whisker-pairing. In normal adult rats, neurons in the D2 barrel column show the greatest response to stimulation of the D2 whisker, and a smaller, but equally smaller, response to stimulation of the D1 and D3 whiskers. Pairing of D2 and D1 whiskers for 7 days causes a significant increase in response to the intact D1 whisker by cells in the D2 barrel column and a decrease in response to the trimmed D3 whisker. This response bias is the most important indicator of plasticity. Following cortical NE depletion, there is a significant reduction in responses of D2 barrel column cells to stimulation of D2 and its neighboring whiskers but RFs can be mapped. Most importantly, the same whisker-pairing produces no shift toward greater responses to the intact D1 whisker. The results strongly support the idea that NE facilitates the synaptic modification necessary for whisker-pairing plasticity in adult rat cortex. (NIH grants NS-25907 and NS-09929)

538.9

EFFECTS OF CHOLINERGIC DEPLETION ON GAD IMMUNOREACTIVITY IN RAT PMBSF CORTEX P. Herron[1], L. Zhang[1], Z.C. Li[1], and J.B. Schweitzer*[,2]. Depts. of Anatomy and Neurobiology[1] and Pathology[2], School of Medicine, University of Tennessee, Memphis., Memphis, Tennessee 38163.

Immunocytochemical methods were used to examine the distribution of puncta and fibers immunoreactive for glutamic acid decarboxylase (GAD) in the posteromedial barrel subfield (PMBSF) cortex after lesions of cholinergic neurons in nucleus basalis of Meynert (NBM). ACh neurons in NBM were selectively lesioned with intraventricular injections of an immunotoxin, 192 IgG-saporin. After seven to nine weeks post-injection survival times, animals were perfused with zinc aldehyde fixative, 30 μm tangential sections were processed for GAD immunoreactivity, and measurements were made using the NIH Image program.

Cholinergic depletion caused selective changes in the morphology of GAD+ barrels in the PMBSF cortex. The GAD immunoreactivity of puncta and fibers were significantly reduced in septa and the outer perimeter of barrel walls. The length, width, and area of barrels were reduced 10 to 20% in cholinergic depleted animals compared with controls. The optical density of GAD immunoreactivity in hollows was not affected by this treatment. These results suggest that long-term cholinergic depletion may lead to the loss of inhibitions in barrel walls and septa but not in hollows.

Supported by Alzheimer's Association (PH), NSF IBN 95-11872 (PH & JBS).

538.10

EFFECTS OF CHOLINERGIC DEPLETION ON THE CORTICAL FUNCTIONS IN FISHER HYBRID RATS Z.C. Li, J.B. Schweitzer, L. Zhang, P. Herron*. Dept of Anatomy and Neurobiology, School of Medicine, University of Tennessee, Memphis., Memphis, Tennessee 38163.

The effects of cholinergic depletion (CD) on evoked neuronal activities of posteromedial barrel subfield (PMBSF) in young and aged male Fisher hybrid rats were examined by extracellular recordings. We tested 110 cells for mean firing rate (MFR), receptive field (RF) size to standard vibrissal deflections of 3 degrees.

We found: 1. CD did not affect the RF size in either young or aged animals compared to controls. 2. In aged rats, CD can significantly increase the MFR of PMBSF neurons to vibrissal deflections ($P<.05$). In young animals, CD treatment caused no difference in the MFR. 3. CD did not affect the MFRs of PMBSF neurons to deflections of the strongest surrounding whisker in either young or aged animals compared to controls.

The above results demonstrated that cholinergic depletion may impair selected cortical processings in the aged animals and suggested that cholinergic innervation to the cortical neurons may play an important role in information processing in the somatic sensory cortex.

Supported by Alzheimer's Association (PH), NSF IBN 95-11872 (PH& JBS).

538.11

COMPARISON BETWEEN THALAMOCORTICAL AND INTRACORTICAL SYNAPTIC RESPONSES EVOKED *IN VITRO*. Y. Amitai* and Z. Gil, Dept. of Physiology, Faculty of Health Sciences, Ben-Gurion University, Israel, 84105.

We explored differences in the properties of converging afferent inputs to a single cortical neurons in the barrel area. Thalamocortical slices were prepared from mature mice. Either thalamocortical (TC) or horizontal, layer 5 intracortical (IC) axons were stimulated, and responses in layer 5 neurons were recorded. Monosynaptic EPSPs had latencies less than 1.8 ms and low shape variance. Disynaptic thalamocortical IPSPs had latencies longer than 1.8 ms. All neuronal types, as defined by intrinsic firing patterns, received both TC and IC monosynaptic input. The shape parameters of monosynaptic EPSPs from the two inputs did not differ significantly. The rate-of-rise of EPSPs varied considerably across cells, but TC and IC-EPSP rate-of-rise onto single cells were correlated. The relative threshold for activation of excitation and inhibition were strikingly different between the two tracts: TC stimulation induced $GABA_A$-dependent IPSPs at stimulus intensities equal to, or less than, those required for evoking excitation in 35% of the cells. In contrast, the threshold response to IC stimulation was always an EPSP, and only stronger stimuli could generate di- or polysynaptic IPSPs.

We suggest that postsynaptic factors may tend to equalize the waveforms of TC- and IC-generated EPSPs in individual neurons. The TC pathway much more effectively activates feedforward inhibitory circuits than the horizontal IC pathway.

This work was supported by the BSF, grant no. 91-00197, and the ISF, grant no. 80/95-1.

538.12

THE INFLUENCE OF INTRACORTICALLY EVOKED INHIBITION ON DIRECTIONAL SENSITIVITY OF NEURONS IN CAT S-1. A.A.Alexandrov* St.Petersburg State University, St.Petersburg, Russia.

Firing activity of the cortical neurons *in vivo* can be inhibited by glutamate, ejected microelectrophoretically 200-300 μm apart from a recording neuron. This inhibition was shown to be associated with glutamatergic excitation of the inhibitory GABA-ergic interneurons. In a single unit study of somatosensory cortex we investigated effects of this kind of inhibition on a properties of neuronal receptive fields such as directional sensitivity. Deflection of vibrissa coused spiking activity of S-1 neurons, which was often more intense in one direction of deflection than in the other directions. Distant application of glutamate during vibrissa testing usually suppressed responses in one direction of deflection more than in the other, e.g. there were cases when responses to some directions were completely blocked while responses to the other directions were not changed. Neurons without directional sensitivity might became directional-sensitive during glutamate application. Some directional-sensitive neurons become tuned to another directions of vibrissa deflection. Our data supports role for intacortical inhibition in formation of directional sensitivity of the S-1 neurons.

538.13

MODULATION OF TEMPORAL DYNAMICS OF SPONTANEOUS AND EVOKED UNIT ACTIVITY IN RAT SI BY PHARMACOLOGICAL ACTIVATION OF HOMOTOPIC SII. M. E. Jackson* and L. J. Cauller, GR41, Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083-0688.

Anatomical studies have demonstrated projections from supragranular neurons in rat SII to homotopic regions of SI. The purpose of this study was to determine the ability of the SII to SI projection to modulate the responses of SI neurons during peripheral somatic stimulation. Spontaneous and evoked extracellular multiunit and singleunit recordings were made with glass micropipettes in the supragranular layers of the forepaw representation in SI of urethane or Nembutal anesthetized rats. Extracellular activity was then recorded during slow infusion of the excitatory amino acid agonists L-glutamic acid and kainic acid through glass micropipettes inserted into homotopic areas ipsilateral SII. Pharmacological activation of supragranular neurons in SII altered both the frequency and temporal dynamics of spike trains recorded in SI. This effect was reversed by slow infusion of the excitatory amino acid antagonist kynurenic acid. Inactivation of SII by infusion of 2% lidocane had no effect in the anesthetized rat.

Supported by a grant from the Whitehall Foundation.

538.14

FUNCTIONAL CONNECTIONS BETWEEN BARREL-COLUMNS IN RAT CORTEX: RECORDINGS OF MULTIPLE SINGLE NEURONS. M. Lebedev*, G. Mirabella, and M.E. Diamond. Cognitive Neuroscience Sector, International School for Advanced Studies, Trieste, 34014 Italy.

This study addresses the relationships between barrel-columns in the vibrissal area of rat SI cortex. Normal rats were compared to rats receiving 3 days of whisker pairing (all whiskers on the right side of the snout were clipped except D2 and one neighbor, D1 or D3). The subjects were anesthetized and an array of up to 8 microelectrodes was inserted into the cortical region related to the caudal vibrissae of rows C-E. The array was advanced through cortex in steps of 100 μm. At each electrode tip, 3-6 individual neurons were isolated using spike waveform discrimination, yielding a sample of about 20-30 simultaneously-recorded single neurons. Spontaneous activity and activity evoked by controlled vibrissa deflection were recorded. Receptive field shifts previously described were confirmed -- column D2 neurons gave heightened responses to the paired neighboring whisker. Cross-correlation analysis indicated that potentiated connections between neurons in the two paired columns could offer an explanation for receptive field shifts. For example, the average value of cross-correlation between neuron pairs in column D2 and D1 was more than twice as great after vibrissae D2/D1 were paired than in normal controls. The average time jitter between events in the two paired cortical columns decreased, suggesting more secure transmission of information between paired cortical columns. (Supported by NINDS 32647, the Whitehall Foundation, and M.U.R.S.T.)

538.15

RECURRENT EXCITATION AND ITS CONTROL IN A MODEL OF RAT WHISKER BARREL CORTEX. J.A. Hartings, D.J Pinto, D.J. Simons*. Depts. of Mathematics, Neurobiology and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

We used a previously constructed model of a whisker barrel to examine how recurrent excitation, feedforward inhibition, and network-dependent inhibition differentially modulate cortical responses to strong (temporally coherent) versus weak (temporally dispersed) afferent signals. The model consists of interconnected populations of excitatory and inhibitory units, which both receive direct inputs in the form of pre-recorded thalamic spike trains. By systematically varying connection strengths among populations we found that the positive feedback provided by recurrent excitation enhances inherent neuronal non-linearities, disproportionately amplifying cortical responses to strong inputs. Engagement of inhibitory units by this recurrent excitation confers a non-linear property to the network inhibition, and, with a brief temporal delay, this acts powerfully to counterbalance the explosive nature of the recurrent excitation. Feedforward inhibition (thalamus to inhibitory units) operates in a strict linear fashion, effectively reducing the amount of thalamic input and hence the engagement of recurrent excitation. The dynamics of synaptic integration creates a 'window of opportunity' for temporally coherent inputs to engage the positive feedback mechanisms of the network, producing a strong excitatory response. In this fashion, spatial contrast, e.g., principal vs adjacent whisker stimulation, is enhanced. Supported by NIH NS19950/NSF IBN9421380.

538.16

THALAMOCORTICAL RESPONSE TRANSFORMATIONS OF VELOCITY AND AMPLITUDE IN THE RAT WHISKER BARREL SYSTEM. D.J Pinto*, J.C. Brumberg, D.J. Simons. Depts. of Mathematics, Neurobiology, and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

Our previous modeling studies suggested that barrel neurons are more sensitive to the velocity of whisker deflection than to deflection amplitude. To examine this prediction, single unit recordings using glass micropipettes were obtained in fentanyl-sedated adult rats from thalamic (barreloid) neurons. The stimulation protocols entail moving the principal whisker in a randomly interleaved combination of five velocities and three amplitudes. Large and medium amplitude deflections produced barreloid responses of roughly the same magnitude, which decreased as deflection velocity diminished from very fast to very slow. Low amplitude deflections produced responses that were somewhat smaller and that showed a more pronounced decline as velocity was decreased. Regardless of deflection amplitude, responses were more temporally dispersed at slower velocities. Population histograms were constructed from the thalamic unit responses and were used as input to our simulated barrel. Simulated responses depend more strongly on input dispersion than input magnitude. Moreover, the simulations lead to quantitative predictions about barrel neuron responses. These predictions are being tested directly by examining the responses of barrel neurons to whisker stimuli identical to those used to generate the thalamic input to the simulated barrel. Supported by NIH NS19950 and NSF IBN9421380.

538.17

LAMINAR ANALYSIS OF MULTIPLE WHISKER INTERACTIONS IN RAT BARREL CORTEX. J.C. Brumberg*, D. J Pinto, H.T. Kyriazi, D.J. Simons. Depts. of Neurobiology and Mathematics/Statistics, University of Pittsburgh, Pittsburgh, PA 15261.

To assess the effects of multiple whisker interactions (up to 11), single unit recordings using glass micropipettes were obtained in fentanyl-sedated adult rats from neurons within different laminae of rat barrel cortex and from thalamic (barreloid) neurons. Using a condition-test paradigm, the columnar or principal whisker (PW) was deflected alone or in combination with adjacent and/or far whiskers (two arcs or rows from the PW). Consistent with previous intra- and extracellular data, layer IV barrel neurons displayed the earliest response onsets to PW deflections, and they had the smallest excitatory receptive fields. While the strength of inhibitory interactions among neighboring whiskers was roughly equivalent throughout the column, barrel neurons showed the greatest loss of surround inhibition during microiontophoresis of the $GABA_A$ antagonist bicuculline. In addition, barrel neurons showed the greatest receptive field expansion. Compared to barrel neurons, thalamic neurons displayed larger excitatory receptive fields and much less surround inhibition, even when up to 5 neighboring whiskers were stimulated along with the PW. Results suggest that: (1) inhibitory circuitry within the barrel strongly constrains receptive field size of layer IV neurons, (2) *intercolumnar* processing creates the larger receptive fields seen in the non-granular layers, and (3) *intracolumnar* connections originating in a barrel account for most of the between whisker-evoked inhibition observed throughout its column. Supported by NIH NS19950 and NSF IBN9421380.

538.18

DIFFERENTIAL MODULATION BY MOVEMENT OF SOMATOSENSORY CORTICAL RESPONSES ACROSS CUTANEOUS RECEPTIVE FIELDS. Richard Courtemanche[1]*, Gong-Duo Sun[2], Marie-Thérèse Parent[1] & Yves Lamarre[1]. [1]Dép. de Physiologie & CRSN, Univ. de Montréal, C.P. 6128, Succ. Centre-Ville, Montréal (Qc) Canada H3C 3J7; [2]Dept of Physiology, Nanjing Univ., Nanjing, People's Republic of China.

Recent studies have reported that rapid modifications of cutaneous receptive fields (RFs) can take place at multiple levels in the dorsal column-lemniscal pathway, following anesthesia or deafferentation. Complete gating during movement of the somatosensory response in areas 3b and 1 to an air-puff stimulus on the skin while preserving movement-induced activity has previously been observed. This could be explained by rapid movement-related modifications or displacement of cutaneous RFs at some level in the dorsal column-lemniscal pathway. This possibility was explored by recording the activity of 35 neurons in areas 3b and 1 of one female *Macaca mulatta* monkey during elbow flexions and at rest. Equal pressure air-puff stimuli were given at one (9/35 neurons) or multiple sites (26/35) within and on the edges of RFs located on the contralateral forearm. Response latencies for all cells ranged between 12-28 ms (mean:16 ms). Post-stimulus time histograms showed that 32/35 neurons had decreased responses to stimulus during movement compared with stimulus during rest. So far, air-puffs given at RF edges during movement have not produced response increases compared with air-puffs given at rest: RF shifts have not been found. However, while 9/26 neurons presented a uniform response decrease, 17/26 showed non-uniform decreases across multiple sites in the RF. This latter sample displayed proportions of decrease during movement compared to rest that varied across different regions of the RF. This argues against a simple suppression of information during movement, and offers evidence for more complex mechanisms of information processing in the somatosensory cortex and along the dorsal column-lemniscal pathway during movement. Supported by various MRC and FCAR grants.

PAIN MODULATION: PHARMACOLOGY—GABA AND NMDA RECEPTORS

539.1

SPINAL MECHANISMS OF ANTI-ANALGESIA: LEARNED SAFETY SIGNALS BLOCK NON-OPIATE AS WELL AS OPIATE ANALGESIAS. M. McGorry*,B. Schwartz, D. Sisk, E.P. Wiertelak[1], S.F. Maier,& L.R. Watkins, Dept. Psych., U CO at Boulder, Boulder, CO 80309 & [1]Macalster College, St. Paul, MN.

We have previously shown that learned safety signals (light cues) can abolish analgesia produced by systemic morphine as well as by intrathecal (IT) μ & δ opiate agonists [Sci., *256* (1992) 830; Brain Res. *634* (1993) 214]. This raises the question of whether safety signals modulate only opiate analgesias, or whether non-opiate analgesias can be affected as well. Thus we tested the ability of learned safety signals to modulate non-opiate analgesias produced by IT serotonin (5HT) & GABA agonists.

Detailed methods of establishing & testing safety signals are described in the articles cited above. After extinguishing the danger signals associated with the context, baclofen (GABA-B agonist), α-methyl-tryptamine (5HT-2 agonist), or phenylbiguanide (5HT-3 agonist) was injected epidurally under brief halothane anesthesia. Drugs found to be partial opiate analgesics were preceded by 15 mg/kg naltrexone s.c. to allow testing only of the non-opiate component of the analgesias. Safety signals abolished non-opiate analgesias mediated by GABA-B & by 5HT-3 receptors. No reversal of 5HT-2 analgesia was observed. Testing is continuing with 5HT-1 & GABA-A agonists.

Thus, anti-analgesia can profoundly modulate opiate, GABA, & 5HT mediated analgesias. These data extend the concept of anti-analgesia from meaning merely anti-opiate effects to the recognition that multiple analgesia systems can be profoundly modulated in this fashion. Supported by NIH NS31569 & Undergrad. Research Opport. Prog.

539.2

INTERACTION BETWEEN $GABA_B$ RECEPTOR ACTIVATION AND NEUROKININ-1 RECEPTOR GENE EXPRESSION IN RAT SPINAL CORD DURING NOCICEPTION. Kenneth E. McCarson* and S. J. Enna Dept. of Pharm/Tox/Therap., Univ. of Kansas Med. Ctr., Kansas City, KS 66160-7417 USA.

Gamma-aminobutyric acid (GABA) agonists for both $GABA_A$ and $GABA_B$ receptors induce analgesia in animal models of acute and chronic nociception, including the formalin test. It has been reported that substance P (SP) and the neurokinin-1 receptor (NK-1R) are involved in mediating the nociceptive responses to formalin; NK-1R antagonists inhibit various aspects of formalin-induced nociception and NK-1R gene expression is increased during formalin-induced nociception. Since the antinociceptive properties of $GABA_B$ receptor agonists may be due to their ability to reduce SP release evoked from spinal cord tissue, the present study was undertaken to examine the regulation of NK-1R gene expression by baclofen during neuronal activation of the spinal cord dorsal horn by formalin-induced nociception. Total RNA samples prepared from the lumbar spinal cords of rats receiving a drug pretreatment of baclofen (4mg/kg, s.c.) or saline followed by injection of formalin (5%, 100μl) into the right hindpaw were assayed for NK-1R mRNA using solution hybridization - nuclease protection assays 6 hours after hindpaw treatment. Baclofen pretreatment completely abolished the flinching response to formalin during ten minutes of the late phase of the formalin test, but did not induce significant muscle weakness as measured in the inclined screen test. Preliminary results indicate the expression of NK-1 mRNA in the ipsilateral lumbar dorsal horn of the spinal cord is increased 6 hours following formalin treatment as compared to tissue obtained from sham treated rats. Pretreatment of animals with baclofen substantially reduced the formalin-induced increase in NK-1 mRNA levels in the ipsilateral spinal cord dorsal horn but had no significant effect on NK-1 mRNA levels in sham-treated rats. These results suggest that baclofen exerts its analgesic action, at least in part, by modifying tachykinin transmission through the NK-1R, and that neurokinin receptor expression and turnover may be regulated by the degree of nociceptive activation of the hindpaw and spinal cord dorsal horn.

539.3

EFFECTS OF $GABA_A$ RECEPTOR ANTAGONIST ON TRIGEMINAL (V) SUBNUCLEUS CAUDALIS NOCICEPTIVE NEURONES IN NORMAL AND NEONATALLY CAPSAICIN (CAP)-TREATED ADULT RATS. C.Y. Chiang*, C.L. Kwan, J.W. Hu and B.J. Sessle. Faculty of Dentistry, University of Toronto, Toronto, Ont. M5G 1G6, Canada.

Our previous studies have demonstrated that NMDA and segmental modulatory mechanisms may be involved in the CAP-induced neuroplastic changes in the receptive field (RF) properties of V caudalis nociceptive neurones in adult rats. Since GABA is involved as an inhibitory neurotransmitter in segmental modulation, the aim of this study was to test whether the $GABA_A$ receptor antagonist bicuculline (BIC) can influence the properties of caudalis nociceptive neurones in normal (n=5) and CAP (n=7) rats. Single neurone activity was recorded extracellularly in urethane/α-chloralose anesthetized and paralysed adult rats, and neurones were classified as wide dynamic range (WDR) or nociceptive specific (NS). RF properties were tested in each neurone before and for 60 min after BIC (3-5 μg/10 μl, i.t.) application, and any BIC-induced changes were expressed as a percentage (mean±S.E.M.) of control (i.e., pre-application) values. A total of 17 nociceptive neurones were studied (3 WDR, 3 NS in normal rats; 5 WDR, 6 NS in CAP rats). Consistent with our earlier findings, tactile and pinch RFs were expanded in CAP rats compared to normal rats. BIC application generally produced in both groups of rats seizure-like neuronal discharges with a 18-30 s latency and 3-10 min duration. BIC was also found to induce changes in RF properties that peaked at 5 min and lasted for 30 min. Compared to control values (100%), the peak changes in normal and CAP rats included, respectively, decreases in response threshold (3.5±1.4%; 14±8.3%), and increases in responses to mechanical threshold stimulation (4037±3252%; 1495±998%) and in responses to pinch (656±523%; 231±34%). Another noteworthy change was the appearance of a tactile RF in the NS neurones of both normal and CAP rats. Furthermore, the tactile RF of WDR neurones in normal and CAP rats expanded, respectively, to 633±379% and 593±383% of control. While the pinch RF of WDR and NS neurones in normal rats was expanded to 307±85%, the pinch RF in CAP rats was reduced to 29±8% of control. These findings suggest that the tactile and pinch RF properties of caudalis nociceptive neurones are under GABAergic control and that CAP-induced C-fibre depletion may alter this modulatory mechanism.

Supported by NIH grant DE-04786.

539.4

ADRENOCEPTORS (α_2) MODULATE NMDA-EVOKED RESPONSES OF NEURONS IN THE MEDULLARY DORSAL HORN . K.-M. Zhang*, X-M. Wang, A.M. Peterson, D.E. Brown, W.Y. Chen and S.S. Mokha Department of Physiology, Meharry Medical College, Nashville, Tennessee 37208.

Activation of α_2-adrenoceptors has been reported to be involved in antinociception in animal and human studies. Microiontophoretically applied norepinephrine has been reported to produce reduction in the noxious stimuli evoked responses of neurons in the spinal dorsal horn. No comparable studies have been performed in the medullary dorsal horn, and the modulation of NMDA-evoked responses by activation of α_2-adrenoceptors remains unknown. The present study, therefore, investigated the modulation of NMDA-evoked responses of neurons in the medullary dorsal horn by activation of α_2-adrenoceptors. Extracellular single-unit recordings were made in the superficial and deeper dorsal horn of the medulla (trigeminal nucleus caudalis) in anesthetized rats (urethane, 1.5 g/kg, i.p.) using the central barrel of a seven barrel microelectrode. The drug barrels contained solutions of the following drugs: NMDA (50 mM, pH 8.2); DL-2-Amino-5-Phosphonovaleric acid (AP-5, 50 mM, pH 8.0); Clonidine (100 mM, pH 4.5) and Idazoxan (100 mM, pH 4.5). All drugs were ejected with positive current except NMDA and AP-5 which were ejected with negative current. Clonidine reduced the NMDA-evoked responses of 86% of nociceptive-specific (NS) neurons, 75% of wide dynamic range (WDR) neurons and 40% of low threshold (LT) neurons in the superficial dorsal horn. In the deeper dorsal horn, clonidine inhibited NMDA-evoked responses of 100% of NS neurons, 86% of WDR neurons and 20% of LT neurons. The facilitatory effect of clonidine were observed in 14% of NS, 25% of WDR, 60% of LT neurons in the superficial dorsal horn and 40% of LT neurons in the deeper dorsal horn. Idazoxan, α_2-adrenoceptor antagonist, reversed the inhibitory effect of clonidine in 12/16 neurons. It is suggested that α_2-adrenoceptor activation produces a predominantly inhibitory modulation of the NMDA-evoked responses of nociceptive neurons, and facilitation of low threshold neurons in the medullary dorsal horn.

Supported by NIH (DE10903; RR03032) and NSF (IBN-9109247; HRD-9255157)

539.5

MICROINJECTION OF SUBSTANCE P INTO THE KÖLLIKER FUSE (KF) NUCLEUS INHIBITS OR FACILITATES NMDA-EVOKED RESPONSES OF NEURONS IN THE SUPERFICIAL AND DEEPER DORSAL HORN OF THE MEDULLA. X-M. Wang*, K.M. Zhang and S.S. Mokha. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

We have been investigating the modulation of excitatory amino acid evoked responses by activation of α_2-adrenoceptors. Kölliker Fuse nucleus (KF) is one of the regions which may contribute to the noradrenergic modulation of somatosensory information in the dorsal horn of the medulla (trigeminal nucleus caudalis). No previous study has investigated the descending modulation of NMDA-evoked responses by stimulation in KF. The present study, therefore, investigated the effects of microinjection of substance P into KF on the NMDA-evoked responses of neurons in the superficial and deeper dorsal horn of the medulla. Extracellular single unit recordings were made from 23 neurons with the central barrel of a seven barreled microelectrode in rats anesthetized with urethane (1.5 g/kg, i.p.). The other barrels contained freshly made solutions of NMDA (50 mM, pH 8.0), AMPA (5mM, pH 8.0), clonidine (100 mM, pH 4.5), idazoxan (100 mM, pH 4.5) and CNQX (5 mM, pH 8.0). NMDA, AMPA, and CNQX were ejected with negative current whereas clonidine and idazoxan were ejected with positive currents. Neurons were characterized nociceptive specific (NS), wide dynamic range (WDR), low threshold (LTH) using natural stimuli (brush, pinch, squeeze, heat). Substance P (3.7 or 37 pmol) was microinjected through a guide cannula placed stereotaxically 1 mm dorsal to KF. Microinjection of substance P into KF produced inhibition of NMDA-evoked responses of neurons in the deeper dorsal horn whereas it produced inhibition or facilitation in the superficial dorsal horn. The inhibitory effect was antagonized by microiontophoretically applied idazoxan on 7/8 neurons. Clonidine produced an inhibitory effect on some (6/7) of these neurons. All neurons (6) facilitated by stimulation in KF were located superficially, and facilitation was blocked by CNQX in 2/2 neurons. We conclude that α_2-adrenoceptors and AMPA receptors mediate the descending modulation from KF.

Supported by NIH (DE10903;RR03032) & NSF (IBN-9109247; HRD-9255157)

539.6

OPIOID RECEPTORS (μ,δ) MODULATE THE NMDA-EVOKED RESPONSES OF TRIGEMINOTHALAMIC NEURONS IN THE RAT. S.S. Mokha* and X-M. Wang, Department of Physiology, Meharry Medical College, Nashville, Tennessee 37208.

We reported recently that activation of μ and δ opioid receptors produces a predominantly inhibitory modulation of the NMDA-evoked responses of nociceptive neurons (unidentified as to their projections) in the superficial and deeper dorsal horn of the medulla (Zhang et al. 1996, Brain Res.). However, the functional significance of EAA receptors in mediating the responses of physiologically characterized trigeminothalamic neurons and the opioid-receptor mediated modulation of EAA receptor-evoked responses of these neurons remains unknown. The present study was , therefore, designed to investigate the modulation of NMDA-evoked responses of trigeminothalamic neurons by activation of μ- and δ-opioid receptors. Extracellular single-unit recordings were made in the superficial and deeper dorsal horn of the medulla (trigeminal nucleus caudalis) in anesthetized rats (urethane, 1.5 g/kg, i.p.), using glass-coated platinum-plated tungsten microelectrode glued to a seven-barrel micropipette at an angle of about 20^0. One barrel of the seven-barrel microelectrode contained 2 M NaCl for automatic current balancing. The remaining barrels were filled with freshly made solutions of the following drugs: NMDA (50 mM, pH 8.2); D,L-2-amino-5-phosphonovaleric acid (AP-5, 50 mM, pH 8.0); [D-$Pen^{2,5}$]-Enkephalin (DPDPE, 20 mM, pH 4.0); DAMGO (20 mM, pH 4.0) and naloxone hydrochloride (10 mM, pH 4.5). NMDA and AP-5 were ejected with negative current while DPDPE, DAMGO and naloxone were ejected with positive current. Microiontophoretic application of NMDA activated 18/19 trigeminothalamic neurons. Administration of DAMGO, a selective μ opioid receptor agonist, reduced the NMDA-evoked responses in 77% of trigeminothalamic neurons. DPDPE, a selective δ opioid receptor agonist, produced inhibition of NMDA-evoked responses in 36% of neurons. The predominantly inhibitory modulation of NMDA receptor mediated responses of nociceptive trigeminothalamic neurons by activation of μ and δ opioid receptors may provide a neural mechanism for the antinociceptive actions of opioids.

Supported by NIH (DE10903; RR03032) and NSF (IBN-9109247;HRD-9255157)

539.7

REDOX MANIPULATION OF NMDA RECEPTORS IN VIVO: ENHANCEMENT OF NMDA-ELICITED BEHAVIORS AND EFFECT ON DYNORPHIN-INDUCED ALLODYNIA. T.M. Laughlin*[1], and G.L. Wilcox[1,2]. [1]Dept. of Pharmacology and [2]Program in Neuroscience, University of Minnesota, Minneapolis, Minnesota 55455.

Sulfhydryl reducing agents, such as dithiothreitol (DTT), potentiate NMDA receptor-evoked currents in vitro whereas oxidizing agents, such as 5,5'-dithio-bis-(2-nitrobenzoic acid) (DNTB), attenuate these currents. Reducing agents modulate current by reducing disulfhydrly bridges at the redox modulatory site located in the extracellular domain of the NMDA receptor. This involvement of the redox modulatory site involvement in neurotoxicity has been previously studied; here we examined the effect of this redox modulatory site on nociceptive spinal cord signaling in mice.

Drugs were diluted in 0.9% saline and administered intrathecally (i.t.) to male ICR mice. NMDA i.t. to mice induces caudally directed biting and scratching behaviors, which are counted during the first minute after injection. A five minute pretreatment with DTT (10, 30 nmol, i.t.) shifted the NMDA dose-response curve to the left, suggesting enhancement of receptor function through reduction of the redox modulatory site. Five minute pretreatment with 10, 30, and 50 nmol DTT potentiated the response to 0.3 nmol NMDA by 140, 160, and 230 percent, respectively. Increasing the 30 nmol DTT pretreatment time to 30 minutes produced additional enhancement of NMDA response. In addition to NMDA-induced behavior, we also tested the effect of DTT on tactile and cold allodynia induced by dynorphin A (1-17) (3 nmol, i.t.).

NMDA-induced behavior is a model used to study acute nociception. Potentiation of this behavior by DTT suggests that endogenous reducing agents may up-regulate NMDA receptor function, increasing responses to endogenous glutamate. Furthermore, enhancement of NMDA receptor function by endogenous reducing agents may enable spinal cord neurons to modulate receptor function in chronic neuropathic pain states. (Supported by NIDA/K02-DA-00145 & NIDA/R01-DA-04274 to GLW, T32 DA07097 supports TML)

539.8

Antisense Mediated Downregulation of Spinal NMDA Receptors: Behavioral Consequences. Mary G. Garry, Ph.D.* Lloyd P. Walton, B.S., Harbhej Singh, M.D., and Jay Yang, M.D., Ph.D. Department of Anesthesiology, University of Texas Southwestern Medical Center, Dallas, TX 75235

N-Methyl-D-Aspartate (NMDA) receptors at the level of the spinal cord are known to have a role in pain processing and are believed to be involved in the development of morphine tolerance. Due to the toxicity of NMDA antagonists and the need for long-lasting therapies for chronic pain, we have used an antisense approach in an attempt to downregulate spinal NMDA-R1 receptors in the rat. In *in vitro* studies, we determined that PC-12 cells (which express functional NMDA receptors) incubated for 3 days in 100 μg of an 18-Mer oligodeoxynuclotide (CAGCAGGTGCATGGTGCT) showed a decrease in NMDA receptor immunoreactivity when compared to normal cells. We then applied the antisense probe spinally in rats to determine whether this tool would be effective in reducing the pain behavior typically observed following intrathecal (i.t.) administration of NMDA. Briefly, male rats were implanted with i.t. cannulas to the lumbar level. Two days following recovery from implantation, baseline behavioral data was collected (# of caudally directed bites, scratches, and licks/min [BSL]). Next, an antisense probe (100μg/10μl/day) or vehicle was administered i.t. for 3 days. To determine the downregulation of spinal NMDA receptors *in vivo*, i.t. NMDA (3nmol/10μl) was administered. Mean baseline BSL's were 3.57±0.99/min (n=14). In control animals, the # of BSL behaviors increased to 24±4.7/min (n=3) in response to NMDA. In the antisense treated animals, the NMDA response was reduced to 8.6±2.46 BSL's/min (n=3). In addition, this response was observed to be dose-related (20-100μg/day for 3 days). In summary, these data indicate that an antisense approach is a useful tool in the downregulation of NMDA receptors in the spinal cord; resulting in altered behavioral responses to NMDA. Studies are currently underway to determine the usefulness of this approach in the reduction of chronic pain and in the development of morphine tolerance. This work was supported by the Sid W. Richardson Foundation.

539.9

EFFECTS OF D-CYCLOSERINE PRETREATMENT ON THE INDUCTION OF PERSISTENT HINDLIMB FLEXION IN SPINALIZED RATS. M.F. Anderson* Department of Psychiatry, St. Elizabeth's Medical Center of Boston, Boston, MA 02135.

Unilateral stimulation (2mA, 7ms, 100Hz, 60min) across the upper hindlimb in the spinalized rat produces persistent hindlimb flexion. In this model, the N-methyl-D-aspartate (NMDA) antagonist, MK-801, effects a dose dependent reduction in flexion. After spinalization at T-7 of urethane anesthetized Long Evans rats, wound clips were applied bilaterally to the musculature of the right upper hindlimb and animals were pretreated with one dose of either sterile water (vehicle control) or D-cycloserine (0.1, 1.0, and 10.0 mg/kg, i.p.). After a 30min waiting period, current (2mA, 7ms, 100 Hz, 1 hr) was delivered across the clips. Following stimulation, flexion was not significantly different between the experimental and control groups; except, in the 10.0 mg/kg D-cycloserine group where flexion was significantly increased. These data suggest that induction of persistent hindlimb flexion in spinalized rats may be enhanced by agonists to the strychnine-insensitive glycine binding-site associated with the NMDA receptor. (Support: St. Elizabeth's Medical Center of Boston)

539.10

EFFECT OF INTRATHECAL (IT) MK801 IN A RAT MODEL OF POSTOPERATIVE PAIN. P.K. Zahn, T.J. Brennan*, G.F. Gebhart, Departments of Anesthesia and Pharmacology, University of Iowa, Iowa City, IA 52242

We have developed and characterized a rat model for postoperative pain. An incision causes reliable, quantifiable mechanical hyperalgesia that is inhibited by systemic and IT morphine. The purpose of this study is to examine the effect of MK801, a noncompetitive N-Methyl-D-Aspartate (NMDA) receptor antagonist, on mechanical hyperalgesia in this model.

Rats with IT catheters were anesthetized with 2% halothane and underwent an incision as described (Brennan et al., Pain 64: 493). Two hours later, withdrawal threshold to punctate stimulation was determined by applying calibrated von Frey filaments adjacent to the wound. Each filament was tested once starting with 15 mN and continuing to 522 mN until a withdrawal response occurred. Response frequency to application of a 5 mm diameter plexiglas disk (attached to a 400 mN von Frey filament) directly to the wound was measured to assess a nonpunctate stimulus. Rats were tested at 10 min, 30 min, and 1 and 2 hrs after surgery and on postoperative day 1.

In the saline treated group (n=5), the median withdrawal threshold decreased from 522 mN before surgery to 24 mN 2 hrs later. Hyperalgesia was persistent; the median withdrawal threshold remained less than 95 mN for 2 hrs. After administration of 4, 14, or 40 nmol MK801 IT, the median withdrawal thresholds remained less than 80 mN for the next 2 hrs. The mean response frequency for nonpunctate hyperalgesia in the saline treated group (n=5) increased from 0±0% before surgery to 100±0% 2 hrs later and was maintained for the next 2 hrs. In separate groups of animals (n=5 per group), after IT administration of 20 and 40 nmol MK801, the mean response frequencies were 80±13% or greater for 2 hrs. Similar results were observed on postoperative day 1 using these same doses of MK801.

Unlike IT and systemic morphine, IT MK801 did not modify mechanical hyperalgesia to punctate or nonpunctate stimuli in this rat model of postoperative pain.

539.11

INHIBITION OF ACUTE THERMAL HYPERALGESIA BY DNQX IS SYNERGISTIC WITH THE ANTINOCICEPTIVE EFFECT OF MK-801 BUT NOT WITH THAT OF THE NONCOMPETITIVE NMDA ANTAGONIST CPP, C.S. Hornfeldt* and A.A. Larson. Department of Veterinary PathoBiology, University of Minnesota, St. Paul, MN 55108.

N-methyl-D-aspartate (NMDA) and non-NMDA excitatory amino acid (EAA) receptors are thought to play a role in nociceptive transmission. We tested the hypothesis that both types of receptors are involved in acute thermal hyperalgesia. Hyperalgesia was induced by exposure of the tail of ether-anesthetized mice to a radiant heat source for 50 sec, a time sufficient to induce redness, but no blister. Twenty-five min later, 1 mg/kg of naloxone was administered i.p., unmasking a significant decrease in the tail-flick latency 5 min later when the tail was immersed in a water bath maintained at 46°C. Activity at NMDA and non-NMDA receptors was found to be necessary for thermal hyperalgesia as intrathecal injections of either MK-801 (dizolcipine), 6,7-dinitroquinoxalinedione (DNQX) or the competitive antagonist (±)-3-(carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), injected 5 min prior to testing, each reduced the degree of hyperalgesia in a dose-dependent fashion. The effects of MK-801 plus DNQX and of CPP plus DNQX were determined using isobolographic analysis. Antinociception produced by DNQX plus MK-801 was synergistic whereas the effect of DNQX plus CPP was merely additive. These data indicate that the mechanism of action of MK-801 differs from that of CPP in this model of hyperalgesia in spite of their common ability to inhibit NMDA-induced activity. We postulate that MK-801 induces its anti-hyperalgesic effect by a mechanism that does not involve a simple inhibition of the NMDA receptor complex. The effect of MK-801 that is not due to antagonism of NMDA activity appears to be important for its ability to induce antinociception (Supported by NIDA 04090 to A.A.L.).

539.12

ACTIVATION OF NMDA RECEPTORS MEDIATES THE ENHANCEMENT OF FORMALIN-INDUCED PAIN BY INTRATHECAL BICUCULLINE M. Kaneko* and D.L. Hammond, Dept. of Anesthesia & Critical Care, Univ. of Chicago, Chicago, IL 60637

Intrathecal (i.t.) pretreatment with bicuculline (BIC), a $GABA_A$ receptor antagonist, enhances the second phase pain behaviors produced by injection of formalin in the hind paw of the rat (Kaneko and Hammond, *World Congress on Pain*, 1996). This study investigated the role of N-methyl-D-aspartate (NMDA) receptor activation in the enhancement of formalin-induced pain behaviors by BIC. Sprague-Dawley rats were prepared with an i.t. catheter. One week later, rats were pretreated with either saline, 0.3 μg BIC, or 0.3 μg BIC and 0.3 μg of D-(-)-2-amino-5-phosphonovalerate (APV), followed 10 min later by a s.c. injection of formalin (0.25 - 2.5 %). The number of flinches and weighted pain scores were recorded for 60 min. In saline-treated rats, the concentration of formalin that produced the half-maximal number of flinches in the second phase (FED_{50}) was 0.93 (95%CL: 0.84-1.03) %. BIC reduced the FED_{50} to 0.44 (0.37-0.52) %. In rats in which APV was coadministered with BIC, the FED_{50} was increased to 0.76 (0.67-0.86) %. APV similarly attenuated the enhancement of weighted pain scores induced by i.t. administration of BIC. This dose of APV by itself had no effect on formalin-evoked pain behaviors. These findings suggest that the enhanced responses to formalin in the late phase are mediated, in part, by an activation or "unmasking" of NMDA receptors in the spinal cord. (Supported by USPHS grant DE11423).

539.13

SPINAL GABA AGONISTS DIMINISH ALLODYNIA CAUSED BY NEUROPATHIC INJURY. TP Malan, Jr., HP Mata, J Lai*, F Porreca. The University of Arizona Pain Institute, Tucson, AZ 85724

GABA agonists diminish thermal hyperalgesia caused by nerve injury. However, sensitivity of nociceptive responses to antinociceptive drugs may depend on the specific sensory modality tested. For example, after spinal nerve root ligation thermal hyperalgesia is diminished by intrathecal (i.th.) morphine, while allodynia is insensitive to supramaximal i.th. doses of this opioid. This may be because thermal stimuli are carried by C-fibers, which contain presynaptic opioid receptors, while allodynia may be carried by A_β fibers, which do not express opioid receptors. We hypothesized that because GABA receptors are found presynaptically on small unmyelinated fibers and large myelinated fibers (as well as postsynaptically), allodynia would be sensitive to intrathecal GABA agonists.

After approval by the Animal Care and Use Committee, male Sprague- Dawley rats (250-350g) were implanted with an i.th. catheter. After full recovery they underwent spinal nerve root ligation surgery. After 7 days they received isoguvacine (7.5 μg, i.th.), baclofen (0.5 μg, i.th.) or vehicle. Allodynia was assessed every 5 min by measuring withdrawal threshold to tactile stimulation using von Frey filaments.

Neuropathic injury decreased withdrawal threshold to tactile stimuli by 80%. After neuropathic injury, isoguvacine ($GABA_A$ agonist) reduced allodynia (increased withdrawal threshold) by 50% of the maximum possible effect (MPE). Baclofen ($GABA_B$ agonist) decreased allodynia by 100% of MPE.

Allodynia caused by neuropathic injury is sensitive to GABA agonists, supporting the hypothesis that sensitivity of different sensory modalities to pharmacologic agents may depend on the fiber types encoding the afferent input and the distribution of drug receptors. Strategies directed at increasing inhibitory control systems may prove useful in modulating neuropathic pain.

Supported by The University of Arizona and The University of Arizona Foundation.

539.14

HISTOGRANIN ATTENUATES CHRONIC PAIN INDUCED BY PERIPHERAL NEUROPATHY. FORMALIN-INDUCED NOCICEPTION, AND DIRECT APPLICATION OF NMDA. J.B. Siegan*, A.T. Hama, and J. Sagen. Dept. Anatomy and Cell Biology. University of Illinois at Chicago, Chicago, IL 60612.

Work in our laboratory has demonstrated that transplantation of adrenal medullary tissue into the spinal subarachnoid space reduces pain sensitivity. The mechanism for the beneficial effects of the transplants in alleviation of persistent pain is unknown. A 15 amino acid peptide, histogranin, that was isolated from adrenal medullary tissue has been shown to antagonize NMDA receptor activation both *in vitro* and *in vivo*. The goal of the following studies was to determine whether direct intrathecal application of the stable histogranin analog [Ser^1] HN (SHG) may account for the effects of adrenal medullary transplants and mimic these transplants in alleviating hypersensitivity in three models. All rats were implanted with intrathecal catheters for SHG application. Group 1: rats were pretreated with SHG (1.0μg or 4.0μg) prior to i.t. injection of NMDA (20, 10, 5 nmol); behavioral tests were performed to measure hypersensitivity. Group 2: Chronic constriction injury was induced by unilateral loose ligation of the sciatic nerve. Two weeks following ligation animals were treated with SHG (1.0μg, 2.0μg, or 4.0μg) and behavioral tests were performed to determine abnormal pain sensations. Group 3: 5% formalin was injected s.c. in animals either pretreated with SHG or saline, and flinches were counted every 5 minutes following injection. Results demonstrated that SHG attenuates NMDA hypersensitivity at higher doses, and abolishes this hypersensitivity at lower doses. SHG attenuated the tonic phase of formalin induced nociception, compared to saline pretreated animals. Neuropathic pain symptoms were also significantly alleviated by SHG in sciatic nerve ligated animals. These results parallel findings with adrenal medullary transplanted animals, and support the hypothesis that adrenal medullary transplants can produce agents which directly inhibit NMDA-mediated events in the spinal cord. Supported by NS25054.

540.1

ADL 2-1294, A PERIPHERALLY SELECTIVE OPIATE ANALGESIC. D.L. DeHaven-Hudkins*, L. Cortes Burgos, H. Nagasaka and T.L. Yaksh. Adolor Corporation, Malvern, PA 19355 and Dept. Anesthesiology, Univ. Calif. San Diego, La Jolla, CA 92093.

ADL 2-1294 is an opiate agonist with affinity for the μ (K_i = 3 nM), δ (K_i = 48 nM) and κ (K_i = 1156 nM) subtypes of the cloned human opiate receptor, and poor central bioavailability. The efficacy of ADL 2-1294 was established in a rat model of inflammatory pain which measures blood pressure changes in response to knee compression following injection of a 4% kaolin/4% carrageenan mixture into the knee joint cavity. Injection of 0.3 mg ADL 2-1294 into the intraarticular space of the knee results *in* potent antinociception that is antagonized by naloxone, whereas injection into the contralateral knee joint or via the i.m. route fails to inhibit compression-induced changes in blood pressure. ADL 2-1294 potently inhibits formalin-induced flinching during the late phase following i.paw injection (A_{50} = 6 μg). The lack of effect of ADL 2-1294 on early phase flinching or following injection into the contralateral paw confirms the peripheral nature of the analgesia observed. Local injection of ADL 2-1294 also produces antinociception against Freund's adjuvant- (ED_{50} = 20 μg) or tape stripping-induced hyperalgesia (ED_{50} = 71 μg), as evidenced by decreased paw pressure thresholds. In all animal models examined, the analgesic potency of ADL 2-1294 was comparable to or better than that of morphine. ADL 2-1294 has potential therapeutic utility as a peripherally selective opiate analgesic which lacks many of the side effects generally associated with such opiates.

540.2

PRESYNAPTIC MODULATION BY A μ-OPIOID RECEPTOR AGONIST AT PRIMARY AFFERENT TERMINALS IN THE RAT SPINAL DORSAL HORN REVEALED BY OPTICAL IMAGING WITH A VOLTAGE-SENSITIVE DYE K. Murase*, K. Oda, M. Tanifuji, J. B. Park§, and P. D. Ryu§. Dept. Info. Sci., Fukui Univ., 3-9-1 Bunkyo, Fukui 910, Japan, and §Dept. Vet. Pharm. Toxicol, Seoul Nat'l Univ., Suwon, Korea.

μ-opioid receptors (MOR) exist at primary afferent C-fiber terminals. Evidence suggests that the MOR activation may lead to a reduction in Ca^{++} influx into the terminals, and thus, diminishing transmitter release. We here report that, by a direct recording at the terminal regions with an optical method, neuronal activities of terminals are reduced by a selective MOR agonist Tyr-D-Ala-Gly-N-Methyl-Phe-Gly-ol(DAMGO), and that this effect of DAMGO is abolished by Ca^{++} channel antagonists.

Transverse slices of the rat spinal cord were stained with a voltage-sensitive dye RH-482. The light absorption change at the dorsal horn evoked by dorsal root stimulation was recorded by a high-resolution imaging system (SD1001, Fuji Micro. Dev.). Postsynaptic components of the optical response could be eliminated by adding excitatory amino acid receptor antagonists APV (30 μM)+CNQX (5 μM) in the perfusate. Because, the optical response in APV + CNQX was not modified by a further addition of ω-conotoxin GVIA (ω-CgTx, 1 μM) and ω-agatoxin IVA (ω-Aga, .2 μM), that are known to block synaptic transmissions by inactivating presynaptic P and N-type calcium channels.

DAMGO suppressed the optically-recorded presynaptic activities in APV + CNQX at the superficial laminae dose-dependently (29%, 24% & 5% at 1, .3 & .1 μM, respectively). This effect of DAMGO was absent in the presence of ω-CgTx and ω-Aga.

Support by Grant-in-aid #07680868 from Jpn Min. Edu. Sci. Cul.

540.3

DELIVERY OF ENKEPHALINS TO MOUSE SENSORY NEURONS BY A HERPES VIRUS ENCODING PROENKEPHALIN. S.P. Wilson*, D.C. Yeomans†, M.A. Bender§ and J. Glorioso§. Dept. of Pharmacology, Univ. of South Carolina Sch. of Med., Columbia, SC 29208; †Dept. of Anatomy and Cell Biology, Univ. Illinois, Chicago, IL 60612; §Dept. of Molecular Genetics and Biochemistry, Univ. of Pittsburgh, Pittsburgh, PA 15261.

A recombinant herpes simplex type 1 virus (KPE) containing a CMV immediate-early promoter-human proenkephalin cassette in the thymidine kinase locus was constructed and purified. Tissue culture cells infected with KPE synthesized proenkephalin by Western blot analysis. This virus and a similar virus containing the lac Z reporter gene (KZ) were then used to infect female Swiss-Webster mice (6 wks) to test functional expression *in vivo*. Application of viral suspensions to the abraded hairy or glabrous surface of the hindpaw resulted in numerous neurons expressing the transgene in ipsilateral dorsal root ganglia L3-L5 at 4-35 days postinfection. Mice infected with KZ or KPE were then tested for foot withdrawal responses to noxious radiant heat. In both normal mice and capsaicin-sensitized mice, systemic naloxone decreased foot withdrawal latencies at 3-5 days following infection with KPE but not with KZ. These effects were observed at low heating rates, mediated by C fibers, but not at high rates of heating, mediated by Aδ fibers. These results indicate that infection of small-diameter cutaneous afferents with a proenkephalin-encoding herpes virus results in opioid-mediated, C fiber-specific antinociception. (Supported by NSF Grant IBN 94-09201)

540.4

ANTINOCICEPTIVE EFFECTS OF μ, δ, and κ OPIATE AGONISTS AND ANTAGONISTS ON RAT FOOT WITHDRAWAL RESPONSES MEDIATED BY Aδ OR C NOCICEPTOR ACTIVATION Y. Lu, V. Pirec, and D. C. Yeomans* Dept. of Anatomy and Cell Biology, University of Illinois at Chicago. Chicago, Illinois 60612.

The purpose of this study was to determine if μ,δ, or κ opiate agonists preferentially attenuate nociceptive responses mediated by the activation of Aδ or C fiber nociceptors. Aδ or C fiber mediated responses were assessed by measuring latencies for foot withdrawals evoked by high or low rate skin heating respectively. Intrathecal application of the δ agonists DPDPE or DSLET produced dose-dependent antinociception for responses to both high and low rate skin heating. These effects were antagonist reversible and showed approximately the same potency and dose response slope for both heating rates. The κ agonist U50488 did not produce antinociception on either test using doses up to 500 nmoles. The mu opioid agonists morphine and DAMGO also produced dose-dependent, antagonist reversible antinociception for both Aδ and C mediated responses. However, although μ opioids appeared to be more potent for C fiber mediated responses, the slopes of the dose-response lines for the high and low heating rates for both morphine and DAMGO were significantly different, precluding direct ED_{50} comparisons. One possible explanation for the slope differences is that μ opioids act presynaptically on C-fiber terminals and postsynaptically on both Aδ and C evoked activity in dorsal horn neurons. Supported by USPHS Grant DA08256 (DCY).

540.5

SPINAL μ-, δ- AND κ-OPIATE RECEPTORS MEDIATE INHIBITION OF THE NOCICEPTIVE TAIL WITHDRAWAL REFLEX EVOKED BY INTENSE PERIPHERAL ELECTRICAL STIMULATION IN THE RAT. V.V. Romita* and J.L. Henry, Depts. of Physiology & Psychiatry, McGill University, McGill University, Montreal, QC H3G 1Y6

Opioids have been implicated in the antinociception evoked by activation of high threshold primary afferents. This study determined whether spinal opiate receptors mediate antinociception provoked by intense peripheral electrical stimulation in the tail-flick test. Conditioning electrical stimulation with 2 ms square pulses, at 4 Hz for 20 min at 20 times threshold (to elicit muscle twitch) to previously defined meridian points in the hindlimb inhibited the withdrawal reflex during the stimulation and the inhibition persisted for greater than one hour after the end of the stimulation in anaesthetized intact rats (n=8) and for up to 15 min in unanaesthetized spinal rats (n=12). In control anaesthetized intact (n=12) and unanaesthetized spinal rats (n=14) placement of electrodes with no stimulation produced no effect. In spinal rats, preadministration of naloxone (25 mg/kg; i.p.) completely blocked the evoked inhibition (n=10). In intact animals both naloxone (n=8) and ß-FNA (10 nmols, i.th.; n=9) antagonized the inhibition during the stimulation and the persistent inhibition by 50-60 %. Tipp[ψ] (10 nmols, i.th.; n=7) and nor-BNI (10 nmols, i.th.; n=13) decreased the inhibition during the stimulation by 30 % and 56%, respectively. Both antagonists completely blocked the persistent effect and even facilitated the withdrawal. The data suggest that the three types of opiate receptor differentially mediate the evoked antinociception. Spinal activation of μ-, κ- and, to a lesser extent, δ-opiate receptors partly mediate the evoked inhibition during the stimulation, while the persistent inhibition is dependent on activation of δ- and κ- and to a lesser extent μ-receptors. *Supported by Canadian MRC*

540.6

SPINALLY ADMINISTERED OPIOIDS WITH AGONIST ACTIVITY AT KAPPA$_2$ OPIOID RECEPTORS ARE ANTIHYPERALGESIC DURING PERIPHERAL INFLAMMATION. J. Ho, A. J Mannes, R. Dubner and R. M. Caudle*. Neurobiology and Anesthesiology Branch, National Institute of Dental Research, NIH, Bethesda, MD 20892.

Previous work demonstrated that activation of kappa$_2$ opioid receptors inhibits the flow of current through N-methyl-D-aspartate (NMDA) receptors (J. Neurosci. 14:5580). These findings suggested that kappa$_2$ opioid receptor activation could be used to modify NMDA receptor-mediated responses. Numerous studies have demonstrated that intrathecally administered NMDA receptor antagonists inhibit hyperalgesia in rats, but do not influence normal pain sensation. These studies indicate that NMDA receptors are involved in hyperalgesia.

The present study examined spinally administered kappa$_2$ agonists during inflammation of the rat hindpaw to determine if these agents were antihyperalgesic like NMDA receptor antagonists or analgesic like conventional opioids. Paw withdrawal latencies to heat were used to measure pain thresholds. Bremazocine and GR89,696, opioids with kappa$_2$ agonist activity, inhibited the hyperalgesia associated with hindpaw inflammation. They did not elevate the latency of the inflamed paw above pre-inflammation levels nor did these agonists elevate the threshold of the non-inflamed paw. The ED50's were 155 and 2 nMols for bremazocine and GR89,696 respectively. The antihyperalgesic effect of these agents was blocked by co-administration of naloxone. The selective mu agonist, DAMGO, and the delta agonist, DPDPE, elevated the paw withdrawal latencies of *both* the inflamed and non-inflamed paws. These agonists also elevated paw withdrawal latencies above pre-inflammation levels. The kappa$_1$ selective agonist, U69,593, had no effect on either paw. These findings suggest that kappa$_2$ agonists are selective and effective antihyperalgesic agents that may act by inhibiting the flow of current through NMDA receptors.

Supported by the National Institute of Dental Research.

540.7

SPINAL CORD ENDOGENOUS OPIOID MEDIATION IN ILLNESS-INDUCED & CONDITIONED HYPERALGESIAS.

E. P. Wiertelak*, Department of Psychology, Macalester College, St. Paul, MN 55105.

Intraperitoneal (IP) administration of lithium chloride (LiCl; emetic) or lipopolysaccharides (LPS; pyretic) induces a potent hyperalgesic state as measured by the tailflick test. Moreover, animals can learn to activate hyperalgesia in response to signals for impending illness, a phenomenon known as *conditioned hyperalgesia*. To investigate whether LiCl, LPS, or conditioned hyperalgesia mechanisms rely on spinal opiate activity, rats were implanted with intrathecal (IT) catheters and allowed one week post-surgical recovery. Parallel microinjection and testing methods were employed for the study of LiCl, LPS, and conditioned hyperalgesias to define the opiate natures of the resultant hyperalgesias, and allow for an initial comparison of the spinal opiate involvement in each event.

Conditioned hyperalgesia was acquired by rats in response to internal cues (taste) paired with illness (conditioned taste aversion). Compared to vehicle controls, IT injections of naltrexone reversed conditioned hyperalgesia, suggesting specific opiate involvement in the spinal cord. Intrathecal injections of naltrexone also completely reversed the illness-induced hyperalgesia resulting from IP LiCl or LPS, as compared to vehicle controls. Further IT studies using specific opiate receptor-subtype antagonists (CTOP, Naltrindole, Binaltorphimine) are in progress. To date, the data indicate these hyperalgesias involve common spinal systems. Supported by grant DA09289-02 from the National Institute on Drug Abuse at the National Institutes of Health.

540.8

PAIN FACILITATION INDUCED BY OPIATES: AN OPIOID OPPOSING PROCESS BLOCKED BY THE NMDA ANTAGONIST MK 801. J.P. Laulin, A. Larcher, E. Célérier, M. Le Moal* and G. Simonnet, INSERM U. 259, rue Saint Saëns, 33077 Bordeaux, France.

Several trends of evidence suggest that the development of tolerance to opiates would result in the activation of compensatory systems rather than in the desensitization of opiate receptors. Such compensatory systems, acting as opioid-opposing systems, would mask the effects of opiates like analgesia by inducing opposite effects such as hyperalgesia or allodynia. This hypothesis also implies that these opioid-opposing systems might be activated even after a first administration of opiates. Recently, we have demonstrated the reality of this phenomenon by studying the variations of the rat nociceptive threshold using the tail-flick latency test to a thermic stimulus (water bath at 52°C). We have observed that a single administration of heroin (2.5 mg/kg) induced a 2 hours analgesia followed by a hyperalgesia of similar duration. Moreover, the administration of naloxone (1 mg/kg) during the analgesic phase did not only reverse the analgesia but also induced a clear hyperalgesia, indicating that opioid-opposing systems were simultaneously activated. In addition, tolerance could develop very rapidly since a second administration of heroin, as soon as 2 hours after the first injection, elicited an analgesic effect 3 times lower than the first one. According to previous works which have reported that tolerance could be prevented by chronic treatment with NMDA antagonist, the effect of MK 801 on opioid-opposing systems activation by heroin administration was studied. Pretreatement of the animals with an injection of MK 801 (0.15 mg/kg) resulted in a significant increase in both amplitude and duration of the analgesic effect of acute heroin (1 mg/kg); MK 801 also blocked in a dose-dependant manner (0.1-0.3 mg/kg) the hyperalgesia provoked by the administration of naloxone during the heroin-induced analgesia. At last, acute tolerance observed for a second injection of heroin, was totally prevented by a previous administration of MK 801 (0.15 mg/kg). Similar results were obtained with morphine. In conclusion, taken together, these results indicate that potentiation of analgesia and prevention of tolerance could be attributed to the blockade by MK801 of compensatory systems recruitment. These data also suggest that glutamate, via NMDA receptors involvement, plays a crucial role in the activation of opioid-opposing systems by opiates.

This work was supported by an INSERM and Université de Bordeaux II.

540.9

DIFFERENTIAL CONTRIBUTON OF SUPRASPINAL AND SPINAL OPIOID RECEPTORS TO THE ANALGESIC ACTION OF SYSTEMIC MORPHINE IN THE FORMALIN TEST IN THE RAT C. Loo and A.I. Basbaum*, Depts. Anat., Physiol. and W.M. Keck Fdn. Ctr. for Integrative Neurosci., UCSF, San Francisco.

The analgesia produced by systemic injection of morphine in acute nociceptive test results from a synergistic action at spinal and supraspinal sites (Yeung and Rudy, 1980). The relative contribution of each site to the analgesia, particularly in models of persistent pain is, however, not clear. To address these questions we studied the reversal of morphine analgesia produced by injections of i.c.v. and/or intrathecal (i.t.) naloxone methiodide (10ng-10µg), a quaternary opiate antagonist. Morphine (10mg/kg) was injected 10 min prior to a hindpaw injection of formalin (5%; 50µl). To selectively study the reversal of the analgesia in the tonic phase of the formalin test, we injected the antagonists in the interphase, i.e. before the onset of phase 2 pain behavior. Icv injections were made via a 3rd ventricle cannula, i.t. injections by lumbar puncture. Phase 2 formalin behavior was scored (time weighted method; paw favoring-1; lifting-2; licking-3) for up to 80 min after formalin.

The naloxone methiodide potency for reversal of phase 2 analgesia was greatest by the icv route. Thus, 10 µg icv produced an 81.9% reversal; 10 µg i.t. only produced a 35.7% reversal (compared to rats that received saline). Furthermore, the minimal dose for producing some antagonism was 10 ng icv, but 100 ng was required i.t. When different doses were combined, we did not detect synergistic reversal. Importantly, complete reversal of phase 2 analgesia (100%) could *only* be produced by simultaneous injections (e.g. 10 µg icv and i.t.). This indicates that multiple sites must be accessed by systemic morphine to produce complete analgesia in this test.

Since it is unclear whether or not different populations of spinal cord nociresponsive neurons are regulated by supraspinal or spinal morphine, we are also studying the spinal cords of these rats for expression of the fos protein when analgesia is reversed by concomitant icv and i.t. antagonist injections. Futhermore, this evidence for additivity rather than synergy in a tonic pain test is being tested with simultaneous microinjections of morphine. Support: DA 08377 & NS21445.

540.10

LOSS OF ANTIALLODYNIC AND ANTINOCICEPTIVE SPINAL/SUPRASPINAL MORPHINE SYNERGY IN NERVE-INJURED RATS. D. Bian, M.H. Ossipov*, M.L. Nichols, T.P. Malan, Jr. and F. Porreca, Univ. Arizona Pain Institute, Tucson, AZ 85724.

It is well known that the antinociceptive potency of morphine is markedly influenced by a substantial multiplicative interaction at spinal and supraspinal sites. It has recently been shown, however, that *i.th.* morphine is not effective in suppressing tactile allodynia in animals with an L5/L6 nerve ligation injury, and that the opiate loses both antinociceptive potency and efficacy in the tail-flick test in these animals. It is conceivable that nerve ligation injury alters central nociceptive processing in a manner to alter opioid-mediated descending inhibitory mechanisms resulting in a loss of the spinal/supraspinal synergy observed with morphine. To test this hypothesis, nerve injury was produced by unilateral ligation of the L5 and L6 spinal roots of the sciatic nerve of rats. Response to acute nociception was measured by determining the warm-water (52°C) tail-flick latency. Tactile allodynia was measured by probing with calibrated von Frey filaments. Morphine was given *i.th.*, *i.c.v.* and in a 1:1 ratio of *i.th.* plus *i.c.v.* Data were converted to % maximal possible effect (%MPE). In sham-operated rats, the antinociceptive (tail-flick) A_{50}'s of *i.th.*, *i.c.v.*, and *i.th.* plus *i.c.v.* morphine was 1.9 (1.0 - 3.4) µg, 3.2 (2.3 - 4.5) µg and 0.30 (0.2 - 0.6) µg, respectively, and in nerve-ligated rats the A_{50} values were 3.7 (1.4 - 10) µg, 1.5 (0.3 - 6.9) µg and 4.5 (3.1 - 6.3) µg. Isobolographic analysis indicated that the antinociceptive interaction between *i.th.* and *i.c.v.* morphine was synergistic in sham-operated rats and additive in nerve-ligated rats. The antiallodynic A_{50} values of *i.c.v.* morphine alone and with 1:1 *i.th.* morphine were 1.9 (1.3 - 2.8) µg and 0.82 (0.57 - 1.2) µg, representing a non-significant 2.3-fold shift in the *i.c.v.* morphine dose-response curve which was not synergistic; *i.th.* morphine alone was inactive. These data indicate a loss of spinal/supraspinal antinociceptive and antiallodynic synergy of morphine in the nerve-injured rat and may reflect an altered role of descending modulatory systems mediated by supraspinal morphine. The loss of synergy may underly the perceived resistance of clinical neuropathic pain to opioid therapy. Supported by USPHS Grants from the NIDA.

540.11

SUPRASPINAL/SPINAL ANTINOCICEPTIVE SYNERGY OF SELECTED OPIOID DELTA AGONISTS IN MICE. M.H. Ossipov, K.P. Mayfield*, T.W. Vanderah, J.M. Lashbrook, T. P. Malan, Jr. and F. Porreca, University of Arizona Pain Institute at the Health Sciences Center, Tucson, AZ 85724.

A multiplicative antinociceptive interaction of morphine activity at spinal and supraspinal sites has been clearly established and is probably responsible, in part, for the clinical utility of this compound in normal dose-ranges. While synergistic actions of opioid µ receptor agonists have been shown, it is unclear whether a similar interaction exists for opioids acting via the delta (δ) receptor. Response to acute nociception was measured by determining the warm-water (55°C) tail-flick latency before and after intracerebro-ventricular (*i.c.v.*), intrathecal (*i.th.*) or *i.th.* plus *i.c.v.* (1:1 fixed ratio) injection of the peptidic opioid δ agonists [D-Ala^2, Glu^4]deltorphin (DELT), [D-Pen^2, D-Pen^5]enkephalin (DPDPE) or the non-peptidic, SNC-80. The δ receptor selectivity of the highest studied *i.c.v.* or *i.th.* doses of these agonists was confirmed by showing antagonism with naltrindole (δ antagonist) but not with nor-binaltorphimine (κ antagonist) or β-funaltrexamine (µ antagonist). The respective A_{50} (± 95% C.L.'s) values for DELT given *i.th.*, *i.c.v.* and *i.th.* plus *i.c.v.* were 78 ± 40 µg, 6.1 ± 1.9 µg and 6.6 ± 2.4 µg, respectively. The respective A50 (± 95% C.L.'s) for *i.th.*, *i.c.v.* and *i.th./i.c.v.* DPDPE were 17 ± 7.4 µg, 13 ± 3.5 µg and 7.1 ± 2.7 µg, while these respective values for SNC-80 were 110 ± 19 µg, 140 ± 25 µg and 155 ± 16 µg. Isobolographic analysis revealed that the spinal/supraspinal antinociceptive interaction for DELT and for DPDPE to be synergistic. In contrast, the spinal/supraspinal antinociceptive interaction for SNC-80 was found to be sub-additive. These data indicate that a multiplicative antinociceptive interaction between opioid δ agonists given *i.th.* and *i.c.v.* can be demonstrated for some, but not all, opioid δ agonists. The sub-additive interaction found with SNC-80 may represent different preferential sites or mechanisms of action of this compound. Such analysis will be important in determining the potential clinical application of compounds acting through opioid δ receptors. Supported by the NIDA.

540.12

MICROINJECTION OF MORPHINE IN THE A7 CATECHOLAMINE CELL GROUP PRODUCES HYPERALGESIA. J. E. Holden* and H. K. Proudfit, Dept. of Pharmacology, University of Illinois at Chicago, Chicago, Il 60612.

The A7 catecholamine cell group contains noradrenergic neurons that innervate the spinal cord dorsal horn and stimulation of these neurons produces potent antinociception. We have previously identified methionine-enkephalin-containing (mENK) neurons in the ventromedial medulla (VMM) that project to tyrosine hydroxylase-immunoreactive neurons in the A7 cell group. The present experiments determined the role of these mENK neurons in the modulation of nociception. The first experiment determined the effects of microinjecting the excitatory amino acid, monosodium glutamate, into the A7 area in female Sprague-Dawley rats. Glutamate produced a significant antinociceptive effect ($p < .05$). The second experiment determined the effect of microinjecting the non-selective opioid receptor agonist, morphine sulfate (MS), into the A7 area. MS produced a significant hyperalgesic effect compared to saline controls ($p < .0001$). This hyperalgesic effect was reversed by microinjection of the opioid antagonist, naltrexone, in the A7 cell group, which indicates that the MS effect was mediated by opioid receptors.

These results confirm previous observations that activation of neurons in the A7 cell group produces antinociception and lead to the conclusion that MS produces hyperalgesia by inhibiting neurons in this region. These findings suggest that mENK neurons in the VMM facilitate nociception by inhibiting neurons in the A7 cell group.

This work was supported by PHS Grants DA3980 from the National Institute on Drug Abuse and TR2 NR07075 from the National Institute of Nursing Research.

540.13

INESCAPABLE SHOCK (IS) POTENTIATES MORPHINE ANALGESIA 24 HRS LATER: THE ROLE OF MU OPIOID RECEPTORS IN THE DORSAL RAPHE NUCLEUS (DRN).

SE Hammack*, CE Hartley, LC Sutton, SF Maier, & LR Watkins. Dept Psych, U Colorado, Campus Box 345, Boulder, CO 80309.

Inescapable tailshock (IS), but not identical escapable tailshock, potentiates morphine analgesia 24 hrs later as measured by the tail flick (TF) test. Previous experiments have shown that IS potentiates analgesia to a broad range of systemic morphine doses as well as to morphine microinjected into discrete sites in the raphe bulbospinal pathway. The present experiments characterize the involvement of mu receptors in the dorsal raphe nucleus (DRN), a site believed to mediate many of the effects produced by IS.

Rats implanted with DRN cannulae received either 100 IS (1mA, 5 sec, 1 min ITI) or restraint. Baseline TF latencies were measured 24 hrs later followed by microinjection of the mu receptor agonist DAGO (0.3 µg) or equivolume vehicle (1 µl saline). Fifteen min later, 3 TF latencies were measured every 10 min for 1 hr. Neither IS nor restraint groups given saline vehicle exhibited analgesia. IS groups exhibited a profound potentiation of DAGO analgesia when compared with restraint controls.

These data suggest that mu opioid receptors in the DRN are critical to IS induced potentiation of morphine analgesia. Ongoing experiments are aimed at further characterizing the opioid receptor subtypes involved both at the time of IS and 24 hrs later. Supported by NIMH grant MH50479 and the Undergraduate Research Opportunities Program.

540.14

INESCAPABLE SHOCK (IS) POTENTIATES MORPHINE ANALGESIA 24 HRS LATER: THE ROLE OF THE MU OPIOID RECEPTORS IN THE LUMBOSACRAL SPINAL CORD.

CE Hartley*, SE Hammack, LC Sutton, SF Maier, & LR Watkins. Department of Psychology, U Colorado, Boulder, CO 80309.

We have recently shown that IS, but not restraint or identical escapable shock, produces a marked enhancement of analgesia 24 hrs later when morphine either is delivered systemically or microinjected into discrete sites within the raphe bulbospinal pathway. The present experiments replicate and extend previous findings by examining the involvement of mu opioid receptors in lumbosacral spinal cord.

Adult male albino rats were exposed either to 100 inescapable tailshocks (IS; 5 s, 1 min variable ITI, 1 mA, delivered by fixed fuseclip electrodes) or to an equivalent period of restraint. Rats were then tested for pain responsivity using the tailflick (TF) test in a novel environment 24 hrs later. After baseline TF assessment, rats were briefly exposed to a short-acting inhalant (methoxyflurane) to allow a single epidural drug injection to be made. Each rat received either 0.25 µg morphine, 18.75 ng DAGO, or equivolume vehicle (10-15 µl saline). Full recovery from anesthesia occurred within 1-2 min. Beginning 15 min after injection, 3 TF latencies were assessed each 10 min for 60 min. Analgesia was not observed in any saline groups. Likewise, restraint groups given morphine or DAGO failed to exibit analgesia. In contrast, profound morphine and DAGO analgesia was present in IS groups.

These data demonstrate a striking potentiation of morphine and DAGO analgesia 24 hrs after inescapable tailshock and suggest that mu opioid receptors in the lumbosacral spinal cord are critical to this phenomenon. Ongoing experiments are aimed at further characterizing the opioid receptors involved at the time of IS and testing. Supported by UROP & NIMH grant MH50479.

540.15

ANTINOCICEPTION PRODUCED BY MICROINJECTION OF DELTA OPIOID RECEPTOR AGONISTS INTO THE VENTROMEDIAL MEDULLA OF THE RAT. S. N. Thorat* and D. L. Hammond. Dept. of Anesthesia & Critical Care, Univ. of Chicago, Chicago, IL 60637.

The role of supraspinal ventromedial medullary sites, such as the n. raphe magnus (NRM) and n. reticularis gigantocellularis pars alpha (NGCpα), at which selective delta opioid receptor agonists may act to produce antinociception was investigated in adult male Sprague-Dawley rats. Animals were stereotaxically implanted under ketamine/xylazine anesthesia with a guide cannula aimed at either NRM or NGCpα. One week later, either [D-Ala^2, Glu^4]deltorphin (DELT; delta-2 agonist) or [D-Pen^{2,5}]enkephalin (DPDPE; delta-1 agonist), were microinjected into the NRM or NGCpα. The nociceptive threshold was determined at 0, 15, 30, 45 and 60 min after the microinjection of DELT or DPDPE using the tail-flick and 55°C hot-plate tests. Administration of DELT (0.02 - 2.0 nmol/0.25 µl) or DPDPE (0.64 - 5.8 nmol/0.25 µl) into NRM or NGCpα dose-dependently increased the tail-flick latency, with a peak effect occurring at 30 min and 45 min, respectively. At the highest dose tested, DELT increased the tail-flick latency to a maximal value of 10.6 ± 0.8 sec, whereas DPDPE increased the tail-flick latency to a maximal value of 10.1 ± 0.8 sec. Microinjection of DELT or DPDPE did not produce any significant antinociception in the hot-plate test. These results provide evidence that activation of delta-1 or delta-2 opioid receptors in the ventromedial medulla is sufficient to produce antinociception in the tail-flick test. The lack of effect in the hot plate test may be a result of the high intensity thermal stimulus applied in the hot-plate test or the administration of an inadequate dose. (Supported by USPHS grant DA 06736).

540.16

PERIAQUEDUCTAL GRAY INHIBITION OF DORSAL HORN NEURONS: THE CONTRIBUTION OF ENDOGENOUS OPIOIDS ACTING VIA MU RECEPTORS. D. Budai* and H.L. Fields. Depts. of Neurology and Physiology and the Keck Center for Integrative Neuroscience, University of California, San Francisco, CA 94143.

Activation of neurons in the midbrain periaqueductal (PAG) inhibits both dorsal horn neurons and behavioral responses to noxious stimuli. Although PAG elicited behavioral antinociception can be antagonized by naloxone, no descending opioid-mediated inhibition of dorsal horn neurons has been demonstrated. In the present study, we examined the contribution of spinal mu opioid receptors to the descending modulation of somatosensory processing. Extracellular recordings were made from single nociresponsive dorsal horn neurons of the rat in response to iontophoretically applied excitatory amino acids (EAA; NMDA or kainic acid) or to noxious heat delivered to the tail. Descending inhibition was evoked by the activation of ventral PAG cells using microinjection of 30-50 ng bicuculline (BIC). After BIC administration, responses of nociceptive dorsal horn neurons to noxious heat were decreased by 60-80% within approximately 15 minutes. The inhibition lasted for 40-50 minutes. This inhibition was transiently reversed by iontophoretic application of the opioid antagonists naloxone and CTOP. In the absence of PAG BIC, naloxone but not the selective mu opioid receptor antagonist, CTOP, had an excitatory influence on dorsal horn neurons. Responses to iontophoresed EAAs were also influenced by PAG BIC. However, the inhibition of the EAA responses did not consistently follow that of the heat responses. This is the first demonstration that endogenous opioids acting via spinal mu opioid receptors contribute to brainstem control of spinal dorsal horn neurons. This inhibition may result in part from presynaptic inhibition of afferents to dorsal horn nociresponsive neurons. Supported by PHS grant, DA 1949.

540.17

OPIOID RECEPTOR MEDIATED PAIN FACILITATION EVOKED BY PAG ß-ENDORPHIN IS MODULATED BY STRESS AND SPINAL CHOLECYSTOKININ A.A. Hawranko*, D.L. Smith, P.J. Monroe, and D.J. Smith. Depts. of Anes. and Pharm./Tox. West Virginia University, Morgantown, WV 26506

Although opioids are usually associated with pain inhibition, under certain conditions they may produce pain facilitation (JPET 265:286, 1993). Moreover, the pain facilitory effect of opioids may be modulated by behavioral states such as anxiety (Neurosci. Abs. 21:1169, 1995). A region of the brain associated with the integration of pain and stress is the periaqueductal gray (PAG). This area contains ß-endorphin (ßE), and is a site of origin of opioid receptor-activated pain facilitory processes. In the current study, the participation of spinopetal cholecystokinin (CCK) neuronal processes in the pain facilitory effect of ßE from the PAG was evaluated. Antinociception (tail flick test) occurring from the microinjection of ßE into the PAG is enhanced by co-administration of the opioid receptor antagonist CTP at doses lower than those required to block antinociception. This is consistent with the notion that opioid receptors involved in pain facilitory function have a high affinity for opioid drugs. Furthermore, the intrathecal administration of proglumide, a CCK receptor antagonist, also enhances the response to ßE. As previously shown, noxious stress reduces the antinociceptive potency of ßE, and causes a dose of ßE which is ineffective in non-stressed rats to become clearly anti-analgesic. Like in the non-stressed rat, each of these actions is reversed by CTP, or is attenuated by proglumide. Thus, an opioid activated CCK-dependent pain facilitory system dampens the overall antinociceptive potency of ßE, and is highly prominent during stress. It may provide a basis for increased pain sensitivity with opioid agonists, and paradoxical analgesia associated with low doses of opioid antagonists.

Supported by NIH Training Grant 5 T32 GM07039 and WVU Medical Corp.

540.18

NALOXONE REDUCES THE VENTRAL PERIAQUEDUCTAL GRAY-INDUCED INHIBITION OF DORSAL NEURONS IN RATS. Q. Shen, Y.B. Peng, Huw Rees*, W.D. Willis. Department of Anatomy and Neurosciences and The Marine Biomedical Institute, The University of Texas Medical Branch at Galveston, Galveston, Texas 77555-1069

We tested the effects of naloxone hydrochloride, a specific opiate antagonist, on the responses and ventral periaqueductal gray-induced inhibition of dorsal horn cells of the lumbar spinal cord in rats anesthetized with pentobarbital sodium (50 mg/kg, i.p.,). Naloxone was administered through microdialysis fiber with its open membrane placed in the dorsal horn. Extracellular single unit responses were recorded from the dorsal horn neurons in the vicinity of the microdialysis fiber. Naloxone was tested on nociceptive dorsal horn neurons. Significant increases of the background activity and responses to brush, press and pinch stimuli were observed in all tested cells. There was also a significant block of the ventral periaqueductal gray-induced inhibition of activities to brush, press and pinch (from 40% to 18%, 66% to 32%, 70% to 36%, respectively) in these cells. These results suggest that the opioid receptors in the spinal cord are involved in the ventral periaqueductal gray-induced inhibition of dorsal horn neurons. (This study was supported by NIH Grants NS 09743 and NS11255)

540.19

NALOXONE-INDUCED HYPOALGESIA: EVIDENCE FOR THE EXPRESSION, BUT NOT ACQUISITION, WITH THE TAIL-FLICK RADIANT HEAT TEST. H. Foo* . Psychology, Northern Territory University, Darwin, NT 0909, Australia.

Previous research has shown that rats given pairings of naloxone in combination with exposure to the heated floor of the hot-plate apparatus come to acquire hypoalgesia. This hypoalgesia has been evidenced by a decrement in nociceptive responding across assays for acute pain (eg. hot-plate test) and for chronic pain (eg. formalin test). The present experiments constitute an investigation of 'naloxone-induced hypoalgesia' with the tail-flick radiant heat test. Experiment 1 showed that rats exposed to pairings of naloxone and a heated floor were hypoalgesic when then tested for their latencies to flick their tails in response to the application of radiant heat, providing further evidence for the generality of the hypoalgesic response that have been detected previously. Experiment 2 examined whether rats given pairings of naloxone and exposure of radiant heat to the tail come to acquire hypoalgesic response. In contrast to the finding of hypoalgesia in rats that have been given naloxone in combination with immersion of their tails into heated water (cf. Rochford & Stewart, 1987), the present investigation found no evidence for hypoalgesia in rats injected with naloxone in advance of exposure of their tails to radiant heat. The discrepancy in these findings is explained in terms of procedural differences that were employed in the two studies. (This research was supported by a grant from the Northern Territory University)

540.20

THE EFFECT OF PLACENTA INGESTION ON KAPPA-OPIOID ANTINOCICEPTION IN RATS. J.M. DiPirro, T.M. Vanderwerf, and M.B. Kristal*. Behavioral Neuroscience Program, Dept. of Psychology, University at Buffalo, Buffalo, NY 14260.

Ingestion of POEF (Placental Opioid-Enhancing Factor), found in placenta and amniotic fluid, produces a dramatic enhancement of opioid/opiate-mediated antinociception in the rat, but alone does not produce antinociception. POEF ingestion was found to produce enhancement of morphine-induced antinociception but left morphine-induced hyperthermia unmodified, in the same rats. In an attempt to determine whether this differential effect was due to opioid-receptor specificity, we undertook a series of studies to evaluate the effect of POEF ingestion on antinociception produced by i.c.v. injection of preferential opioid-receptor agonists. Initial studies showed that POEF ingestion enhances δ-mediated antinociception and inhibits μ-mediated antinociception. The present study investigated the effect of POEF on the antinociception produced by the preferential kappa-receptor agonist spiradoline (U-62,066).

Ten minutes after ingesting placenta or control substance (1.0 g), rats were injected with spiradoline (200 nM/ 4 μl, i.c.v.) or vehicle. Antinociception (hindpaw-lick/jump latency) was assessed using a 52°C hot-plate algesiometer 20 minutes after i.c.v. injection.

The results indicate that, unlike δ- and μ-antinociception, κ-antinociception appears to be unaffected by POEF. Future studies will determine whether this finding is related to spiradoline dose or the absence of κ-receptor involvement in POEF enhancement.

Supported by NSF grant IBN 91-23748, and funds supplied by the office of the Dean of the Faculty of Social Sciences, awarded to M.B.K.

PAIN MODULATION: PHARMACOLOGY—OPIATES II

541.1

ANALGESIC ACTION OF DIHYDROCODEINE (DHC) IS NOT DUE TO FORMATION OF DIHYDROMORPHINE (DHM) I. Jurna*, J. Baldauf and W. Fleischer. Dept. of Pharmacology, Univ. of the Saarland, D-66421 Homburg, and Mundipharma, D-65549, Limburg, Germany.

The analgesic efficacy of DHC ranges between those of morphine and codeine. DHC is assumed to act by its O-demethylated product, DHM. To test this hypothesis, experiments were carried out on rats in which pain-related nociceptive activity was elicited in neurons of the thalamus VPL and VPM by electrical stimulation of afferent C fibers in the sural nerve. DHC injected i.v. depressed evoked nociceptive activity in a dose-dependent way, the ED_{50} being 0.47 mg/kg. Blocking O-demethylation by pretreatment with metyrapone or cimetidine failed to reduce the effectiveness of DHC. Since, in addition, DHC was twice as potent as DHM (ED_{50} 0.97 mg/kg i.v.), it is concluded that DHC produces analgesia by itself.

541.2

CROSS-TOLERANCE TO MORPHINE ANALGESIA IN RATS INJECTED WITH ETHANOL, BUT NOT IN RATS THAT ORALLY SELF-ADMINISTERED ETHANOL. A. L. Vaccarino, R. L. Bell, L. C. Couret, Jr., G. A. Olson, R. D. Olson & J. H. McLean*. Department of Psychology, University of New Orleans, LA 70148.

Previous studies have shown cross-tolerance between morphine- and ethanol-induced analgesia. In the present study, we examined the effects of ethanol pretreatment (oral self-administration vs ip injections) on subsequent tolerance to ethanol-induced analgesia (EIA) and cross-tolerance to morphine-induced analgesia (MIA). In experiment 1, male Long-Evans rats were injected with ethanol (2.5 g/kg, ip) or an equal volume of saline once a day for 2 days. On day 3, the animals were tested for either EIA (2.5 g/kg, ip) or MIA (10 mg/kg, ip) in the hotplate test (52°C, 60 sec cut-off period). Rats pretreated with ethanol showed tolerance to EIA and cross-tolerance to MIA. In experiment 2, rats were given increasing amounts of ethanol added to their drinking water over a 15-day period. The concentrations of ethanol were five days of 5%, five days of 10% and five days of 15%. A control group of rats had plain tap water to drink. On day 16, all animals were given plain tap water to drink. On day 17, the animals were tested for EIA (2.5 g/kg, ip) or MIA (10 mg/kg, ip) in the hotplate test. Rats allowed to self-administer ethanol showed tolerance to EIA, but did not show cross-tolerance to MIA. Thus, when ethanol is experimenter-administered it is cross-tolerant with MIA, but when ethanol is self-administered it is not cross-tolerant with MIA.

541.3

(-)-NORPSEUDOEPHEDRINE ENHANCES THE ANALGESIC EFFECTS OF MORPHINE P. Nencini*, S. Fraioli, D. Perrella, V. Sorana, T. Pascucci, C.V. Nucerito, Institute of Medical Pharmacology, University of Rome "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy

Like amphetamine cocaine and other psychomotor stimulants, weak amphetamine-like agents, such as phenylpropanolamine and ephedrine potentiate the analgesic effects of morphine. In the present study we investigated the ability of (-)-norpseudoephedrine (NPE), apparently less active in terms of amphetamine-like properties of its enantiomer phenylpropanolamine, to influence both the analgesic effects of morphine and its discriminative stimulus properties. In male mice NPE (5.6-10.0-17.0 mg/kg i.p.) did not prolong the latency to lick or to remove paws from a plate warmed at 54°C. However, at the dose of 17 mg/kg it significantly potentiated the analgesic effect of 3.2 mg/kg of morphine (ANOVA of the area under the curve from 0 to 120': F,11,90=9.574, p=0.001). These results were replicated in rats by use of the formaline test, which measures the numbers of hindpaw flinches produced by injecting 50 μl of formaline into the dorsal surface of the paw. NPE 17 mg/kg increased the effect of sub-analgesic doses of morphine (0.56 and 1.0 mg/kg), as measured by the area under the curve of the late nociceptive phase, from 25' to 60' (ANOVA: F,8,53=5.098, p=0.001). In rats trained to discriminate between 0.5 mg/kg of amphetamine and saline in a two-lever operant behavior reinforced by water access, NPE induced a dose-dependent increment of drug lever responding from 0% at 1.0 mg/kg to 100% at 32.0 mg/kg. In contrast, NPE did not generalize for the morphine discriminative stimulus up to the dose of 56.0 mg/kg, which produced a substantial reduction of the response rate. However, when given in combination, NPE attenuated the discriminative stimulus properties of morphine and potentiated its reduction of the response rate. In conclusion, NPE enhances the analgesic effect of morphine in mice and rats. This NPE effect is probably mediated by its amphetamine-like properties and does not reflect a generalized enhancement of morphine's actions. Supported by a 60% grant of the Italian Ministero della Università e della Ricerca Scientifica.

541.4

SEASONAL VARIATION IN ANALGESIC THRESHOLDS TO MORPHINE AND MELATONIN ANALGESIA IN AMPHIBIANS. C. W. Stevens* and L. L. Deason, Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK 74107-1898.

Seasonal variation in the analgesic effects of opioids have been noted in mammals as well as the analgesic effect following melatonin administration. For the past three years, morphine (100 nmol/g, s.c.) was administered monthly to separate groups of Northern grass frogs, *Rana pipiens*. Nociceptive thresholds (NT) and analgesic effects were estimated using the acetic acid test. Morphine was most effective during Spring and Summer months. Baseline NT also varied seasonally, but did not account for the differences seen in morphine effectiveness. Systemic administration of melatonin (10-1000 nmol/g, s.c.) gave an ED50 of 455 nmol/g and melatonin effects were not blocked by naltrexone (100 nmol/g, s.c.) pretreatment. To examine the effect of photic input, optic nerves were sectioned and light blocked to the pineal. Blinded animals had increased NT compared to sham-operated controls. Frogs placed in 24h total darkness also showed an increased NT compared to control group in the lab. These studies suggest that there may be a seasonal variation in opioid sensitivity and that melatonin may regulate baseline nociceptive thresholds in this species. Supported by the Whitehall Foundation and NIH grant DA07326.

541.5

SPINAL OPIOID PHARMACOLOGY IN THE FROG: CHEMICAL, THERMAL AND MECHANICAL SENSITIVITIES S. Willenbring* and C.W. Stevens. Dept. of Physiology and Pharmacology, Oklahoma State Univ., College of Osteopathic Medicine, Tulsa, OK 74107

We have previously demonstrated the use of chemical (topical acetic acid), thermal (radiant heat) and mechanical (von Frey filament) stimuli as quantifiable behavioral response assays in the northern grass frog (*Rana pipiens*). These three stimulus modalities appear to be neurologically distinct in that (1) all three are sensitive to systemic morphine, yet (2) only thermal and chemical responses are diminished by adrenergic analgesia, while (3) only mechanical and chemical responses become hypersensitive following nerve injury. The present study assessed changes in all three sensory modalities after spinal administration of various opioid receptor subtype agonists: μ agonists morphine and fentanyl (10 and 30 nmol, each); δ agonists DADLE, DSLET, DPDPE and deltorphin (30 and 100 nmol, each); κ agonists CI-977 and U50488 (30 and 100 nmol, each). Significant elevations of all three sensory thresholds were observed to varying degrees in each subtype category, though not with every drug in each category. Effects of all agonist drugs were abolished by prior systemic administration of the non-specific opioid receptor antagonist naltrexone (100 nmol/g). In the naltrexone pretreated morphine group, thermal and mechanical thresholds were significantly lower than control. It appears that all three sensory modalities are altered by activation of opioid receptors; however, variations within and between subtype categories suggest that these mechanisms may not be specifically or exclusively mediated via classical opioid receptors. (support: NS09732, SW; DA07326, CWS)

541.6

BEHAVIORAL EXPERIENCE DIFFERENTIALLY ALTERS THE ANTINOCICEPTIVE EFFECT OF MORPHINE AND (±)-EPIBATIDINE IN MICE. A.W. Bannon*, K.L. Gunther, and M.W. Decker. Abbott Laboratories,Neuroscience Discovery, D-47W, AP-9A, Abbott Park, IL 60064-3500

In this study, open field exposure and measurement of rectal body temperature (i.e., behavioral experience) prior to antinociceptive testing was found to significantly reduce (41%) the jump latency of saline treated animals compared to animals that were not exposed to the behavioral experience (i.e., behaviorally naive). In behaviorally naive animals, a significant reduction (24%) in jump latency was observed following treatment with a dose of naltrexone that attenuates the antinociceptive activity of morphine. In general, prior behavioral experience did not alter the response observed following morphine treatment. In contrast, prior behavioral experience significantly increased the jump latency 197% of animals treated with (±)-epibatidine, a compound acting a nicotinic cholinergic receptors, compared to behaviorally naive mice treated with the same dose of (±)-epibatidine. To assess the level of stress induced by the behavioral experience, plasma corticosterone levels were determined in groups of mice following activity monitoring and measurement of rectal body temperature at the time when analgesia testing was started. Following the behavioral experience, corticosterone levels were significantly increased to 127% of control (i.e., behaviorally niave animals). Overall, these results indicate prior behavioral experience may alter the baseline response of control animals and differentially modulate the antinociceptive activity of opiates and non-opiates. (supported by Abbott Laboratories)

541.7

COMPARISON BETWEEN ANALGESIC AND IMMUNE EFFECTS OF MORPHINE AND TRAMADOL *IN MICE*, Paola Sacerdote, Mauro Bianchi, Barbara Manfredi and Alberto E. Panerai*. Dept. Pharmacology, University of Milano, Via Vanvitelli, 32, 20129 Milano, Italy

Tramadol is a centrally acting opiate analgesic drug mainly used for the treatment of acute pain that shows moderate selectivity for the μ-opioid receptor. It is well known that morphine, the classical μ receptor agonist, exerts an immunosuppressive action in several species. Since the interference of tramadol with the immune function has not been investigated yet, we compared the effects of the acute administration of morphine (2.5, 5.0, 10, and 20 mg/kg s.c.) and tramadol (1.0, 5.0, 10, 20, 40 and 80 mg/kg s.c.) on nociceptive thresholds (by hot-plate test) and immune responses (ConA-induced splenocytes proliferation and natural killer activity) in the mouse. Moreover, we evaluated the effects of the chronic administration (2 weeks) of morphine (10 mg/kg s.c.) and tramadol (20 mg/kg s.c.) on the same parameters. After acute administration, the antinociceptive effect of morphine was evident at all doses, while the immunosuppressive effect was evident starting at 10 mg/kg. After chronic treatment, tolerance developed for both the antinociceptive and immunosuppressive effect of morphine. Acute tramadol induced an analgesic effect at the doses of 20, 40, and 80 mg/kg, while, at difference from morphine, it induced a stimulation of immune responses. This effect was evident also at non-analgesic doses. Moreover, tolerance did not develop to the antinociceptive effect of tramadol, but it developed to its immunostimulatory effect. Our data indicate that the pharmacodynamic profiles of tramadol and morphine are totally different. The lack of immunosuppression observed after tramadol administration could be taken into consideration in the choice of pain treatments, e.g in post-operative pain.

541.8

ACUTE OPIOID TOLERANCE IN MOUSE SPINAL CORD YIELDS RESULTS COMPARABLE TO THOSE OBSERVED IN CHRONIC OPIOID TOLERANCE MODELS. C.A. Fairbanks*[1], K.F. Kitto[1], G.L. Wilcox[1,2] [1]Department of Pharmacology, [2]Graduate Program Neuroscience, U of MN, Minneapolis, Minnesota.

The mechanistic similarity between acutely and chronically-induced morphine tolerance has been previously proposed but remains largely unexplored. The present experiments sought to examine the modulation of acutely-induced spinal morphine tolerance by three receptor systems: **I** the NMDA receptor, **II** the imidazoline$_1$ receptor, **III** the α_{2A} adrenergic receptor. Acute tolerance to morphine was induced in male ICR mice (15-20 g) by intrathecal (i.t.) injection of morphine (40 nmols).

Antinociception was detected via the hot water (52.5° C) tail flick test. **I** Morphine tolerance attenuation was tested using NMDA receptor antagonists (competitive and non-competitive respectively) dizolcipine (MK801) and LY235959. **II** Attenuation of tolerance was also tested through co-pretreatment of morphine with agmatine, an imidazoline$_1$ receptor agonist with no activity at the α_2 drenergic receptor. **III** Selective activation of α_{2A} adrenergic receptors was accomplished by administration of a mixture of α_2 adrenergic agonist UK,14-304 or dexmedetomidine and α_{2C} antagonist prazosin (1 μM). Mice with a mutation (D79N), created by gene targeting strategies, that selectively disrupts function of the α_{2A} subtype permit examination of this subtype in opioid tolerance. The α_{2A} receptor relationship with acute spinal morphine tolerance was evaluated by comparison of this D79N mutant strain against the wild-type counterparts. **I** Coinjection (i.t.) of the NMDA antagonists MK801 (1 μg) or LY235959 (1 ng) with morphine (40 nmols) attenuated acute tolerance to morphine measured 8 h later. **II** Coinjection (i.t.) of agmatine (4 nmols) with morphine (40 nmols) also attenuated the development of tolerance. **III** Acute tolerance to morphine was not accompanied by cross tolerance to UK,14-304 or dexmedetomidine; However, cross-tolerance was observed through selective activation of α_{2A} receptors.

The results of these experiments are in accordance with previous results reported in models of *chronically*-induced morphine tolerance. This agreement persists across three independent receptor systems. This study attests to the powerful predictive value of *acute* induction as a model for opioid tolerance. (Supported by NIH/K02-DA-00145 & NIH/R01-DA-04274, HL43671, ADAMHA training grant T32 DA07234 (NIDA).

541.9

THE DEVELOPMENT OF MORPHINE TOLERANCE AND DEPENDENCE IN RATS WITH CHRONIC PAIN-LIKE BEHAVIOR FOLLOWING SPINAL CORD ISCHEMIA J.-X. Hao*, W. Yu, X.-J. Xu and Z. Wiesenfeld-Hallin, Karolinska Institute, Dept. Clin. Neurophysiol., 141 86 Huddinge, Sweden

It is unclear whether or not patients with chronic pain develop tolerance and dependence to opiates. Data from experimental studies have also yielded conflicting results. We have examined the development of tolerance and dependence to morphine injected onto the spinal cord in a rat model of chronic pain following spinal cord injury, where the animals exhibit an allodynia-like response to mechanical and cold, but not heat, stimuli. Ten μg intrathecal (i.t.) morphine completely relieved the marked allodynia-like response to innocuous mechanical stimuli. The analgesic effect of this dose of morphine injected twice daily was, however, diminished within a few days. Tolerance to the antinociceptive effect of morphine assessed with the tail flick test also developed similarly in rats with chronic pain and normal controls. Both groups exhibited similar spinal signs of naloxone-precipitated withdrawal after three weeks of i.t. morphine treatment. The results suggest that the presence of chronic pain did not prevent the development of morphine tolerance and dependence, even when morphine was used to treat the chronic pain itself.

Supported by the Swedish Medical Research Council and Astra Pain Control AB.

541.10

MORPHINE-INDUCED ANTINOCICEPTION: A COMPARATIVE STUDY OF MALE AND FEMALE RATS, P.E. Stewart* and J.A. Gasiewski. Dept. of Physiology & Pharmacology, Philadelphia College. of Osteopathic Medicine, Philadelphia, PA 19131

Data obtained from both preclinical and clincal investigations show that gender can affect pharmacological responses. In this exploratory study, the influence of gender on opioid-mediated antinociception in rats was investigated. Following baseline tail-flick latency (TFL) measurements, male (250-300g, 7-8 weeks) and age- or weight-matched female Sprague-Dawley rats were injected (s.c.) with morphine sulfate. The dose range of morphine was 0.3 to 10.0 mg/kg (1 ml/kg injectate volume). TFL measurements were obtained at 10, 20, 40, 60, 80, 100, and 120 minutes after injection of morphine. The resulting ED_{50} (95% confidence interval) values of 0.66 mg/kg (0.59-0.74 mg/kg) for males, 0.74 mg/kg (0.69 - 0.80 mg/kg) for age-matched females, and 1.16 mg/kg (0.65 - 1.86 mg/kg) for weight-matched females were not statistically different ($P > 0.12$). Likewise there was no difference in the response with respect to duration ($P = 0.97$). One explanation for finding no difference may be that the present study did not monitor the estrus cycle. In fact, the animal testing order may have minimized any influence of a particular day of the estrus cycle. Future studies will address the influence of the estrus cycle on the antinociceptive action of systemic morphine. Supported by intramural funding sources from PCOM.

541.11

EFFECTS OF VIP 11-28 AND NALOXONE ON ANALGESIA IN RATS. D. Conde and B. R. Komisaruk*. Institute of Animal Behavior, Rutgers-The State University of NJ, Newark, NJ 07102.

The analgesia produced by vaginocervical mechanostimulation (VS) is mediated in significant part by the pelvic nerve (Cunningham, et al. 1991), which terminates in spinal cord (Chinapen, et al, 1992) in regions of VIP concentration (Basbaum and Glazer, 1983; Kawatani et al, 1986; Nadelhaft, 1983). VIP is released into spinal cord in response to VS (Komisaruk, et al, 1989). Furthermore, VIP administration has been reported to produce analgesia (Komisaruk et al, 1988). Incubation of VIP, a 28-amino acid peptide, with spinal cord homogenates yields several fragments, one of which is VIP 11-28 (Barbato, et al, 1988). Intrathecal (IT) surgery and ovariectomies were performed in 250-320 g rats at least 2 weeks before administration of VIP 11-28 or lactated Ringer's solution. Both groups were tested immediately after IT injection and every 10 minutes thereafter for one hour. IT administration of VIP 11-28 (16.4 μg in 5 μl lactated Ringer's) produced a significant elevation of TFL at 30 and 60 minutes post injection (18.7% and 24.7%, respectively) compared to baseline preinjection values, and 16.8% and 17.5%, respectively, over the control group at the same time periods. Preinjection TFL did not differ significantly between the groups, and the control group showed no significant changes in TFL across the 60 minute test period, supporting our earlier studies with this peptide fragment. Pretreatment with naloxone (10mg/kg IP) did not significantly attenuate the TFL-elevating effect of VIP 11-28. The present findings indicate that VIP11-28 exerts an analgesic effect that is, at least in part, independent of the endogenous opiate system. Support: NIH-GM08223-11

541.12

EFFECTS OF PRENATAL MORPHINE EXPOSURE ON ANALGESIA PRODUCED BY VAGINOCERVICAL STIMULATION IN RATS. S. T. Cunningham*[1], Ilona Vathy[2] and B. R. Komisaruk[1]. [1]Inst. Animal Behavior, Rutgers, The State Univ. NJ, Newark, NJ 07102 [2] Dept. Psychiat./Nsci. Albert Einstein Col. Med., Bronx, NY 10461.

Vaginocervial stimulation (VS) induces analgesia, as measured by (1) increased latency to flick tail away from a radiant heat source (TFL) and (2) elevated vocalization threshold (VOC-T) to electroshock of the tail. Opiatergic (Hill and Ayliffe, 1981; Steinman et al., 1983) and mono-aminergic (Crowley et al., 1977; Cunningham et al., 1995) mechanisms modulate this analgesia. The present study investigated the effects of prenatal morphine exposure on analgesia produced in adult female rats by: morphine administration, VS, and dopamine receptor antagonists + VS. In expt 1, in response to adult morphine treatment (1 mg/kg SC), rats treated with saline prenatally showed a 124% *greater* increase in TFL than rats that received morphine prenatally (although both groups did show significant analgesia to morphine), supporting previous findings by Vathy et al (1992; 1995). In expt 2, VS produced a significantly (196%) greater increase in TFL in the prenatal *morphine*-treated rats than in the saline controls. In expt 3, all rats were challenged with 0.05 mg/kg IP of the D1 antagonist SCH 23390, the D2 antagonist spiperone hydrochloride, and distilled water, in counterbalanced order. Consistent with previous results (Cunningham and Komisaruk, 1995; Crowley et al., 1977), SCH 23390 produced a dose-dependent potentiation of VOC-T during VS, with no significant effect on TFL. Spiperone produced a dose-dependent increase in TFL and VOC-T during VS. However, prenatal morphine treatment did not differentially affect the potentiation of VS-produced analgesia by these dopamine receptor antagonists. We conclude that the potentiation of VS-produced analgesia by prenatal morphine is evidently due to factors other than alterations in endogenous opioid or dopaminergic mechanisms. Support:5S06GM08223-11 (BRK); DA05833 (IV)

541.13

ESTROUS CYCLE AND OVARIECTOMY ALTER NOCICEPTIVE BEHAVIORS AND MORPHINE-INDUCED ANALGESIA IN THE FORMALIN TEST AND A NOVEL ELECTRICAL STIMULATION PARADIGM. M. Vincler[#], W. Maixner[#], G. Essick*, and A.R. Light[+]. Departments of Pharmacology[#], Prosthodontics*, and Physiology[+], University of North Carolina, Chapel Hill, NC 27599-7545. The role of ovarian hormones in nociception and morphine-induced analgesia has been examined extensively. Pain sensitivity thresholds have been shown to fluctuate during estrous cycling and in response to ovariectomy using some thermal and electrical paradigms. However, the results of these studies have been inconclusive and contradictory. Our laboratory is examining estrous cycling and ovariectomy (OVX) on nociceptive behaviors and morphine-induced analgesia using two behavioral paradigms: the formalin test and a novel electrical stimulation paradigm. Results from the electrical stimulation paradigm show that phase of estrous alters the latency to bar press and reflex force ($p<.01$), but not early vocalization ($p>.05$). Specifically, animals exhibited the following behaviors across shock intensities: faster latencies during proestrus than during metestrus; greater reflex force during proestrus than during both estrus ($p<.01$) and metestrus ($p<.05$). The effects of OVX and morphine-induced analgesia on these behaviors are currently being investigated. In the formalin test, preliminary results have indicated that OVX did not modify behavioral responses (flinch/shake and lick/bite) when compared to sham OVX animals. However, when phase of estrous cycle was considered, significant differences emerged. Proestrus animals exhibited significantly less flinch/shake responses compared to OVX animals in Phase 1 and Phase 2 of the formalin test. Diestrus animals exhibited significantly shorter durations of lick/bite responses compared to animals in proestrus, metestrus, or OVX animals in Phase 1, but not Phase 2 of the formalin test. Preliminary results suggest that OVX animals are more sensitive to the analgesic effects of morphine (1mg/kg and 3mg/kg) than sham OVX animals. The significance of these differences are currently being investigated. *Supported by NIDA grants DA04420 (A.R.L.) and DA07244-04 (L. D.).*

541.14

ACTIVATION OF PERIPHERAL CCK_B- BUT NOT CCK_A-RECEPTORS INHIBITS LOCAL OPIOID ANALGESIA IN INFLAMMATION. L. Zhou*[1,2], M. Schäfer[1,2], C. Stein[2]. [1]Preclin. Pharmacol., DIR/NIDA/ NIH, [2]Dep. of Anesth., Johns Hopkins Univ., Baltimore, MD 21224.

There is abundant evidence that cholecystokinin (CCK) attenuates opioid analgesia within the central nervous system. Recent studies showed that dorsal root ganglion neurons in the rat also express CCK receptors which are transported both to central and peripheral nerve terminals. The present study investigated whether CCK modulates peripheral opioid analgesia in inflamed tissue. In Wistar rats, a painful hindpaw inflammation was induced by Freund's adjuvant and nociceptive thresholds were assessed by a paw pressure test. The peripheral antinociceptive effects of intraplantar (i.pl.) [D-Ala^2,N-Me-Phe^4,Gly-ol^5]-enkephalin (DAMGO, 10 μg) and fentanyl (1.5 μg i.pl.) were dose-dependently inhibited by $CCK_{desulfated}$ (0.001-1.0 μg i.pl.), a primary CCK_B-agonist. $CCK_{sulfated}$, a CCK_A-agonist, did not reverse DAMGO- or fentanyl-induced peripheral analgesia. The anti-opioid effect of $CCK_{desulfated}$ was dose-dependently antagonized by the CCK_B receptor antagonist L365,260 (0.05-5μg i.pl.), but not by the CCK_A receptor antagonist L364,718. At these doses, CCK agonists alone did not alter baseline thresholds in inflamed or noninflamed paws. These results indicate that activation of peripheral CCK_B- but not CCK_A-receptors attenuates the local antinociceptive effects of μ-receptor selective opioid agonists in inflamed tissue. The most likely mechanism for the anti-opioid effect of CCK is an interaction of opioid and CCK_B-receptors on sensory nerve terminals at the second messenger level.

541.15

DAMGO INHIBITS PROSTAGLANDIN E_2-INDUCED POTENTIATION OF A TTX-RESISTANT NA^+ CURRENT IN RAT SENSORY NEURONS *IN VITRO*. M. S. Gold* and J. D. Levine Depts. of Medicine, and Oral Surgery and Division of Neuroscience, UCSF, Box 0452A, San Francisco CA 94143-0452

We have tested the hypothesis that the μ-opioid agonist,. [D-Ala^2,N-Me-Phe^4,Gly^5-ol]enkephalin (DAMGO), inhibits prostaglandin E_2 (PGE_2)-induced modulation of a tetrodotoxin-resistant voltage-gated Na^+ current (TTX-R I_{Na}) in putative nociceptors *in vitro*. Patch-clamp electrophysiological techniques were used on cultured dorsal root ganglion neurons from the adult rat. PGE_2 (1 μM) induced a 103 +/- 22.8 % increase in peak TTX-R I_{Na}. The PGE_2-induced increase in TTX-R I_{Na} in the presence of 1 μM DAMGO (24.9 +/- 7.7%), was significantly less than that induced by PGE_2 alone. In contrast, when DAMGO was applied after PGE_2, PGE_2-induced increase in TTX-R I_{Na} (85.3 ± 19.6%) was not significantly different than the increase in the current induced by PGE_2 alone. Preapplication of naloxone (10 μM) blocked DAMGO-induced inhibition of the PGE_2-induced increase in TTX-R I_{Na}. DAMGO, alone, had no effect on peak TTX-R I_{Na} (1.4 +/- 1.5 % of baseline). Our observation that DAMGO prevents PGE_2-induced potentiation of TTX-R I_{Na} is consistent with the suggestion that modulation of TTX-R I_{Na} underlies the hyperalgesic agent-induced increase in the excitability of nociceptors associated with sensitization and hyperalgesia. Furthermore, our data suggest that inhibition of hyperalgesic agent induced modulation of TTX-R I_{Na} may be a novel mechanism underlying opioid-induced antinociception.

This work was supported by NIH grants NS21647 and NS07265

541.16

NOCICEPTIN OR ANTINOCICEPTIN: POTENT SPINAL ANTINOCICEPTIVE EFFECT OF ORPHANIN FQ/NOCICEPTIN IN THE RAT. X.-J. Xu*, J.-X. Hao and Z. Wiesenfeld-Hallin, Karolinska Institute, Dept. Clin. Neurophysiol., S-141 86 Huddinge, Sweden.

An endogenous agonist of an orphan receptor that has similarities to opioid receptors has been recently isolated from brain (Reinscheid et al., Science, 270:1995; Meunier et al., Nature, 377:1995). It has structural similarities to opioid peptides, but has negligible affinity for μ, δ and κ opioid receptors and has very high affinity for the G-protein-coupled orphan receptor LC132. Nanomolar concentrations of the peptide, termed orphanin FQ (Reinscheid et al., 1995) or nociceptin (Meunier et al., 1995), inhibited forskolin-stimulated adenylyl cyclase, caused motor deficits when injected intrathecally (i.t.) and hyperalgesia when injected intracerebroventricularly (i.c.v.) in mice. Thus, it was concluded that this peptide has a pro-nociceptive function. We examined the function of this peptide in more detail with electrophysiological and behavioral techniques in rats.

In contrast to the hyperalgesic effect of nociceptin/orphanin FQ reported after i.c.v. injection in mice, we found a dose-related inhibition of the flexor reflex in decerebrate, spinalized, unanesthetized rats. The antinociceptive effect of this peptide was not reversed by large doses of naloxone (opioid antagonist), atipamezole (α_2 adrenoceptor antagonist) or bicuculline (GABA-A receptor antagonist), indicating that it did not exert its effect via the most important spinal inhibitory systems. We also tested the effect of i.t. nociceptin/orphanin FQ in behaving animals and found a potent, dose-related antinociception on the tail flick test, with no signs of motor impairment. No effect of nociceptin/orphanin FQ was found on spinal blood flow with laser Doppler flowmetry. Thus, this peptide may represent a new class of analgesics.

Supported by the Swedish Medical Research Council, the European Commission's Biomed 2 programme and Astra Pain Control AB.

541.17

CIRCUITRY UNDERLYING "ANTI-OPIOID" ACTIONS OF ORPHANIN FQ (OFQ) IN THE ROSTRAL VENTROMEDIAL MEDULLA (RVM). M.M. Heinricher*, S. McGaraughty and D. Grandy. Div. Neurosurgery and Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

The rostral ventromedial medulla (RVM) has received considerable attention in efforts to understand the mechanisms of opioid analgesia. Two classes of physiologically identifiable RVM neurons with distinct responses to opioids have been characterized. "Off-cells," are invariably activated, although indirectly, by opioids, and there is strong evidence that this activation is crucial to opioid antinociception. "On-cells," thought to enable or facilitate nociception, are directly inhibited by opioids. "Neutral cells" are unaffected by opioid administration, and their role in nociception has not been determined.

OFQ, the newly identified endogenous ligand of the "orphan" opioid receptor LC132 has been shown in mice to reverse opioid analgesia when given icv. The aim of the present study was to characterize the effects of OFQ on identified RVM neurons, and to determine whether OFQ modifies the well-defined direct and indirect effects of opioids within this region. To this end, single cell recording was combined with local infusion of OFQ in the RVM of rats lightly anesthetized with barbiturates. Local infusion of OFQ profoundly suppressed the firing of all three cell classes and blocked opioid-induced activation of off-cells. Local OFQ also blocked the antinociceptive effects of DAMGO infused within the RVM, but not of morphine given systemically.

Insofar as activation of off-cells is sufficient to produce a behaviorally measurable antinociception, these results suggest that OFQ blocks the antinociceptive effects of opioids acting within the RVM by preventing activation of this cell class.

Supported by grants from NIDA (DA05608 and DA08562) and the National Headache Foundation.

541.18

INTRACEREBROVENTRICULAR INJECTION OF ORPHANIN FQ INDUCES HYPERALGESIA AND ATTENUATES ACUPUNCTURE ANALGESIA IN RATS. C.B.Zhu, X.L.Zhang, S.F.Xu, X.D.Cao and G.C.Wu*, State Key Lab. of Medical Neurobiology, Shanghai Med. Univ. Shanghai 200032, China.

Orphanin FQ(OFQ) is a newly discovered heptadecapeptide which is distinct from other endogenous opioid peptide in pain modulation. In the present study, the effects of OFQ on the response of rats to noxious stimulation and acupuncture analgesia were reported. The steadily increasing current was applied at tail tip of rats as noxious stimulation. The smallest intensity of the current provoking the tail-flick was recorded as pain threshold(PT). Electroacupuncture (EA) was applied at "Zu-San-Li"(St 36) and "Kun-Lun"(UB 60)points.

The results showed that the OFQ at a dose of 0.1 μg/20 μl(55 pmol, icv) has no effect on rats' response to noxious stimulation. At a dose of 1 μg/20 μl(0.55 nmol), OFQ induces a significant decrease of PT which lasted for about 2 hr($P<0.01$, *vs* control rats). EA induced obvious increase of PT in control rats, while in OFQ(1 μg/20 μl)-treated rats, the EA-induced increase of PT was much lower($P<0.01$). When the dose of OFQ was added up to 10 μg/20 μl (5.5 nmol), the rats showed an obvious decrease not only in pain threshold 10-30 min after OFQ injection but also in muscular tone 30 min later. In addition, 10 μg OFQ induced ataxia in most rats.

After blocking the biogenesis of OFQ receptor with repeated icv injection of antisense oligonucleotide, the rats displayed significant increase in PT compared with saline-treated animals($P<0.01$). In these rats, EA showed a better analgesic effect than in control rats($P<0.01$). When the OFQ was administered (1 μg/20 μl) to antisense-oligo-treated rats, it showed no effect on the response of rats to noxious stimulation. In another set of experiments, the rats were icv injected with decapeptide(Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln)(1 μg/20 μl), a fragment of OFQ, the animals displayed no change in pain threshold. Taken together, these data indicate that the newly discovered OFQ induces hyperreaction of rats to noxious stimulation and attenuates acupuncture analgesia.

541.19

EFFECTS OF CENTRALLY ADMINISTERED ORPHANIN FQ ON TAIL-FLICK LATENCY AND MOTOR ACTIVITY IN RATS
K.R. Gogas*, Julie Thompson and John C. Hunter, Department of Analgesia, Institute of Pharmacology, Neurobiology Unit, Roche Bioscience, Palo Alto, CA 94304

Orphanin FQ (ORFQ), a seventeen amino acid polypeptide isolated from rat brain that has some sequence similarity to the opioid peptides, has been suggested as the endogenous ligand for the orphan receptor ORL-1 (Opioid-like receptor 1; Reinscheid et al., 1996; Meunier et al., 1996). Intracerebroventricular (icv) administration of ORFQ in mice has been associated both with hyperalgesia in the tail-flick and hot-plate tests and with reductions in motor activity (Reinscheid et al., 1996; Meunier et al., 1996). We have therefore tested the effects of icv ORFQ on tail-flick latency and motor activity in rats to determine whether the purported hyperalgesic and motor effects seen in mice can be extended across species. Male Sprague-Dawley rats (90-120 g; Harlan) were used for all experiments. For the tail-flick study, animals received direct icv injections of either vehicle (PBS) or ORFQ (0.01-10 nmol in 2 μl; n=7-8) one hr after baseline testing. At 10 and 30 min post-dose, tail-flick latencies were again assessed for each animal. Data analysis showed that none of the ORFQ treated groups were significantly different from vehicle in either the changes from pre-dose or actual post-dose latency times. To assess the effects of ORFQ on motor activity, reverse-light cycled animals received icv injections of ORFQ (0.01-10 nmol) and were immediately placed into standard perspex transfer cages equipped with San Diego Instruments Automated Cage Activity Systems. Their activity was monitored over three 15 min intervals. Analysis of the motor activity data showed that the total activity of animals receiving doses of 1.0 nmol and 10 nmol ORFQ was significantly lower than vehicle during the first 15 min interval only. While the finding that icv ORFQ inhibits motor activity indicates that this peptide is biologically active, these results provide no support for the hypothesis that ORFQ is involved in the central mediation of nociception in rats.

PAIN MODULATION: PHARMACOLOGY—AMINO ACIDS, ANESTHETICS, ANTIDEPRESSANTS

542.1

ANTAGONISM OF BACLOFEN ANTINOCICEPTION BY AN aODN TO THE K^+-CHANNEL CODING MBK1 GENE C. Ghelardini, N. Galeotti, ^S. Capaccioli, ^A. Quattrone, ^L. Papucci and A. Bartolini Depts of Pharmacology and ^Pathology, I-50134 Florence, Italy, SPON: Eur. Brain and Behav. Soc.

The stimulation of $GABA_B$ receptors provokes the opening of several K^+ channels in central neurones by a PTX-inhibitable mechanism (Gähwiler and Brown, 1985). The antinociception induced by the $GABA_B$ agonist baclofen was antagonized not only by PTX pretreatment (Hoehn et al., 1988), but also by K^+-channel blockers (Ocaña et al., 1993).To further investigate the role of K^+-channels as intracellular effectors in baclofen-induced antinociception, the effects produced by antisense-mediated switching off of the K^+-channel expression were examined in mice. A specific aODN targeting the translation start region of the MBK1 K^+-channel mRNA was designed. Mice were randomly assigned to an antisense, degenerated or vehicle group. Each group received a single i.c.v. injection on days 1, 4 and 7. The effect of the aODN treatment was evaluated 72 h and 7 days after the last i.c.v. injection by using the mouse hot-plate test. The aODN (1-2-3 nmol per injection) produced, at 72 h, an antagonism of the baclofen (4 mg/kg s.c.) induced antinociception. The degenerated and vehicle treatments did not produce any antagonism of baclofen induced antinociception in comparison with naive and saline i.c.v. injected mice. This observation ruled out the possibility that the antagonism exerted by aODN could be due to an aspecific sequence-independent action on cerebral structures. A quantitative RT-PCR study demonstrated a reduction of mRNA levels only on the aODN treated group. Mice receiving the aODN treatment did not show any modification of spontaneous motility and motor coordination tested respectively by the Animex apparatus and the rota-rod. The modulation of central K^+-channels represents, therefore, an important intracellular step in the antinociception induced by baclofen.

This work was supported by grants from MURST.

542.2

INHIBITION OF PHOSPHOPROTEIN DEPHOSPHORYLATION ENHANCES THE RESPONSES OF CAT SPINAL DORSAL HORN NEURONS TO PERIPHERAL NOXIOUS AND NON-NOXIOUS STIMULI AND TO NMDA AND QUISQUALATE.
V. Radhakrishnan* and J.L. Henry. Departments of Physiology and Psychiatry, McGill University, Montreal, Quebec.

Protein phosphorylation plays an important role in various neuronal functions. Phosphorylated proteins are dephosphorylated by protein phosphatases. The role of dephosphorylation in spinal sensory mechanisms is not known. Therefore, in the present study we tested the effect of okadaic acid, an inhibitor of protein phosphatases PP-1 and PP-2A, on the responses of spinal dorsal horn neurons to various peripheral stimuli and to the excitatory amino acids NMDA and quisqualate. Single neuronal activity was recorded extracellularly from L_5-L_7 segments of the spinal dorsal horn using multibarrelled electrodes in chloralose-anesthetized cats. Okadaic acid, NMDA and quisqualate were applied by iontophoresis. Okadaic acid did not affect the on-going activity of 34 (30 WDR and 4 non-nociceptive neurons) of the 37 neurons tested; the remaining three WDR neurons that showed an increase in excitation were not included in this study. The responses to noxious thermal stimulation (n=9) and to pinch (n=4) were enhanced by okadaic acid by 30-70%. The responses of 4 non-nociceptive and 2 WDR neurons to hair stimulation were also enhanced (20-40%), while the response of one WDR neuron was depressed (25%). The responses to a train of high intensity electrical stimulation of the superficial peroneal nerve were enhanced in 5 (20-40%), depressed in one (30%) and unaffected in 3 WDR neurons. The responses to iontophoretic application of NMDA (n=5) and quisqualate (n=2) were also enhanced (60-90%). In general, the results indicate that okadaic acid facilitates the responses of spinal dorsal horn neurons to both noxious and non-noxious stimulation and to the excitatory amino acids NMDA and quisqualate. Thus, inhibition of dephosphorylation appears to increase the responses of dorsal horn neurons to various excitatory inputs. A corollary is that the protein phosphatases PP-1 and PP-2A may play a role in limiting the responses of dorsal horn neurons to sensory stimuli. *(Supported by MRC and by NINDS)*

542.3

CYCLOOXYGENASE INVOLVEMENT IN EXCITATORY RESPONSES TO SYNAPTIC INPUTS, EXCITATORY AMINO ACIDS AND SUBSTANCE P IN RAT SPINAL DORSAL HORN NEURONES *IN VIVO*. G.M. Pitcher and J.L. Henry*, Depts. Physiology & Psychiatry, McGill Univ., Montreal (Quebec) H3G 1Y6 Canada.

The purpose of this electrophysiological study is to examine the effects of inhibition of cyclooxygenase (COX), which synthesizes prostaglandins from arachidonic acid, on responses of rat spinal dorsal horn neurones to synaptic inputs and to iontophoretic application of NMDA, AMPA, Quis and substance P. Male Sprague Dawley rats (325-350 g) were anaesthetized (Na-pentobarbital; 50 mg/kg) and the cord was cut at T9. Single unit extracellular spikes were recorded using multibarrelled micropipettes. Neurones were classified functionally on the basis of their responses to natural cutaneous stimuli. Responses to these synaptic inputs and to excitatory amino acid and NK-1 receptor agonists were similar to those reported previously. The COX inhibitor, indomethacin (2.0-10.0 mg/kg, i.v.), preferentially depressed the excitatory responses to glutamate (n=4/4), Quis (n=8/9) and AMPA (n=7/8) but failed to alter the response to NMDA (n=6/6). Indomethacin also depressed the excitatory responses to substance P (n=5/5). In addition, indomethacin attenuated synaptically elicited nociceptive responses of WDR neurones (n=6/6), including both the initial, fast component and the late, slow afterdischarge. Responses of non-nociceptive neurones to hair stimualtion, innocuous touch and pressure were unaffected by indomethacin (n=9/9). The data indicate a selective effect of inhibition of COX on nociceptive responses of dorsal horn neurones in the rat. The depression of responses of these neurones to Quis, AMPA and substance P suggests that the depression of the synaptically elicited responses is mediated at least partially via an action in the spinal cord.

(Supported by the Canadian MRC, the McGill University Faculty of Medicine and the Royal Victoria Hospital Research Institute)

542.4

NITRIC OXIDE RELEASE INTO THE KNEE JOINT CONTRIBUTES TO THE DEVELOPMENT OF HEAT HYPERALGESIA AND INFLAMMATION IN AWAKE ARTHRITIC RATS. W. D. Willis, N. B. Lawand and K. N. Westlund*, Marine Biomedical Inst., Univ. Of Texas Medical Branch, Galveston, TX 77555.

Acute peripheral inflammation depends on the release of chemical mediators which produce edema formation as a result of plasma protein extravasation from the local microvasculature. Nitric oxide (NO) has been identified as a potent vasodilator "endothelium-derived relaxing factor" which is present in the endothelial cells lining the blood vessels and in neuronal tissues. NO causes vasodilatation by relaxing vascular smooth muscle via stimulation of guanylate cyclase. Previous studies have shown that inflammatory mediators induce the formation and release of NO. The aim of the present study is to investigate the involvement of NO in the peripheral processing of nociceptive information and the development of inflammation.

Male Sprague Dawley rats were anesthetized with a short acting barbiturate (Brevital) and injected intraarticularly with a mixture of 3% kaolin and carrageenan. Paw withdrawal latency to radiant heat was tested before and at 4hrs after induction of inflammation. Immediately after the test, a nitric oxide synthase inhibitor (L-NAME; 30μg) was injected directly into the knee joint. PWL to heat stimuli was then tested at 5, 6, 7, and 8 hrs post induction of inflammation. Assessment of joint inflammation was done by measuring the knee joint circumference. Our findings show that L-NAME injected intraarticularly blocked the development of heat hyperalgesia and the inflammation on the treated side. In another experiment, nitric oxide was found to increase in the knee joint after inflammation as measured by a NO sensitive electrode. These results suggest that endogenous NO is released at the site of the inflammation, inducing vasodilatation, edema formation and mediating peripheral nociceptive transduction. (Supported by NIH grants NS 28064, NS 09743 and NS 11255).

542.5

THE EFFECTS OF EXCITATORY AMINO ACIDS (EAAs) ON THE DISCHARGE OF ARTICULAR SENSORY RECEPTORS IN RATS. N. B. Lawand*, K. N. Westlund and W. D. Willis. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77555-1069.

Previous behavioral experiments have shown that intraarticular injection of EAAs produce hyperalgesia and mechanical allodynia in awake rats (Lawand et al., 1996), indicating that hyperalgesia depends, in part, on EAAs that act on the peripheral terminals of primary afferent fibers. The aim of the present study is to examine the effects of these amino acids on the discharges of articular mechanoreceptors in the knee joint of anesthetized rats.

Extracellular recordings were made from the medial articular nerve in rats anesthetized with pentobarbital. Glutamate and aspartate were applied intraarterially close to the joint in concentrations of 10^{-5} M up to 10^{-3} M. Response characteristics to mechanical or chemical stimulation of articular sensory receptors were identified. Following administration of the amino acids an excitation of the primary articular afferents occurred and the responses of these fibers to mechanical stimuli of the joint was enhanced. These results provide evidence for the involvement of excitatory amino acid receptors in peripheral nociceptive transduction and suggest an important role for these receptors in the development of hyperalgesia associated with joint infammation. (Supported by NIH grants NS 09743, NS 28064 and NS 11255).

542.6

A POSSIBLE ROLE FOR THE NMDA RECEPTOR-NITRIC OXIDE CASCADE IN THE ROSTRAL VENTROMEDIAL MEDULLA IN MEDIATING SECONDARY HYPERALGESIA. M.O. Urban*, S.V. Coutinho and G.F. Gebhart. Department of Pharmacology, University of Iowa, Iowa City, IA 52242.

We have recently demonstrated an involvement of descending pain facilitatory systems from the rostral ventromedial medulla (RVM) in a model of secondary hyperalgesia produced by mustard oil (a C-fiber excitant) measuring the spinal tail-flick reflex (TF) in awake rats. The present experiments were designed to determine a potential role for NMDA receptors and nitric oxide in the RVM in mediating this hyperalgesia. Topical application of mustard oil (20 μl, 100%) to the skin of the left hind leg produced a facilitation of the TF that was dose-dependently inhibited by microinjection of the NMDA receptor antagonist APV (1-1000 fmol) in the RVM 5 min prior to mustard oil. Injection of the nitric oxide synthase inhibitor L-NAME into the RVM (100-1000 nmol) produced a similar inhibition of mustard oil hyperalgesia while the inactive enantiomer D-NAME was without effect. Additionally, NADPH-diaphorase histochemical staining revealed an increase in positive cells in the RVM following topical application of mustard oil. Injection of NMDA (1-60 pmol) alone into the RVM produced a facilitation of the TF that had a short duration (12 min) and was inhibited by prior injection of L-NAME (1000 nmol). These data suggest that activation of NMDA receptors in the RVM produces a facilitation of spinal nociception via nitric oxide and support a potential role for the NMDA receptor-nitric oxide cascade in the RVM in mediating secondary hyperalgesia produced by mustard oil.

Supported by DA 02879

542.7

DOSE RESPONSE EFFECTS OF AP-5 AND NBQX ON CHRONIC CENTRAL PAIN FOLLOWING SPINAL CORD HEMISECTION. A.D. Bennett*, M.D. Christensen and C.E. Hulsebosch. Dept. of Anat. and Neurosci. and Mar. Biomed. Inst., Univ. of Texas Medical Branch, Galveston, TX 77555

Spinal cord injury (SCI) often results in dysesthesias in human patients. We recently developed a SCI mammalian model of chronic central pain in which the spinal cord is hemisected at T11 in rats and results in the development of mechanical allodynia and thermal hyperalgesia similar to that seen in humans. Excitatory amino acids could play an important role in the development of chronic central pain behavior observed after spinal hemisection. Our studies were designed to test if AP-5 and NBQX, NMDA and non-NMDA receptor antagonists, respectively, were effective in reducing behavioral symptoms of chronic central pain in rats in a dose response manner. We used accepted tests of mechanical and thermal allodynia to measure the responses to a variety of somatosensory stimuli. Supraspinal responses were tested using thermal stimuli. Our data validate earlier studies of the temporal development of both mechanical and thermal allodynia in both forelimbs and hindlimbs 21 days after hemisection and the maintenance of allodynia which persists for months. Intrathecal injections of graded doses in 10 μl volume of AP-5 or NBQX (from 200 μM to 5 mM) resulted in inhibition of mechanical and thermal allodynian in a dose dependent manner with presurgical values achieved at 5 mM doses. We interpret these data to support the involvement of NMDA and non-NMDA receptors in the development of chronic central pain. (Supported by NIH grant NS 11255 and the Kent Waldrep National Paralysis Foundation.)

542.8

BEHAVIORAL AND ELECTROPHYSIOLOGICAL EFFECTS OF DEXTRORPHAN IN A PRIMATE MODEL OF PERIPHERAL NEUROPATHY. K. Gondesen*, S.M. Carlton, H. Rees, and W.D. Willis. Dept. of Anatomy and Neurosciences, Marine Biomedical Institute UTMB, Galveston, TX 77555-1069

This study addresses the efficacy of Dextrorphan (DEX), a non-competitive NMDA antagonist, in attenuating behavioral and electrophysiological abnormalities previously documented in a monkey model of peripheral neuropathy.

In an anesthetized monkey (Macaca fascicularis), one L7 spinal nerve was tightly ligated and a sham operation performed on the contralateral side. A small laminectomy was performed and a 4 French catheter was placed in the intrathecal space near the lumbar enlargement and connected to a small reservoir implanted subcutaneously. Compared to presurgery levels, the animal demonstrated an increased sensitivity (allodynia) on the plantar surface of the experimental foot to mechanical stimulation with von Frey hairs and brushing with a camel hair brush 4 days post-surgery. On subsequent days, a 500ul bolus injection of 10, 50 or 100nm of DEX was delivered IT. The first test period of each day established baseline; following administration of DEX, testing was repeated at 15, 30, 60, 120 and 180 min intervals. A bolus of 50 and 100nm DEX produced a decreased response to von Frey hairs and brush on the experimental foot throughout the testing period. Responses remained decreased, taking up to 2 days to recover. The lowest dose had various effects ranging from very little or no change to increased responses. No change in muscle tone and no cognitive effects were observed at any dose.

In electrophysiology experiments, 3 doses of DEX were applied through a microdialysis fiber (0.1, 0.3 and 1mM). Recordings were made from WDR spinothalamic tract (STT) cells. DEX inhibited the response of STT cells to graded cutaneous stimuli (brush, press, pinch and squeeze). The inhibition was dose dependent.

These results suggest that DEX alleviates pain behaviors resulting from nerve injury. At a cellular level, DEX modulates the central processing of STT cells. Therefore, DEX may be a suitable therapeutic drug for the treatment and management of neuropathic pain. NS11255, NS27910.

542.9

OPIATES AND OPIATE RECEPTORS IN PERIPHERAL SENSORY AXONS. S. M. Carlton*, S. Zhou, and R. E. Coggeshall. Department of Anatomy and Neuroscience and Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77555-1069.

Cutaneous nerves contain opiate receptors and peripheral opiates relieve inflammatory pain. We now ask what proportion of unmyelinated cutaneous axons contain opiate receptors, whether any of the opiates themselves can be found in normal cutaneous unmyelinated axons and whether peripheral opiate ligands can block noninflammatory pain.

For the anatomical studies, blocks of skin were taken from normal rats perfused with mixed aldehydes and immunostained for either enkephalin peptide or the μ and δ opiate receptors. For the behavioral studies, rats were briefly anesthetized with halothane and the glabrous skin of the 3rd hind toe was injected with glutamate (20μl), either alone or co-injected with the μ receptor ligand DAMGO or the δ receptor ligand DPDPE.

The anatomical studies showed that 32% of peripheral cutaneous unmyelinated axons express the μ opiate receptor (N=3), 30% express the δ receptor (N=1) and 17% were immunoreactive for the enkephalin peptide (N=3). The behavioral studies demonstrated that peripherally administered glutamate resulted in significant pain behaviors and these behaviors were attenuated by DAMGO but not DPDPE.

We conclude that both opiate receptors and the opiate enkephalin are present in normal rat unmyelinated cutaneous axons and that peripherally administered opiate ligands can relieve glutamate-induced pain. It remains to be determined whether opiates and their receptors are colocalized and whether the proportions of axons expressing opiates and opiate receptors change in various pain states. Supported by NIH NS27910, NS10161, NS11255.

542.10

PERIPHERAL GLUTAMATE AND SUBSTANCE P RECEPTORS PLAY A ROLE IN PAIN BEHAVIORS. S. Zhou* and S. M. Carlton. Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, TX 77555.

Our previous work has shown that injection of glutamate (GLU) or its agonists into rat glabrous skin induces mechanical allodynia and mechanical hyperalgesia. It is unknown what role, if any, peripheral GLU receptors plays in the generation of heat hyperalgesia and spontaneous pain. Furthermore, it has been previously reported that substance P (SP) can enhance GLU-induced activity in the spinal cord. The presence of SP receptors on peripheral axons suggest that a similar mechanism may exit in the skin. In the present study we demonstrate a role for NMDA, non-NMDA and SP receptors in peripheral pain mechanisms.

A total of 120 male Sprague-Dawley rats (320-380g) were used in this study. Mechanical (von Frey filaments, bending forces of 6.7, 10.4, 18.8 and 39.2 mN) or thermal (44°C hot water) stimuli were applied to the tail of each rat. GLU (RBI, 5mM), SP (RBI, 0.01mM), phosphate buffered saline (PBS) and combinations of Glu+SP, Glu+MK-801 (0.01mM) and Glu+CNQX (0.1mM, 0.3mM) were injected (20 ul) subcutaneously, 5.5-6cm from the tip of the tail.

The results show: 1) injection of GLU alone results in the generation of mechanical allodynia, mechanical and heat hyperalgesia, 2) MK-801 and CNQX reduced these GLU-induced behaviors, 3) compared to the injection of GLU alone, SP+GLU resulted in enhanced pain behaviors, including the generation of spontaneous pain (tail flicks in the absence of any stimuli).

The data indicate that peripheral NMDA and non-NMDA receptors play a role not only in mechanical allodynia and hyperalgesia, but also in heat hyperalgesia. Furthermore, similar to central mechanisms, SP can potentiate GLU-induced pain in the periphery. (Supported by NS11255 and 27910 to SMC).

542.11

PERIPHERAL NMDA RECEPTORS PLAY A ROLE IN CAPSAICIN HYPERALGESIA. C.E. Prioleau*, R.E. Coggeshall and S.M. Carlton. Dept. of Anatomy and Neurosciences, Univ. of TX Medical Branch, Galveston, TX 77555

Anatomical evidence from our laboratory documents the presence of NMDA receptors on unmyelinated axons in the glabrous skin. Activation of these receptors by subcutaneous injection of glutamate or NMDA results in pain behaviors. Intradermal capsaicin activates unmyelinated cutaneous fibers and produces pain. The present study investigates the role of peripheral NMDA receptors in capsaicin pain.

Male Sprague-Dawley rats were briefly anesthetized with halothane and the glaborous skin of the third hind toe was injected with 10μl of either phosphate buffered saline (PBS, n=5) or the glutamate antagonist MK-801 (.001, .01, or .05mM, n=2 for each dose). Five minutes later, 10μl of capsaicin (1, 10, or 100 μg, n=3 for each dose) was given at the same site.

Compared to baseline and vehicle control scores (n=5), rats injected with PBS+capsaicin showed increased spontaneous pain as measured by an increased number of flinches, and mechanical allodynia and hyperalgesia as measured by increased responses to stimulation with von Frey filaments. These nocifensive behaviors increased in a dose dependent fashion and remained localized to the area of the injection site. Rats injected with MK-801+capsaicin showed a dose-dependent reduction in the nocifensive behaviors to near baseline levels.

These data indicate that activation of peripheral NMDA receptors contributes to the pain induced by capsaicin injection and suggests that local application of glutamate antagonists may be clinically useful for the treatment of pain. Supported by NS11255 (SMC and REC) and NS27910 (SMC).

542.12

PERIPHERAL NMDA RECEPTORS CONTRIBUTE TO THE PAIN BEHAVIORS OBSERVED IN THE RAT FORMALIN TEST. E. M. Davidson, S. L. Knock* and S. M. Carlton. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77555.

Previous work in our laboratory has demonstrated the presence of peripheral NMDA receptors on unmyelinated axons in the glabrous skin of the rat. Moreover, glutamate or NMDA injected peripherally have been shown to induce pain behaviors which are attenuated by MK-801, an NMDA antagonist. The present study investigated the involvement of NMDA receptors in the pain behaviors observed in the formalin test, a well-known and frequently used pain model.

Male Sprague-Dawley rats were briefly anesthetized with halothane and the glabrous skin of the 3rd hind toe was injected with either 30μl phosphate buffered saline (PBS, n=5) or MK-801 (0.001mM, n=6). Ten minutes later, 15μl of 5% formalin was injected in the same area. Pain behaviors were assessed for 30 minutes post-injection by measuring time spent licking and/or elevating the injected paw, and counting flinches during 5 minute intervals.

Compared to baseline, rats injected with PBS+formalin demonstrated significant increases in both pain behaviors. In contrast, rats treated with MK-801+formalin showed less time licking/elevating the injected paw compared to PBS+formalin, however, the number of flinches was not significantly different between the 2 groups. MK-801+formalin rats showed decreased pain behavior during the second but not the first phase of the formalin test.

These results suggest that peripheral NMDA receptors play a role in pain perception, contributing to formalin-induced pain and possibly inflammatory pain. (Supported by NS11255 and NS27910 to SMC).

542.13

ROLE OF EXCITATORY AMINO ACID RECEPTORS IN NERVE-INJURY INDUCED PAIN. M.L. Nichols, D. Bian, S. Wegert, M.H. Ossipov, T.P. Malan, Jr. & F. Porreca*. Univ. Arizona Pain Institute, Health Sciences Center, Tucson, AZ 85724.

The present studies examine the antiallodynic effects of different classes of intrathecal (*i.th.*) excitatory amino acid (EAA) antagonists in a nerve ligation model of neuropathic pain in rats. Nerve injury was produced by tight ligation of the L5/L6 spinal nerves, resulting in stable and long-lasting tactile allodynia shown by a decreased threshold to paw withdrawal elicited by probing with von Frey filaments. Threshold response to thermal nociceptive stimuli were evaluated using the warm-water (55°C) tail-flick test or the radiant-heat induced foot-flick test in sham-operated or nerve-injured rats. In nerve-ligated rats, *i.th.* morphine did not alter tactile allodynia (up to 100 μg) and did not alter the withdrawal threshold to von Frey filaments in sham-operated controls. In nerve-injured rats, hyperalgesia could be demonstrated in the foot, but not the tail and *i.th.* morphine produced antinociception in both endpoints with decreased potency and in the tail-flick test with decreased efficacy. *I.th.* MK801, a non-competitive NMDA antagonist, did not affect allodynia at non-behaviorally toxic doses (i.e., up to 3.5 μg) or produce antinociception in tail-flick or foot-flick endpoints. Pretreatment with *i.th.* MK-801, however, restored antiallodynic efficacy of *i.th.* morphine and increased the antinociceptive potency and efficacy of i.th. morphine in the tail-flick test. Presuppression of foot hyperalgesia with *i.th.* MK-801 did not alter the potency of *i.th.* morphine to suppress the foot-flick response. Allodynia was unaffected by *i.th.* metabotropic antagonist, (L)-AP3. In contrast, CNQX, a competitive AMPA antagonist, produced dose-related antiallodynic actions with an A_{50} value (95% C.L.) of 0.7 (0.4-1.3) μg; no behavioral toxicity was evident in CNQX treated rats over this dose range. These data suggest that (a) allodynia associated with peripheral nerve injury results, in part, from activation of EAA receptors and that such activity limits the antiallodynic and antinociceptive effects of opioids, and (b) that blockade of AMPA receptors can directly block the evoked allodynia associated with nerve injury. Supported by USPHS Grants.

542.14

SUBCUTANEOUS CO-INJECTION OF LIDOCAINE AND DEXTROMETHORPHAN GIVE A PROLONGED ANALGESIC BLOCK. J. K. Birknes[1], B.S., M. Vladimirov[1], M.D., Ph.D., Donald Johnson[2], Ph.D., F. S. Caruso[2], Ph.D., and G. R. Strichartz[1], Ph.D.* (1)Pain Research Group, Dept. of Anesthesia Research Laboratories, Harvard Med. Sch., Brigham and Women's Hospital, Boston, MA 02115 and (2)Algos Pharmaceuticals Corp., Collingwood Plaza, 4900 Route 33, Neptune, NJ 07753-6804

The effectiveness for cutaneous analgesia of dextromethorphan hydrobromide (DM·HBr: F.W.=370.3), a noncompetitive antagonist of the N-methyl-D-aspartate receptor, and lidocaine hydrochloride (LID·HCl: F.W.=270.8), alone and in combination, was investigated in conscious unanesthetized male Sprague- Dawley rats and Dorcet-cross sheep. The cutaneous trunci muscle reflex (CTMR), characterized by twitches of the lateral thoraco-spinal muscles in response to a brisk pinch of the overlying skin, was used as an indicator of nociception. The degree and duration of CTMR inhibition was estimated at regular intervals after subcutaneous injection of 0.6 mL of drug solution in the rat. The dose dependence of duration for LID showed that 1.25 mg/ml led to an incomplete block of the CTMR with partial inhibition lasting for 26.3 ± 2.0 min ($\bar{X}$ ± S.E., n=15), while 1.75 mg/ml DM produced a complete block of CTMR for 62.5 ± 6.5 min and partial inhibition for 270.0 ± 41.8 min (n=6). Co-injection of LID and DM at these doses resulted in a complete block for 112.5 ± 19.6 min and a partial inhibition of the CTMR for 18.7 ± 3.0 hours (n=6). Similarly, in the sheep, subcutaneous injection of 7 mL of 20 mg/ml LID abolished the CTMR for 30 min, with full recovery occurring at 420 min (n=8). Co-injection of this LID dose and 7 mg/ml DM abolished CTMR (94.4 ± 5.6% inhibition) for 180 min with complete recovery at 22 hrs (16.7 ± 8.3% inhibition, n=9). Altogether these results show a 4-6 fold potentiation in duration of LID induced analgesia by DM. We conclude that this may represent a new mechanism of peripheral analgesia that would be promising for clinical application. *Supported by a grant from Algos.*

542.15

VOLATILE GENERAL ANESTHETICS AFFECT A NEURAL PATHWAY IN *DROSOPHILA*. Meiqiu Lin* and Howard A. Nash. Lab. of Molecular Biology, National Institute of Mental Health, Bethesda, MD 20892.

The identity of the physiologically relevant target(s) of general anesthetics remains controversial. In previous studies we have shown that a neural component of the startle response of *Drosophila* is sensitive to the general anesthetic halothane and that its sensitivity is affected by genetic alteration of a potassium channel. The sensitive component contributes to the long-latency (4.34 ± 0.06 msec) response, measured as the time between application of an electrical stimulus across the fly's eyes and the appearance of an evoked potential in the muscles that effect the escape behavior. In contrast, responses with intermediate (2.23 ± 0.04 msec) or short (1.43 ± 0.02 msec) latencies are much less sensitive to halothane. To localize the portion of the brain that is unique to the long-latency response, we determined the positions of stimulating electrodes that permit the occurrence of this response and compared them to positions which only permit shorter responses. The long-latency response is obtained only when electrodes remain in the retinal layer and fail to penetrate into the optic lobe. This strongly suggests that the anesthetic-sensitive component lies in the visual pathway that connects the lamina with the Col A neurons that innervate a descending giant fiber. Three other general anesthetics: methoxyflurane, enflurane, and desflurane, are also differentially effective in inhibiting the long-latency response. As with halothane, their potency correlates well with their effectiveness in inducing gross behavioral changes.

This research is supported by National Institute of Mental Health.

542.16

PRESENCE OF INHIBITORY CUTANEOUS RECEPTIVE FIELDS PREDICTS DIFFERENTIAL EFFECT OF INTRAVENOUS LIDOCAINE ON HEAT-EVOKED SPINAL NEURONAL RESPONSES. T.J.Ness*. Dept. Anesthesiology, University of Alabama at Birmingham (UAB) Birmingham, AL 35294

Intravenous lidocaine has previously been demonstrated to have a differential inhibitory effect on two classes of neurons excited by a visceral stimulus, colorectal distension (NS Absts 20:135.13). One feature which differentiated these two neuronal groups was the presence or absence of inhibitory cutaneous receptive fields.

The present study examined the effects of intravenous lidocaine on lumbosacral spinal neurons excited by radiant heating of the hind paw. Extracellular recordings were made from sixteen L3-L5 dorsal horn neurons in spinally transected, decerebrate rats. Neurons were characterized for excitatory and inhibitory responses to nonnoxious (brush, tap) and noxious (pinch, heat) cutaneous stimuli; eight neurons were inhibited by noxious cutaneous stimuli applied to nonsegmental sites; the other eight had only excitatory receptive fields.

Consistent (± 20% on 3 trials) excitatory responses to 50°C, 15 s radiant heating of the ipsilateral hind paw were determined. Cumulative doses of intravenous lidocaine (0.25, 1 and 4 mg/kg) were administered at 16 minute intervals and repeat heating trials performed every 4 minutes. Similar to studies of neurons excited by colorectal distension, intravenous lidocaine produced a dose-dependent inhibition of heat-evoked neuronal responses that was significantly greater in neurons without inhibitory cutaneous receptive fields.

Supported by a Koller Grant from the Am. Soc. Regional Anesthesia.

542.17

SPINAL GABAPENTIN IS ANTINOCICEPTIVE IN THE RAT FORMALIN TEST. N. Shimoyama, M. Shimoyama, A.M. Davis, C.E. Inturrisi and K.J. Elliott*. Dept. of Pharmacology, Cornell U. Med. Coll. and Pain Research Program, Dept of Neurology, MSKCC, NY, NY 10021.

Anticonvulsants have analgesic properties and are used in the management of pain in patients (Elliott, 1995). Gabapentin is a new anticonvulsant (MacDonald, 1994) that interacts with a CNS L-type Na^+-independent amino acid transporter (Su, 1995). Clinical experience (KJE) and case reports suggest gabapentin has analgesic properties in patients (Mellick 1995, Segal 1996). To determine whether gabapentin has antinociceptive properties through a spinal action, pretreatment with gabapentin prior to the formalin test, a model of central sensitization, was examined. Gabapentin, 6, 20, and 60 ug, or saline in a volume of 5 ul was administered intrathecally 10 minutes prior to the injection of intraplantar formalin to SD rats. In the saline-pretreated animals, formalin produced a stereotypical behavioral response consisting of flinching and licking of the injected paw. Gabapentin at the dose of 6 ug, did not affect flinching or licking behavior as compared to saline control. Gabapentin at 20 ug and 60 ug decreased flinching and licking behaviors as compared to saline control in a dose-dependent manner. At 20 ug, gabapentin pretreatment decreased flinching by 50% and licking by 30%. At 60 ug, gabapentin decreased flinching by more than 60% and decreased licking by more than 90%. These results indicate that spinal gabapentin is antinociceptive, possibly via an enhancement of inhibition through a gaba-ergic mechanism. Supported in part by NIDA Grants DA00255, DA01457, DA00198 and the VZV Foundation.

542.18

EFFECTS OF ANTIDEPRESSANTS ON THE C-FIBER-EVOKED NOCICEPTIVE REFLEX IN RATS C. Mestre[b]*, T. Pelissier[a], A.Hernandez[a] and A. Eschalier[b] Dept. of Anesthesia & Critical Care, Univ. of Chicago, USA; [a]Dept. of Pharmacol., Faculty of Medicine, Univ. of Chile, Santiago, Chile; [b]Dept. of Pharmacol., Faculty of Medicine, Clermont-Ferrand, France.

Results from experimental and clinical studies indicate that antidepressants have specific analgesic effects which appear to be independent of the antidepressant efficacy. To determinate the site of this antinociceptive action, the effect of clomipramine (CMI) and desipramine (DES), two antidepressants which inhibit the reuptake of serotonin and norepinephine, respectively, was studied in male Sprague-Dawley rats, after intravenous (i.v.), intrathecal (i.t.) or intracerebroventricular (i.c.v.) administration. The C-fiber-evoked reflex was examined. Electromyographic activity was measured in the biceps femoris muscle, elicited by electrical stimulation of the ipsilateral sural nerve receptive field in urethane anesthetized rats. The results demonstrate that, following i.v. administration, both antidepressants produced a dose-dependent depression of the C-fiber reflex (-50±9 and -43±5% for CMI and DES, respectively). I.t. administration of the highest dose of DES inhibited the C-fiber reflex (-32±5% *vs.* i.t. saline), whereas CMI produced only a small decrease (-16±3% *vs.* i.t. saline). After i.c.v. injection, only CMI produced a dose-dependent depression of the C-fiber reflex (-91±7% *vs.* i.t. saline). The results suggest that these drugs mediate their antinociceptive effects via distinct central site depending on their monoaminergic profile. Antidepressants that inhibit serotonin reuptake may act at the supraspinal level, probably by activating the bulbospinal inhibitory pathway, whereas antidepressants that inhibit noradrenaergic reuptake may act at a spinal site.

542.19

ANTAGONISM OF CLOMIPRAMINE AND AMITRIPTYLINE INDUCED ANALGESIA BY AN AODN TO THE MBK1 GENE. N. Galeotti, C. Ghelardini, ^S. Capaccioli, ^A. Quattrone, ^L. Papucci and A. Bartolini Depts of Pharmacol. and ^Pathol., I-50134 Florence, Italy, SPON: Eur. Brain and Behav. Soc.

PTX-sensitive G-proteins are responsible for inhibition of adenylate cyclase activity and modulation of several K^+ and Ca^{2+} channels (Hille, 1994). The tricyclic antidepressant (TCAs) drugs clomipramine and amitriptyline are able to produce antinociception by activating the serotoninergic system that provokes the activation of a PTX sensitive G-protein (Galeotti et al., 1996). In order to investigate whether K^+-channels are involved in clomipramine and amitriptyline induced antinociception, the effects produced by an aODN to the K^+-channels were examined in mice. A specific aODN targeting the translation start region of the MBK1 K^+-channel mRNA was designed. Mice were randomly assigned to an antisense, degenerated or vehicle group. Each group received a single i.c.v. injection on days 1, 4 and 7. The effect of the aODN was evaluated 72 h and 7 days after the last i.c.v. injection by using the mouse hot-plate test. The aODN (1-2-3 nmol per injection) produced, at 72 h, a dose-dependent antagonism of clomipramine (25 mg/kg s.c.) and amitriptyline (20 mg/kg s.c.) induced antinociception. The antagonism of TCAs induced analgesia disappeared at 7 days indicating a lack of irreversible damage or toxicity by the aODN treatment. The degenerated and vehicle treatments did not produce any antagonism of the TCA induced antinociception in comparison with naive and saline i.c.v. injected mice. This observation ruled out the possibility that the antagonism exerted by aODN could be due to an aspecific sequence-independent action on cerebral structures. Moreover, a quantitative RT-PCR study demonstrated a reduction of mRNA levels only on the aODN treated group. These results indicate that the modulation of K^+-channels is an important intracellular step in clomipramine and amitriptyline antinociception.

This work was supported by grants from MURST.

543.1

ENTRAINMENT OF RHYTHMIC BURSTING INDUCED BY BLOCK OF SYNAPTIC INHIBITION IN THE NEONATAL RAT SPINAL CORD E. Bracci, M. Beato, L. Ballerini* and A. Nistri, Int Sch Adv Studies (SISSA), 34013, Trieste, Italy.

Spinal networks are able to provide the basic features of motor rhythmic patterns which are modulated by proprioceptive and sensory inputs. A highly regular pattern of motoneuronal bursting develops in the rat spinal cord *in vitro* after simultaneous block of $GABA_A$ and glycine receptors by bicuculline (20 μM) and strychnine (1 μM), respectively. Such bursts, comprising several intraburst oscillations, appear spontaneously with an average duration of about 7 s and an average frequency of about 2/min. We studied if dorsal root stimulation could entrain such a rhythmic bursting. Bursts were recorded either intracellularly from individual lumbar motoneurons or extracellularly from lumbar ventral roots. A single stimulus (0.1 ms; 5-10 V) to a lumbar dorsal root during interburst quiescent period elicited a burst with duration and oscillatory structure dependent on the interval from the end of the last spontaneous burst: closer stimulations elicited shorter and less structured events. Trains of stimuli were able to entrain bursting at frequencies ranging from 0.05 to 1.0 Hz. When stimulations were delivered at higher frequencies they still elicited a short reflex response. At such stimulation rates, however, bursts developed at low frequency independently of external inputs. In the range of intervals in which the entrainment was possible, burst duration and number of intraburst oscillations decreased as stimulation frequency increased. At the maximum frequency compatible with entrainment, burst duration was reduced to 25-8 % of the one of spontaneous events. Thus, in the absence of synaptic inhibition, spinal networks exhibited a high degree of adaptability of their rhythmic motor response to dorsal root synaptic inputs, although at high stimulation rates they generated bursts unrelated to reflex activity. Supported by Telethon (#502)

543.2

SIMULATION OF SPINAL SEGMENTAL REFLEXES: THE ALTERNATE GOLGI REFLEX D. P. Bashor* and S. M. Merkel. Dept. of Biology, UNC Charlotte, NC 28223

A simulation system was used to investigate the neuronal interactions of stretch (IA), classical Golgi tendon (IB), and "alternate" IB reflexes. The simulation consisted of 14 cell and 6 fiber populations (100 elements each) representing a pair of antagonistic motoneuron (MN) populations with their associated paired IN populations: Renshaw, IA and IB INs, and excitatory and inhibitory IN pools. A pair of Alternate IB IN populations provided MN excitation. Fiber inputs included paired IA and IB afferents, tonic descending excitation to all cell populations, and a "modulator" used to vary the excitability of the Golgi-related populations. The force output of the MNs was estimated by an "activity index", the product of fraction of cells active and the mean spike frequency. In a representative experiment, the baseline agonist-antagonist activity index ratio was 10:10. Activation of agonist IA afferents alone changed this ratio to 34:4. The agonist IB fibers were then activated at the same time as the IA afferents. By modulating the relative excitability levels of the "classical" IB IN and the "Alternate" IB IN populations, a range of activity index ratios were achieved, from those in which agonist IB activation reduced the agonist-antagonist difference ("classical" effect), to those in which IB activation increased the agonist-antagonist difference ("alternate" effect). Supported by UNC Charlotte.

543.3

CHEMICAL MICROSTIMULATION IN BRAIN LOCOMOTOR REGIONS IN LARVAL LAMPREY. A. Hagevik*, A. Oxner McGaha, and A.D. McClellan, Div. of Biological Sciences, Univ. of Missouri, Columbia, MO 65211

In the present study, chemical microstimulation, using pressure ejection of excitatory amino acids (EAA) or their agonists, was used to map brain locomotor regions in larval lamprey (Petromyzon marinus). In vitro brain/spinal cord preparations (n = 10) were used, and locomotor activity was recorded from ventral roots. During the experiment, effective stimulation sites were marked with Alcion blue to determine their proximity to descending brain neurons and the trigeminal system, which were labeled prior to the experiment with HRP.

Diencephalon. Microstimulation with EAA or their agonists in the lateral part of the diencephalon, near the Di cell group, elicited spinal locomotor activity but usually with delays greater than 10 s.

Mesencephalon. Chemical microstimulation with D-glutamate, L-glutamate, kainate, or quisqualate in the lateral part of the mesencephalon as well as near the MRN cell group elicited spinal locomotor activity with short latency.

Rhombencephalon. The most effective locomotor regions were in the lateral areas of the rostral rhombencephalon in the alar plate. However, short latency locomotor activity could also be elicited in or near the ARRN and sometimes the MRRN. Stimulation in the PRRN usually elicited tonic spinal motor activity.

In summary, in the mesencephalon, effective chemical microstimulation sites probably represent higher order locomotor control centers, such as the mesencephalic locomotor region (MLR). In the rhombencephalon, lateral locomotor regions may correspond to sensory pathways (e.g. trigeminal) that have inputs to brainstem locomotor centers. At least some of the descending neurons that activate spinal locomotor networks appear to reside in the ARRN and MRRN. The functional relationship between these various areas is yet to be determined.

Supported by NIH Grant NS 29043 and APA Grant MB1-9108 awarded to A.D.M.

543.4

ESTIMATION METHODS IN LOCOMOTION NETWORK ACTIVITY. J. Tabak, C.R. Murphey, and L.E. Moore*. Phys. & Biophys., UTMB, Galveston, TX 77555, and Eq. Neurobiol., CNRS URA 256, Univ. Rennes 1, France.

In previous studies using the simple neural network model of Roberts & Tunstall (1989, 1995), we demonstrated critical influences of the voltage-dependent activation of NMDA receptor channels in controlling fictive swimming of Xenopus embryos, including stabilization of alternating versus synchronous activity, rhythmic activity without inhibition and struggling-like behavior. Nonlinear estimation methods were applied to single cell and network responses in order to more quantitatively determine the role of cellular electrotonic structure, voltage-dependent channel kinetics and synaptic properties. Current-clamp responses and frequency-domain driving point admittance measurements were fitted to a Rall-type Hodgkin-Huxley model to estimate both the passive electrotonic structure of the cell and the voltage-dependent channel activation kinetics.

1) In analysis of model-generated data, frequency domain method proved more accurate in obtaining estimates in a lumped soma/dendritic cable model with potassium and sodium voltage-gated conductances. 2) Problems of non-uniqueness for either the time- or frequency-domain analysis alone can be significantly reduced by simultaneously fitting of both time- or frequency-domain data. 3) Comparisons across data sets from individual cells showed a) changes in leakage resistance with time attributed to membrane recovery from microelectrode penetration, or b) discrepancy for the activation kinetics of the potassium conductance, suggesting that a current having different effects on the two kinds of measurements was left out of the model.

Cluster analysis of solutions reveals relationships between the parameters, and sensitivity analysis shows which parameters are likely to be well estimated. Network "training" methods were also used in order to obtain estimates of synaptic parameters from measurements of swimming activity.

Supported by R01-MH45796, US and CNRS & MESR, France.

543.5

STEPPING MOVEMENTS IN PARAPLEGIC PATIENTS INDUCED BY EPIDURAL SPINAL CORD STIMULATION

Y Gerasimenko[1], WB McKay, FE Pollo, MR Dimitrijevic*
Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas
[1]Pavlov Institute of Physiology, St Petersburg, Russia.

In this report, we summarize our findings in five complete paraplegic patients recorded during sustained tetanic stimulation of the posterior structures of the lumbar spinal cord. Subject ages ranged from 18 to 43 and spinal cord lesions were between C5 and T7. They all retained spinal motor neuron reflex excitability. Stimulation was delivered through a 4 electrode catheter (electrodes 1 or 3 cm apart) introduced into the epidural space between the T11 and T12 vertebrae. By stimulating the posterior portion of the lumbar spinal cord with single stimuli at 1 Hz, we documented the location and adjusted it to be within the L2-L3 segments by eliciting muscle twitches from the quadriceps and adductor muscles.

Stepping movements and electromyographically recorded patterns of motor unit activity were observed most frequently when the cathode was over L2-L3 spinal cord segments, stimulus frequency was 20 to 50 Hz, pulse width between 0.3 and 1 ms and a strength of 5.5 to 8.5 mA was provided. The frequency of stepping movements and EMG bursts elicited was about 0.3 Hz. In addition, we found that the interval between the onset of tetanic stimulation and the appearance of movements was in the range of 5 to 7 seconds. After cessation of stimulation, rhythmic EMG activity could persist for up to 8 seconds.

These observations are consistent with our previous report that stepping movements can be obtained with continuous tetanic input to the spinal cord in paraplegics (Rosenfeld et al, *Abstr* 21:688, 1995).

Funding provided by the V.L. Smith Foundation for Restorative Neurology and The Kent Waldrep Institute for Paralysis Research.

543.6

MOTOR OUTPUT ELICITED BY CONTINUOUS EPIDURAL STIMULATION IN PARALYZED SPINAL CORD INJURED HUMANS

FE Pollo*, WB McKay, Y Gerasimenko[1], MR Dimitrijevic
Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas; [1]Pavlov Institute of the RAS, St Petersburg, Russia.

Five spinal cord injured subjects with neurophysiologically documented complete lesions between C5 and T7 were studied 15 to 65 months post injury. Catheters containing 4 electrodes, 1 or 3 cm apart, were introduced into the epidural space overlying the lumbosacral spinal cord. Electrical stimulation ranging in frequency from 1 to 80 Hz and amplitude of up to 30 mA was applied. Stimulus rates below 20 Hz brought muscle 'twitch' responses with no patterning. However, stimulus frequencies above 20 Hz elicited a variety of movements and motor unit activation patterns. Movements produced included uni-and bilateral withdrawal, extensor, cross-extensor and stepping movements in the supine position. Patterns of motor unit activation were categorized as: tonic activation of one or more muscle groups; bursting of one or more muscle groups at a frequency of 0.3 to 0.5 Hz that could be simultaneous or reciprocal about a single joint, proximal or distal, or multiple joints, alternating between the two lower limbs with patterns resembling those recorded during locomotion.

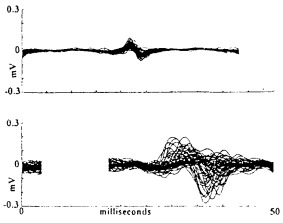
These results suggest that depending on the stimulus site and parameters, multi-muscle activation patterns resembling those recorded during ambulation and stepping movements can be elicited in humans paralyzed by spinal cord injury.

Funding provided by the V.L. Smith Foundation for Restorative Neurology and The Kent Waldrep Institute for Paralysis Research.

543.7

CENTRAL MODULATION OF EMG RESPONSES TO SPINAL CORD STIMULATION. AM Sherwood*, JA Halter, DC Lee, WB McKay, Y Gerasimenko[1], MR Dimitrijević Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston TX 77030, ([1]from: Pavlov Institute, St Petersburg, Russia).

Continuous stimulation of the posterior aspect of the lumbosacral spinal cord through epidural electrodes has been shown to elicit alternating patterns of locomotor-like EMG activity in spinal cord injured humans [Rosenfeld et al., *Abstr* 21:688, 1995]. We studied this motor output in five paralyzed spinal cord injured individuals, whose lesions were from C5 to T7, sustained 18 to 65 months before the study. Surface electrodes were used to record over major muscle groups of the legs. Spinal cord stimulation (SCS) through electrodes at vertebral level L1 activated the spinal motor neuron pool, recordable as surface electromyographic activity. Two sets of 100, successive, superimposed, tibialis anterior responses to stimulation with a 25 ms period (at t=0) are shown. For clarity, portions of the traces with responses to stimuli at t=+25 ms (upper) and at t=-25 ms (lower), respectively, were excluded. Synchronous responses (upper) suggest relatively direct activation of the motor pool, which were also constant in amplitude. Changing conditions, here, changing stimulus current from 4.1 ma (upper) to 5.1 ma (lower), resulted in less synchronous, lower amplitude, delayed (~8 ms), less consistent responses. The lower trace activity was taken from the midst of a 2 second burst, representative of burst patterns with periods of 5-10 s.



We suggest that a spinal pattern generator activated by the SCS was responsible for modulation of the response.

Supported by: *Vivian L. Smith Foundation for Restorative Neurology* and *Kent Waldrep Institute for Paralysis Research.*

543.8

CENTRAL PATTERN GENERATOR IN HUMAN: MOTOR RESPONSES EVOKED BY AND SPINAL CORD EVOKED POTENTIALS RECORDED FROM EPIDURAL ELECTRODES AT DIFFERENT LOCATIONS

MR Dimitrijević, JA Halter*, Y Gerasimenko[1], AM Sherwood and WB McKay Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston TX 77030, ([1]from: Pavlov Institute of Physiology, St Petersburg, Russia).

A previous study has demonstrated that it is possible to elicit patterned activity resembling stepping in the lower extremities of chronic spinal cord injured humans through continuous stimulation of the posterior aspect of the lumbosacral spinal cord using epidural electrodes [Rosenfeld et al., *Soc.Neurosc.Abstr* 21:688, 1995]. Furthermore, the location of the epidural electrodes and the stimulus intensity were found to significantly effect the character of the patterned motor activity. In this study, we extend these early observations and also examine the character of the motor responses to single stimuli and the properties of spinal cord evoked potentials (SCEPs) recorded via the epidural electrodes. As described in a companion study (Pollo et al., this volume) the motor responses in five paralyzed spinal cord injured individuals were examined. SCEPs were recorded from the epidural electrodes resulting from electrical stimulation to the tibial nerve at the popliteal fossae (duration: 0.5ms, rate: 3.1pps and supramaximal to soleus activation). In one case, with the cathode at the lower T12 region and anode at the upper T12 region, the motor responses resulting from spinal cord stimulation at low rates (1pps) could be seen to recruit proximal musculature preferentially whereas stimulation at higher rates (30pps) was seen to preferentially recruit patterned activation of the distal musculature. The SCEPs showed a maximal postsynaptic potential present between the cathode and anode. Correlation was seen in the amplitudes of SCEPs with left versus right tibial nerve stimulation and the activation of left versus right motor responses at 1pps whereas the patterned activity evoked by higher stimulus rates sometimes showed a opposite relation. *Supported by the Vivian L. Smith Foundation for Restorative Neurology* and *Kent Waldrep Institute for Paralysis Research.*

543.9

Reflex Pathway Modulation in FDL and EDL Motoneurons Differs During Fictive Scratching Versus Locomotion in the Cat. A.M. Degtyarenko, E.S. Simon*, T. Norden-Krichmar and R.E. Burke. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892

Our laboratory has shown that disynaptic cutaneous EPSPs (central latency ≤ 2.0 ms) in flexor digitorum longus (FDL) motoneurons (MNs) by low-threshold (2xT) superficial peroneal (SP) afferents are powerfully enhanced during the early flexion phase of fictive locomotion (*Exp Br Res* 83:489, 1991). Last year, we reported that disynaptic IPSPs from SP are similarly enhanced in extensor digitorum longus (EDL) MNs during the flexion phase (*SN Abstr* #171.4, 1995). In contrast, disynaptic EPSPs produced by the medial plantar nerve (MPL) are suppressed during the flexion phase of locomotion in both FDL and EDL MNs. All three sets of disynaptic PSPs are due to distinct sets of last-order interneurons. We have found that modulation of these cutaneous reflex pathways is quite different during fictive scratching. The disynaptic components of SP and MPL PSPs are markedly suppressed throughout rhythmic fictive scratching in both FDL and EDL MNs. Trisynaptic (latencies > 2.0 ms) SP PSP components (EPSPs in FDL and IPSPs in EDL MNs) exhibit some modulation with scratching phase but are nevertheless smaller than during rest. However, fictive scratching does not suppress transmission in all disynaptic pathways; disynaptic group I EPSPs exhibit similar enhancement during the flexion phase of both fictive locomotion and scratching in both FDL and EDL MNs, during periods when the motoneurons are depolarized and active. These observations suggest that different spinal circuits are involved in the generation and transmission of rhythmic neural output during fictive locomotion versus scratching.

543.10

Patterns of Reflex Modulation are Markers for the Structure of the Central Pattern Generator for Locomotion in the Cat. R. E. Burke*, A. M. Degtyarenko and E. S. Simon. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892-4455

Our laboratory has demonstrated robust enhancement of disynaptic EPSPs in flexor digitorum longus (FDL) and of disynaptic IPSPs in extensor digitorum longus (EDL) motoneurons during the early flexion phase in well-developed fictive locomotion after stimulation of low threshold afferents in the superficial peroneal (SP) nerve (*Exp. Brain Res. 71*:568, 1988; *83*:489, 1991; SN Abstr #171.4, 1995). Such stereotyped associations between cyclic motoneuron activity and cutaneous disynaptic pathway modulation results from convergence of state-dependent output from the locomotor central pattern generator (CPG) onto specific sets of last-order interneurons. The patterns provide information about the structure of the CPG as well as about the organization of reflex pathway interneurons. For example, FDL motoneurons occasionally fire strongly during the extensor phase of fictive stepping, with or without the normal bursting during early flexion, and with no change in cyclic activity in other muscles. Disynaptic SP EPSPs are not enhanced during such extensor phase firing and exhibit degrees of flexor phase enhancement that vary directly with the level of remaining flexor phase FDL activity. This suggests that CPG output to the FDL and the SP pathway interneurons is tightly linked during early flexion and can be bypassed under some locomotor states. In addition, we have found that the same patterns can often be found during bouts of fictive stepping that are irregular or incompletely developed. These "natural experiments" provide examples that allow identification of specific subfractions of the flexion phase during fictive locomotion.

543.11

RHYTHMICAL EMG ACTIVITY RECORDED IN JAW OPENER AND CLOSER MUSCLES, USING JAW ATTACHED ISOLATED BRAINSTEM PREPARATION IN VITRO. S.TANAKA, M.KOGO*, T.MATSUYA and S. H. CHANDLER [1] The First Dept. of Oral & Maxillofacial Surgery, Osaka Univ., Faculty of Dentistry. [1] Dept.of Physiological Science, Univ. of California, Los Angeles. 90024.

Our previous study showed rhythmical trigeminal activity can be elicited in isolated brainstem preparation in vitro. We used 0 to 2-day-old neonatal Splague Dawley rats deeply anesthetized with halothane. The preparation and recording were performed in the recording chamber with modified Krebs solution,which were equilibrated with 95% O2 and 5% CO2. Bath application of NMA and bicuculline elicited rhythmical jaw movements. Rhythmical EMG activities were recognized in jaw opener (digastric) and jaw closer (masseter) muscles. Bilateral synchrony was recognized in each muscle. However, no synchrony was found between digastric and masseter activities. It is likely that two coupled rhythm generating circuits related to trigeminal activity exist in the brainstem: One for jaw closing (masseter) and the other for jaw opening (digastric).

Supported by a Grant from the Ministy of Education and Science of Japan (No.07457487)

543.12

GASP-LIKE ACTIVITY IN THE TRANSVERSE RHYTHMIC SLICE PREPARATION OF MICE. J.M. Ramirez*, P. Telgkamp, F.P. Elsen and D.W. Richter. Department of Physiology, University of Göttingen, 37073 Göttingen, FRG.

The transverse brainstem slice containing the pre-Bötzinger complex (pBC) has been used as an *in vitro* model for studying the central respiratory system of rats (Smith et al. 1991. Science 254: 726) and mice (Funk et al. 1994. J. Neurophys. 72: 2538; Ramirez et al. 1996. J. Phys. 429: 599). This preparation generates spontaneous rhythmic activity that can be recorded as output-activity from hypoglossal (XII) rootlets. It is assumed that this activity reflects the eupneic respiratory motor output which is in phase with inspiration. We performed selective lesioning experiments in medullary slice preparations obtained from 2 to 3 week old mice (P14-21). At this age anatomical landmarks, such as the nucleus ambiguus, are clearly visible under the dissecting microscope. Following removal of the pre-Bötzinger complex of one side, rhythmic activity was abolished in 5, but persisted in 4 preparations. In these preparations, the amplitude of XII rhythmic activity was decreased, but the shape of integrated activity was not significantly changed. Upon removal of the pre-Bötzinger complex of both sides, rhythmic XII activity ceased in all examined cases. In 5 out of 9 pre-Bötzinger complex lesioned preparations, bursts of hypoglossal activity could still be elicited in response to hypoxia or during recovery from hypoxia. However, these bursts were different from those generated during eupneic conditions: the onset of XII activity was much steeper and the amplitude of integrated XII activity exceeded that observed under eupneic conditions. We never observed such high-amplitude, gasp-like bursts in the presence of the pre-Bötzinger complex. Thus, we hypothesize that the respiratory center inhibits gasp activity under normoxic conditions. This hypothesis is supported by the finding that similar bursts were generated following blockade of GABAergic inhibition with 20μM bicucculline.

Supported by the DFG and SFB to JMR and DWR.

543.13

OPTIMIZING THE TORQUE-TIME CURVE IN HUMAN PARALYZED MUSCLE USING FORCE-FEEDBACK CONTROL R.K. Shields*, Y.J. Chang. Physical Therapy Graduate Program, University of Iowa, Iowa City, IA 52242.

Four stimulation strategies have been described when activating paralyzed muscle. According to the force-frequency curve, increasing the frequency of stimulation (HF) causes increased force on the linear segment of the curve (Shields and Chang, 1996). Reducing the frequency (LF) is effective when neuromuscular transmission is compromised (Jones, 1981). A variable frequency stimulation (VFT) enhances force by a catch-like property (Binder-Macleod, 1992; Chang and Shields, 1995) while a constant frequency (CFT) is the usual method for activating paralyzed muscle. At what point one strategy becomes more effective than the other during repetitive activation (fatigue) has not previously been assessed. We used real time force-feedback to trigger each strategy so that the optimal method to activate paralyzed muscle during fatigue could be determined. Six chronically paralyzed individuals (> 1 year) and two acutely paralyzed individuals (< 6 weeks) had the soleus muscle activated with a 10-pulse CFT (15 Hz) train using a fixed 1on, 2 off duty cycle for 120 consecutive contractions. When the force dropped 10% the stimulation frequency either increased 15% (HF), decreased 10% (LF), or a variable frequency train (VFT) (6 ms doublet followed by 15 Hz train) was applied. Only one strategy was studied during a given experiment. At least a 2% change was required before the new strategy was accepted. In the chronically paralyzed group, the HF strategy was 16% and 9% more effective than the CFT and VFT, respectively, during the first 40 contractions (16%) The VFT was 20% more effective than the HF strategy towards the end of the protocol. The LF strategy was rarely (2%) accepted. These findings indicate that the most effective strategy to attenuate fatigue of paralyzed muscle depends on the muscle's activation history. The mechanism each strategy uses to overcome fatigue will be discussed.

543.14

SPINAL INTERNEURONS INVOLVED IN THE ESCAPE BEHAVIOR IDENTIFIED BY IN VIVO CALCIUM IMAGING IN ZEBRAFISH. E. Forbell, N.F. Cambronero, S. Ross and J.R. Fetcho*. Dept. Neurobiology and Behavior. SUNY at Stony Brook, NY 11794.

Seven classes of interneurons in larval zebrafish spinal cord have been identified morphologically in a study by Bernhardt et al. (JCN 302:603). We have begun to study the functional roles of these interneurons in the transparent, larval fish by using calcium imaging techniques. We focused initially on the question of whether one class of spinal interneuron, the circumferential descending interneurons (CiD cells) were involved in escape behaviors. These neurons have an axon which extends ventrally from the soma to a region near the Mauthner axon and then runs caudally and dorsally in an axonal bundle with the axons of other CiD cells. The neurons are very similar morphologically to the descending interneurons (DIs) in goldfish which are monosynaptically excited by the Mauthner cell (which initiates the escape) and in turn electrotonically excite primary motoneurons, thus contributing to the rapid C-bend in the escape behavior. The morphological similarities between the CiDs in zebrafish and the DIs in goldfish led to the prediction that CiDs would also be activated during escapes. To examine this, we backfilled CiD cells by an injection of calcium green dextran into dorsal caudal spinal cord. We imaged the calcium increases in the somata of these cells during escapes elicited by a light, abrupt touch to the contralateral head or tail produced by a piezoelectric device. We found that the CiD cells responded during escapes as expected based upon their similarity to the well studied DIs in goldfish. These observations emphasize the morphological and functional similarities between the spinal organization in zebrafish and goldfish. These similarities will allow imaging studies of activity in populations of identified interneurons in zebrafish which will compliment the data obtained about their synaptic connectivity from intracellular studies in the much larger goldfish. Taken together, these data will provide a more complete view of the rules by which activity in neuronal populations controls behavior. Supported by NIH NS26539.

543.15

ESTIMATION OF COUPLING STRENGTH FOLLOWING REGENERATION OF THE LAMPREY SPINAL CORD. T. Kiemel and A. H. Cohen*. Department of Zoology, University of Maryland, College Park, MD 20742.

Data of fictive swimming from isolated lamprey spinal cords bathed in D-glutamate were analyzed under four conditions: (i) medial tracts lesioned 8–10 months previously with acutely lesioned lateral tracts ($n = 4$); (ii) lateral tracts lesioned 8–10 months previously with acutely lesioned medial tracts ($n = 5$); (iii) intact medial tracts with acutely lesioned lateral tracts ($n = 5$); and (iv) intact lateral tracts with acutely lesioned medial tracts ($n = 5$). All lesions were performed just rostral to the first dorsal fin. Data from regenerates was previously published.

Activity was recorded from one ventral root rostral and one ventral root caudal to the lesion site. Bursts were detected and a sequence of burst times for each root was calculated. Burst times were fitted by a model of two noisy phase oscillators using a maximum-likelihood approach. Parameters in the model include the strength of descending and ascending coupling across the lesion site, the amount that one oscillator slows down or speeds up the other oscillator as a function of the relative phase between the oscillators. In the case of previously-lesioned tracts, coupling strength provides a quantitative measure of the quality of regeneration.

In one animal with previously-lesioned medial tracts and one animal with previously-lesioned lateral tracts, coupling strength was not significantly different from zero, indicating no evidence of regeneration in these animals. All other previously-lesioned tracts had coupling strengths significantly different from zero. These results corresponded exactly with a statistical test for regeneration based on the crosscorrelation of spike trains. Coupling strengths of previously-lesioned tracts tended to be less than the coupling strength of intact tracts and the coupling strengths of medial tracts, whether previously-lesioned or intact, tended to be less than the coupling strength of lateral tracts.

Supported by NIH NS16803 to AHC.

543.16

EFFECTS OF ACETYLCHOLINE ON FICTIVE SWIMMING AND SPINAL NEURONS IN THE LAMPREY. J.T. Buchanan* & K.A. Quinlan. Department of Biology, Marquette University, P.O. Box 1881, Milwaukee, WI 53201-1881.

Motoneurons of the lamprey spinal cord are considered to be passive output elements of the locomotor system. However, it has been reported recently (Perrins & Roberts, *J. Neurophysiol.* 73: 1013, 1995) that in the *Xenopus* embryonic spinal cord, motoneurons synaptically interact with spinal interneurons and may participate in rhythmogenesis. As a first step toward assessing the possible involvement of motoneurons in rhythmogenesis in lamprey, we have examined the effects of acetylcholine on fictive swimming and on spinal neurons.

Fictive swimming was induced in the spinal cord/notochord preparation by superfusion with N-methyl-D,L-aspartate (0.2 mM), and ventral root activities were monitored with suction electrodes. Application of acetylcholine (0.01-1 mM) to the bath decreased the mean cycle period of fictive swimming and induced or enhanced slow modulation (period ≈20 sec) of the swim rhythm. Mecamylamine (0.02 mM), a nicotinic receptor antagonist, could block acetylcholine action, and could suppress slow modulatory rhythms present in the absence of added acetylcholine.

Acetylcholine (10 mM) was also applied locally to the cord surface during intracellular microelectrode recordings from motoneurons and proposed locomotor interneurons (lateral interneurons and CC interneurons). In the presence of tetrodotoxin, acetylcholine produced depolarizations and increases in membrane conductance of these cells. These actions were blocked by mecamylamine or *d*-tubocurarine. In normal Ringers, local application of acetylcholine elicited a barrage of ipsps in CC interneurons, indicating that the acetylcholine strongly excited inhibitory interneurons synapsing upon nearby CC interneurons.

These results suggest that motoneurons and interneurons of the lamprey spinal cord possess excitatory nicotinic acetylcholine receptors, and stimulation of these receptors during fictive swimming can alter the fictive swim cycle period.

543.17

SURVEY OF BIOPHYSICAL PROPERTIES OF DESCENDING BRAIN NEURONS IN LARVAL LAMPREY. D.T. Rouse, X. Quan, and A.D. McClellan*, Div. of Biological Sciences, Univ. of Missouri, Columbia, MO 65211

Reticulospinal (RS) neurons integrate synaptic inputs from many sources and contribute significantly to the descending control of locomotion. The biophysical properties of RS neurons are important determinants of how synaptic inputs will be transformed to a particular firing pattern. In the present study, intracellular recordings were made in in vitro brain/spinal cord preparations (n = 29) from larval lamprey (Petromyzon marinus) to determine the biophysical properties of identified and non-identified RS neurons (n = 131).

Spike frequency adaptation (SFA): SFA was observed in almost all of the descending neurons, including Muller cells.

Bursting: During constant depolarization, some descending neurons, particularly in the PRRN, fired a short burst, while at higher current intensity these neurons fired several short bursts.

Delayed excitation (DE): About 24% of the descending neurons showed a delay in firing (max. = 160-350 ms) in response to a depolarizing current pulse when preceded by a hyperpolarizing pulse. DE was observed in some of the neurons in the MRN, ARRN, and MRRN, and was seen reliably in Muller cells M1 and M2.

Post inhibitory rebound (PIR): About 10% of the descending neurons fired one or a few action potentials following a hyperpolarizing current pulse. PIR was observed in some neurons in the ARRN, MRRN, and PRRN.

In summary, RS neurons in the lamprey are heterogeneous in their biophysical properties which presumably reflects the type of integration needed by each cell. A determination of the ionic and pharmacological bases for these properties will provide insight into the mechanisms by which these cells integrate synaptic inputs and activate the spinal locomotor networks.

Supported by NIH Grant NS 29043 and APA Grant MB1-9108 awarded to A.D.M.

543.18

NORADRENALINE MODULATES RECIPROCAL GLYCINERGIC INHIBITION AND REDUCES SWIMMING FREQUENCY IN XENOPUS LAEVIS TADPOLES. J.R. McDearmid and K.T. Sillar*. Gatty Marine laboratory, School of Biological and Medical Sciences, University of St Andrews, Fife KY16 8LB, Scotland.

The biogenic amine noradrenaline (NA) has been shown to modulate locomotor rhythm generation in vertebrates, but little is known of its mechanism of action. We have examined the effects of NA on fictive swimming in α-bungarotoxin immobilized *Xenopus laevis* tadpoles. At 1-20μM, NA slows the swimming frequency by up to 50%. Reciprocal mid-cycle glycinergic inhibition is a fundamental component of the synaptic drive which influences cycle period in the central pattern generator of several vertebrate systems. We therefore studied the effects of NA on this component of fictive swimming by recording intracellularly from myotomal motoneurons in the spinal cord. We find that NA reversibly enhances the size and duration of glycinergic mid-cycle ipsps.

To investigate a possible presynaptic site of action for NA, tetrodotoxin (TTX) was applied to block Na^+-dependent action potentials. Under these circumstances, spontaneous, strychnine-sensitive ipsps are recorded which reflect the quantal release of glycine from the terminals of spinal glycinergic commissural interneurons. NA increased the rate of occurrence of the spontaneous ipsps without affecting the distribution of their amplitudes. These data provide evidence for a direct facilitatory action of NA on the glycine release mechanism.

In conclusion these results show that NA is capable of reducing the frequency of swimming in *Xenopus* tadpoles. We propose that this effect is mediated, at least in part, through the presynaptic facilitation of glycine release from commissural interneurons. This enhances the mid-cycle inhibitory component of the swimming cycle and thereby delays the onset of the next cycle.

Supported by the BBSRC and the Royal Society of London.

543.19

ANTAGONISTS UNMASK COMPONENTS OF EXCITATORY SYNAPTIC DRIVE TO MOTORNEURONS DURING SWIMMING IN *XENOPUS* TADPOLES. F.Y. Zhao and Alan Roberts*, School of Biological sciences, University of Bristol, Bristol BS8 1UG, UK.

To investigate the contributions of chemical and electrical synaptic input during swimming locomotion in hatchling *Xenopus* tadpoles, we made intracellular recordings from motorneurons during fictive swimming in immobilised animals. We then used a microperfusion system to apply antagonists locally to the recorded neuron to block specific synaptic components (dihydro-β-erythroidine (DHβE) for nicotinic ACh receptors (nAChR); kynurenate (Kyn) for glutamate (GluR); DHβE plus Kyn for nAChR and GluR; Cd^{++} for all chemical synapses). Tonic depolarization has no electrical component and is mainly glutamate dependent. ACh and an unidentified component play minor roles. Glutamate and ACh provide most of the fast EPSP underlying spikes but there is also a clear electrical component. We have proposed that the relative contributions of glutamate, ACh and electrical synapses will change as a function of longitudinal position and the frequency of fictive swimming. These proposals have been tested by measuring the effects of antagonists in motorneurons at different longitudinal positions during swimming at different frequencies.

Supported by the Wellcome Trust.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CIRCUITRY AND PATTERN GENERATION III

544.1

SENSORY NEURON ACTIVATION OF MODULATORY PROJECTION NEURONS. DM Blitz* & MP Nusbaum. University of Pennsylvania School of Medicine, Department of Neuroscience, Philadelphia, PA 19104.

The neural network located in the stomatogastric ganglion (STG) of the crab *Cancer borealis* produces the pyloric and gastric mill rhythms. STG network activity is modulated by projection neurons whose somata are located in the other ganglia (CoGs & OG) of the stomatogastric nervous system. Several of these projection neurons have been identified and their effects on the STG network characterized. However, little is known about the behaviorally-relevant pathways whereby these neurons are activated. We found recently that a pair of previously identified stretch receptors, the gastro-pyloric receptor cells (GPR1&2: Katz et al, J Neurophysiol 62:558, 1989) activate a subset of the projection neurons that influence the STG network. Specifically, GPR activates modulatory commissural neuron 1 (MCN1: Coleman & Nusbaum, J Neurosci 14:6544, 1994) and commissural projection neuron 2 (CPN2: Norris et al, J Neurophysiol 72:1451, 1994). Either tonic or rhythmic stimulation of GPR co-activates MCN1 & CPN2, resulting in the activation of a distinct gastric mill rhythm from the STG network. The effects of GPR can outlast its stimulation for up to several minutes.

Interestingly, while low frequency stimulation of GPR (5Hz) excites MCN1 & CPN2, higher frequency stimulation (20-30Hz) has mixed excitatory & inhibitory effects on these neurons. GPR contains ACh, 5-HT (Katz et al, J Neurophysiol 62:558, 1989) and an allatostatin-like peptide (Skiebe & Schneider, J Exp Biol 194:195, 1994). We are working to determine the relative contributions of each GPR transmitter to the strong, long-lasting influence of GPR on MCN1 & CPN2.

Supported by IBN9309804, NS29436 & HFSP (MPN).

544.2

SYNAPTOTAGMIN-LIKE IMMUNOREACTIVITY IN THE STOMATOGASTRIC NERVOUS SYSTEM OF THE CRAB, *CANCER PRODUCTUS*: A MARKER FOR SYNAPTIC NEUROPIL. J. M. Parrish, D. Goerlitz, K. Graubard*. University of Washington, Seattle, WA. 98195.

Synaptotagmin is an abundant integral membrane protein found in synaptic vesicles, making it a good antigen for a synapse-specific antibody. The *Drosophila* synaptotagmin gene has been cloned and an antiserum (DSYT2) made to the highly conserved cytoplasmic domain (Littleton et al., *Development* **118**: 1077-1088, 1993). Using confocal microscopy, we have found that the DSYT2 antibody (gift of Dr. Hugo Bellen) reveals synaptotagmin-like immunoreactivity in the stomatogastric nervous system (STNS) of the marine crab, *Cancer productus*.

The synaptotagmin-like staining on crab PD muscle fibers and in the STNS corresponds to known synaptic regions. Terminal varicosities on PD muscles stain similarly to those on crayfish muscle reported by Cooper et al. (*Brain Res.* **703**: 214-216, 1995). In the STNS, synaptotagmin-like labelling is extensive and punctate. In the stomatogastric ganglion (STG) the label is intense and densely packed in the peripheral neuropil, while the core is almost devoid of staining. Neuropilar staining extends beyond the STG itself into the adjacent *stn* and *dvn* nerves. Patches of neuropil in the *sons* and at the *stn-son* junction also label. Some fibers in the *stn* and *dvn* stain near the STG and decrease in number as distance from the ganglion increases.

In preparations double-labeled for synaptotagmin and for the neuromodulator cholecystokinin (CCK_{C36}), preliminary results show that anti-CCK_{C36} labeled profiles coincide with a subset of the total synaptotagmin-like labelling in the peripheral STG neuropil. Other preparations containing Lucifer Yellow-filled neurons were labeled with anti-synaptotagmin. Primary and secondary neurites occasionally traverse labeled regions. Fine neurites, however, nearly always lie in regions of synaptotagmin-like staining, often at the very edge of the neuropil border. Fine neurites that trail into the *dvn* and *stn* also lie in anti-synaptotagmin labeled regions. Supported by NIH grant NS15697 (to KG).

544.3

TWO NOVEL TACHYKININ-RELATED PEPTIDES FROM THE CRAB NERVOUS SYSTEM. AE Christie[1]*, CT Lundquist[2], DR Nässel[2] & MP Nusbaum[1]. [1]Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA 19104. [2]Department of Zoology, Stockholm University, S-10691 Stockholm, Sweden.

Previous studies have shown that tachykinin-related peptides (TRPs) are likely to be present in the stomatogastric nervous system (STNS) of the crab *Cancer borealis* (Goldberg *et al.*, Cell Tiss Res, 252:515, 1988; Blitz *et al.*, J Comp Neurol, 354: 282, 1995). We recently isolated two novel TRPs from the crab CNS that show strong amino acid sequence similarity to several insect TRPs, including locustatachykinin (LomTK), a peptide that modulates neural network activity in the crab stomatogastric ganglion (STG). The CNS from 160 *C. borealis* were used for extraction via an acidified solvent. Four reverse-phase HPLC column systems were used to obtain pure peptides. A cockroach hindgut muscle contraction bioassay and a radioimmunoassay utilizing LomTK I antiserum were used to monitor all collected fractions, as was done previously for other TRPs (Muren & Nässel, in press, NY Acad Sci USA). We isolated two novel peptides and had their amino acid sequences determined by Edman degradation. The sequences were confirmed by mass spectroscopy and chemical synthesis to be APSGFLGMR-NH_2 and SGFLGMR-NH_2. We named these peptides *Cancer borealis* tachykinin-related peptide Ia and Ib (CabTRP Ia and Ib), respectively.

Superfusion of synthetic CabTRP Ia (10^{-6} M) to the isolated *C. borealis* STG excited both the pyloric and the gastric mill systems. CabTRP Ia increased the frequency and intensity of ongoing pyloric motor patterns. It also excited most gastric mill neurons. A full gastric mill rhythm was not elicited, but some gastric mill rhythm-like activity was evident. We are continuing to examine the actions of CabTRP Ia on the STG motor patterns and to determine the tissue distribution of this peptide in the crab STNS. Supported by F32-NS09718 (AEC), IBN-9309804 & HFSP (MPN) and the Swedish Natural Science Research Council (DRN).

544.4

TWO PROCTOLIN/GABA NEURONS ELICIT DISTINCT MOTOR PATTERNS FROM THE SAME NEURAL NETWORK. M Bartos* & MP Nusbaum. Univ of Pennsylvania, Sch of Med, Dept of Neurosci, Philadelphia, PA, 19104.

The rhythmically active neural network in the crustacean stomatogastric ganglion (STG) generates the gastric mill and the pyloric rhythms. In the crab, *Cancer borealis,* modulatory neurons project from other ganglia (COGs, OG) to innervate the STG and elicit distinct versions of the gastric mill and the pyloric rhythm. Two of these modulatory neurons, MPN and MCN1, contain both GABA and the neuropeptide proctolin. Previously, Nusbaum & Marder (J Neurosci 9:1591, 1989) showed that MPN activation and proctolin superfusion elicited the same pyloric rhythm. MCN1 also elicits a pyloric rhythm (Nusbaum et al, J Neurosci 12:2706, 1992), but we have found that this rhythm is different from that produced by MPN stimulation or proctolin application.

Like MPN stimulation and proctolin application, MCN1 stimulation enhances the pyloric cycle frequency of slow rhythms (<1 Hz) but does not alter the frequency of fast rhythms. The activity levels and phase relationships of the pyloric motoneurons, however, are different during MCN1 stimulation than during times of MPN activity or proctolin application. For example, MCN1 elicits a stronger activation of the PD and VD neurons and a weaker activation of IC. These differences appear to result at least partly from the fact that MCN1 contains a second neuropeptide, CabTRP-Ia (Christie et al, this volume), that is not found in MPN. There is also electrical coupling between MCN1 and several pyloric neurons. We are extending these studies to determine whether the presence of CabTRP-Ia, and the electrical coupling, is sufficient to account for the different pyloric rhythms elicited by MCN1, MPN and proctolin. Supported by NS-29436, IBN-9309804 and HFSP (MPN).

544.5

AMINE EFFECTS ON GLUTAMATE RESPONSES IN THE PYLORIC MOTOR NETWORK. B. R. Johnson* and R.M. Harris-Warrick. Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Dopamine (DA), serotonin (5HT) and octopamine (Oct) each have a unique, distributed spectrum of effects on graded synaptic strength in the pyloric network of the stomatogastric ganglion from the spiny lobster, *Panulirus interruptus*. This modifies the motor pattern generated by the pyloric network. We examined amine effects on the inhibitory responses of pyloric neurons to iontophoresis of glutamate (Glu), one of two pyloric transmitters, to determine the synaptic sites of amine modulatory action. DA and Oct strongly enhanced the Glu response in the PY and LP neurons, DA strongly reduced the Glu response in the VD and PD neurons, and 5HT reduced the Glu response in VD. The amines had only weak effects on the Glu responses of the other pyloric neurons. DA enhanced the Glu response of LP and PY even after pharmacological block of I_A and I_h, currents that DA reduces and enhances, respectively, and which account for some of DA's effects on neuronal activity. These results suggest sites of amine action. For example, despite DA inhibition of PD and VD responses to Glu, DA enhances synaptic Glu input to these cells, suggesting a predominant action of DA to enhance presynaptic release onto these cells. Both synaptic and iontophoretic Glu input to LP and PY is enhanced by DA and Oct, suggesting a post-synaptic mechanism on these cells. Supported by NIH grant NS 17323 and the Human Frontier Science Program.

544.6

FOOD EXTRACTS STIMULATE THE FEEDING MOTOR PROGRAM IN HELISOMA, IN PART BY ACTIVATING DOPAMINERGIG INTERNEURON N1A. A.D. Murphy*, E.M. Quinlan and B.C. Arnett. Dept. Bio. Sci., Univ. Illinois at Chicago, Chicago, IL 60607.

Watermelon extract potently stimulates feeding when applied to intact snails and also when perfused into the oral cavity of semi-intact preparations. Feeding is mediated by the triphasic feeding motor pattern (FMP). Dopamine (DA) is the most potent and reliable neuromodulator that is known to activate FMP and feeding behavior. Electrical stimulation of interneuron N1a also can evoke FMP. Double staining of physiogically characterized neurons N1a, by injection of Reactive Red #4 and use of the FaGlu histochemical stain for DA, indicated that interneuron N1a contains DA. Watermelon extract activated neuron N1a, FMP, and feeding behaviour in semi-intact preparations. These dopaminergic effects are partially antagonized by Sulpiride and by Haloperidol, DA antagonists that previously have been shown to be effective for some molluscan DA receptors.
Supported by NSF grant BNS 91-21374.

544.7

GLUTAMATE'S ROLE IN THE EXPRESSION OF LEECH SWIMMING: GATING CELL ACTIVATION OF THE OSCILLATOR. M. S. Thorogood*, S. L. Melson and P. D. Brodfuehrer. Department of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

Previous studies have demonstrated a role for L-glutamate in initiation of the swim motor program of *Hirudo medicinalis*. First, focal application of glutamate or one of its agonists excites the swim-gating interneuron, cell 204. Second, 6,7-dinitroquinoxaline-2,3-dione (DNQX) blocks input from swim-trigger interneuron, cell Tr1, to cell 204. Third, the direct connection from mechanosensory cells to cell Tr1 is similarly antagonized by DNQX. Here we ask if glutamate is required for the expression of fictive swimming. Initially, we monitored the effect of non-NMDA antagonists on DP nerve-induced swim episodes. In the presence of 10^{-4} M DNQX, the ability of DP nerve stimulation to evoke swimming disappeared. Cell 204 also lost its ability to initiate swimming following DNQX exposure. We then examined glutamate's involvement at the level of the swim oscillator, which includes cells 208, 115 and 28. In the absence of disynaptic connections, focal application of 10^{-3} M kainic acid, a glutamate agonist, resulted in excitation of cell 208. In addition, exposure of the nerve cord to 10^{-4} M DNQX or 10^{-5} M 6-cyano-7-nitroquinoxaline-2,3-dione resulted in elimination of the monosynaptic connection from cell 204 to each of the three swim oscillator interneurons. The direct signal from cell 208 to the dorsal exciter motoneuron cell 3, however, persists in the presence of 10^{-4} M DNQX, suggesting that this synapse is not glutamatergic. Thus, glutamate is involved at multiple levels of the leech swim motor program: in initiation of swimming via the cell Tr1 swim-initiating pathway and in expression of swimming via cell 204's output to the swim oscillator. (NSF Grant IBN-95-14617 and a Whitehall Foundation Grant).

544.8

EFFECTS OF METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION ON FICTIVE LOCOMOTION IN THE LAMPREY P. Krieger*, A. El Manira and S. Grillner. Nobel Institute for Neurophysiology, Dept. of Neuroscience, Karolinska Institutet 171 77 Stockholm, Sweden.

Activation of group I metabotropic glutamate receptors (mGluRs) with the specific agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) increases the frequency of the locomotor rhythm and causes a depolarization of neurons in the spinal cord. The effect on locomotor-driven EPSPs were studied in split-bath experiments where only the rostral part of the spinal cord was activated by NMDA, while DHPG was applied to the caudal part. DHPG depolarizes neurons in the caudal part, thus increasing the excitability of the neurons for the same given locomotor drive. These effects are all blocked by the group I mGluR antagonists (+)-MCPG and (RS)-1-aminoindan-1,5-dicarboxylic acid. The effects of DHPG are similar to those seen with the mGluR agonist 1S,3R-ACPD. The group III agonist L-AP4 causes a small reduction of the locomotor frequency. L-AP4 reduces the amplitude of IPSPs evoked by stimulating crossing axons and in some cases L-AP4 also reduces the amplitude of locomotor-driven EPSPs. In combination with our previous results, this suggests that activation of group I mGluRs have an excitatory effect on network neurons and that activation of presynaptic group II and III mGluRs causes a decrease in transmitter release.
Supported by the Swedish Medical Research Council, project no. 11562

544.9

MODULATORY ACTION OF INHIBITORY NEUROTRANSMITTERS ON THE MUDPUPPY (*Necturus maculatus*) LOCOMOTOR PATTERN. K. Jovanović, T. Petrov, J.J. Greer* and R.B. Stein. Division of Neuroscience, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

Inhibitory amino acids such as glycine and GABA play an important role in generating and/or coordinating rhythmic motor patterns in a wide variety of species. To further investigate their role in locomotion of walking amphibians, we used an *in vitro* preparation isolated from the mudpuppy. The preparation consisted of the first five segments of the cervical spinal cord and the right limb attached by the brachial plexus. During NMDA-induced locomotion rhythmic motor output (EMG) was recorded unilaterally from the forelimb flexor and extensor muscles. After a well-defined walking pattern was established, the effects of neuroactive substances such as glycine, strychnine, and variety of $GABA_A$ and $GABA_B$ receptor agonists and antagonists were observed. Use of nipecotic acid, a GABA uptake blocker, indicated that an endogenous release of GABA occurs during NMDA-induced locomotion. The results of this study suggest that GABAergic and glycinergic systems are active during NMDA-induced locomotion in the mudpuppy. This activity appears not to be essential for the locomotor rhythmogenesis but may play a role in mediating reciprocal antagonism that coordinates the locomotor pattern. Glycine and $GABA_A$ receptor activation affects rate control, flexor-extensor antagonism and regular EMG burst pattern whereas $GABA_B$ receptor activity primarily affects the frequency of the locomotor rhythm. The preliminary results obtained by using immunohystochemical techniques indicate that glycine and GABA-immunoreactive cell bodies ranging from 15-20 μm in diameter are located within the central grey matter 200-400 μm below the dorsal surface of the spinal cord. Further investigation should provide more insight to the connections of these cells with the rest of the neuronal network underlying locomotion in the mudpuppy. Supported by MRC and NCE.

544.10

DRUG EFFECTS ON MOTION SICKNESS SYMPTOMS IN CAT, J.B. Lucot*. Dept. Pharmacology, Wright State Univ., Dayton, OH 45435.

The motion sickness rating scale of Suri, et al (Aviat. Space Environ. Med, 50:614,1979) uses observable signs which correlate with vomiting. While not nausea, both they and nausea reflect activity in the neural circuitry underlying vomiting that is different from the motor act of vomiting.

With no effect on vomiting, combined, but not separate, block of alpha and beta adenoceptors increased defecation, while amphetamine increased urination and salivation. Peripheral muscarinic block decreased salivation and defecation while increasing urination and salivation. Peripheral muscarinic block decreased salivation and defecation while increasing urination. Scopolamine additionally decreased licking and increased panting. Drugs that decrease vomiting and appear to decrease symptoms by an effect on the neural path for vomiting include scopolamine, 5-HT1A agonists, a histamine synthesis inhibitor and NMDA channel blockers. Blocking NK-1 receptors has no effect on symptoms even at doses abolishing vomiting.

The most common symptom (licking) seems a motor act associated with vomiting. Other common symptoms reflect parasympathetic activity. Pharmacological studies of motion sickness must consider direct drug effects on autonomic function to avoid false negative and false positive results.

NASA/Lilly/Pfizer

544.11

HEPES BUFFER SUPPRESSION OF SPONTANEOUS NEURONAL NETWORK ACTIVITY. S. R. Laufer*, G. W. Gross and D. C. Tam. Center for Network Neuroscience, Dept. of Biological Sciences, Univ. of North Texas, Denton, Tx 76203.

Neurons and glial cells both express multiple pH regulatory mechanisms that require bicarbonate or CO_2, or both, to regulate intracellular pH. HEPES-buffered media is commonly employed in place of standard HCO_3-CO_2 buffer to maintain extracellular pH but does not provide the intracellular buffering power of HCO_3. We compared the spontaneous activity of cultured spinal cord networks under their normal bicarbonate-CO_2-buffered minimal essential medium (NMEM) at pH=7.35 with activity under HEPES (20mM)-buffered medium (HMEM). Murine spinal cord neurons were cultured on an array of 64 photoetched microelectrodes. Replacing the NMEM with HMEM elicited immediate changes in the activity particularly an overall reduction in the amplitude and the number of integrated bursts. This becomes more profound with the duration of exposure to the HMEM. The suppressive effects on activity were not immediately reversed by returning the culture to NMEM. After recovery of activity a second 20 minute exposure to HMEM produced even greater inhibition than the first. The inhibitory effects continued to increase after removal of the HMEM. A 20 minute HMEM exposure typically blocked recovery of the initial spontaneous network activity for hours. Burst data analysis revealed wide alterations in all six integrated burst parameters measured: rate, amplitude, duration, period, interval, and mean burst area. The results suggest that HEPES buffered media, even for a relatively brief time, can produce marked inhibition of spontaneous neuronal activity accompanied by disruption of normal network activity patterns. (Supported by ONR N00014-94-1-0686)

544.12

NITRIC OXIDE: DISTRIBUTION AND EFFECTS ON SPONTANEOUS RHYTHMIC ACTIVITY IN EMBRYONIC CHICK SPINAL CORD. P.A. Carr*, P. Wenner and M.J. O'Donovan. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892.

The complete complement of neurochemicals responsible for mediating the spontaneous episodic activity observed in isolated embryonic chick spinal cord remains to be determined. Results in cat suggest that nitric oxide (N.O.) may be utilized by a subpopulation of locomotor-related spinal neurons (Carr et al. Brain Res. Bull. 37:213-218, 1995). Here, we have examined the possible role of N.O. in chick spinal rhythmic activity. NADPH-diaphorase histochemistry was used to examine the presence and distribution of the N.O. synthesizing enzyme, N.O. synthase, in the developing chick spinal cord. The distribution of NADPH-diaphorase histochemical reaction product was examined in transverse sections of perfusion fixed spinal cord from embryos between embryonic day 4 (E4) and 18 (E18) of incubation. Weak to moderate labeling of blood vessels was observed at most ages. Putative neuronal labeling was first observed in lamina IV (large, clustered cells), the lateral funiculus and in the vicinity of the central canal (small cells) in E9-E14 animals. In older animals (E15-E18), large labeled cells could be also be observed in Hoffman's nucleus and the ventral horn in addition to small cells distributed throughout the dorsal horn. A rostrocaudal survey of the spinal cord of E10 animals revealed labeled cells (lamina IV, central canal region and dorsal lateral funiculus) confined to sections spanning mid-thoracic to mid-lumbosacral segments. To investigate the effect of N.O. on spontaneous rhythmic activity, an N.O. synthase inhibitor was bath applied to the E10 chick embryo spinal cord preparation. Results suggest that inhibition of N.O. synthase may decrease the frequency of spontaneously occurring episodes of electrical activity in the chick spinal cord. Supported by the NIH and MRC (Canada).

544.13

EVOKED SYNAPTIC POTENTIALS ARE TRANSIENTLY DEPRESSED FOLLOWING RHYTHMIC ACTIVITY IN THE EMBRYONIC CHICK SPINAL CORD. B. Fedirchuk* and M.J. O'Donovan. Laboratory of Neural Control, NINDS, NIH, Bethesda MD 20892.

Rhythmic activity in the spinal cord of the embryonic chick is episodic and neural excitability is decreased following a bout of rhythmic activity. One mechanism that could contribute to this decreased excitability is a reduced efficacy of transmission through synaptic pathways. In order to assess this possibility, experiments were conducted on isolated E10 to E11 embryonic chick spinal cords. Electrical stimulation of the ventrolateral tract (VLT) or a dorsal root (DR) at the lumbosacral levels was used to evoke postsynaptic potentials in lumbosacral motoneurons and interneurons which were recorded as compound potentials in the ventral roots (VR) and VLT respectively. These potentials were examined before and after rhythmic episodes.

The amplitudes of VLT and DR-evoked potentials recorded from both the VR and VLT were decreased after either spontaneously occurring or electrically evoked rhythmic activity. This reduction of the evoked potentials persisted for minutes after a rhythmic episode indicating that there was a prolonged reduction in the efficacy of these synaptic pathways. The amplitudes of both the early and late components of the VR and VLT potentials could be reduced, and the reduction of the later portion resulted in a marked decrease in the duration of the potential. In some examples, only the late component was affected indicating that interposed interneurons may make polysynaptic pathways more susceptible to this modulation. Repetitive antidromic activation of motoneurons in the absence of rhythmic activity was not effective in altering the VLT-evoked VR potential suggesting that the modulation is produced by; 1) postsynaptic effects initiated by rhythmic activity but not antidromic stimulation, and/or 2) presynaptic effects. Experiments investigating these possibilities are continuing. Supported by the NIH and the MRC of Canada.

544.14

SPONTANEOUS RHYTHMIC ACTIVITY IN SPINAL CORD PREPARATIONS FOLLOWING SURGICAL ABLATION OF MOTONEURONS. P. Wenner* and M.J. O'Donovan. Lab. Neural Control, NINDS, NIH, Bethesda, MD 20892-4455.

In the isolated chick embryo spinal cord preparation rhythmic activity consisting of multiple cycles of depolarizing potentials occurs spontaneously in motoneurons and many interneurons. Previous experiments designed to specifically abolish discharge in motoneurons demonstrated that spinal cord pieces devoid of motoneuronal activity were still competent to generate a single (non-regenerative) spontaneously occurring depolarizing potential. Recently, similar results were observed in preparations of the dorsal half of the spinal cord, which did not contain motoneurons. While these experiments suggest that motoneuron activity was not necessary for spontaneous activity to occur, they did not address a possible motoneuronal role in shaping the pattern of activity (i.e. regenerative activity consisting of multiple cycles). To do this experiments were performed in which the motor column was surgically removed. Spinal cord preparations composed of 2 lumbosacral segments (LS1-2 or LS3-4) were produced in which the motor column on one side was surgically ablated using a fine tungsten needle. These preparations were then hemisected and extracellular recordings were obtained using suction electrodes placed on the ventral white matter. Both the control and lesioned hemicords produced spontaneously-occurring episodic activity consisting of multiple cycles. This demonstrates that the motor nucleus is not necessary for regenerative activity. The number of cycles in a given episode was reduced in the lesioned hemicord compared to that of the control hemicord. Motoneurons may therefore play a role in the number of cycles generated per episode, but it is clear from histological examination of the tissue that even in the cleanest motoneuron lesions, some interneurons were destroyed. This interneuronal damage could also account for the observed differences between control and lesioned hemicords.

544.15

ELECTROMYOGRAPHIC ANALYSIS OF SPONTANEOUS MOVEMENTS IN THE EMBRYONIC ZEBRAFISH (*DANIO RERIO*). Uri Cohen, Anne Donevan*, and Michael J. O'Donovan. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892.

The zebrafish is widely recognized as an excellent model system for studying vertebrate neuronal development but little attention has been devoted to the emergence and function of spontaneous neural activity within the spinal cord and brain. We have begun to examine this question by recording the spontaneously occurring electrical activity from muscles in the body wall of zebrafish embryos. Electrical activity was recorded from the muscle using a suction electrode applied to the skin. Vigorous electrical signals were recorded, clustered into bursts that coincided with movements of the animal. The electrical activity was reversibly depressed following bath application of curare (10-100μM) indicating its origin from muscle. Our initial recordings have been from 1-2 day old embryos and we have found great variability in the burst frequency between embryos. This variability may be due to inter-embryo variation, developmental changes in the episode frequency, and/or sensitivity of the activity to environmental factors. Typically bursts were brief (< 1 sec) but occasionally much longer discharges were observed that were organized sometimes into multiple cycles. In some experiments bursts were organized into couplets.

These data indicate that it is feasible to quantify the early activity of the developing zebrafish using electromyography. Further experiments are in progress to establish developmental changes in the activity and the role of the spinal cord in its genesis. *Supported by NIH and the Howard Hughes medical scholars program.*

544.16

HOMEOSTATIC REGULATION OF RHYTHMIC NEURAL OUTPUT IN THE DEVELOPING CHICK SPINAL CORD. Nikolai Chub and Michael J. O'Donovan*. Lab. Of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Spontaneous rhythmic activity in the isolated cord of the chick embryo is generated by a network comprised of several classes of neuron including glutamatergic, GABAergic and possibly cholinergic types. Following bath application of *either* excitatory blockers (AP-5 100μM, CNQX 20μM, DHβE 100μM) *or* inhibitory blockers (bicuculline 50μM, Strychnine 20μM) spontaneous activity was blocked for a period of time (1-1.5hr) but then recovered in the presence of the antagonists. Combined application of the antagonists was not associated with recovery over a 4 hour monitoring period. The efficacy of the antagonists was assessed during recovery by their ability to block glutamatergic or inhibitory reflex pathways. Once recovered, the rhythmicity was remarkably similar to control activity, although it occurred more slowly. Following excitatory blockade the alternating pattern of flexor and extensor discharge was preserved. These findings suggested that spontaneous rhythmic activity in the chick cord can be supported by predominantly inhibitory *or* excitatory networks. This idea was confirmed by changes in the equilibrium potential (EP) of the synaptic drive measured under voltage clamp in ventrally located neurons. Following excitatory blockade the EP of the synaptic drive became ~10mV more negative with respect to control and approached the GABA EP. In the presence of inhibitory antagonists the EP of the synaptic currents became ~25mV more positive. These findings suggest that the rhythmic output of the spinal cord is resistant to major pharmacological perturbations and exhibits a form of short term plasticity that may reflect homeostatic regulation of the network output. *Funded by NIH.*

544.17

Mg^{++} REVERSIBLY BLOCKS SPONTANEOUS LOCOMOTOR ACTIVITY PRODUCED BY LARVAL BULLFROG CENTRAL NERVOUS SYSTEM *IN VITRO*. Shehzad H. Choudry and G.R. Davis, Jr.* Department of Biology, Wofford College, Spartanburg, SC 29303

Previous studies have shown that the isolated central nervous system (CNS) of larval bullfrogs (Rana catesbeiana) produces spontaneous episodes of locomotor activity. However, these results were obtained from preparations maintained in Ringer's solution that did not include Mg^{++}, a natural component of extracellular fluid. Does spontaneous locomotor activity depend upon the omission of Mg^{++} from the bathing solution?

Larvae (Taylor & Kollros Stage IV to XI) were anesthetized with Tricaine and the CNS was removed to a chamber containing Mg^{++}-free Ringer's solution maintained at 4-14°C. Suction electrodes were applied bilaterally to ventral roots which innervate axial swimming muscles. Seventeen of 19 preparations produced episodes of spontaneous locomotor activity. Nine of these spontaneously active preparations were subsequently placed in Ringer's that included 1.6 mM Mg^{++}. Spontaneous locomotor activity was immediately abolished in six of nine preparations, while in two preparations the activity was eliminated within seven minutes. One preparation produced a single episode after 20 min. in Mg^{++}-Ringer's. All nine preparations resumed spontaneous locomotor activity 15-90 min. after being returned to Mg^{++}-free Ringer's. These results suggest that Mg^{++} reduces the excitability of the isolated CNS, perhaps by 1) attenuating synaptic transmission by competing with Ca^{++} and/or 2) via a mechanism involving NMDA receptors. (Support: Howard Hughes Medical Institute grant to Wofford College)

544.18

SULPHORHODAMINE-LABELLED CELLS IN BRAINSTEM OF THE IN VITRO NEONATAL RAT FOLLOWING ELECTRICALLY-EVOKED LOCOMOTION. D.M.Fyda, S.Hochman, & L.M.Jordan*. Dept. of Physiol., Univ. Manitoba, Winnipeg, Manitoba R3E 0W3.

Electrical stimulation of the medioventral medulla (MED) evokes locomotion in the in-vitro neonatal rat brainstem-spinal cord preparation (Atsuta et al., *J. Neurophysiol.*, 64:727, 1990) at a time when most supraspinal inputs to at least the thoracic cord are intact (by E20.5). Electrical stimulation (150uA, 0.5ms, 10Hz) of the MED evoked locomotion which was sustained for 1-1.5 hr in the 1-3 day old neonatal rat brainstem-spinal cord preparation (post-collicular decerebration) with hindlimbs attached. During locomotion the activity-dependent label sulphorhodamine (0.0005%) was present in the bath and enabled us to assess the brainstem distribution of neurons activated during or following locomotion. Brainstem labelling occurred ventrolaterally from the trapezoid bodies to the inferior olive and extended across the pontine transverse fibers. Ventral labelling was more prominent ipsilateral to the stimulation site although the vigor and rhythmicity of locomotion appeared more prominent in the hindlimb contralateral to the stimulation site. Dorsally, the lateral reticular formation and lateral lemniscus, the rubrospinal tract and pontine trigeminal nucleus were also labelled. Labelling appeared to be specific to the locomotion-evoking site since similar stimulation in ventromedial brainstem sites which failed to produce locomotion evoked labelling only in the stimulation site itself with only sporadic labelling occurring throughout the brainstem. Preparations which remained undisturbed in the presence of the sulphorhodamine showed virtually no brainstem labelling. These results provide a first attempt at localizing the brainstem areas activated during electrically-evoked locomotion in the in-vitro neonatal rat brainstem-spinal cord preparation and suggest possible pathways which may be involved in the initiation or maintenance of locomotion in the neonatal rat. Supported by the Medical Research Council of Canada.

544.19

LOCOMOTOR-LABELLED NEURONS ARE DIFFUSELY DISTRIBUTED IN THE NEONATAL RAT LUMBAR SPINAL CORD. C. Cima*, D. Fyda, L. Song, M. Sawchuk, L.M. Jordan & S. Hochman.

Our principal objective is to identify and characterize the spinal neurons which encode the locomotor rhythm. Sulforhodamine is a fluorescent dye thought to be taken up endocytotically in synaptically-active neurons. We used bath incubation of sulforhodamine (0.0001 - 0.0005%) in the isolated intact neonatal rat spinal cord (P0-P4) to identify synaptically-active neurons during evoked locomotor activity. Previously, Kjærulff et al (*J. Physiol.* 478:265, 1994) used sulforhodamine to label neurons active during locomotor activity induced by serotonin and NMDA (5.0 - 7.5 μM) in elevated $[K^+]_o$ (7 mM). However, we have observed that identical concentrations of NMDA and $[K^+]_o$ label a significant fraction of spinal neurons in intermediate laminae even in the absence of locomotion. Hence we have used brainstem electrical stimulation (medio-ventral medulla: 150 μA, 0.5 ms, 10 Hz) or bath application of serotonin alone (10-20 μM) to evoke locomotion in order to identify activity-labeled neurons more directly related to the observed behavior.

Serotonin-induced locomotor activity was monitored by recording from L2 and L5 ventral roots which reflect activity in flexors and extensors respectively (Cazalets et al *J. Neurosci.* 15:4943, 1995). Locomotor frequency remained relatively constant (typically at 0.1 Hz) for the duration of sulforhodamine incubation (2.5 - 4 hours). Locomotor activity was associated with a diffuse distribution of spinal neuronal labelling dominated by cells in intermediate laminae. Control values of labeling were observed when bath applied serotonin did not evoke locomotor activity. Similar labelling patterns were observed with brainstem electrical stimulation-evoked locomotor activity. In comparison to Kjærulff et al (1994), a much reduced number of activity-labeled neurons were observed.

Future studies will involve histochemical and electrophysiological characterization of locomotor-labelled neurons. *Supported by the Manitoba Health Research Council.*

544.20

The number of postsynaptic currents necessary to produce locomotor-related cyclic information in neurons in the neonatal rat spinal cord. Morten Raastad*, Bruce R. Johnson and Ole Kiehn. Division of Neurophysiology, University of Copenhagen, Denmark.

Synaptic transmission by activation of ionotropic receptors is the main form of neuronal communication in neural networks such as those responsible for mammalian locomotion. To better understand how synaptic signalling contributes to network activity, we analysed synaptic input to interneurons that may participate in locomotor networks in the isolated spinal cord from neonatal rats. Locomotor related activity was induced by bath application of NMDA in combination with 5-HT, or by muscarinic agonists, and monitored by recording motoneuron bursts from the ventral roots L2 and L5. Intracellular, voltage clamp recordings were made from neurons close to the central canal and in lamina VII by using the tight-seal recording technique. In many cells that received locomotor-related cyclic modulation of their membrane currents, putative unitary postsynaptic currents (PSCs) could be identified. The strength of the synaptic signals could be expressed by their relative contribution to the total cyclic current modulation. We therefore estimated the number of synaptic currents needed to account for the observed locomotor related current fluctuations in 16 cells. The average cyclic current modulation was calculated as the difference between the current integral (charge) during the first and second half of the locomotor related cycles. This modulation index was divided by the average charge of the PSCs. Between 17 and 250 average PSCs per second were needed to produce the observed cyclic current modulation in different cells. In most cells, 90% of the charge of an average PSC was delivered within 17 to 25 ms. In some cells, the excitatory PSCs had a faster time course, down to 5 ms. We conclude that very few (1-4) synapses need to be active simultaneously in order to produce the observed cyclic current modulation, giving the individual PSCs a powerful influence on the postsynaptic cell. Supported by a Mobility Grant from EU (MR), the Danish Research Academy (BRJ) and OK is a Hallas-Møller senior research fellow.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS IX

545.1

CONTEXTUAL FEAR CONDITIONING POTENTIATES ACOUSTIC STARTLE RESPONSES IN RATS. P.W. Frankland*[1], C. Dockstader[2], R.J. McDonald[2], [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 and [2]Department of Psychology, University of Toronto, Toronto, Ont., Canada.

The acoustic startle reflex is a short-latency motor response to a loud and unexpected noise that is modifiable by experience. For example, startle amplitudes are potentiated in the presence of a cue that has previously been paired with an aversive event such as the delivery of foot shock (e.g., Davis et al., 1993). Conversely, startle amplitudes are attenuated in the presence of a cue that has previously been paired with a pleasurable event such as the delivery of food (Schmid et al., 1995). The modification of startle responsivity in the presence of these cues appears to be amygdala mediated.

Here we test whether exposure of rats to a context where they previously received shock potentiates acoustic startle responses. Rats (n=12) were initially tested for startle in two sessions. On each of two consecutive days rats were placed in a startle chamber and presented with 40 startle-eliciting stimuli. The mean startle amplitude of each rat in the second of these two sessions served as a pre-training baseline. One day following the this session, rats were placed back in the startle chamber where they received 0 (n=4), 1 (n=4) or 3 (n=4) 0.6 mA shocks during a 5 minute period. One day following this context training, they were placed back in the startle chamber and presented with 40 startle stimuli. In the no shock and 1 shock conditions, startle amplitudes post-training were not significantly different from the pre-training baseline. However, in the 3 shock condition, post-training startle amplitudes were significantly increased compared to pre-training baselines. These results suggest that contextual fear stimuli as well as cued fear stimuli potentiate acoustic startle responses. The effects of amygdala and hippocampal lesions on potentiation of startle are presently being examined. (Work supported by Connaught grant to RJM).

545.2

AN ANALYSIS OF CONTEXTUAL, AUDITORY AND GENERALIZED AUDITORY-CUE FEAR, J. W. Rudy*and C. R. Pugh. Department of Psychology, University of Colorado, Boulder, Co. 80309

A number of variables such as damage to the hippocampal formation, age, strain, and post-conditioning isolation have been shown to influence contextual but not auditory-cue fear conditioning. These dissociations have led to the view that contextual and auditory-cue fear conditioning are mediated by different neural systems. In the present experiments we extend this analysis to the study of generalized auditory-cue fear. We report that several of the variables that affect contextual fear conditioning also influence generalized auditory cue fear. Specifically, more generalized fear was found when (a) rats were isolated in a novel environment than when returned to their home cages immediately after conditioning, (b) when the retention interval was 3 hrs rather than 24 hrs, (c) in 18-day-old compared to 25-day-old rats and (d) when scopolamine was injected following the conditioning trials. Moreover, exposure to the auditory cue 24-hours prior to conditioning eliminated both the isolation and scopolamine effects. These results suggest that similar memory processes are involved in contextual and auditory-cue fear conditioning. To observe the similarity, however, one must measure generalized auditory-cue fear conditioning, because variables that influence contextual fear conditioning have no effect on direct auditory-cue fear conditioning. The results also support the view that both contextual and auditory-cue fear conditioning depend on two independent processes, one that constructs a representation of the stimulus and another that associates the representation with the aversive outcome. We suggest that it is the stimulus construction process that is commonly affected by the variables we investigated.

545.3

A SELECTIVE ROLE FOR CORTICOSTERONE IN FEAR CONDITIONING. C. R. Pugh*, M. Fleshner, D. Tremblay and J. W. Rudy. Department of Psychology, University of Colorado, Boulder, Co. 80309

There is evidence that optimal long-term memory may depend on a basal level of corticosterone. We examined the contribution of corticosterone to contextual fear conditioning which rats display to the static features of the environment in which shock occurs and to auditory-cue fear conditioning they display to a phasic tone paired with shock. The Type II glucocorticoid antagonist, RU 38486 impaired contextual fear conditioning. Compared to sham operated and normal control rats, adrenalectomized rats showed reduced long-term contextual fear conditioning. However, neither their short-term contextual fear nor their auditory-cue fear conditioning was impaired. Contextual fear conditioning in adrenalectomized rats with corticosterone replacement matched the level of controls. Moreover, rats exposed to the context prior to adrenalectomy showed normal long-term contextual fear conditioning. Dehydoepiandrosterone (DHEA), a "functional" glucocorticoid antagonist produced the same pattern of results as adrenalectomy. Thus, corticosterone's role in fear conditioning is selective: It appears to contribute to the neural processes that support the construction of a representation of the context. These results add to a growing literature indicating that contextual fear conditioning depends upon two processes. One process constructs a representation of the context while the other associates that representation with the aversive experience. These conclusions are similar to those derived from the effects of hippocampal-formation damage and suggest that corticosterone may be exerting its effects through the hippocampal formation.

545.4

DIFFERENTIAL ANTEROGRADE EFFECTS OF ELECTROCONVULSIVE SHOCK ON CONTEXTUAL LEARNING, CONDITIONED AND UNCONDITIONED FEAR. M.G.M. Oliveira*, O.F.A. Bueno and E.B. Gugliano. Dept. Psicobiologia, UNIFESP-EPM, São Paulo, SP, Brasil, CEP 04023-062.

Distinct amnestic treatments have differential effects on contextual learning (CL) and classically conditioned fear (CF) response in rats. This has been shown after paradoxical sleep deprivation, hippocampal lesion and chronic pre-training electroconvulsive shock (ECS) administration. The purpose of the present study was to investigate the effects of a unique ECS administered 1h, 2h or 4h before training on CL, CF and on unconditioned fear (UF). Rats were trained in a step-through inhibitory avoidance apparatus comprised of a dark shock compartment interconnected with a white safe compartment. After entering the dark box they received 5 paired tone (90dB/1sec)-shocks (1 mA/1sec) 15 sec apart. Twenty-four hours later half the animals were submitted to the step-through inhibitory avoidance test in the same apparatus (CL test), and the other half was placed in a new environment (open-field arena) where freezing time was measured for 8 min. During the 4th min the same tone as presented during training sounded. Freezing after the tone was a measure of CF while that before tone was a measure of UF. Fourty eight hours after training, the animals submited to CL test were tested on the open-field and vice-versa. ECS when administered 1h, 2h or 4h before training impaired inhibitory avoidance performance as compared to control animals. This is interpreted as an anterograde amnesia for CL. When administered 1h or 2h before training ECS decreased UF, suggesting an anxiolytic effect of ECS, as well as decreased freezing after tone. As no interaction (tone x group) was found, this latter decrement is better interpreted as due to the anxiolytic rather than to an amnestic effect of ECS on this task.

Finantial support: AFIP and CNPq.

545.5

Memory Deficits and ECS: Conditioned fear and neurochemistry vary with stimulus laterality. Laura Fochtmann*, Andrew Francis, James McCaughran Dept. of Psychiatry, SUNY-Stony Brook, Stony Brook, NY 11794-8101.

Electroconvulsive therapy (ECT) is effective in severe mood disorders but its use is limited by memory disruption. In patients, cognitive side effects vary by memory task and are less with unilateral (UL) vs. bilateral (BL) stimuli. Our studies of conditioned fear response (CFR) compare memory effects of BL vs UL electroconvulsive shock (ECS) in CF-1 mice.

CFR was induced over 5 trials (CS-US) by pairing a tone (CS) with aversive footshock (US). Mice then received sham ECS, a single BL ECS 24 hr prior to retention testing, or 6 daily BL or UL ECS. Retention of CFR was manifested by reduced locomotion in parallel extinction trials (no US) in either the training-box without CS (context) or in a simulated home cage during the CS (cue). A course of BL ECS eliminated the contextual CFR. A course of UL ECS or a single BL ECS did not abolish the CFR but did decrease the CFR relative to sham ECS. In contrast, the cue CFR was reduced in all ECS groups. Thus, the CFR discriminates the memory disruption induced by chronic BL ECS from that of chronic UL ECS or a single BL ECS. Since CFR memory deficits depend on the specific task, the number of ECS and electrode placement, the data also suggest that CFR can model the memory effects of ECT.

Quantitative autoradiography was used to measure changes in receptors in the amgdala and hippocampus with these ECS schedules since these structures are linked to the cued and contextual components of the CFR. With BL vs UL ECS, we also observe distinct changes in glutamatergic and catecholaminergic receptors. These may relate to the differential disruptions of memory with clinical use of BL and UL ECT.

Supported by a grant from NIMH (R29-MH46040).

545.6

EFFECTS OF LIDOCAINE INJECTION INTO THE HIPPOCAMPUS ON CONTEXTUAL FEAR CONDITIONING INDUCED WITH FOOTSHOCKS OF HIGH AND LOW INTENSITIES. R. A. Prado-Alcalá*[3], A. Vazdarjanova[1], G. L. Quirarte[3], and J. L. McGaugh[1,2]. [1]Center for the Neurobiology of Learning and Memory and Departments of Psychobiology and [2]Pharmacology, University of California, Irvine, CA 92717-3800, and [3]Medicine School, National University of México, México, D.F., 04510.

Contextual fear conditioning is readily induced when rats are given inescapable footshocks (UCS) in a distinct environment (CS). Lesions of the hippocampus produce both anterograde and retrograde amnesia of contextual fear conditioning. This experiment examined the effects of bilateral infusions of 1μL of 2% lidocaine into the dorsal hippocampus administered immediately after contextual conditioning. Rats were placed inside a trough-shaped alley and given 5 consecutive footshocks of low or high intensities. Freezing behavior was measured before footshock delivery as well as 24 h after training. In agreement with previous lesion studies, the posttraining infusions of lidocaine produced a significant retention deficit in the animals trained with low footshock; in marked contrast, these infusions were ineffective when high footshock intensity was used during training. These data indicate that the involvement of the dorsal hippocampus in memory consolidation of contextual fear conditioning is dependent upon footshock intensity during training.

Research supported by USPHS MH12526 from NIMH and NIDA (JLM) and by DGAPA-UNAM (RAPA and GLQ).

545.7

FORNIX LESIONS AND OLFACTORY FEATURE POSITIVE AND FEATURE NEGATIVE CONDITIONAL DISCRIMINATIONS IN PAVLOVIAN CONTEXTUAL FEAR CONDITIONING. G. D. Gale*, S. G. Anagnostaras, & M. S. Fanselow. Department of Psychology, University of California, Los Angeles, CA 90095.

The hippocampus has been implicated in the processing of conditional, contextual, and configural cues during learning. We examined the acquisition of fear to a context when one feature of the context signaled either the presence or absence of shock. Female Long-Evans rats with bilateral lesions of the fornix or sham lesions were trained on either a feature positive discrimination (POS) where an ammonia odor served as the cue for shock, or a feature negative discrimination (NOS) where the *absence* of the ammonia odor served as the cue for shock. During discrimination training POS subjects received separate sessions in which 3 unsignaled footshocks (2-s, 1 mA; 60-s ITI) were presented in the presence of ammonia and no shocks were presented in the absence of ammonia; NOS subjects also received separate sessions during which shocks were presented in the *absence* of ammonia and no shocks were delivered in its presence. Neither NOS or POS subjects reliably learned to discriminate the shock context based on the olfactory cue. Relative to NOS subjects acquisition of conditioned fear to the shock context was enhanced in POS subjects, suggesting that olfactory cues enhance the rate of contextual fear conditioning. In POS subjects fornix lesions but not sham lesions blocked generalization decrement to the absence of ammonia. Renewal of conditioned fear in the presence of the olfactory cue following extinction sessions was unaffected by fornix lesions in both NOS and POS subjects. Taken together these results suggest that oflactory cues enhance the acquisition rate of contextual fear conditioning but do not enhance discriminibility. Supported by NIMH grant (MH39786) to MSF.

545.8

EXCITOTOXIC DORSAL HIPPOCAMPUS LESIONS AND PAVLOVIAN FEAR CONDITIONING IN RATS. S. Maren*†§, G. Aharonov†, and M. S. Fanselow†. †Dept. of Psych., Univ. of Calif., Los Angeles, CA 90095-1563; §Dept. of Psych., Univ. of Mich., Ann Arbor, MI 48109-1109 .

Electrolytic lesions of the dorsal hippocampus (DH) have been reported to produce deficits in both the acquisition and expression of conditional fear to contextual stimuli in rats. To assess whether damage to DH neurons is responsible for these deficits, we examined the effects of selective *N*-methyl-D-aspartate (NMDA) lesions of the DH on both the acquisition and expression of fear conditioning in rats. In Experiment 1, rats were placed in observation chambers and given three tone (90 dB, 2 kHz, 10 s)-footshock (1 mA, 2 s) pairings with a 74 s ISI. Seven days before or 7, 28, or 100 days after conditioning bilateral lesions were made in the DH using NMDA (20 μg/μL, 0.8 μL/side); control rats received sham surgery with no drug infusion. Following conditioning or surgery, fear to the context of the conditioning chamber was assessed by returning the rats to the chambers and scoring freezing; fear to the tone was assessed in a different chamber the following day. Results showed that DH lesions produced severe retrograde deficits in context fear when made up to 28, but not 100, days following training; DH lesions made before training did not affect contextual fear conditioning. Tone fear was modestly impaired by DH lesions at all training-to-lesion intervals. In Experiment 2, retrograde, but not anterograde, DH lesions produced modest deficits in context fear using an unsignaled shock procedure. In Experiment 3, anterograde electrolytic DH lesions produced modest deficits in context fear using both signaled and unsignaled shock procedures. This pattern of results reveals that neurons in the DH are not required for the acquisition of context fear and have only a minimal role in expressing context fear acquired with unsignaled shocks. However, DH neurons have a critical and time-limited role in the expression of contextual fear acquired with signaled shocks. We suggest that this pattern of results was obtained because context fear can be mediated by conditioning to either unimodal cues (hippocampus-independent) or a configure of polymodal background cues (hippocampus-dependent). Supported by NIMH grant (MH39786) to MSF and an NRSA (MH11061) to SM.

545.9

TIME-LIMITED RETROGRADE AMNESIA OF CONTEXTUAL FEAR CONDITIONING AFTER ELECTROLYTIC DORSAL HIPPOCAMPAL LESIONS IN RATS. S. G. Anagnostaras*°, S. Maren°†, & M. S. Fanselow°. °Dept. of Psychology, University of California, Los Angeles, CA 90095 and †Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI 48109.

As with other hippocampal-dependent learning, we have reported that electrolytic lesions of the dorsal hippocampus (DH) produce a severe deficit in contextual fear if made 1 day, but not 28 days, after a signaled fear conditioning session. Here we examine retrograde amnesia (RA) of contextual fear produced by DH lesions in a within-subjects design and the effect of signaling on the temporal gradient of RA. Unlike our previous reports, rats had both a remote and recent memory at the time of the lesion. In Exp. 1, animals were given 10 shocks (2-s, 1-mA; 60-s ITI; *unsignaled training*: no tones) in one context (*remote*) and 10 shocks in a different context 40 days later (*recent*), followed by DH lesions 1 day later. Relative to sham-operated controls, DH animals exhibited a mild RA for both *remote* and *recent* contextual fear memory with no evidence of a temporal gradient. For Exp. 2, we repeated the study with *signaled training*; animals were given 10 tone (10-s, 85-dB, 2 or 8-kHz)-shock (2-s, 1-mA; 70-s ITI) pairings in one context (*remote*) and 10 tone-shock (a different tone) pairings in a different context 50 days later (*recent*), followed by DH lesions 1 day later. Relative to controls, DH rats exhibited no deficit in *remote* contextual fear, but *recent* contextual fear memory was practically abolished. Thus, the temporal gradient of RA of contextual fear produced by DH lesions depends critically on the way that contextual fear is induced at the time of conditioning. When shocks are signaled during training (even though signals are not present during testing), DH lesions produce a severe, but time-limited RA. When shocks are unsignaled during training, the same lesions produce a mild, temporally-stable RA. These results are consistent with those reported by Kim & Fanselow (1992, *Science*, 256) who observed time-limited RA after a signaled conditioning session. Signaled training may be required to fully engage the hippocampus in contextual fear conditioning because the context serves as a less valid or "background" predictor of the shock in this condition. Supported by NIMH grant (MH39786) to MSF.

545.10

NMDA ANTAGONISTS BLOCK THE FACILITATORY EFFECTS OF CONTEXT PREEXPOSURE ON ONE-TRIAL CONTEXTUAL FEAR CONDITIONING. D. L. Stote* & M.S. Fanselow. Department of Psychology, University of California, Los Angeles, CA, 90095.

In one-trial contextual fear conditioning, a rat is placed in a novel chamber and after a specified interval receives an aversive footshock. When tested at a later time for conditional fear the rat freezes in response to these chamber or "contextual" cues. One factor that contributes to the level of fear in this procedure is the length of the placement-to-shock interval used during training. An immediate placement-to-shock interval results in no appreciable fear and intermediate placement-to-shock intervals (e.g. 10-30 sec) result in low levels of fear. In contrast, intervals of 90 sec or more evoke high levels of contextual fear. When rats are preexposed to the context 24 hours prior to training they show high levels of fear at intermediate placement-to-shock intervals compared to non-preexposed controls. It seems that without preexposure the intermediate intervals do not allow sufficient time for the animal to form a representation of the context prior to the footshock. This suggests that there are 2 processes involved in context conditioning: forming a representation of the context and forming an association between that representation and shock. NMDA mediated processes have been implicated in contextual fear conditioning. Intracerebroventricular application of the NMDA antagonist APV attenuates acquisition of conditional fear to the training context but it is not known if this is due to a disruption of either contextual representation formation or the context-shock association. To answer this question, we administered d,l-APV (5 µg, ICV) or MK801 (.1 mg/kg IP) during preexposure to a conditioning context. Both NMDA antagonists blocked the facilitatory effects of context preexposure. These results suggest that NMDA receptors are important for the learning that occurs during the context preexposure period. Supported by a NIMH grant (MH39786) to MSF.

545.11

ALTERATIONS IN GAP-43 AS A FUNCTION OF CONTEXTUAL FEAR CONDITIONING IN C57BL/6 AND DBA/2 MICE. Elizabeth A. Young* and Jeanne M. Wehner, Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

C57BL/6 mice perform well on contextual fear-conditioned learning while DBA/2 mice show impaired contextual but normal auditory conditioning. Thus, these strains can be used to dissociate neurochemical changes that are specific to learning. DBA/2 mice show reduced hippocampal protein kinase C (PKC) activity but little is known about specific phosphoprotein substrates in these strains. Hippocampal GAP-43, a presynaptic membrane-associated PKC substrate was quantified using Western blots in hippocampal membranes from naive C57 and DBA mice, after exposure to shock only, or at various times after contextual fear conditioning. Naive C57 and DBA mice were not different in the amount of GAP-43. However, only C57 mice showed significant effects of contextual conditioning. At 3 and 24 hrs. after training, hippocampal membrane-associated GAP-43 was increased 37% and 49% over naive C57s. The shock-only group showed no alterations in GAP-43 at any time tested. These results suggest that GAP-43 may play a role in contextual fear conditioning. (MH-48663 and AA-00141)

545.12

THE EFFECT OF CURRENT INTENSITY ON FOS PRODUCTION OF FEAR CONDITIONED MICE AFTER ACQUISITION. S.Milanovic*[1,2], O.Laban[1], J.Radulovic[1], O.Stiedl[1], F.Henn[2], and J.Spiess[1]. [1]Dept. Mol. Neuroendocrinol., Max Planck Inst. Exp. Med., Goettingen; [2]Central Inst. Mental Health, Mannheim, FRG.

The relationship of behavior induced by fear conditioning and FOS production in the brain of C57BL6/J male mice after acquisition has been investigated. Mice were placed into the conditioning chamber, and after 182 s, a foot-shock (ES) of constant current (2s) and different current intensities (0.2, 0.7, 1.3 or 2.0 mA) was applied (context/ ES groups). Another group of mice (context group) was only exposed to the conditioning chamber for 182 s. The brains of mice were analyzed immunohistochemically for the production of FOS 30, 60, 90 and 180 min after acquisition. Additionally, mice of the context/ ES groups and the context group were tested for contextual retention 24 h after acquisition. The freezing score of the context/ ES (0.7-2.0 mA) groups was markedly increased in comparison to the corresponding values of the context/ES (0.2 mA) or context group. When data on mice of the context/ ES (0.2 mA) group were analyzed individually, it was found that 60% of the animals associated context (CS) with foot shock (US). The activity of the context/ ES (0.7-2.0 mA) groups was significantly lower than the activity of the context or the context/ES (0.2 mA) group. The only brain region that showed differences in FOS production between mice of the context/ ES (0.2mA) group, the context group and all other groups was the nc.amygdaloideus medialis. In this nucleus of mice of the context group, FOS production was apparent 30 min after acquisition, reached the maximum at 60 min and returned to the baseline at 180 min. FOS production was significantly higher at all time points in the same nucleus of mice of the context/ES (0.7-2.0 mA) groups compared to mice exposed to context only and did not return to the baseline after 180 min. The FOS production pattern of the context/ ES (0.2 mA) group was either similar to the context group or the context/ES (0.7-2.0 mA) groups. This split may be related to the individual differences found in the retention tests of the same group. *(Supported by the Max Planck Society and the Central Inst. Mental Health)*

545.13

MK-801 DISRUPTS THE ACQUISITION AND EXPRESSION OF CONDITIONED FEAR IN THE ACOUSTIC STARTLE PARADIGM. T. Haralambous, J. Cranney and G. Paxinos*. School of Psychology, The University of New South Wales, Sydney, 2052, Australia.

The non-competitive NMDA receptor antagonist, dizocilpine (MK-801) disrupts freezing in the acoustic startle paradigm (Cranney et al., *Neurosci. Abs.* vol.20, p.1009, #413.16). This study attempted to determine whether this disruption by MK-801 was due to a block of the acquisition of conditioned fear (as opposed to this disruption of freezing resulting from possible sensorimotor or state dependent effects). Four groups (n=12) of rats were administered either MK-801 (0.1 mg/kg, s.c.) or saline 15 min prior to conditioning (Day 1), test (Day 2), or both. The conditioning phase consisted of 3 startle stimulus sessions (50 min apart) of 10 (115-dB, 5 ms-rise/fall, 30 to 90-s ISI) white noise stimuli, which commenced 60-s after exposure to the context. As expected, MK-801 disrupted the acquisition of conditioned fear, but in addition it appeared to disrupt the expression of conditioned fear. Other possible explanations for this apparent block of expression of conditioned fear include an interaction of either the sensorimotor effects or state dependent learning effects of MK-801, with a block of conditioned fear acquisition. Alternatively the results could be explained in terms of the anxiolytic actions of MK-801.

Supported by ARC and NH&MRC.

545.14

HIPPOCAMPAL LESIONS ATTENUATE LATENT INHITION OF CONTEXTUAL FEAR CONDITIONING IN THE RAT. D.Lukes, J.Cranney*, M.Kiernan[1] and R.F.Westbrook. School of Psychology, University of New South Wales, Sydney, 2052, and [1] Charles Sturt University, Bathurst,2795, N.S.W., Australia.

Latent inhibition refers to the relative difficulty with which a pre-exposed stimulus may come to serve as a conditional stimulus. Four groups of male, Wistar rats were used (total N=48). Two groups were bilaterally lesioned by infusing n-Methyl-d-Aspartate (NMDA) in fields CA1 and the dentate gyrus of the dorsal hippocampal formation. Two further groups received corresponding sham operations. One group from each surgical condition received ten, 20-min pre-exposures in the to-be-conditioned environment. The two remaining groups received no pre-exposure. All rats were then conditioned to the environment using a single footshock (1.0 mA, 1.0s). The next day all rats were replaced in the conditioning environment and observed for freezing in the absence of footshock. Sham operated rats which received pre-exposure froze significantly less than did subjects comprising the three other groups. Among non pre-exposed animals, no significant difference for conditioned freezing was observed between lesioned and sham rats. Results were taken as indicating that NMDA lesions constrained to fields CA1 and the dentate gyrus of the dorsal hippocampus are able to attenuate latent inhibition of contextual fear conditioning. However, such conditioning in the absence of pre-exposure was not affected by these lesions in the present preparation. Further research into the effects of hippocampal lesions on contextual conditioning is being undertaken. [Supported by the Australian Research Council.]

545.15

ACCUMBAL LESIONS ATTENUATE CONTEXTUAL FEAR CONDITIONING IN THE RAT. M.J. Kiernan*[1], G. Bailey, J. Sims, D. Lukes and J. Cranney. Psychology, Univ. of New South Wales, Sydney, 2052, and [1]Charles Sturt University, Bathurst, 2795, Australia.

Contextual fear conditioning, as indexed by freezing, occurs in normal rats following the presentation of an aversive unconditioned stimulus in a specific context/environment. two groups of male Wistar rats were used in this experiment. One group (n=12) was lesioned bilaterally by infusing n-Methyl-D-Aspartate (NMDA) into the accumbens nucleus. The other group (n=8) received corresponding sham operations. Following recovery, all rats were conditioned to the environment using three 1-mA, 1-s footshocks (ISI=20 s).The next day rats were replaced in the conditioning environment and observed for freezing in the absence of footshock. The lesioned rats displayed significantly less freezing than did the sham rats. Current experiments are exploring alternative explanations of the results, including possible deficits in (a) the formation of contextual representations, (b) general conditioning, or (c) motivation. There are no differences in locomotor and exploration measures. A model of the role of the accumbens nucleus in contextual conditioning will be presented. [Supported by the Australian Research Council.]

545.16

LESIONS OF THE DORSAL HIPPOCAMPUS OR BED NUCLEUS OF THE STRIA TERMINALIS (BNST) BLOCK FREEZING BUT NOT ENHANCEMENT OF THE STARTLE REFLEX IN A CONTEXT PREVIOUSLY PAIRED WITH SHOCK. K. A. McNish*, J. C. Gewirtz, and M. Davis. Depts. of Psychiatry and Psychology, Yale Univ. Sch. of Med., New Haven, CT 06508.

The roles of the dorsal hippocampus and BNST were assessed in the expression of contextual fear conditioning using two measures of conditioned fear: freezing and enhancement of the acoustic startle reflex. A discriminative contextual fear conditioning paradigm was developed which demonstrated both conditioned freezing and enhanced startle in a context previously paired with footshock, relative to a context in which footshock had never been presented. Both the degree of freezing and magnitude of startle enhancement were directly related to the shock intensity used during training (0, 0.3, 0.6, 1.2 mA). Post-training lesions of the dorsal hippocampus or the BNST produced a complete blockade of freezing to an aversive context, but no attenuation in the enhancement of startle. These data suggest that the hippocampus and BNST may form part of the same circuitry involved in freezing to an aversive experimental context. The sparing of context-induced enhancement of startle following lesions of either the hippocampus or BNST suggests that there may be residual fear to the experimental context in the absence of these structures. Alternatively, performance effects of these lesions on either startle or freezing could account for the lack of correlation between the two measures. [Supported by MH 47840, MH 00004 and AFOSR F49620-93-1-0293 DEF].

545.17

MEDIAL PREFRONTAL CORTEX LESIONS AND THE ACQUISITION AND EXTINCTION OF CONTEXTUAL FEAR CONDITIONING. F.A. Guarraci* and S.L. Young. Dept. of Psychology, University of Vermont, Burlington, VT 05405.

Morgan, Romanski and LeDoux (1993) found that medial prefrontal cortex (mPFC) lesions prolong extinction to discrete conditioned fear stimuli, but have no impact on the extinction of background contextual cues. In the present experiments we tested the effects of mPFC lesions, on foreground context conditioning.

Forty-eight Long-Evans rats were used. In Experiment 1, sixteen rats were placed in operant chambers for 180s and then received 1 footshock daily for 5 days. Freezing behavior was measured to assess conditioned fear. Rats were matched according to freezing and given either electrolytic lesions of the mPFC or sham lesions. Following recovery, all rats were exposed to the contextual cues for 330s daily for 8 days. During conditioning, all rats acquired contextual fear. The lesions had no affect on the rate of extinction but reduced the level of freezing during testing. These results suggest that the mPFC may be important for the retrieval of contextual fear.

In Experiment 2, we compared the effects of mPFC lesions on the acquisition or retrieval of conditioned contextual fear. Thirty-two rats received mPFC or sham lesions either before conditioning or the day after conditioning. All rats were placed in operant chambers for 180s and then received 3 footshocks, 60s apart. During conditioning, all rats acquired contextual fear. During extinction, all rats were freezing less than 1% of the time by day 3. However the rats with mPFC lesions made after conditioning froze significantly less on days 1-2 of extinction training. Interestingly, rats with mPFC lesions made prior to conditioning were not significantly different from either sham lesioned group.

In conclusion, our results indicate that the mPFC may be involved in the retrieval of recently learned, emotionally significant events, and similar to the findings of LeDoux and colleagues, is not important to the acquisition or extinction of contextual fear. Supported by the Department of Psychology, University of Vermont.

545.18

DORSAL HIPPOCAMPAL LESIONS PRODUCE ANTEROGRADE BUT NOT RETROGRADE AMNESIA OF LATENT INHIBITION TRAINING INDEPENDENT OF CONTEXTUAL MEMORIES. S. L. Young* and R. J. Frohardt. Department of Psychology, University of Vermont, Burlington, VT 05405.

The present experiments examine the role of the dorsal hippocampus in latent inhibition. The latent inhibition procedure consisted of a training phase of nonreinforced exposure to a tone followed by a phase of tone-shock pairings.Tone preexposure retarded acquisition of the conditional response, freezing, showing that the training procedure produced latent inhibition. Hippocampal lesions block latent inhibition. Since latent inhibition may be a phenomenon that depends upon the animal associating contextual cues with the tone, the lesions may disrupt latent inhibition by interfering with contextual learning. Therefore, we examined whether latent inhibition is subject to the same sorts of manipulations as contextual fear conditioning.

Hippocampal lesions produce both anterograde and retrograde amnesia of context conditioning. We asked if hippocampal lesions produce both retrograde and anterograde amnesia of latent inhibition. Subjects were given hippocampal lesions or sham surgery before or after latent inhibition training. Lesions given before but not after training blocked latent inhibition suggesting that the effect of the lesions on latent inhibition may not be due to disruption of contextual associations.

In a separate experiment, we gave the animals an opportunity to learn the experimental context before assessing the effect of the lesion on latent inhibition. We preexposed the animals to the experimental context or an alternate context 28 days before hippocampal lesioning or sham surgery. This procedure prevents the learning deficit caused by hippocampal lesions in context conditioning. However, the context preexposure did not alter the impact of hippocampal lesions on latent inhibition. These results also indicate that the effect of dorsal hippocampal lesions on latent inhibition may not be due to disruption of a context-tone association. This research was supported by UVM Psychology Department.

545.19

LESIONS OF THE HIPPOCAMPUS IMPAIR ACQUISITION BUT NOT RETENTION OF INHIBITION. S.C. Benoit, T.L. Davidson, and L.E. Jarrard*. Purdue Univ., West Lafayette, IN; Washington and Lee Univ., Lexington, VA 24450.

Davidson, Benoit, and Jarrard (1995) found that the capacity of hippocampal rats to inhibit responding to contextual cues depended on the number of unsignalled food Unconditioned Stimuli (USs). Both control and ibotenate lesioned hippocampal animals showed similar conditioned activity in the absence of unsignalled USs but activity of hippocampal rats was greater than controls when unsignalled USs had been delivered in the training context. To determine whether this was a deficit in learning or in performance, the present experiment employed a similar training procedure except that rats were lesioned after training was completed. Rats (N = 32) were given 15 daily training sessions during which a 10-sec tone CS predicted the delivery of a food US. Half of these animals also received 3 unsignalled presentations of food per session (Group 3 US) while the other half received no unsignalled USs (Group 0 US). Following training and recovery from surgery, hippocampal and control rats were given 14 sessions of extinction (i.e., no food was delivered). Results of extinction testing showed that there was no difference between control and hippocampal animals in responding to the context. If lesions produced only a performance deficit, elevation of responding would be expected. However, relative to controls, hippocampal animals in both 0 and 3 free US groups showed less extinction to the tone suggesting that hippocampal animals had difficulty learning inhibition to the now non-reinforced CS. The pattern of results indicates that removing the hippocampus impairs the rats' ability to learn inhibition to cues that have undergone both excitatory and inhibitory training. Funded by grants from NIH and NSF.

545.20

MULTIDIMENSIONAL EFFECTS OF HIPPOCAMPAL SUBFIELD LESIONS ON ATTENTIONAL PERFORMANCE OF A SERIAL REACTION TIME TASK IN RATS. **K. L. Stacey, A. M. Bratt, S. P. Kelley, R. M. Chase and G. Mittleman***. University of Memphis, Memphis, TN 38152.

The purpose of this experiment was to compare the relative attentional contributions of different hippocampal subfields. A serial reaction time task (5CHOICE) was used which required animals to successfully discriminate the location of a randomly presented 3W target light in the presence of different types of distractors. Animals with lesions of subiculum, fimbria-fornix, dentate gyrus, and whole hippocampus were compared to unoperated controls for decrements in performance across different distractor modalities (3W light and 105dB white noise), different temporal distractor positions (2.5 s before or concurrent with the target), and different distractor durations (1s and 5s) within eight 30-minute sessions. Comparisons to baseline and across lesions were made using percent correct, percent incorrect, percent misses, correct latency, incorrect latency, and response bias as dependent measures. Rats with lesions of the subiculum and the entire hippocampal formation displayed heightened distractibility, increased strategy use, and impaired habituation as well as showing an abnormal insensitivity to changes in distractor modality. Rats with fimbria-fornix or dentate gyrus lesions showed no deficits under any experimental conditions. These results indicate that disruption of the perforant path has a greater impact on attentional performance than disruption of fornical input or output pathways and creates attentional deficits that are roughly analogous to those found in schizophrenia.
Funding was provided by the Scottish Rite Schizophrenia Foundation.

546.1

A SEARCH FOR HEMISPHERIC SPECIALIZATION FOR VISUAL MEMORY IN MACAQUES. Robert W. Doty*, Ruiming Fei, Ross D. Rugaber, and Andreas E. Savakis, Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

In human subjects the right hemisphere is commonly more efficient than the left in visual processing. The situation is unclear for macaques. Two male *M. nemestrina*, MA and MV, with optic chiasm, callosum and anterior commissure cut are presently being tested. One or the other eye/hemisphere views a series of 140 images, subtending 21° on a 17-inch color monitor. A "new" versus a "repeat" image for the session requires direction of gaze to points above or below the image, respectively, for 400 msec at >200 msec latency. From 0-3 other images may intervene (the "lag") following initial presentation. After initial training of MA, 3 types of images have been used, randomized from an inventory of 600-1200 items of each type, using R and L eyes on alternate days. The types are: 1) "general" (objects, scenery); 2) nonobjective multicolored patterns; 3) human faces (cropped to elliptical format but including differences in age, sex, race, beard, spectacles). With type 1, N=1278 trials for each hemisphere, d' was identical, 1.78 (ca 81% correct) for each. Again, with type 2) material, N=1452, d' was the same for each hemisphere, 1.61. Human faces were so difficult, however, as to require reduction of "lag" to a single intervening image, in which case, for N=645, d'=1.61 for right, 1.40 for the left hemisphere (p=.18, no significant difference). Animal MV, performing more erratically and not exceeding lag of 1, with N=568 trials for type 1 images, has d'=1.53 for right versus 0.93 for left (p=.002); and for type 3, N=426, d'=1.33 and 0.93 for right versus left (p=.04), suggesting a hemispheric difference. Bias from surgical sites should favor right hemisphere in MA but not MV. Obviously the issue remains unresolved and work continues!

Supported by grant #NS20052 from NINDS.

546.2

CONTEXT-RECALL MEMORY SCANNING IN TWO PRIMATE SPECIES. A.F. Carpenter*, G. Pellizzer and A.P. Georgopoulos. Brain Sciences Center, VAMC, Minneapolis, MN 55417.

Six human subjects and one rhesus monkey performed a visuomotor context-recall memory scanning task. The task required memorizing a sequential list of visual stimuli, then choosing which of them is the correct target for motor response based on the serial order relations between the stimuli. A series of circular targets ("list stimuli") were shown in a random sequence on a monitor; stimuli remained on the screen throughout the entire trial. When one of these stimuli changed color ("test stimulus"), the subject had to move a cursor toward the stimulus that followed the test stimulus in the original sequence. Human subjects performed the task with lists of 1-7 stimuli in a randomized design; the monkey performed the task with lists of 3-5 stimuli presented in blocks.

In both species the mean reaction time increased as a linear function of the number of stimuli in the list, suggesting that subjects "scan" the list items in memory. The slope of this relation was over 3 times higher in the monkey (344 msec/list item) than in the human subjects (101 msec/list item); this implies that the monkey's rate of memory scanning is much slower than that of the humans. When the test stimulus was presented immediately following the last list stimulus, both species exhibited a "recency effect" such that responses were faster and more accurate for targets presented later in the list that for those presented earlier. When a 2 sec delay was imposed between the last list stimulus and the test stimulus, human subjects did not exhibit this recency effect. These findings indicate that (i) monkeys can perform recall (in addition to recognition) memory tasks, and (ii) both monkeys and humans seem to utilize a memory scanning process in performing context-recall tasks, albeit at different rates.

(Supported by NIH grant NS17413)

546.3

WORKING MEMORY IN INFEROTEMPORAL CORTEX: AN EXPERIMENTAL TEST OF ATTRACTOR NETWORK BEHAVIOR. V. Yakovlev[1], S. Fusi[2], D.J. Amit[2], S. Hochstein*[1] and E. Zohary[1]. [1]Dept. of Neurobiology, [2]Racah Institute of Physics, Hebrew University, Jerusalem, 91904, Israel.

An experimental paradigm was designed to critically test the hypothesis that neurons in inferotemporal (IT) cortex operate as an attractor neural network. Single cell activity was recorded in area IT of a macaque monkey performing a delayed match-to-sample task. Stimuli, from a set of 30 geometrical colored images, were shown for 500 ms with an intervening delay period of 5 sec. We determined for each cell the two stimuli that evoked the strongest and weakest visual response, and used them subsequently. The sample stimulus was degraded by random RGB noise, while the test stimulus was always intact. Stimulus degradation modulated both the monkey's behavior and the cells' visual activity: As the degradation level was increased, the monkey's performance deteriorated until it reached chance level. Correspondingly, in most IT neurons higher stimulus degradation led to weaker neuronal response. Several neurons maintained a sustained discharge during the inter-stimulus-interval following the best stimulus Furthermore, in one distinct experiment the delay activity was unaffected at low levels of degradation in marked contrast to a change in the neural response to the same degraded stimuli. Then, beyond a certain level of degradation, the delay activity disappeared. This type of behavior is in accordance with the attractor picture, in which to each distribution of delay activities corresponds a basin of attraction. Stimuli within the basin lead to identical delay activity distribution. Once outside the basin, the delay activity undergoes a major change. Supported by Israel Min. of Science & Arts

546.4

ACTIVITY OF PREFRONTAL (PF) NEURONS DURING AN OBJECT AND SPATIAL MEMORY TASK E.K. Miller*, S.C. Rao, and G. Rainer
Dept. of Brain and Cog. Sciences, MIT., Cambridge, MA 02139

A variety of studies suggest that visual working memory (WM) for objects and for spatial information are processed independently in separate regions of the PF cortex. To determine whether any PF neurons can contribute to both object and spatial WM, we recorded from the lateral PF cortex while a monkey performed a task that required memory for both types of information. The task began with a brief presentation of a sample object at the center of gaze followed by a delay. Then, two test objects were briefly presented at two of four possible extrafoveal locations. One of the test objects matched the sample, the other was a nonmatch. Following another delay, the monkey was required to make a saccade to the remembered location of the match. Thus, this task employed two delays. In the first ('what' delay) the monkey had to retain object information in WM, while in the second ('where' delay) it had to retain spatial information in WM.

We have preliminary results from 46 PF neurons that exhibited high levels of activity during one or both delay intervals (delay activity). Based on MRI data, these neurons were located ventral to the principal sulcus, near the border of areas 46 and 12. Some neurons showed only sample-selective delay activity during the 'what' delay (7/46, or 15%) and other neurons showed only location-selective delay during the 'where' delay (8/46, or 17%). Surprisingly, many neurons (17/46, or 37%) showed both sample- and location-selective delay activity during alternate phases of the task. That is, they exhibited sample-selective delay activity during the 'what' delay **and** location-selective delay activity during the 'where' delay. These findings demonstrate the existence of PF neurons that contribute to both WM for objects and WM for spatial information.

Supported by The Sloan Foundation and the McDonnell-Pew Foundation

546.5

A ROLE FOR PREFRONTAL CORTEX IN BOTH WORKING MEMORY AND ATTENTION: DISSOCIATION BETWEEN DMTS AND DNMTS PROCEDURES. J.M. Williams* & B. Givens. Dept. of Psychology, The Ohio State University, Columbus, OH 43210.

An operant task was designed to separately assess medial septal area (MSA) and prelimbic/infralimbic (PLIL) cortex contributions to working memory and attention/stimulus encoding processes. Given reported differences between delayed match-to-sample (DMTS) and delayed nonmatch-to-sample (DNMTS) procedures, this experiment also examined the contributions of the MSA and PLIL to these two procedures. Rats were tested in an operant chamber with three lights located on the front panel. Levers were located beneath each light. In the sample phase, rats were presented with either a signal in which the center light was illuminated or no signal. To assess the attentional components of the task, signal length was varied (1 to 2 seconds). After the sample stimulus, a delay of 0 or 2 seconds was introduced. This delay constituted the memory component of the task. During the choice phase, a light appeared randomly over either the right or left lever. The opposite side remained unlit. In order to obtain a reward, rats were required to press the lever that either matched (DMTS group) or did not match (DNMTS group) the sample stimulus.

DNMTS results indicated that the MSA is more involved in the working memory components of the task, whereas the PLIL is more involved in the attentional/stimulus encoding components of the task. Results of the DMTS study indicate that there are distinct differences between DMTS and DNMTS procedures, including difficulty of task (as indicated by trials to criterion and post-surgery baseline performance), and PLIL involvement in attention and working memory. In contrast to the DNMTS rats, DMTS rats show delay-dependent and stimulus length-independent deficits following PLIL infusions, indicating that, for the DMTS group, the PLIL is more involved in the working memory components of the task and not the attentional/stimulus encoding aspects of the task. Results suggest that DMTS and DNMTS differences should be considered when assessing the neuroanatomy of cognitive processing and may explain some of the debate as to whether the PLIL is involved in attentional or mnemonic processing.Supported by ABMRF

546.6

POSTTRAINING OR PRETEST INFUSION OF GLUTAMATE ANTAGONISTS INTO THE MEDIAL PREFRONTAL CORTEX IMPAIRS MEMORY FORMATION OR RETRIEVAL IN AN INHIBITORY AVOIDANCE TASK. K. C. Liang* & S.-J. Hu. Dept. of Psychology, Natl'. Taiwan Univ., Taipei, Taiwan, 10764, R.O.C.

The medial prefrontal cortex (mPFC) is implicated in affective functions. We have previously shown that the mPFC is more involved in retrieving a remote (21-day) affective memory than a recent (1-day) one (Liang & Liao, *Soc. Neurosci. Abst., 1995, 21, 1448*). To pursue this finding further, the present study examined the roles of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors in the mPFC in formation and retrieval of affective memory.

Male Wistar rats were bilaterally implanted with cannulae into the mPFC. They were trained on a one-trial step-through inhibitory avoidance task (foot shock 1.75 mA/1 s) and tested for retention either 1 or 21 days later. Five min. prior to the test, rats received infusion (0.5 μL) of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) or 2-amino-5-phosphonovaleric acid (APV) into the mPFC. Results indicated that pretest infusion of CNQX (0.3 μg) into the mPFC had little effect on 1-day retention but impaired 21-day retention, whereas pretest infusion of APV (2.5 μg) had no effect on either test. To examine the role of mPFC in memory formation, pre- or posttraining drug infusion was administered. Lidocaine impaired 1-day retention when infused into the mPFC shortly before and after training, but had no effect if given 4 hrs later. Posttraining intra-mPFC infusion of CNQX (0.3 μg or 1.0 μg) caused a time-dependent memory deficit in both 1- and 21-day tests. Posttraining intra-mPFC infusion of APV at various doses caused no amnesic effect in the 1-day test. However, a marked memory deficit was apparent if rats given 2.5 μg APV were tested 21 days later.

These findings, taken together, suggest that in the mPFC, transmission through AMPA receptors may be involved in processing information of aversive tasks, and NMDA-dependent plasticity may underlie formation of long-term affective memory.

(Supported by a grant NSC-84-2311-B002-025 from the government of R.O.C.)

546.7

THE EFFECTS OF FRONTAL CORTICAL LESIONS IN THE RAT DEPEND ON THE PROCEDURAL DEMANDS OF TASKS TRAINED IN THE RADIAL ARM MAZE (RAM) . M.C. Porter*, B.M. Glode, J.R. Pelletier & R.G. Mair. Dept. Psychol., Univ. New Hampshire, Durham, NH 03824.

Rats were trained on a computer controlled 8 arm RAM task and then given lesions of MDn projection areas or sham surgery. After recovery they were trained on six tasks, beginning with standard RAM, then two versions of a four forced choice procedure, and then three versions of a two choice DNMTS task. Rats with lesions of the medial wall (MW) region or MW combined with cortex dorsal to the rhinal sulcus showed similar impairments. Lesioned animals were not impaired on the two choice DNMTS when the alleys used for training were selected at random on a trial by trial basis. They were significantly impaired: (1) on the standard and four forced choice tasks and (2) when the same alleys were used to train two choice DNMTS on all trials. Training animals in the dark or increasing delays up to 15 minutes had no significant effects on the severity of deficits. Supported by grant RO-1 NS26855 from NINDS to RGM.

546.8

AUDITORY SPATIAL WORKING MEMORY IN BARN OWLS: DISRUPTION BY PHARMACOLOGICAL INACTIVATION OF THE FOREBRAIN ARCHISTRIATUM, E. I. Knudsen* and P. F. Knudsen. Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305-5401.

The central nervous system continuously stores information about the spatial locations of potentially important stimuli in a temporary form referred to as spatial working memory. We are using the barn owl to explore the pathways and mechanisms that mediate auditory spatial working memory. We evaluated auditory spatial working memory using a delayed-response task. First, the owl fixated a zeroing light. Then a noise burst was presented from a hidden, movable speaker. The owl made a stimulus-guided head saccade to the sound source. The noise was turned off and the owl re-oriented to the zeroing light. When the zeroing light was turned off, the owl made a memory-guided head saccade and strike at the location of the speaker.

Neuronal inactivation (muscimol; 0.5 - 1.0 μg) in a region of the barn owl archistriatum that is functionally analogous to the mammalian frontal eye fields caused owls to lose the ability to respond to the remembered locations of auditory stimuli: the owls were unable to make memory-guided head saccades or memory-guided strikes at auditory targets. In contrast, archistriatal inactivation had no effect on stimulus-guided responses to auditory stimuli. The memory-dependent deficit was selective for acoustic events that occurred in the hemifield contralateral to the side that was inactivated. The data demonstrate that in the avian archistriatum, as in the mammalian prefrontal cortex, there exists a region that is essential for the expression of spatial working memory. Supported by NIDCD #RO1 DC00155-16.

546.9

THALAMIC AMNESIA RECONSIDERED: EXCITOTOXIC LESIONS OF THE INTRALAMINAR NUCLEI (ILn), BUT NOT THE MEDIODORSAL NUCLEUS (MDn) DISRUPT OLFACTORY CONTINUOUS DNMTS PERFORMANCE. R.G. Mair*, Y.P. Zhang, J.A. Burk, B.M. Glode, M. Foye, & J.R. Pelletier. Dept. Psychol., Univ. New Hampshire, Durham, NH.

In rats, large lesions of thalamus that include portions of the MDn and the ILn disrupt olfactory continuous delayed non-matching to sample (cDNMTS). To compare the effects of lesioning ILn and MDn, rats were trained to perform this task and then given either sham surgery or an excitotoxic lesions of one of these structures. Surprisingly rats with the ILn lesion were impaired while the rats with MDn lesions were not. None of the lesion groups were impaired on a subsequent olfactory discrimination that used go/no go procedures comparable to cDNMTS. In previous studies of central olfactory system lesions we have observed comparable impairments only after complete lesions of pyriform cortex. While surprising, these findings are consistent with results for a place DMTS task (Burk & Mair, this meeting). Supported by grant RO-1 NS26855 from NINDS to RGM.

546.10

THALAMIC AMNESIA RECONSIDERED: EXCITOTOXIC LESIONS OF THE INTRALAMINAR NUCLEI (ILn), BUT NOT THE MEDIODORSAL NUCLEUS (MDn) DISRUPT PLACE DMTS PERFORMANCE. J.A. Burk*, B.M. Glode, J.R. Pelletier & R.G. Mair. Dept. Psychol., Univ. New Hampshire, Durham, NH 03824.

In rats, large lesions of thalamus that include portions of the MDn and ILn disrupt performance on DNMTS and DMTS based on place cues. In this study, rats were trained to perform a place DMTS task and then given excitotoxic lesions of either (1) the ILn, (2) the MDn, (3) the L-IML site (which affects both ILn and MDn and has previously been associated with DMTS impairments), or (4) sham surgery. The excitotoxic L-IML lesions had effects consistent with previous studies of rats with RF L-IML lesions. Surprisingly ILn lesions caused severe deficits while MDn lesions had little effect. None of the lesion groups were impaired on a subsequent serial reversal learning task based on place cues. While surprising, these findings are consistent results for an olfactory cDNMTS task (Mair et al, this meeting). Supported by grant RO-1 NS26855 from NINDS to RGM.

546.11

DIFFERENT HIPPOCAMPAL - CORTICAL - STRIATAL - THALAMIC CIRCUITS MEDIATE DELAYED VS NON-DELAYED FORAGING IN THE RAT, Stan B. Floresco*, Jeremy K. Seamans and Anthony G. Phillips. Department of Psychology, Univ. of British Columbia, Vancouver, B.C., V6T 1Z4.

The ventral CA1/subiculum (vCA1/sub) region of the hippocampus, the prelimbic cortex (PL) and the nucleus accumbens (NAc) comprise a neural circuit that has been implicated in the efficient performance spatially-mediated foraging tasks. The first series of experiments assessed the nature of the interactions between these brain regions by utilizing a transient lidocaine-induced disconnection procedure. Rats were well-trained on one of two versions of a spatially-cued radial-arm maze task and then received a unilateral lidocaine infusion into the vCA1/sub and a contralateral lidocaine infusion into either the PL or the NAc. Disconnections between the vCA1/sub and the PL severely disrupted foraging during a delayed spatial win-shift (SWSh) task, in which the rat was required to obtain information about the location of food, hold it over a 30 min delay and use this information later to guide foraging. However, similar vCA1/sub-PL disconnections did not disrupt performance of a non-delayed random foraging (RF) task, in which rats were required to locate 4 pellets placed randomly on an 8-arm maze. Conversely, disconnections between the vCA1/sub and the NAc impaired performance on the RF task, but not the delayed SWSh task. These results suggest a functional dissociation between the brain circuits involved in delayed and non-delayed spatially-mediated foraging.

The mediodorsal nucleus of the thalamus (MD) is part of an anatomical loop which includes the PL and the NAc. As such, the MD may be part of a neural circuit that mediates foraging strategies across a delay. In a separate experiment, two groups of well-trained rats received bilateral infusions of lidocaine into the MD prior to either the test phase of the delayed SWSh task, or prior to the RF task. Preliminary data indicate that transient lesions of the MD impair performance during the delayed SWSh task, but not the RF task, a pattern of deficits similar to those observed following transient lesions of the PL (Seamans et al., 1995). These findings suggest the MD may be part of a neural circuit involved in delayed, spatially-mediated foraging, which includes the PL and the vCA1/sub. (supported by NSERC, Canada.)

546.12

DIFFERENTIAL OUTCOMES ENHANCES MEMORY PERFORMANCE ON OPERANT MATCHING-TO-POSITION IN NORMAL AND THIAMINE DEFICIENT RATS.
L. Savage*, S. Pitkin, K. Knitowski, K. Daly, & A. Ganzekaufer. Department of Psychology, SUNY-Binghamton, NY 13905

Numerous studies have demonstrated that rats with Pyrithiamine-induced Thiamine Deficiency (PTD) have extensive diencephalic damage and are severely impaired at acquiring new tasks that require the use of "working memory". The Differential Outcomes (DO) procedure, which correlates specific reinforcers with specific discriminative stimuli, improves performance of normal subjects on various conditional discrimination tasks. Previous research (Savage & Langlais, 1995) revealed that implementing the DO procedure in the T-maze version of Matching-to-Position (MTP) completely eliminated the PTD-induced learning deficit, but not the PTD-induced memory deficit. In the current study we implemented the DO procedure in an operant version of MTP and found that, although it did not enhance learning of the task, it did help improve short-term memory performance in both normal and PTD-treated rats. Additionally, normal and PTD-treated rats were administered i.p. injections of scopolamine and MK-801. Rats, both normal and PTD-treated, trained with the DO procedure continued to have better memory performance than rats trained with a non differential procedure when administered scopolamine, but not MK-801. Using the DO procedure allows brain damaged subjects to utilize different undamaged information processing systems, and affects the influence of amnesic drugs.
This research was supported by a SUNY Faculty Award to L.S.

546.13

ROLE OF MONKEY CEREBELLAR DENTATE NUCLEUS IN PROCEDURAL MEMORY. X. Lu*, O. Hikosaka, and S. Miyachi. Dept. of Physiol., Juntendo Univ. Sch. of Med., Tokyo, 113. Japan

To examine whether the cerebellum is involved in learning and memory of sequential procedures, we trained a monkey on a sequential button press task (2x5 task) (Hikosaka et al., J. Neurophysiol., 74, 1652-1661, 1995). On pressing of a home key, 2 of 16 (4x4) LED buttons (set) were illuminated, and the animal had to press them in a predetermined order which he had to find by trial and error. A total of 5 sets (hyperset) were presented in a fixed order as a trial. An error at any set aborted the trial. In one block, a hyperset was repeated until 10 successful trials were completed. The monkey had learned 22 hypersets daily so that he could perform them with few errors. We asked whether, by the reversible functional blockade of the dentate nucleus (DN) with muscimol, the monkey had difficulty in performing the learned hypersets or difficulty in learning newly generated hypersets, or both.

First, we recorded from neurons in DN through guide tubes which were implanted with the aid of MRIs; many of them showed clear task-related changes only when the ipsilateral hand was used. Among them was a group of neurons that displayed exclusive or enhanced activity changes for the first button press compared with the second press in each set. They were considered as sequence-dependent neurons. We then injected muscimol (1 µl x 5 µg/µl) into the site where such sequence-dependent neurons were recorded. We found that the number of errors for the learned hypersets increased when performed by the ipsilateral ($p<0.001$), but not contralateral ($p>0.05$) hand. In contrast, the number of errors for new hypersets showed no significant increase with either the ipsi- or contralateral hand ($p>0.05$). These results suggest that the cerebellar DN is critical for storage and/or retrieval of long-term procedural memory rather than its acquisition.

546.14

LESIONS OF THE CEREBELLAR NUCLEI IMPAIR SEQUENCE LEARNING IN A SERIAL REACTION-TIME TASK. P.D. Nixon and R.E. Passingham (SPON: Brain Research Association) Department of Experimental Psychology, University of Oxford, Oxford OX1 3UD.

Several groups have claimed that cerebellar patients are impaired on the serial reaction-time task, a test of procedural learning. However, it is not certain that in these studies the pathology was confined to the cerebellum

We therefore trained monkeys pre-operatively on a SRT task with 4 target stimuli. The stimuli lit in a repeating sequence (SEQ). Learning was measured by the decrease in response times for each sequence of 4 moves (STs) compared with the times when the stimuli were lit in random order (RAN). When performance on the SEQ condition was optimal, bilateral lesions were placed in the lateral cerebellar nuclei, using kainic acid; the remaining animals served as unoperated controls.

In the operated animals post-operative STs for the SEQ and RAN conditions were, as expected, considerably increased, This was true both for reaction times and movement times. Unexpectedly, the STs for the RAN condition were slower than would be predicted by the motor impairment alone. However, all the operated animals showed evidence of some retention of the sequence. All the animals were then given a new repeating sequence to learn. The control group took 1400 trials to reach their previous level of performance, whereas the lesion group failed to reach their level after 2000 trials. These data confirm the results obtained for patients.

Supported by a Wellcome Trust Program Grant (038041/Z/93).

546.15

EFFECTS OF MIDLINE AND LATERAL CEREBELLAR LESIONS ON MOTOR AND NON-MOTOR LEARNING IN RATS. C.C. Joyal*, J. Caston and R. Lalonde. Faculté des Sciences de Rouen, Laboratoire de Neurophysiologie sensorielle, Mt-Saint-Aignan, France, 76011 and Groupe de Recherche en Neuropsychologie, Université de Montréal, Canada.

Rats lesioned either in the midline cerebellum, comprising the vermis and fastigial nucleus, or the lateral cerebellum, comprising the cerebellar hemispheres and dentate nucleus, or in the fastigial nucleus only were evaluated in a series of motor and non-motor learning tests. Rats with midline lesions had difficulty in maintaining their equilibrium on a bridge and were slower before turning upward and traversed less squares on an inclined grid. They were not impaired for muscle strength when suspended from a horizontal wire. Rats with lateral lesions had milder deficits on the bridge and were not affected in the other two tests. In the Morris water maze test, rats with lateral lesions were deficient in spatial orientation, whereas rats with midline lesions were deficient in visuomotor coordination. Lateral lesions had no effects on visual discrimination learning. These results illustrate the differential influence of midline as opposed to lateral cerebellar regions on both motor and non-motor behaviors. Fastigial nucleus lesions decreased the time spent in equilibrium and latencies before falling on the bridge and the distance travelled along the inclined grid but had no effect on muscle strength when suspended from the horizontal string. Quadrant entries and escape latencies were higher in rats with fastigial lesions during the hidden platform condition of the Morris water maze but not during the visible platform condition. It is concluded that fastigial-lesioned rats are impaired in equilibrium and spatial orientation but with repeated trials learn to improve their performances. This work was supported by scholarships to C.C. Joyal from CRSNG and FCAR.

546.16

ROLE OF THE INFERIOR OLIVARY COMPLEX IN MOTOR SKILLS AND MOTOR LEARNING IN THE ADULT RAT. L. Rondi-Reig[#], N. Delhaye-Bouchaud[#], J. Mariani[#*], J. Caston[§]. [#]Lab. de Neurobiologie du Développement, Institut des Neurosciences (CNRS URA 1488) Univ. P&M Curie, 75005 Paris, France. [§]Lab. de Neurophysiologie Sensorielle Faculté des Sciences, Univ. de Rouen, France.

In order to determine the role of the inferior olivary complex (IOC) of adult rats in motor learning, we studied the behavior of animals whose IOC was destroyed using i.p. injection of 3-acetylpyridine. Muscular strength, motor coordination and equilibrium of animals were tested using different motor tasks: hanging test, stumbling test and unrotated rod test. The different scores show that 3-acetylpyridine treated rats had motor coordination and static equilibrium deficiencies, whereas their hanging capabilities were intact when compared to controls. Animals were also trained upon an unrotated rod or upon the rod rotating at 5, 10 or 20 rpm. 3-acetylpyridine treated rats were able to maintain their equilibrium on the unrotated rod and at 5 rpm. Moreover after motor training at 5 rpm, rats were able to improve their motor skills and reached the same score as controls. Despite their good motor skills, animals were unable to maintain their equilibrium when rotated at 10 and 20 rpm. These results suggest that the inferior olivary complex is needed for learning of motor sequences involving a high synchronization of movement. (supported by a MRE grant 92.C.0756)

546.17

EFFECTS OF ELECTRICAL STIMULATION AT MEDIAL AND LATERAL PARAFASCICULAR NUCLEUS ON TWO-WAY ACTIVE AVOIDANCE. A.Vale-Martínez, M. Martí-Nicolovius, G. Guillazo-Blanch and I. Morgado-Bernal*. Dept. de Psicobiologia i Metodologia de les Ciències de la Salut, Edifici B, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), SPAIN.

Posttraining parafascicular intracranial electrical stimulation (PF ICS) can have facilitatory, detrimental or neutral effects on 2-way active avoidance acquisition (5 sessions, 1 daily, 10 trials each) and long term retention (LTR) (1 session, 10 trials). To test whether facilitatory effects of PF ICS on this task could be due to the application of the treatment at the medial or lateral parafascicular nucleus (PF), rats were implanted with an electrode at PF. Immediately after each acquisition session, experimental rats were electrically stimulated at the PF for 10 minutes. According to the electrode tip locations, experimental rats were divided in 6 groups: 3 medial PF groups (Anterior-ICS, Central-ICS and Posterior-ICS) and 3 lateral PF groups (Anterior-ICS, Central-ICS and Posterior-ICS). Two control groups were used (Medial control and Lateral control) depending on the electrode implantation laterality.

Our results showed that posttraining medial PF ICS has detrimental effects on 2-way active avoidance that are attributed to the medial PF relations with the limbic system. The same treatment applied at lateral PF confirmed facilitatory (Posterior-ICS), detrimental (Central-ICS) and neutral (Anterior-ICS) effects on this task, depending on the affected antero-posterior area of the nucleus. Facilitatory effects are discussed in terms of PF relations with the subparafascicular nucleus (SPF) and PF projections to the raphe nuclei system. Detrimental effects might be a result of lateral PF relations with the motor system.

DGICYT grant (PB92-0617), DGR grant (1994)

546.18

ROLE OF RAT "BARREL" CORTEX IN TACTILE LEARNING. J.A. Harris, R.F. Westbrook, and M.E. Diamond*, Sch. of Psychology, Univ. of New South Wales, Syndey, 2052 Australia and Cognitive Neurosci. Sector, International Sch. for Advanced Studies, Trieste 34014 Italy.

We are using the gap-crossing task to investigate in what way the rat primary somatosensory "barrel" cortex contributes to the identification of objects contacted by the whiskers. Rats are trained without visual information (under red light) to obtain food by crossing a gap from one elevated platform to another. Trained rats show whisker-dependent crossing, traversing wide gaps (up to 16 cm) where they can detect the goal platform only by projecting the whiskers in front of the snout. The behavior is cortex-dependent: gap-crossing ability is abolished if vibrissal barrel cortex is ablated, and naive rats cannot learn the task after such ablation. We are examining to what extent gap-cross learning is horizontally distributed across cortex. Rats are trained with 4 whiskers on one side of the face. Once the task has been learned, these 4 "trained" whiskers are cut off and 4 new "prosthetic" whiskers are glued to whisker stumps -- either to the stumps of the trained whiskers, to 4 different stumps on the same side of the face, or to 4 stumps on the other side of the face. The rats are then re-tested. If they can immediately use information from prosthetic whiskers attached to "untrained" stumps it means that off-centre barrel-columns (those deprived of whisker input during training) must have participated in the acquisition of the gap-crossing task, or else have access to the learned information. (Supported by NINDS 32647, the Whitehall Foundation, and M.U.R.S.T.).

546.19

THE EFFECT OF AUDITORY SHUTTLE-BOX LEARNING ON THE AMINERGIC TRANSMISSION SYSTEMS OF ASSOCIATIVE BRAIN AREAS OF GERBILS. H. Stark* and H. Scheich, Federal Institute for Neurobiology (IfN), Brenneckestr. 6, 39118 Magdeburg, Germany

Auditory cortex has been shown to be a site of widespread neuronal learning processes even in the context of simple auditory conditioning behavior and parts of the prefrontal cortex are a site of the working memory. In view of their presumed role in determining behavioral and motivational relevance of incoming information we investigated whether the dopaminergic and serotonergic systems are involved in learning activities of both brain areas. Using chronic brain microdialysis technique samples from auditory cortex and medial prefrontal cortex were obtained before, during and after daily footshock avoidance training simultaneously from trained gerbils and passive control animals or pseudotrained animals. Levels of extracellular dopamine (DA), homovanillic acid (HVA) as metabolite of the dopaminergic system as well as 5-hydroxyindoleacetic acid (5-HIAA) as metabolite of the serotonergic system were systematically analyzed. The common response of the dopaminergic systems of sensory and associative brain areas appeared to reflect the initial formation of the behaviorally relevant association exclusively during the first training day while the serotonergic response appeared to correlate with the stress level of animals.

Supported by DFG, STA 464/1-1

546.20

AP-5 and L-NAME block sensory-sensory conditioning in waking rat barrel cortex. M. Maalouf, O.E. Valdivia, A.A. Myasnikov and R.W. Dykes*. Département de physiologie, Faculté de Médecine, Université de Montréal, Montréal, Québec.

Several studies have shown that the behavior of cortical neurons changes during learning, yet, few of them have demonstrated if such changes are a form of cortical plasticity or merely a reflection of subcortical events. Delacour et al. (1987) showed by recording from neurons in barrel-cortex of awake rats that paired stimulation of the principal whisker (named S2) and a non-adjacent one (S1) enhances the response to S1 of some neurons representing S2 in the cortex. We hypothesized that this sensory-sensory conditioning is a form of cortical learning that relies on a mechanism similar to hippocampal long-term potentiation. We recorded a total of 70 barrel cortex neurons during 50 pairings of S1 and S2. In each pairing, the ending of S1 preceded the beginning of S2 by 300 ms and the duration of each was 750 ms. The interval between two pairings was randomly chosen between four preset values (5, 7, 9 or 11 s). Of the 25 neurons that received this conditioning paradigm, 5 showed a significant increase in the response to S1. The behavior of 30 neurons was evaluated during the same conditioning paradigm while applying by microiontophoresis, either the NMDA-receptor antagonist, AP5 (17/30), or the nitrous oxide synthase inhibitor, L-NAME (13/30). Changes in the response to S1 were not seen in any of these cases. Finally, no changes were observed in the activity of (15) neurons while the rats underwent a truly random conditioning paradigm consisting of 50 non-paired stimulations of S1 and S2. We conclude that the changes in neuronal responsiveness are attributable to a cortical mechanism that depends upon NMDA receptors and may involve nitrous oxide as a potential retrograde messenger (Supported by the Medical Research Council of Canada and the Faculty of Medecine of the University of Montréal).

546.21

PARADOXICAL INFANTILE OLFACTORY MEMORIES. R.M. Sullivan*, M. Landers and D.A. Wilson, Department of Zoology, University of Oklahoma, Norman, OK 73019

Neonatal rats can learn to avoid or approach an odor. However, under most circumstances, neonatal rats will learn an odor preference, even when the reinforcer used during conditioning is clearly aversive to the pup. For example, pairing an odor with a moderate shock produces a subsequent preference for the conditioned odor. During the end of the second week of life, conditioning becomes similar to the adult, so that odor and 1mA shock pairing now produces an odor aversion This series of studies further explores this developmental change in olfactory learning.

Pups were given odor (2ul citral) and shock (.7 mA, 1 sec, 3 min ITI) pairings for a single 30 min training session. Olfactory learning was assessed in a 2-odor choice test (citral vs familiar clean bedding odor). **Experiment 1** assessed the age at which pups change from learning a preference to learning an aversion from odor - shock conditioning. The results show that PN9 pups learn/express an odor preference whereas PN10 pups learn/express an aversion, illustrating a developmental change in the representation of this training from appetitive to aversive learning. **Experiment 2** assessed whether pups would express a preference (typical of PN9) or express an aversion (typical of PN10) when trained on PN9 but tested on PN10. The results showed that pups trained on PN9 exhibit a preference when tested on either PN9 or PN10. **Experiments 3 and 4** assessed whether the olfactory memory could be changed by extending the training from PN9 (preference learning) to PN10 or beyond (aversion learning). The results showed that when learning began during the period of preference learning and extended into the period of aversion learning, pups continued to express a preference. Together, these results suggests that once an olfactory memory is formed, the original memory trace continues to be strengthened by subsequent experience, despite dramatic developmental changes in the representation of this training from appetitive to aversive learning. Supported by NIH grant HD33402.

546.22

LEARNING TASK DEPENDENT DIFFERENCES IN INTER-REGIONAL CORRELATIONS, BUT NOT MEAN VALUES, OF FLUORO-DEOXYGLUCOSE UPTAKE IN YOUNG RATS. M.W. Lilliquist*, A. Amsel, & F. Gonzalez-Lima. Dept Psychology & Instit. for Neuroscience, Univ. Texas at Austin, Autsin TX, 78712, USA.

We assessed the pattern of brain activity during performance of two closely-matched learning tasks, using ^{14}C-fluoro-deoxyglucose (FDG) uptake as an index of neural activity. Pre-weanling rat pups were trained to traverse a straight alley runway for food reward, according to one of two schedules of reinforcement. Half of the subjects were rewarded according to a 50% quasi-random "irregular" schedule, which results in greater persistence and greater resistence to extinction. The other half were rewarded in a 50% nonrandom manner, consisting of rewarded and nonrewarded trials in "regular" alternation. Under the regular schedule, animals displayed discrimination by faster running speed on reward trials and slower running speed on nonreward trials. FDG uptake occurred at the end of acquisition, during the last 50-trial training session, at which point the regularly-rewarded subjects were exhibiting asymptotic discrimination. FDG uptake from 50 brain regions was measured in ^{14}C units from autoradiograms developed from serial sections of each brain. Means analysis of within-subject normalized FDG uptake revealed few differences. In contrast, analysis of the correlations between brain regions revealed marked differences between regular and irregular reward schedules. In particular, uptake values in the majority of brain regions measured were highly correlated in the irregularly-rewarded subjects, while only a small minority of regions were correlated in the regularly rewarded animals. The widespread inter-correlations between FDG uptake in brain regions may represent a closer functional coupling of brain activity in animal that faces situations of ongoing inconsistency, in comparison to the more stable situation which occurs after extended training with regularly alternating reward. This work supported by NIAAA grant #AA07052 and NSF grant #IBN9222075.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XI

547.1

SEX DIFFERENCES IN CONDITIONED ANALGESIA AND FREEZING. H.S. Stock*, B.J. Caldarone, D.L. Mongeluzi, & R.A. Rosellini. Dept. of Psychology, University at Albany: SUNY, Albany, NY 12222.

Prior research in our laboratory has shown that males exhibit higher levels of conditioned fear-induced enhancement of morphine analgesia compared to females. One possible explanation for this observed behavior is that context conditioned fear was not present for the females. Another possible explanation is that males and females differ in the stress-induced activation of a system(s) that is involved in pain-inhibition. An experiment was conducted to determine if males and females exhibited differential levels of freezing and conditioned analgesia during exposure to a conditioned fear context. The results of the experiment showed that while conditioned fear males and conditioned fear females exhibited similar freezing levels, conditioned fear males exhibited higher levels of conditioned analgesia compared to conditioned fear females. These findings suggest that the sex difference in conditioned fear-induced enhancement of morphine analgesia may be due to a sex difference in the stress-induced activation of a system(s) involved in pain-inhibition.

Supported by internal university grant to H.S..

547.2

WATER MAZE PERFORMANCE AND SWIM STRESS: TOLERANCE AND SEX DIFFERENCES M.Kavaliers*, T. S. Perrot-Sinal and K.-P. Ossenkopp. Neuroscience Program, University of Western Ontario, London, Canada, N6A 5C1

Performance on a water maze task, whereby individuals have to acquire and retain the location of a submerged hidden platform, involves both "spatial" and "non-spatial" components. This study examined the swim stress-induced analgesia (SSIA) arising from protocols associated with water maze task acquisition (single and repeated 1 min swim trials (22°C) with and without a hidden platform; block of 4 swim trials) in male and female deer mice. Sex differences were evident in the levels of analgesia, the development of tolerance, and the neuromodulatory correlates of SSIA. Males displayed significantly higher initial levels of SSIA (1 min swim) and developed tolerance more readily than females. Presence of the hidden platform facilitated the development of tolerance. There were also sex differences in the neuromodulatory substrates of SSIA. One min swims induced a "non-opioid" (naloxone-insensitive) analgesia that was attenuated by the competitive NMDA antagonist, NPC 12626, in males but not in females. The SSIA arising from the "block" of swims was of a mixed opioid and "non-opioid" nature and insensitive to the NMDA antagonist in females. These various swim associated "non-spatial" factors likely contribute to the expression of sex differences in spatial aspects of water maze performance. (supported by NSERC)

547.3

STRESS FACILITATES AVERSIVE PAVLOVIAN CONDITIONING IN MALES, BUT NOT IN FEMALES. G. E. Wood* & T. J. Shors. Dept. Psychol., Prog. Neurosci., Princeton Univ., Princeton, NJ 08544 -1010.

The effects of gender and inescapable stress on aversive Pavlovian eyeblink conditioning were studied. Male and female Spraque-Dawley rats were analyzed under stressed and unstressed conditions. Stressed subjects were restrained and exposed to 30, 1 s per minute, 1 mA tailshocks, and then returned to their home cage. Twenty four hours later, all subjects received classical conditioning (paired stimuli) or explicitly unpaired stimuli. Conditioned subjects were exposed to an 85 dB white noise burst, which was paired with a 0.7 mA periorbital eyelid shock. During the acquisition, stressed males exhibited facilitated conditioning, whereas stressed females exhibited significantly impaired conditioning relative to their gender-specific unstressed controls. These results suggest that exposure to the stressor used here facilitates acquisition of the conditioned response in males, while suppressing it in females. In addition to the stress effects observed on learning, unstressed females acquired the conditioned response at a facilitated rate compared to unstressed males. No effects of stress or gender were observed in the performance of subjects receiving explicitly unpaired stimuli. These data emphasize the importance of incorporating dimorphic responses into current models of stress and learning.

[McDonnel-Pew Foundation, Whitehall Foundation, NSF (IBN-9511027), ONR (N00014-92-J-1897) to TJS].

547.4

STRATEGIES FOR LEARNING ACROSS THE ESTROUS CYCLE IN FEMALE RATS. D. L. Korol*, J .M. Couper, C. K. McIntyre and P. E. Gold. Department of Psychology and Neuroscience Graduate Program. University of Virginia, Charlottesville, VA 22903.

Fluctuations in hormones across the estrous cycle lead to large changes in synaptic density in certain brain areas. These changes are likely to be accompanied by differences in performance on behavioral tasks. Recently, we and others found that at times of high estrogen, females perform worse in the spatial version of the swim task than at times of low estrogen. Perhaps, differences are due to shifts in behavioral strategy and not to deficits in processing spatial information *per se*. We undertook the following experiments to test whether performance on other spatial tasks exhibits differences across the estrous cycle, and whether rats at low estrogen adopt a spatial strategy while rats at high estrogen adopt a response strategy during learning. Vaginal smears were taken at the same time daily from young adult virgin female Sprague-Dawley rats. In the first experiment rats were trained on an appetitive 2-choice discrimination task in a T-maze, with the opportunity to use either a spatial or a response strategy during training. After criterion performance was reached, a probe test was administered to assess strategy type. At stages of high estrogen, a greater proportion of rats relied on a response strategy, whereas at stages of low estrogen, a greater proportion relied on a spatial strategy. In the next experiment, rats were tested in a Y-maze on a rewarded spontaneous alternation task for 10 consecutive days, i.e. across two estrous cycles. No differences between estrous phases were noted for alternation scores or arm entries. However, a shift from a spatial to a response strategy emerged over the 10 days of testing regardless of stage. The results suggest that fluctuations in gonadal steroids might differentially influence behavioral strategies on different tasks, perhaps by affecting specific neural systems. *Supported by NIA (AGO7648) and NINDS (NS32914).*

547.5

DYNAMICS OF WORKING MEMORY ACROSS THE ESTROUS CYCLE. M.E. Blasberg*, R.W. Stackman, C.J. Langan, and A.S. Clark. Department of Psychology, Dartmouth College, Hanover, NH 03755.

Fluctuations in circulating gonadal steroids have been shown to influence both morphological and physiological features of the CA1 subfield of the hippocampus in female rats. Despite evidence for this plasticity, little is known regarding the functional or behavioral impact of these changes. The purpose of this study was to evaluate spatial working memory on a delayed-non-match to sample (DNMTS) radial arm task across the estrous cycle.

Twenty adult Long-Evans female rats were maintained at 85% body weight and housed in a reverse cycle room (lights off 1300 hrs). Throughout the four months of testing, vaginal smears were collected daily at 1200 hrs and females were tested for sexual receptivity with a stud male daily at 1600 hrs. Working memory testing was conducted daily between 1000-1300 hrs.

No estrous cycle variations in acquisition or post-acquisition performance on the standard 8-arm radial maze task were present. With a 1 hour delay imposed, we observed no influence of estrous cycle day on working memory in the DNMTS task. No significant pattern of changes in correct choices, retroactive, or proactive errors occurred as a function of day of the estrous cycle. Latency per choice, a measure of performance, not memory, was significantly increased on proestrous relative to all other cycle days. The effect of a 4 hr delay on DNMTS performance across the estrous cycle is currently under investigation.

Although synaptic connectivity varies considerably across the estrous cycle, our results to date suggest that spatial working memory is remarkably stable. Supported by NIDA-08574 to ASC.

547.6

EFFECTS OF ESTROGEN TREATMENT ON T-MAZE ALTERNATION IN FEMALE AND MALE RATS. A. J. Fader*, A. W. Hendricson, and G. P. Dohanich. Department of Psychology, Tulane University, New Orleans, LA 70118.

Estrogen influences performance on various tasks of learning and memory in both humans and nonhumans. In the present experiments, the effects of estrogen treatment on reinforced T-maze alternation were examined. Gonadectomized female and male rats were treated daily with intramuscular injections of estradiol benzoate (5 µg/kg) or oil vehicle for 8 days prior to T-maze training and throughout 12 days of training. Estrogen treatment induced a mild, but significant, enhancement of T-maze acquisition in gonadectomized females, but not in gonadectomized males. When administered 15 minutes prior to testing on the thirteenth day, the muscarinic antagonist, scopolamine hydrobromide (0.2 mg/kg), significantly impaired alternation in females and males that were treated with oil. Estrogen treatment counteracted the effect of scopolamine on performance in females, as reported previously with higher doses of estrogen (25 µg/rat for 3 days, Dohanich, Fader, and Javorsky, *Behav. Neurosci. 108:988,* 1994). However, estrogen treatment did not reverse the effects of scopolamine in males. Results indicate that estrogen enhances T-maze acquisition and performance in female rats, but not in male rats. NSF awards BNS-9021447.

547.7

EFFECTS OF ESTROGEN AND ENVIRONMENT ON RADIAL MAZE ACQUISITION. J. M. Daniel, A. J. Fader, A. Spencer, B. E. F. Wee*, and G. P. Dohanich. Department of Psychology, Tulane University, New Orleans, LA 70118.

The independent and interactive effects of estrogen and environment on acquisition of an 8-arm radial maze were determined in adult female rats. During the first 5 days of maze training with all arms baited, intact females reared in a complex environment made significantly more correct choices than intact females reared in isolation or ovariectomized females reared in either environment. During 19 additional days of training, females reared in a complex environment made more correct choices than females reared in isolation. During the first 5 days of training in a second experiment, ovariectomized females implanted with Silastic capsules of estradiol (25%) for 30 days prior to training and reared in a complex environment made significantly more correct choices than females with estradiol implants reared in isolation or females with cholesterol implants reared in either environment. During 19 additional days of training, females with estradiol implants made more correct choices than females with cholesterol implants. Results indicate that estrogen and environment can enhance radial maze acquisition, both independently and interactively. NSF awards BNS-9021447 and BNS-9514816 .

547.8

THE EFFECTS OF ESTROGEN ON PERFORMANCE OF A HIPPOCAMPAL-DEPENDENT TASK. B. Berry*, R. McMahan, and M. Gallagher. Department of Experimental Psychology, University of North Carolina, Chapel Hill, NC 27599.

The density of dendritic spines in the CA1 region of the rat hippocampus has been shown to fluctuate in a manner dependent upon concentrations of circulating estrogen, an effect demonstrated in both ovariectomized and normally cycling animals. We assessed learning based on hippocampal-dependent spatial navigation in female rats at identified points in the estrus cycle corresponding to low (estrus) and high (proestrus) circulating estrogen. With background training in water maze procedures, rats learned the location of an escape platform in the maze in a single session of 8 training trials. A strong spatial bias for the escape platform was also evident in a probe trial used to assess retention of learning 30 min after the training session. This entire protocol was completed in less than an hour. The performance of the two groups of female rats (estrus and proestrus) was indistinguishable on all behavioral measures, irrespective of the stage of estrus cycle during the task. These results indicate that rapid learning and retention for spatial information over a relatively short interval may be preserved despite morphological alterations in hippocampal dendritic spine density in the normally cycling female rat. Supported by K05-MH01149 to M.G. and P01-AG09973.

547.9

ESTRADIOL EFFECTS ON RAT SPATIAL MEMORY, V.N Luine*, J. Rentas, L. Sterbank and K. Beck. Psychology, Hunter College of CUNY, New York, N.Y. 10021.

Estradiol (E_2) affects memory in a number of paradigms in both animals and humans. In order to understand the nature of E_2 effects, several experiments using the 8-arm radial maze, a spatial memory task, were done. Rats were ovariectomized, trained on the maze, and E_2 was given (24 h before testing) via s.c. implanted silastic capsules which generated diestrus E_2 levels. No effects were seen in five regular trials when all arms were baited. With delays of 3-5 h after the 4th choice, the treated rats performed better, the number of correct choices was higher. E_2 was without effect in longer delays. Since these results suggested that E_2 may enhance memory, a four arms baited paradigm was utilized to quantitate working and reference memory. Reference errors (RME) are entrances to unbaited arms and provide a measure of learning while working memory errors (WME) are returns to a previously visited baited arm and provide a measure of memory. Five blocks of five trials each were analyzed, and E_2 was without effect on RMEs. WMEs were lower in blocks 4 and 5 of E_2 treated rats. Finally, E_2 silastics were implanted prior to training and 3 weeks before trials to determine if lack of effects in regular trials was due to the short duration of E_2 treatment. A significant interaction between treatment and trials was seen; E_2 treated rats performed generally better. These results show that estradiol enhances performance of a spatial memory task and suggest that the effects are related to enhancements in memory, not learning. Further, sufficient time (days) may be necessary for estradiol's enhancing effects on memory (RCMI-RR03037)

547.10

SPATIAL LEARNING IN RATS - THE EFFECT OF SEX AND PRIOR FAMILIARIZATION WITH NON-SPATIAL ASPECTS OF THE TASK. T. S. Perrot-Sinal, M. Kavaliers and K.-P. Ossenkopp. Neuroscience Prgm., University of Western Ontario, London, Canada N6A 5C2.

Performance of male and female laboratory rats was examined on a stationary hidden platform task (spatial task) in the Morris water maze after prior familiarization with non-spatial aspects of the task. Non-stationary hidden platform (NSP) training was used to familiarize animals with the general requirements of the water maze task (i.e., swimming, escape via the platform etc.) without providing spatial information (i.e., no extra-maze visual cues available). Performance on the spatial task was assessed in males and females who had received prior NSP training compared to rats with only a baseline swim prior to spatial training. NSP training resulted in faster acquisition of the spatial task in both males and females. Additionally, NSP trained animals showed improved retention of the acquired spatial task. A sex difference favoring males on both acquisition and retention of the spatial task was noted only in animals which had *not* received previous NSP training but not in those with prior NSP training. Moreover, there was an apparent reversed sex difference favoring females on some measures of spatial performance in NSP trained animals. These results suggest that performance on the water maze task, including the expression of sex differences, can be altered by previous familiarization with "non-spatial" aspects of the task.

Supported by NSERC grants to K-PO and MK.

547.11

SEX AND AGE EFFECTS ON DENDRITIC SPINE DENSITY IN CA1 OF THE HIPPOCAMPUS. S.G.Warren*, E.Robinson, E.J.Chesler and J.M.Juraska. Psychology Dept., University of Illinois, Champaign, IL 61820.

The density of dendritic spines and synapses in CA1 of the hippocampus varies across the estrous cycle of young female rats. These rapid morphological changes correspond to the circulating levels of estrogen and progesterone. When female rats age the estrous cycle ceases and they enter a state in which gonadal hormone levels no longer vary on a daily basis (called estropause). Here we investigated the morphological changes that occur with the natural cessation of the estrous cycle in aging female rats. Using Golgi stained tissue, dendritic spine density of young cycling females was compared to 12-15 month old and 22-24 month old estropausal females. In addition, young, middle aged and aged males were examined. Preliminary analysis indicates that young males have a greater density of spines than young females and females in estrus have more spines than 12-15 month old estropausal females. Analysis of the remaining groups is currently underway. Supported by NSF IBN 9310945 to JMJ and SGW was supported by HD07333.

547.12

SPATIAL MEMORY DECLINE IN AGED, NON-CYCLING FEMALE RATS VARIES WITH THE PHASE OF ESTROPAUSE. J.M.Juraska* and S.G.Warren. Psychology Dept. University of Illinois, Champaign, IL 61820.

We have previously reported that spatial memory on the Morris water maze varies with the estrous cycle in young adult female rats. Here we investigated the effects of the natural cessation of the estrous cycle (estropause) in aging female rats. Performance of male rats declines on this task as they reach old age, however little is known about the memory decline of females. Females were tested on the Morris water maze at 22-24 months of age, many months following the cessation of the estrous cycle. Females in persistent estrus were compared to those in anestrus. Those in persistent estrus, who have naturally higher levels of estrogen, performed significantly better than those in anestrus. In a comparison with young cycling females, both aged groups were found to perform poorly relative to females in estrus but not those in proestrus. These data suggest that the lowest levels of estrogen in aged females may be detrimental to spatial learning, while the highest estrogen levels in young adult females is detrimental. Supported by NSF IBN9310945 to JMJ and SGW was supported by HD07333.

547.13

WATER MAZE ESCAPE LATENCIES DECREASE IN OLD RATS WITH LOW POSTNATAL TESTOSTERONE EXPOSURE. K. Schultz*, N. Ward, M. Roberts, A. Wintink, L. Francis & T. Williams. Psychology Department, University of Winnipeg, Winnipeg, MB, Canada R3B 2E9

We have examined the extent to which the allocentric water maze performance of 240 old (1 year), adult (85 days) and adolescent (50 days) male and female Long-Evans rats was effected by pre- and post-natal choline supplementation and by post natal testosterone manipulation. One half of the animals tested had been exposed to enhanced choline levels throughout gestation and for 21 days after birth. Equal numbers of males and females were represented in each group. One half of these males had been gonadectomized one day after birth and half of the females received injections of testosterone on postnatal days three and five. All animals were housed in same sex groups in enriched environments following weaning.

Contrary to expectations, repeated measures analysis of variance revealed that across ages there were no significant choline treatment effects. However, the effects of testosterone on performance varied with sex, age and treatment level. Animals with low testosterone levels (control females, gonadectomized males) showed decreasing escape latencies with age while those with higher testosterone levels (control males, testosterone injected females) exhibited their shortest escape latencies as adults. At one year, these latencies returned to the longer, adolescent levels. These behavioural patterns were reflected in variations in the size of the hippocampal dentate gyrus. Thus, while perinatal manipulations of choline failed to have long term effects on allocentric maze performance, those of testosterone persisted into old age.

(supported by The University of Winnipeg)

547.14

Consequences of estrogen loss and replacement on cognitive function of surgically menopausal monkeys. Mary Lou Voytko* and Jennifer Hinshaw. Dept. of Comparative Medicine, Bowman Gray Schl. of Med., Winston-Salem, NC 27157.

Increased forgetfulness and decreased attention are commonly experienced by postmenopausal women. Whether these cognitive changes are related to the loss of estrogen associated with ovarian failure in menopause rather than to age per se is not clear. To address this issue in a primate model, we determined the effects of estrogen loss associated with ovariectomy and subsequent estrogen replacement therapy (ERT) on performance in several behavioral tasks in young female cynomolgus monkeys (n=5). After pretraining on visual discriminations, delayed response, a visuospatial cued task or a simple reaction time task, monkeys were ovariectomized and then continually reassessed on the tasks over 3 months. At that time, 2 monkeys began ERT and 3 monkeys received placebo. Repeated assessment of the tasks occurred for 5 months. Neither learning, memory or reaction time were affected by estrogen loss or ERT. In contrast, spatial orienting to a cue was disrupted by estrogen loss and these alterations were reversed following ERT but not placebo. These cognitive results are the first to be obtained from a primate model of menopause and suggest that estrogen loss associated with ovarian failure may be contributing to alterations in attention of postmenopausal women. Supported by P50HL45666 and 1R29AG13204.

547.15

EARLY ANDROGEN-INDUCED ALTERATIONS IN STRESS RESPONSIVENESS, MONOAMINERGIC SYSTEM, AND COGNITIVE PERFORMANCE: POSSIBLE BASIS FOR ADHD. J. A. King*, C. F. Ferris, Y. Delville, S. Adolphe, and B. Ray. Department of Psychiatry, University of Massachusetts Medical Center, Worcester, MA 01655.

Numerous repons suggest that attention deficit hyperactivity disorder (ADHD) is more prevalent in males than females. Pharmacological treatment of ADHD is based mostly on alterations in the monoaminergic systems. The spontaneously hypertensive rat was been utilized as an animal model of ADHD by many researchers. These studies were designed to evaluate the effects of testosterone administration on behavior, the neuroendocrine system, and the monoaminergic system in the animal model of ADHD. Neonates were microinjected with testosterone (100 ug) or vehicle into the frontal lobe of the cortex, on postnatal day 10. On postnatal day 45 they were evaluated for their performance on the Morris swim Maze and open field activity, and their plasma levels of adrenocorticotrophin (ACTH) after stress, and dopamine immunoreactivity in the frontal lobe of the cortex. In the water maze, ADHD animals took significantly longer to find the platform than Wistar controls ($p \leq 0.05$). This deficit was enhanced with acute androgen administration. Plasma ACTH levels were increased in the ADHD animals exposed to testosterone ($p \leq 0.05$), whereas dopamine immunoreactivity was decreased. Taken together, these studies suggest that early androgen treatment of ADHD may alter the monoaminergic system in a manner that further facilitates cognitive impairment and may modulate the hypothalamic-pituitary response to stress.

Sponsor: NIMH-MH 457114-01

547.16

SEX AND SEASONAL DIFFERENCES IN RELATIVE HIPPOCAMPAL VOLUME IN PARASITIC AND NON-PARASITIC COWBIRDS. N. S. Clayton*, J. C. Reboreda and A. Kacelnik. Section N.P.B., UC Davis, Davis CA 95616.

In brood parasites, spatial ability to locate and return to hosts' nests may have selected for an enlargment of the hipppocampus relative to the remainder of the telencephalon (RHV) when compared to non-parasitic species; and this enlargement may occur only in the breeding season when searching for hosts' nests. To test this hypothesis, RHV was measured in the breeding and non-breeding season for 3 cowbird species which differ in whether one, both or neither sex search for hosts' nests. In the breeding season, only female shiny cowbirds *(Molothrus bonariensis)* search for host nests and therefore females should have a larger RHV than do males; in Screaming cowbirds *(M. rufoaxillaris)*, both sexes inspect hosts' nests in the breeding season, so both sexes should have a large RHV; and in non-parasitic bay-winged cowbirds *(M. badius)* neither sex searches for hosts' nests, and neither sex should have a large RHV. Birds were captured in Buenos Aires in the breeding (n=36) and non-breeding season (n=26), given a lethal intraperitoneal overdose of sodium pentobarbital (8ug/g), and perfused transcardially with 0.85% heparinized physiological saline followed by 4% paraformaldehyde. Brains were removed, post-fixed for 14 days, dehydrated and embedded in polywax. 40 µm coronal sections were cut, and every fifth section stained with Nissl. To calculate RHV, the area of hippocampus and remainder of the telencephalon was measured in each section and volumes computed using a WACOM digitizing tablet and NIH Image® software . The results support the predictions. In the breeding season, shiny cowbird females had larger RHV than the males, whereas there were no sex differences in RHV in screaming or bay winged cowbirds, but screaming cowbirds had larger RHV than did baywing cowbirds. Comparisons of RHV in the breeding and non-breeding season show that both male and female screaming cowbirds had larger RHV in the breeding season whereas only female shiny cowbirds had significantly larger RHV in the breeding season , and in bay winged cowbirds there was no evidence of a seasonal difference for either sex.

UC Davis New Faculty Start Up Funds.

LEARNING AND MEMORY: PHYSIOLOGY III

548.1

SINGLE UNIT ACTIVITY IN MEDIAL PREFRONTAL CORTEX OF RATS PERFORMING AN OPERANT VIGILANCE TASK. K. Hutchinson*, M. Sarter, and B. Givens. Neuroscience Program. Ohio State University, Columbus, OH 43210.

The medial prefrontal cortex (mPFC) is thought to be critical for sustained attention. To examine this function of the mPFC, single unit activity was recorded from prelimbic cortex in rats performing a vigilance task that required the detection of brief visual signals (25-500ms) and the discrimination of signal from nonsignal events. Under control conditions, neural activity was not well correlated (5/75 cells) with specific task events (stimulus onset or behavioral responses). With the addition of a distractor stimulus (1 Hz flashing houselight), a reliable suppression of neural activity, as well as a decrease in vigilance performance, was observed. The suppression of neural activity by the distractor may be mediated by increases in acetylcholine (see Sarter et al, Cog. Brain Res., 1996). Preliminary attempts to manipulate the cholinergic inputs to the mPFC by local infusion of scopolamine during unit recording suggest an interaction between acetylcholine and suppression of single unit activity produced by the distractor stimulus. Current experiments assess mPFC unit activity before and after local cholinergic deafferentation with 192 IGg Saporin. In control experiments, task related cells showed correlated unit activity to the presence of the light (7/13 cells) in a visual discrimination task, whereas activity correlated to the motor response (9/14 cells) in a variable interval task. The data indicate that the behavioral correlates of mPFC neural activity depend on task requirements and also suggest that cholinergic input may have a role in vigilance-related changes in neural activity. *Supported by ABMRF.

548.2

CONDITIONED DOPAMINE RELEASE WITHIN THE PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS. J.B. Mitchell* P.D. Callahan, B.L. Croce and R.J. Bierwirth. Dept. of Psychology, Boston College, Chestnut Hill, MA 02167.

We investigated the effect of classical conditioning on dopamine (DA) release within mesocorticolimbic terminal fields, the medial prefrontal cortex (mPFC) and nucleus accumbens, core (NAC). Male rats received 8 pairings of a conditioned stimulus (CS; light or tone) with an unconditioned stimulus (UCS; food). During each pairing, the CS was presented and 2 min later the UCS was delivered. The CS was left on for the 10 min during which the subject was allowed to eat. Extracellular DA concentrations were measured within the NAC and the mPFC using high speed chronoamperometry. The electrochemical signal was obtained by applying a +0.55 V pulse, relative to a Ag/AgCl reference electrode, to a carbon fiber electrode, at a rate of 5 Hz. Exposure to a CS that had previously been paired with food elicited an increase in the electrochemical signal within both the NAC and the mPFC. The increase during exposure to the CS was larger within the NAC than within the mPFC ($p < .05$), and the response to the CS was smaller than the response to food within both areas ($p < .05$). Interestingly, the temporal characteristics of the response differed between the two areas. Within the mPFC the signal reached maximum amplitude more quickly (113.2 ± 38.3 sec) than within the NAC (300.8 ± 90.6 sec). The response also returned to baseline more quickly within the mPFC than within the NAC (205.2 ± 36.7 and 414.0 ± 88.4 sec, respectively). That is, although presentation of the food-paired CS increased the electrochemical signal within both the NAC and the mPFC, the NAC showed a larger and longer lasting response while the response within the mPFC corresponded, at least approximately, to the time during training when the subject had experienced the CS prior to UCS delivery. These results indicate that stimuli that have been paired with a naturally rewarding stimulus increase activity within the mesocorticolimbic DA system.
Supported by Boston College.

548.3

ACTIVITY OF DOPAMINE NEURONS DURING LEARNING IN A FAMILIAR TASK CONTEXT. J. Hollerman* and W. Schultz. Institut de Physiologie, Univ. Fribourg, CH-1700 Fribourg, Switzerland.

Midbrain dopamine (DA) neurons were recorded in two monkeys during performance of a simultaneous visual discrimination task using either familiar stimuli, which had established significance, or novel stimuli, which had to be learned by trial and error. Consistent with previous reports, DA neuron responses to liquid reward were reduced during established task performance with familiar stimuli in comparison to reward delivered unpredictably outside the task. However, when novel stimuli were introduced DA neuron responsiveness to task reward was transiently reinstated despite the familiar task context. Responsiveness was most elevated before the subjects exhibited clear signs of learning of the significance of the novel stimuli, but was frequently maintained for a number of additional trials. These data suggest that a decrease in the predictability of reward, even in a familiar context, results in larger DA neuronal responsiveness to reward. Furthermore, a period of consistent stimulus-reward pairing is necessary for DA responses to a predicted reward to disappear. The partial predictability of reward during learning of the novel stimuli was reflected in a depression of activity observed in erroneous trials at the time reward would have been delivered for a correct behavioral response. The influence of temporal predicability was tested using familiar stimuli but varying the reward delay. Activations were increased when reward was delivered earlier or later than anticipated. Depressions were observed at the normal time of rewar only if reward delivery was late. Thus DA neurons respond with activation to reward delivery when its occurrence or the time of its occurrence (or both) is not reliably predicted. In a complementary manner, DA neuronal activity is depressed when a predicted reward fails to occur at (or before) the predicted time. Thus DA neurons signal a mismatch between the prediction of reward and its occurrence. The fact that such mismatches and corresponding DA signals occur most frequently in learning situations may provide the long sought link between DA and learning. (Supported by Swiss NSF, Roche Research Foundation and NIMH MH10282)

548.4

NEURONAL ACTIVITY IN PRIMATE ORBITOFRONTAL CORTEX DURING LEARNING. L. Tremblay* and W. Schultz. Institut de Physiologie, Univ. Fribourg, CH-1700 Fribourg, Switzerland.

On the basis of lesion effects and anatomical connections, a major function of orbitofrontal cortex appears to concern the motivational control of voluntary behavior. As the function of motivation becomes particularly apparent during the adaptation of behavior to new contingencies, we investigated the activity of single orbitofrontal neurons in a learning set situation in which animals repeatedly adapted to new stimulus-response associations within a known task structure.

The underlying task was a delayed go-nogo motor paradigm in which both the behavioral reaction and the obtained outcome were indicated by an initial instruction picture. Animals reacted to a subsequent uniform trigger stimulus with one of three possible reactions involving, respectively, juice-rewarded movement, juice-rewarded non-movement and unrewarded movement. During learning trials, new instruction pictures were used while the rest of the task remained unaltered, and animals had to associate the new instruction pictures with the know behavioral reactions and outcomes. In two monkeys, 148 neurons were studied in both familiar and learning situations. Of these, 95 neurons responded to instruction stimuli, 44 responded to juice reward and 38 showed activity anticipating juice reward. During learning, most responses to instructions increased strongly (56/95). They initially lost selectivity for the behavioral reaction or trial outcome and regained it many trials after the monkey had learned the new significances. Instruction responses often decreased or completely disappeared when animals failed to learn the new instruction pictures (15/95). By contrast, reward-related activations showed little changes during learning. Most reward responses (26/44) and reward anticipatory activations (21/38) showed similar amplitudes, durations and selectivities in learning and familiar trials. Interestingly, reward anticipatory activity occurred initially also in unrewarded trials but abruptly disappeared when this trial type was acquired. These data suggest a participation of orbitofrontal cortex in associating environmental signals with behavioral reactions and predicted outcomes of behavior. Supported by Swiss NSF and FRS of Quebec.

548.5

DECREASE OF GLUTAMATE RELEASE DURING THE DELAYED ALTERNATION TASK PERFORMANCE IN THE DORSOLATERAL PREFRONTAL CORTEX OF THE MONKEY - RELATIONSHIP BETWEEN GLUTAMATE AND DOPAMINE RELEASES

T. Kodama*, M. Watanabe, M. Odagiri and K. Hikosaka Dept. Psychol., Tokyo Metropol. Inst. for Neurosci., Musashidai 2-6, Fuchu, Tokyo 183, JAPAN

We reported, in the companion presentation, the increase of dopamine release during the delayed alternation (DA) task in the dorsolateral prefrontal cortex (PFC) of the monkey. Since glutamate is a major excitatory neurotransmitter in the mammalian CNS, we investigated further the glutamate release during this working memory task, examining also possible relationship between dopamine and glutamate releases.

Two monkeys, which had been trained on both DA and sensory-guided control (CON) tasks, were served for this experiment. Extracellular fluids were obtained from the dorsolateral PFC by in vivo microdialysis method during the animal's task performance. Levels of dopamine and glutamate in the fluids were determined by the high-performance liquid chromatography (EICOM, Kyoto).

Significant decrease of glutamate release was observed during the DA as compared to CON in the dorsolateral PFC. Two way analysis of variance (task by neurotransmitter) indicated that there was a significant interaction between them. The results suggest that there is a mechanism which coordinates glutamate and dopamine releases during the spatial working memory task.

This study was supported by the grant from the Ministry of Education, Science and Culture of Japan (No. 05610081, 0661008).

548.6

INCREASE OF DOPAMINE RELEASE DURING THE DELAYED ALTERNATION TASK PERFORMANCE IN THE DORSOLATERAL PREFRONTAL CORTEX OF THE MONKEY – AN IN VIVO MICRODIALYSIS STUDY

M. Watanabe*, T. Kodama, M. Odagiri and K. Hikosaka Dept. Psychol., Tokyo Metropol. Inst. for Neurosci., Musashidai 2-6, Fuchu, Tokyo 183 JAPAN

Dopamine in the primate prefrontal cortex (PFC) plays important roles in the learning and performance of the delayed response (DR) and delayed alternation (DA) tasks; depletion of dopamine in the PFC induces impairments in DR and DA, and iontophoretic application of dopamine agonist and antagonist modulates task-related activity of PFC neurons. However, there has been no study examining dopamine release in relation to DR or DA in the PFC.

We trained three monkeys on both DA and sensory-guided control (CON) tasks. The animal had to respond to right and left keys alternately being intervened by a 5 s delay period in DA, while it was required to respond to the key which was being indicated by the cue after each 5 s delay period in CON. Thus, the animal had to retain the spatial working memory in DA, but not in CON. Brain extracellular fluid samples were obtained by in vivo microdialysis method from dorsolateral, arcuate, orbital and premotor areas of the frontal cortex.

Significant increase of dopamine release was observed during the DA as compared to CON in the dorsolateral PFC, while no such significant increase was observed in other areas of the frontal cortex. The results indicate that dorsolateral area of the PFC is the focus of the dopamine release during the spatial working memory task.

This study was supported by the grant from the Ministry of Education, Science and Culture of Japan (No. 05610081, 0661008).

548.7

MNEMONIC CODING AND OTHER FUNCTIONAL NEURONAL PROCESSING IN THE MONKEY PREFRONTAL CORTEX.

S. Carlson*, P. Rämä, H. Tanila, I. Linnankoski, H. Mansikka. Inst. Biomedicine, Dept. Physiology, University of Helsinki, Finland.

The role of the prefrontal cortex (PFC) in spatial working memory processing has been demonstrated in many experimental studies on monkeys as well as on humans. Several studies have also shown that the PFC responds to sensory stimulation outside of memory task context. In the present work we recorded single neuron activity in the PFC of monkeys in two experimental situations: while monkeys were performing a delayed alternation task (DA) and while they performed no task but visual, auditory and somatosensory stimuli were presented to them.
The data were obtained from three monkeys trained to perform the DA task. From the total number of neurons recorded 32 % fired in relation to the task performance. Three types of task related neuronal activity were recorded: delay-, delay and movement -, and movement-related activity. The majority (70%) of the delay-related neurons could not be activated by any of the sensory stimuli used and did not fire in relation to the movements of the monkey. On the other hand, the majority of all neurons (67%) responding to stimulation outside of the task context did not fire in relation to the DA task. Furthermore, 42 % of all recorded neurons responded neither to the task performance nor to the various stimuli. These results demonstrated that in addition to its role in working memory processing the PFC exhibits responsiveness to many kinds of visual stimuli and also possesses functions neither related to spatial memory processing nor to simple sensory responsiveness.

548.8

NEURONAL NETWORKS RELATED TO WORKING MEMORY PROCESSES IN THE PRIMATE PREFRONTAL CORTEX REVEALED BY CROSS-CORRELATION ANALYSIS.

S. Funahashi*, S. Hara, and M. Inoue. Dept. of Cognitive Sci., Grad. Sch. of Human & Environment. Studies, Kyoto Univ., Kyoto 606-01, Japan.

To examine neuronal networks related to spatial working memory processes in the primate prefrontal cortex, we analyzed multiple-neuron activity while monkeys performed an oculomotor delayed-response task with 8 cue positions. We isolated 2 or 3 single-neuron activities from multiple-neuron activity, determined each neuron's function in relation to the task events, and made cross-correlograms between pairs of isolated neurons to determine an input-output relationship.

An excitatory correlation was found in the majority of the neuron pairs examined, although some pairs exhibited an inhibitory correlation and the remaining showed no correlation. Most of the cross-correlograms were asymmetrical with peaks at latencies of 1~2 ms, suggesting that the effects are mainly mono-synaptic. An excitatory correlation was found from neurons having a visual response to neurons having delay activation or an oculomotor response and from neurons having delay activation to neurons having delay activation or an oculomotor response. These neuron pairs showed similar tuning characteristics. These results indicate the presence of information flow from neurons which receive sensory input to neurons which output motor information through neurons which store mnemonic information in the prefrontal cortex. Furthermore, the presence of excitatory correlation among neurons having delay activation suggests that these interactions play an important role in processing and integrating information stored in working memory. (Supported by Japanese Ministry of Education Grant No. 07680871, 07252216)

548.9

PREFRONTAL CORTEX UNITS IN AN AUDIO-VISUAL DELAY TASK

Mark Bodner, James K. Kroger, Tony Liang, and Joaquín M. Fuster *. Dept. Psychiatry and Brain Research Inst., UCLA School of Medicine, Los Angeles, CA 90024.

The dorsolateral prefrontal cortex (DPC) is essential for the bridging of temporal gaps between perception and consequent action. The DPC performs this function by supporting the cortical processes of working memory and motor set (Fuster, 1989). Previously, DPC neurons were seen to participate in the memory of visual and spatial stimuli for prospective (delayed) motor response. The main purpose of this research is to examine the role of DPC units in working memory of the auditory modality. Single units are recorded from DPC in monkeys performing an audio-visual task with a delay. On each trial, the animal is presented with a tone of high (3000 cps) or low (240 cps) pitch. After a 10-sec delay (auditory retention period), two color lights (red and green) appear simultaneously in two side-by-side stimulus-response buttons (relative position of colors changing randomly). If pitch was high, the animal must press red for liquid reward; if pitch was low, it must press green. Cells in the region of the sulcus principalis show firing frequency changes related to one of more of the task's events: (a) auditory cue; (b) visual cue; (c) motor response; (d) reinforcement. Auditory "memory cells" have been observed: these cells show sustained elevated firing during the delay. In some, the delay-elevated firing is selective for one tone. In conclusion, DPC units respond to the auditory and visual stimuli that the animal utilizes in a cross-modal and cross-temporal task. Some of those units appear related to the working memory of auditory information. These findings highlight the supramodal character of the functions of DPC and their role in the mediation of cross-temporal contingencies. (NIMH)

548.10

A NEURAL LEARNING MODEL BASED ON THE ACTIVITY OF PRIMATE DOPAMINE NEURONS.

R.E. Suri and W. Schultz*, Institute of Physiology, University of Fribourg, CH-1700 Fribourg, Switzerland.

In physiological recordings from alert monkeys in our laboratory, midbrain dopamine neurons respond phasically to unpredicted rewards or to conditioned stimuli predicting rewards. The activity of dopamine neurons is depressed when the animal does not receive the predicted reward, suggesting that they code a deviation between predicted and actually occurring reward. Dopamine neurons were also recorded in more specific Pavlovian and instrumental conditioning situations: Discrimination between two stimuli, generalisation of conditioned stimuli, delay of predicted reward, reward earlier than predicted, variable CS2-CS1 interval and spatial delayed response behaviour.

A neural network model operating in time steps of 100 msec is presented, which reproduces the responses of midbrain dopamine neurons and the behaviour of the monkey in all these investigated situations. The sensory input is pre-processed by an attention mechanism, which allows only the stimulus with the highest motivational and physical salience to be represented in short term memory. This compressed sensory representation serves as input to two adaptive components of the model, emitting a global predictive reinforcement signal and controlling behavioural output, respectively. The predictive reinforcement signal very much resembles the biological activity of dopamine neurons and serves for learning the motivational salience of stimuli, the stimulus-reward association and the stimulus-action association. It is computed by temporal changes in the prediction of reinforcement (temporal difference learning according to Sutton & Barto 1981).

The model implicates that the diffusely projecting dopamine neurons are involved in attention and predictive learning. We suggest that the model may be successful in learning of behavioural sequences, discrimination between stimuli and generalisation.

Supported by the Swiss NSF and the McDonnell-Pew Foundation.

548.11

REDUCTIONS IN ORBITOFRONTAL GLUCOSE METABOLISM ARE ASSOCIATED WITH MEMORY DEFICITS IN AGED RHESUS MACAQUES.
J.A. Roberts, J.L. Eberling, P.R. Rapp, M.H. Tuszynski, W.J. Jagust*. California Regional Primate Research Center, University of California, Davis, CA 95616; Center for Functional Imaging, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720; Ctr For Behav Neurosci, SUNY - Stony Brook, Stony Brook, NY 11794; Dept. Neurosci., University of California - San Diego, La Jolla, CA 92093-0608.

Aged nonhuman primates show increased variability in a variety of neurobiological measures, including brain volume, brain weight, neurochemical measures, and regional cerebral glucose metabolism (rCMRglc). This variability is paralleled by variability in memory performance. We used positron emission tomography (PET) and ^{18}F-fluorodeoxyglucose (FDG) to evaluate the relationship between rCMRglc and performance on a delayed response (DR) task in 10 aged rhesus macaques. Performance on the DR task was assessed within two weeks of the PET study, and animals were identified as either memory impaired or memory unimpaired based on the comparison of their performance to the performance of young control animals. Using a high-resolution PET scanner, we scanned 6 brain levels. To correct for global scaling effects and to reduce the between subject variance, rCMRglc ratios were constructed by dividing rCMRglc values for each region of interest by rCMRglc value for the entire brain. Unpaired one-tailed t-tests were used to determine if impaired animals showed reductions in rCMRglc ratios relative to unimpaired animals in temporal cortex, prefrontal cortex, orbital frontal cortex or hippocampus. The impaired animals showed signficantly ($p<0.05$) lower rCMRglc ratios in orbitofrontal cortex than the unimpaired animals. No other significant changes were observed. These fingings suggest that behavioral deficits in aged animals may reflect the differential vulnerability of specific brain regions, and support the use of PET to evaluate relationships between brain physiology and behavior in the aged nonhuman primate model. (Supported by NIH grants AG07793 and AG10606).

548.12

POSITION SENSITIVITY OF HIPPOCAMPAL AND INFEROTEMPORAL UNITS.
Anna Nowicka*[1,2], James L. Ringo[1] and Stephen O'Neill[1]. [1]University of Rochester, Rochester NY 14642; [2]Nencki Institute of Experimental Biology, 02-093 Warsaw, Poland.

In a visual discrimination task, head-restrained macaques had their eye movements monitored with the scleral search coil technique. The monkeys had to move their eyes to one of five different positions to maintain gaze on an image. The image was then extinguished and the monkeys maintained fixed gaze on the target position in the dark. About 20% of single units recorded from the hippocampus showed statistically significant sensitivity to target position in the dark. A similar fraction were significantly affected by target position in the light, (even though fixating the same visual images). A slightly lower fraction of inferotemporal units also showed target position sensitivity.

The five positions were on a horizontal line, consisting of a center position and 10 and 20 degrees right and left of it. The fields appeared in a variety of orderly types, some showing greater activity for targets in the middle (inverted U shaped), greater activity for the side targets (U shaped), greater activity for right side target, or greater activity for left side targets. No units showed patchy fields. For most of these cells, the firing rates differed by a factor of two or more between the positions with most and least activity. Supported by grant NS26526 from NINDS.

548.13

TIME-DEPENDENT INCREASES IN DISCRIMINATIVE AVOIDANCE RESPONSES AND SHIFTS OF TRAINING-INDUCED NEURONAL ACTIVITY IN LIMBIC THALAMUS.
M. Gabriel* and J. H. Freeman, Jr. Department of Psychology and Beckman Institute, University of Illinois, Urbana, IL 61801

Neuronal discharges in response to a conditional stimulus in various layers of cingulate cortex and nuclei of limbic thalamus (anterior and mediodorsal nuclei) attain maximum (peak) frequency in particular stages (early, intermediate or late) of discriminative avoidance learning (Gabriel *et al., Behav. Brain Res.*, 46 (1991) 175-185). Here we asked whether the area-specific peaks of training-induced neuronal activity (TIA) change with the passage of time alone, as in memory consolidation, or require the repetition of training trials (practice). Multi-unit activity was recorded in the cingulate cortex and the anterodorsal (AD), anteroventral (AV) and mediodorsal (MD) thalamic nuclei as rabbits learned to prevent a foot-shock by stepping in an activity wheel after one tone (CS+) and to ignore a different tone (CS-). Two groups of rabbits were given a single session of training (S1, 120 trials) followed by a second session (S2) either immediately or 48 hours later. The frequency of avoidance CRs increased from S1 to S2 significantly more in the group given a 48-hour delay than in the group given a 0-hour delay ($p < 0.03$), replicating the "incubation effect" (Gabriel, *J. Comp. Physiol. Psychol.*, 66 (1968) 412-416). AD and MD thalamic TIA increased from S1 to S2 in the 0-hour group but not in the 48-hour group ($p < 0.02$, $p < 0.01$). AV thalamic TIA increased from S1 to S2 in the 48-hour group but not in the 0-hour group ($p < 0.01$). Significant time-dependent TIA changes were not found in either the anterior or posterior cingulate cortex. Thus, changes of thalamic TIA, highly similar to previously found trial-related changes, can occur with the passage of time alone (Support: NIH NS26736).

548.14

EFFECTS OF ENTORHINAL CORTEX LESIONS ON DISCRIMINATION LEARNING, RESPONSE TO CONTEXT AND TRAINING-INDUCED NEURONAL ACTIVITY IN RABBITS.
J. H. Freeman, Jr.*, A. Weible, J. Rossi and M. Gabriel. Department of Psychology and Beckman Institute, University of Illinois, Urbana, IL 61801

Neuronal activity was recorded in the cingulate cortex, basolateral amygdala and hippocampus as rabbits learned to prevent a foot-shock by stepping in an activity wheel after one tone (CS+) and to ignore a different tone (CS-) after receiving either sham surgery or electrolytic lesions in the entorinal cortex. The surgery groups acquired avoidance CRs at the same rate. Discriminative (greater activity during the CS+ than the CS-) and excitatory (increased activity from pretraining) neuronal activity was seen in the entorhinal cortex during an intermediate stage of learning ($p < 0.01$). Entorhinal cortex lesions produced a deficit in the magnitude of CS-elicited neuronal discharges in the basolateral amygdala ($p < 0.01$) but enhanced neuronal activity in the hippocampus ($p < 0.01$). The training-related development of neuronal excitation and discrimination in the anterior and posterior cingulate cortex was unaffected by lesions in the entorhinal cortex. Both surgery groups showed significantly fewer CRs during an extinction test in a novel context relative to the original context ($p < 0.02$). Unlike the hippocampus, the entorhinal cortex does not play a role in the detection and behavioral response to novel background stimuli. However, the lesion group showed fewer inter-trial responses during the standard extinction test ($p < 0.01$), indicating an impairment in conditioning to the context. Amygdaloid neuronal discrimination was also impaired in the lesion group during the standard extinction test ($p < 0.05$). These results indicate that the entorhinal cortex and its connections with the amygdala are involved in conditioning to background stimuli. (Support: NIH grant NS26736).

548.15

NEURAL REPRESENTATION OF REWARD CONTINGENCY IN THE RAT ANTERIOR CINGULATE CORTEX.
K.Takenouchi[1], H. Nishijo[2], T.Uwano[2], M.Takigawa[1]* and T.Ono[2]. Dept. Physiol.[2], Fac. Med., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, and Dept. Neuropsychiatr.[1], Fac. Med., Kagoshima Univ., Kagoshima 890, Japan.

Neuronal activity was recorded from the anterior cingulate cortex (AC) of behaving rats during configural associative learning. The rats were trained to lick a protruding spout just after a conditioned stimulus (CS) to obtain reward [intracranial self-stimulation (ICSS), or sucrose solution] or to avoid shock. The CSs included both elemental (auditory or visual stimuli) and configural (simultaneous presentation of auditory and visual stimuli predicting reward outcome opposite to that predicted by each stimulus presented alone) stimuli. Of 62 AC neurons responding during the task, 38 and 4 responded differentially and nondifferentially to the CSs, respectively. Of the 38 differential neurons, 33 neurons showed excitatory (n=10) and inhibitory (n=23) responses selectively to the CSs predicting reward, and were located mainly in the Cg1 and the Cg3 of the rostral part of the AC, respectively. The remaining 20 neurons responded mainly during ICSS and/or ingestion of sucrose, and were located sparsely in the Cg1 of the rostral part of the AC and densely in the Fr2 of the caudal part of the AC. Analysis by the multidimensional scaling of 38 differential CS-related neurons categorized the CSs into 3 groups based on reward contingency regardless of physical characteristics of the stimuli in a two dimensional space; the 3 CSs (2 elemental and 1 configural) predicting sucrose solution, the 3 CSs (2 elemental and 1 configural) predicting no reward, and the lone CS predicting ICSS. The results suggest that the AC is topographically organized, and plays an important role in the processes from recognition of reward availavility of conditioned stimuli to execution of licking behavior.

548.16

REWARD CONTINGENCY-RELATED NEURONAL RESPONSES IN THE RAT LATERAL SEPTAL NUCLEI.
N. MATSUYAMA[1], H. NISHIJO[2], T. UWANO[2], R. TAMURA[2]*, T. ASAKURA[1] and T. ONO[2].
[2]Dept. Physiol., Fac. Med., Toyama Med. and Pharmaceu. Univ., Toyama 930-01, and [1]Dept. Neurosurg., Fac. Med., Kagoshima Univ., Kagoshima 890, Japan.

It has been suggested that the septal nuclei (SP) integrate information from the limbic system such as the amygdala, cingulate, entorhinal cortex, and hippocampal formation, and send those information to the lateral hypothalamus. In the present study, neuronal activity was recorded from the rat lateral SP during associative learning and discrimination of sensory stimuli (CSs) associated with or without reinforcements: sucrose solution and intracranial self-stimulation (ICSS). Sensory stimuli included auditory (1200, 2800, 4300 Hz), visual (light) and configural (simultaneous presentation of tone and light) stimuli. Of 174 SP neurons recorded, 51 responded with excitation (n=32) or inhibition (n=19) in one or more phases of the task. Of these 51 neurons, 14 responded selectively to CSs predicting reward (CS^+-related), 4 to CSs predicting nonreward (CS^--related), 9 nondifferentially to all CSs, and 15 responded mainly during the ingestion/ICSS phase. Activity of the CS^+- and CS^--related neurons changed flexibly based on the reward-contingency rather than physical characteristics of sensory stimuli. The responsive SP neurons were located mainly in the dorsal and intermediate parts of the lateral SP. These results suggest that the lateral SP is deeply involved in discrimination of reward-contingency of stimuli.

548.17

CHOLINERGIC FOREBRAIN MECHANISMS IN CORTICAL MAP REORGANIZATION. M.P. Kilgard* and M.M. Merzenich. Keck Center, UCSF, San Francisco, CA 94143.

Extensive frequency discrimination training can result in a several fold increase in the area of owl monkey auditory cortex representing the trained frequency. Electrical stimulation of the rat basal forebrain paired with tone presentation can increase single unit evoked responses for >30 minutes. The goal of this study was to determine whether electrical stimulation of the rat cholinergic basal forebrain (100-150uAmp 20 pulses 100Hz train) paired with tone presentation (250msec 50dB SPL) results in enduring auditory cortex reorganization. Rats were chronically implanted with bipolar electrodes and received 300-500 pairings per day for ~15days. The auditory cortex was then mapped under barbiturate anesthesia using 80-120 microelectrode penetrations. Multi-unit tuning curves were generated at each penetration site. Our preliminary findings demonstrate that pairing of stimulation of basal forebrain with tones was sufficient to drive largescale, enduring (>24 hours) cortical reorganization. The area of the cortical surface responding to the paired frequency was increased by >2.5-fold over naive controls. Sites near the middle of the expanded region often showed long latencies of 60-80msec, compared to 12-25msec for controls. Future studies will examine the effect of basal forebrain stimulation on the representation of more complex auditory stimuli, such as AM and FM modulated stimuli. [Supported by NIH grant NS-10414, Hearing Research, INC., and NSF predoctoral fellowship.]

548.18

AN OLFACTORY ASSOCIATIVE MEMORY TASK INVESTIGATING CHOLINERGIC MODULATION IN THE RAT PIRIFORM CORTEX. Bergman, R.E.*, De Rosa, E. and Hasselmo, M.E., Harvard University, Department of Psychology, Cambridge, Ma.

This behavioral study was conceived to investigate the role of acetylcholine (ACh) in learning an associative odor memory task. The two experimental tasks were specifically designed to compare learning odor pairs with and without shared components. In each of the experimental tasks two different odor cues were simultaneously presented from two separate sniff ports in a 16-channel flow dilution olfactometer. Rats were required to label each presented odor pair with a behavioral response - 'short' or 'long' - in order to receive reinforcement. The nonoverlapping (NO) task was comprised of odor pairs without shared components. An example of a NO task would be, odor pair AB was reinforced if short response, CD reinforced if long response, EF short etc. In the overlapping (O) task, comprised of odor pairs with shared components, every odor pair component was then labeled with a short and a long response e.g. AB short, AF long, EB long , EF short etc. The above study was designed to test the prediction that muscarinic cholinergic antagonists will decrease the rate of acquisition for the O task. This was predicted by two lines of evidence: (1) electrophysiological studies in the piriform cortex that have demonstrated that ACh selectively suppresses synaptic transmission at intrinsic fibers but not afferent fibers (Hasselmo & Bower, *J. of Neurophys.*, 67, 1222) and (2) computational modeling of these physiological findings suggesting that these effects of ACh may act to reduce interference between overlapping representations of information stored in memory (Hasselmo *et al* , *J of Neurophys.*, 67, 1230). The model predicts that a loss in cholinergic modulation would impair the learning of associations in odor pairs with overlapping components because the loss of cholinergic suppression would allow recall of previous stored associations to interfere with storage of new associations. We hypothesize that the muscarinic antagonist scopolamine should increase interference in the O task causing a greater deficit in acquiring the O task than the NO task.

548.19

MODELING THE FUNCTIONAL ROLE OF ACETYLCHOLINE IN THE HIPPOCAMPUS. G.V. Wallenstein, B.P. Wyble and M.E. Hasselmo*. Dept. of Psychology and Prog. in Neuroscience, Harvard University, Cambridge, MA 02138.

A biophysical model of area CA3 was employed to study how septal ACh interacts with cellular conductances to shape population behavior. Cholinergic effects were modeled by (i) blocking $I_{K(leak)}$; (ii) suppressing the adaptation current, $I_{K(AHP)}$; and (iii) reducing excitatory synaptic transmission between pyramidal cells. Under low $I_{K(AHP)}$ conditions, accurate completion (of a degraded input pattern) was impaired due to an increase in pyramidal cell firing not unique to the given input pattern. Moderate levels of the excitatory synaptic conductance ($\bar{g}_{AMPA}$), indirectly reduced the burst refractory period in pyramidal neurons and promoted the recruitment of large numbers of these cells into globally-synchronous firing resembling epileptiform-like events observed *in vitro*. Subsequently reducing $\bar{g}_{AMPA}$ to approximately 15% of its normal value resulted in successful completion, demonstrating accurate associative memory. An investigation of the $\bar{g}_{K(AHP)}$ - $\bar{g}_{AMPA}$ parameter space revealed the transition boundaries between these functionally different forms of behavior.

The effects of ACh were also investigated in a large-scale network model of the hippocampus. Systemic administration of scopolamine was modeled by blocking the cellular effects of acetylcholine, resulting in memory impairments replicating data from studies on human subjects. This blockade of cholinergic effects impaired the encoding of new input patterns (decreasing delayed free recall from 42% to 3%), but did not impair the delayed free recall of input patterns learned before the blockade. The impairment was selective to the free recall but not the recognition of stored items. The model contained simulations of entorhinal cortex, dentate gyrus, regions CA3 and CA1. Separate vectors representing item and context were presented sequentially to entorhinal cortex. Self-organization in the dentate gyrus formed sparse representations of these patterns, and associations between context and item representations were formed in region CA3. One context pattern was associated with each list item during presentation. Reactivation of context during retrieval caused a subset of the items to be recalled in order of strength. Attractor dynamics controlled activity within parts of the model, allowing recall and preventing the simultaneous activation of multiple items. - NIMH grant R29 MH52732 and ONR grant NO 0014-93-1-0595.

LEARNING AND MEMORY: PHYSIOLOGY IV

549.1

DEVELOPMENT OF A TECHNOLOGY THAT ALLOWS GENE KNOCKOUT ONLY IN THE FOREBRAIN.

Cindy W. Tom[1,2], Joe Z. Tsien[1], Chong Chen*[1,2], Mark Mayford[3], Eric R. Kandel[3] and Susumu Tonegawa[1,2]. [1]H.H.M.I., [2]Ctr for Learning & Memory, MIT, Cambridge, MA 02139, [3]Ctr for Neurobiol. & Behavior, Columbia Univ., New York, NY 10032.

The conventional gene knockout technology results in ubiquitous deletion of the gene of interest throughout the body. It is often desirable to restrict the deletion to cells of a specific tissue or type. We report our progress in developing such a tissue/cell type-restricted gene targeting method. We used the α-Cam kinase II promoter to drive Cre expression because this promoter allows for selective expression of a transgene in forebrain regions. Several Cre transgenic lines were generated and crossed to another transgenic mouse line, provided by Mercer & Anderson, in which the expression of LacZ transgene depends on Cre/LoxP-mediated recombination. Histological analysis of brain sections stained with X-gal allows identification of several Cre transgenic lines, in which Cre/LoxP-mediated recombination is restricted to several forebrain regions. These Cre transgenic lines should be useful to target gene deletion to the forebrain or its subregions.

Supported by NIH R35-C8-53874 grant and a HHMI grant.

549.2

BEHAVIORAL AND ELECTROPHYSIOLOGICAL ANALYSIS OF MICE LACKING THE CREM GENE.

Julie A. Blendy*[1], Jeffrey H. Kogan[2], Gunther Schutz[1] and Alcino J. Silva[2]. [1]German Cancer Research Center, University of Heidelberg, D-69120 Heidelberg, Germany. [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

The cAMP signaling pathway has been implicated in a number of biological processes. CREB and CREM are members of the leucine zipper transcription factor family which are activated in response to increases in intracellular cAMP levels. Previous studies have shown that mice with a targeted mutation of the CREB gene have deficient long-term memory. In addition, hippocampal long-term potentiation (LTP) is impaired in these mice (Bourtchuladze et al., Cell 79:59-68, 1994).

We have recently generated mice with a null mutation of the CREM gene. These mice appear healthy, and exhibit no obvious impairment of growth or development. However, the males are sterile due to an arrest of spermatogenesis. To begin to address the role of CREM in central nervous system function we tested these mice in a contextual fear conditioning task in which animals are trained to associate a particular context with an aversive stimulus. Conditioned animals, when exposed to the context, refrain from all but respiratory movements. This behavior, termed freezing, can be used to determine the degree of learning and memory in an animal. A single 3 min training trial resulted in similar levels of freezing in wild type and mutant mice immediately following the shock. In addition, there appears to be no difference in freezing when mice are tested 30 minutes post training. Furthermore, when mice are returned to the same context 24 hours later, wild type and mutant animals do not display significant differences in freezing behavior. These results are in contrast to the earlier findings with CREB mutants which show deficient long-term (24 hour) memory. The CREM mice will be further examined in the spatial version of the Morris water maze and electrophysiological studies of synaptic transmission and LTP in hippocampal brain slices will be conducted.

(Supported by grants from the Whitehall, Beckman, Klingenstein, Merck and McKnight Foundations)

549.3

EFFECTS OF INTER-TRIAL INTERVAL ON THE MEMORY AND THE LONG-TERM POTENTIATION DEFICITS OF CREB MUTANT MICE.
Jeffrey H. Kogan*[1], Jodi Coblentz[1], Zach Marowitz[1], Julie A. Blendy[2], Gunther Schutz[2], and Alcino J. Silva[1]. [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA. [2]German Cancer Research Center, University of Heidelberg, D-69120 Heidelberg, Germany.

Studies in Aplysia, Drosophila and mice indicate that the transcription factor CREB plays a key role in both long-term memory and synaptic plasticity. We have previously shown that mice with a targeted disruption of the α and the δ isoforms of CREB are deficient in both long-term memory and hippocampal long-term potentiation (LTP). Findings in Aplysia and Drosophila suggest that increases in CREB activity can eliminate the requirement for multiple training sessions with long intervals between training trials to elicit long-term memory. We have found that CREB mutant mice, bred into a C57BL/6x129Sv background, trained in the spatial version of the Morris water maze at two trials/day with extended inter-trial intervals perform normally. In contrast, mutants trained at two trials/day with short inter-trial intervals perform poorly compared to controls. Furthermore, CREB mutants intensively trained in the water maze (12 trials/day for 5 days) perform as well as controls and have normal long-term memory when tested at 28 days.

We have also found that long, but not short, inter-trial intervals can elicit normal contextual long-term memory in CREB mutant mice bred into a C57BL/6 background. In addition, control levels of both early-, and late-, CA1 LTP can be induced in CREB mutant neurons with spaced tetani (3X10 theta bursts at 10 min intervals), but not with massed tetani (3X10 theta bursts at 1 min intervals). In controls, both of these tetani can trigger a late-LTP that is protein synthesis-dependent. On the other hand, protein synthesis-independent LTP, induced with a single tetanus (100 Hz/1 sec, 100 μsec pulse width) is identical in mutants and controls. These results indicate a role for CREB-mediated transcription in both LTP and in the formation of long-term memory. *(Supported by grants from the Whitehall, Beckman, Klingenstein, Merck and McKnight Foundations)*

549.4

THE NF1 MUTATION AFFECTS HIPPOCAMPAL SYNAPTIC FUNCTION AND HIPPOCAMPAL-DEPENDENT LEARNING.
[1]N. Fedorov, [1]P. Frankland, [1]R. Bourtchuladze, [1]Z. Wang, [1]Z. Marowitz, [1]F. Lee, [1]G. Lazlo, [2]T. Jacks and [1] A. J. Silva.*[1]Cold Spring Harbor Laboratory, New York 11724, [2]Massachusetts Institute of Technology, Boston MA, 02139.

Approximately half of children with neurofibromatosis type I (NF1) have learning disabilities of unknown etiology. These cognitive deficits are due to heterozygous mutations in NF1, a GTPase activating protein. Since NF1 is highly expressed in the hippocampus, we concentrated our studies of NF1 (heterozygous) mice on learning and memory tasks sensitive to lesions of this structure (water maze). Our studies showed that NF1 mice have specific spatial learning deficits, even though we and others could not identify any significant brain neuroanatomic pathology. NF1 mice, as NF1 patients, do not have a complete loss of spatial abilities. Instead, we found that the NF1 mice need much more training than littermate controls to reach comparable levels of performance. Control tests indicated that vision, motor coordination, and motivation are normal in these mice. Interestingly, similar to NF1 patients, memory seems to be unaffected by the NF1 mutation in mice: Once trained the NF1 mutants retain spatial information as well as controls. Electrophysiological studies in the hippocampal CA1 region of NF1 mice showed that PPF, PTP, synaptic depletion rates, levels of axonal depolarization (pre-synaptic volleys), and the probability of neurotransmitter release are normal in these mutants. However, for a given stimulation strength, the slope of the EPSPs is smaller in the mutants (>40%), suggesting that the NF1 mutation affects post-synaptic function. These results suggest that a deficit in synaptic transmission may underlies the learning abnormalities in NF1 patients.
This work was supported by the Whitehall, Merck and Beckman Foundations.

549.5

THE INVOLVEMENT OF THE NF1/RAS PATHWAY IN HIPPOCAMPAL FUNCTION.
[1]A. J. Silva, [1]K.P. Giese, [1]P. Chen, [2]W. Edelmann, [2]R. Kucherlapati, [1,3] P. Chapman,*[4]R. Kuhn, and [1]N. Fedorov.
[1]Cold Spring Harbor Laboratory, New York 11724, [2]Albert Einstein College of Medicine, Bronx, NY 10461, [3]Cardiff University of Wales, Cardiff CF13US, UK,[4]University of Köln, 50931 Köln, FRG.

Our behavioral and electrophysiological studies have implicated the NF1 GTPase activating protein in hippocampal function. NF1 is known to regulate ras, a family of small GTP-binding proteins. Our electrophysiological studies showed that mice lacking N-ras (an isoform present in the brain) have larger hippocampal EPSPs than controls (>150%). Interestingly, NF1 mutant mice, which presumably have higher levels of active ras, show smaller EPSPs than controls. In both mutants, synaptic plasticity appears normal (PPF, LTP and its reversal). Behavioral studies found that both NF1 mice and N-ras mutants show clear deficits in the hidden-platform version of the water maze, but not on the visible-platform test. These results indicate that a disruption of N-ras results in abnormal hippocampal function. To further investigate the role of the ras signaling pathway in learning/memory we generated mutant mice deficient for the guanine-nucleotide releasing factor (GRF). GRF is expressed exclusively in brain and is one of the rate-limiting factors for ras activation. With the mutants described above, it is impossible to restrict the mutations to chosen tissues and to specific time-windows. To overcome these limitations we have been developing an inducible gene targeting approach. It uses the Cre recombinase fused with the ligand-binding domain (LBD) of modified steroid receptor proteins. Upon ligand-driven induction the mutation should be restricted to the specific region(s) where the fusion construct is expressed (i.e. in the hippocampus) and to a chosen time (i.e., in adults after induction). We are currently testing this methodology in mice.
This work was supported by the Whitehall, Merck and Beckman Foundations.

549.6

MEMORY INTEGRATION IN COMPLEX LEARNING: A NOVEL NETWORK MODELING APPROACH.
Y. M. Yufik, M. Voloshin[(1)], V. Zhukarev*[(2)], Inst. Medical Cybernetics, Inc. Potomac[(1)], MD 20854, UMD NJ/RWJ MS[(2)], Piscataway, NJ 08854.

Neuropsychological processes underlying human capability to integrate diverse partial experiences into coherent memory structures are not fully understood. A new model called Virtual Network (VN) (Yufik, 1994; Yufik & Sheridan, 1995) defines memory integration mechanisms in complex learning. Learning produces a hierarchy of networks in the associative cortex. Formation of networks is accompanied by their spontaneous self-partitioning into flexible, internally cohesive and weakly coupled subnets, or packets. The principles of network self-partitioning have been derived from the studies of neuronal ensembles (Bechtereva, 1988) and the models of motor control mechanisms in basal ganglia (Voloshin, 1994). The VN model treats associative packets and networks as physical structures subject to the laws of thermodynamics. Packet stability is determined by the balance of associative forces inside the packet and at the boundaries, and is influenced by the specific and non-specific (emotional) performance feedback represented as the "temperature factor." The model explains successfully some main features of complex learning in norm and pathology, and predicts the degree of cognitive complexity of learning and control tasks. A new method of computer memory management derived from the VN model demonstrates better than two orders of magnitude improvement in the performance of control algorithms in comparison with conventional memory.

Sources of funding: NASA, U.S. Army, NSF.

549.7

SPATIAL LEARNING AND HIPPOCAMPAL LONG-TERM POTENTIATION IN MICE LACKING ZFP37.
M. Nosten-Bertrand[1], S. Davis[2], C. de Zeeuw[4], B. Koekkoek[4], D. Michalovich[3], N. Faucon Biguet[1]*, J. Mallet[1], S. Laroche[2], F. Grosveld[3], N. Galjart[3] (1) LGN, CNRS UMR9923, Hôpital de la Pitié Salpêtrière, 75013 Paris, France; (2) CNRS URA 1491, Univ. Paris-Sud, 91405 Orsay, France; (3) Dept. of Cell Biology and Genetics and (4) Dept. of Anatomy, Erasmus Univ., 3000 DR Rotterdam, The Netherlands.

Murine Zfp37 is a member of a large family of C2H2-type zinc finger proteins. It is characterised by two domains: the zinc finger region at its C-terminus, which may be involved in nucleic acid binding or in protein-protein interactions, and a so-called KRAB domain at its N-terminus, which could function as a transcriptional repressor region. An important target of Zfp37 expression are neurons of the adult CNS. To investigate the function of this gene, mutant mice expressing bacterial beta-galactosidase instead of Zfp37 were generated. Homozygous mutant animals are healthy, fertile and no abnormal housing behavior was detected.

Since Zfp37 is highly expressed in the hippocampus, we decided to investigate spatial learning and memory tested in the watermaze in Zfp37 homozygous knock out mice. Preliminary data show that the Zfp37-deficient mice are impaired in spatial learning compared with their wildtype littermates. Their ability to express long-term potentiation in the perforant path input to the dentate gyrus and the Schaffer-commissural input to the CA1 in vivo is currently under investigation. (Supported by CNRS)

549.8

CONTROLLING LEARNING AND MEMORY USING TETRACYCLINE-REGULATED EXPRESSION OF A Ca^{2+}-INDEPENDENT CaMKII TRANSGENE.
M.E. Bach,* E.R. Kandel, R.D. Hawkins, and M. Mayford. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032.

To gain temporal control over when a gene is expressed in genetically modified mice and thereby circumvent developmental effects we employed the tetracycline regulatable tTA system. When the tetracycline analog doxycycline is administered in the drinking water, expression of a Ca^{2+}-independent CaMKII transgene is blocked. When the drug is withdrawn, expression is restored. Two lines of transgenic mice were generated. In the first line moderate transgene expression was found in the striatum, amygdala, cortex, and the CA1 region of the hippocampus, whereas in the second line the transgene was strongly expressed but only in the striatum and lateral amygdala. Both lines were assessed on the conditioned fear task in which a novel context and a distinct cue are paired with an aversive stimulus. If learning occurs, then both the context and cue will elicit fear as evidenced by the behavior freezing. Transgenic mice in the first line did not exhibit a conditioned fear deficit whereas in the second line a severe impairment in both context and cued conditioning was observed. When mice in the second line were treated with doxycycline to shut off the transgene, no learning impairment was observed. However, when we reactivated the transgene and then assessed fear retention six weeks later a severe deficit in freezing was observed. We next measured unconditioned freezing in the second line and found transgenic mice overexpressing CaMKII froze similarly to wild-type mice. This suggests that the conditioned fear impairment observed in mice overexpressing CaMKII may be due to a deficit in learning or memory consolidation and not a performance deficit. These results also demonstrate that the transgene does not exert its effect on learning and memory through an indirect effect on neuronal development. Supported by HHMI & NIMH.

549.9

THE ROLE OF THE AUTOPHOSPHORYLATION OF αCaMKII AND OF THE POTASSIUM CHANNEL SUBUNIT Kvß1 FOR LEARNING AND MEMORY, K.P. Giese*[1], N.B. Fedorov[1], S. Li-Rong[2], D. Reuter[3], T. Leicher[3], J.F. Storm[2], O. Pongs[3] and A.J. Silva[1]. [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA, [2]Institute for Neurophysiology, Oslo N 0317, Norway, [3]Center for Molecular Neurobiology, 20246 Hamburg, Germany.

The α-isoform of the Ca^{2+}/calmodulin-dependent kinase II (αCaMKII) can autophosphorylate at T286 which leads to trapping of Ca^{2+}/calmodulin (CaM) and gives rise to CaM-independent activity. To test whether this autophosphorylation event is important for learning/memory, we generated mutant mice with a point mutation in the αCaMKII gene (T286A) leading to the inactivation of the autophosphorylation site. Homozygous mutants are impaired in cued and contextual learning, whereas heterozygotes are normal in fear conditioning. These results indicate that the autophosphorylation of αCaMKII at T286 is necessary for some forms of learning/memory.

To study if the modulation of voltage-gated K^+ channels is involved in learning/memory, we generated mutant mice lacking the modulatory K^+ channel subunit Kvß1. The presence of Kvß1 leads to an A-type inactivation of some voltage-gated K^+ channels. The Kvß1-deficient mice are normal in open-field behavior, and have normal contextual and spatial learning. However, reversal learning in the Morris water maze is impaired in these mutants. These results suggest that the modulation of A channels affects some forms of learning/memory.

(supported by an EMBO fellowship, by a stipend from the Deutsche Forschungsgemeinschaft and by the NIH grant AG13622-01)

549.10

IMPAIRED MOTOR COORDINATION IN MICE LACKING A GTP-BINDING PROTEIN SUBUNIT. W. Sun*[1], S. Offermanns[2], J. J. Kim[1], R. F. Thompson[1], and M. I. Simon[2]. [1]Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520. [2]Division of Biology, California Institute of Technology, Pasadena, CA. 91125.

Heterotrimeric G proteins are GTP-binding proteins involved in cellular signal transduction. They relay signals from cell surface receptors to intracellular effectors. Cells can respond to a variety of hormones, peptides, and neurotransmitters through G-proteins. $G\alpha_q$ is a recently cloned α subunit of the G protein family. It is insensitive to pertussis toxin and activates phospholipase C (PLC), leading to an increase in intracellular inositol triphosphate (IP_3) and diacyglycerol. Several brain neurotransmitters receptors have been linked to $G\alpha_q$, including metabotropic glutamate receptors, serotonin receptors, and muscarinic receptors. Mice lacking the gene coding for $G\alpha_q$ were generated by homologous recombination. Mice deficient in $G\alpha_q$ show markedly progressive ataxia. Homozygous mutants show clearly abnormal walking patterns as compared to those of heterozygotes and wildtypes. Impairments in motor coordination was further manifested in stationary and rotating rod tests. In the stationary rod test, homozygotes do poorly when compared to heterozygotes and wildtypes, although they show some improvement with repeated trials. In the rotating rod (10 rpm) test, the homozygotes performed poorly and showed little improvement with repeated trials whereas the heterozygotes and the wildtype mice did better and showed significant improvement. Eye-blink conditioning on the mice is currently in progress.

Supported by NSF (IBN-9215069), NIH (AG05142), NIMH (MH52194), ONR (N00014-95-1-1152) and the Sankyo Co., Ltd.

549.11

SUBSTITUTION OF MITOCHONDRIAL DNA (mtDNA) INDUCES NEURAL AND BEHAVIORAL MODIFICATION IN MICE, P.L. Roubertoux*, F. Maarouf, I. Le Roy, R. Moutier, M.-C. Busnel and M. Carlier. URA CNRS 1294, Génétique, Neurogénétique, Comportement, CDTA, CNRS and Université, Orléans, France, 45071 Orléans cedex 2.

The mtDNA makes up less than 0.2 % of the total DNA in mouse. It is entirely transmitted by the mother. The laboratory strains of inbred mice share mtDNA from identical origin *M. m. domesticus* origin except NZB that has mtDNA from *M. m. brevirostris* origin. Two congenic strains for mtDNA have been developed: NZB with mtDNA from CBA/H and CBA/H with mtDNA from NZB. The congenic strains have now reached 25 and 26 backcross generations respectively. The mtDNA transfer was checked by PCR amplification and Alu 1 digestion. The primers (from Dr. Yonekawa) are specific of the D. loop region of the mtDNA, that presents polymorphisms between NZB and CBA/H. The effects of mtDNA substitution appeared for motor behaviors (measured from birth to adulthood) and underlying brain structures. These effects were always in interaction with nuclear genotype.

549.12

PRENATAL ACUTE EXPOSURE TO ORGANIC LEAD ENHANCES LTP IN THE 15-DAY OLD PUP HIPPOCAMPUS *IN VIVO*. A.Z. Elliott* & V. Miletic. Dept. Comp. Biosci. & Environ. Tox. Cntr., Univ. Wisconsin, Madison, WI 53706

We examined whether prenatal and postnatal acute exposure to triethyl lead (TEL) modifies the expression of long-term potentiation (LTP) in electrophysiological recordings in the young rat hippocampus *in vivo*. Pups were given a single subcutaneous dose of TEL (0 or 5 mg/kg) *in utero* on gestation day 18 (E18) or on postnatal days 1, 8 or 15 (P1, P8, or P15, respectively). On P15, a male and a female from each litter were anesthetized with urethane (1.2-1.5 g/kg), and prepared for conventional recording of hippocampal field potentials. A bipolar stimulating electrode was lowered into the CA3 region, and the glass recording electrode (0.5-2MΩ) was positioned in the CA1 region until a maximal response to the CA3 stimulus was observed. LTP was induced by tetanic stimulation (one 400ms train of five 50ms pulses at 50Hz), and recordings of field potentials were repeated at 0.5, 1, 2, 3, and 4 hours post-tetanus. The population spikes (PS) for all pups exposed to TEL postnatally (P1, P8, or P15) were similar to that of controls. However, pups exposed to TEL *in utero* showed an enhancement of LTP (200% above controls at 2 hours). The enhancement of LTP by TEL is the opposite effect of that seen by inorganic lead, previously reported to attenuate LTP. These data indicate that the molecular form is important in assessing the effect of lead on LTP. Further, E18 is a critical time of exposure at which pups are most sensitive to TEL. The data further suggest that the alteration of LTP may contribute to lead's neurotoxic action in the developing rat hippocampus.

(Supported by NIH NS21278).

549.13

NEW ROLES FOR NMDA CHANNELS IN NETWORKS WITH THETA/GAMMA OSCILLATIONS, O. Jensen, M. Idiart* and John E. Lisman, Center for Complex Systems, Brandeis University, Waltham MA02254-9110.

We have proposed that gamma oscillations serve as a timing mechanism to subdivide a theta cycle so that a network can keep ~7 memory patterns active at the same time, in accord with psychophysical measurements (Science 267:1512, 1995). We now extend this model by adding modifiable recurrent collaterals. This allows us to study the transition from short-term to long-term memory based on physiological Hebbian rules. Our analysis shows that kinetics of the NMDA channels is crucial: since different memories are active in different gamma cycles, the life-time of NMDA channels relative to the period of a gamma oscillation (~25 ms) will determine the type of memory formation. NMDA channels with a short life-time (<25 ms) will build up auto-associations among the active patterns in STM. Short-lived NMDA channels have been found in some regions of cortex. Such networks will have the properties of attractor network. Remarkably, such networks also provide very reliable short-term memory for unique lists of known items. NMDA channels with long life-times, as found in the hippocampus, are not suitable for the formation of auto-associative memory. Rather they function to form hetero-associations among the active memories, e.g., to link different memories in a sequence. We show that the CA3 region of the hippocampus is well suited for the storage and retrieval of sequences. A surprising finding is that NMDA-mediated synaptic transmission would function well in sequence recall, suggesting that some forms of recall may be sensitive to NMDA channel blockers. Finally we apply our model to study the hippocampal place cell problem. We hypothesize that the hippocampus stores the path as a sequence during learning. During readout the current position stimulates recall of locations to come. The model predicts that the average phase advance of place cells firing is one gamma period per theta cycle.

549.14

N-(2-CHLOROETHYL)-N-ETHYL-2-BROMOBENZYLAMINE HYDROCHLORIDE REDUCES THE AMPLITUDE OF AUDITORY EVOKED POTENTIALS IN A PAIRED-TONE PARADIGM IN THE ALBINO RAT. C.M. Specht* and D.W. Shucard. Departments of Neurology and Psychology, SUNY at Buffalo, 100 High St. (D-6), Buffalo, NY 14260.

The brain stem nucleus locus coeruleus is the primary source of forebrain norepinephrine and the sole source of neocortical and hippocampal norepinephrine. In addition, it has been widely implicated as modulating behavioral measures of attention, vigilance, arousal, and memory. Our laboratory has focussed on fast habituation (FH) as an index of cognitive activity in terms of the amount of attention devoted to the processing of sequential auditory stimuli. In the paired-tone paradigm, FH is demonstrated when the amplitude of the auditory evoked potential (AEP) in response to the second tone of the pair is reduced relative to that of the first. This project was designed to determine whether the locus coeruleus is involved in FH.

Male Sprague-Dawley rats were injected with a single dose (50 mg/kg, i.p.) of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4; Sigma), a neurotoxin that lesions noradrenergic neurons arising form the locus coeruleus. They were tested one week later.

Pre-lesion baseline AEPs in response to pairs of acoustic stimuli demonstrated FH. After DSP-4 lesioning of the locus coeruleus, however, the overall amplitudes of the AEPs in response to both tones were reduced compared to baseline.

It appears that the widely divergent projections of the locus coeruleus may modulate the phenomenon of FH. These data increase the validity of FH as a measure of attention in the brain by associating the phenomenon with a physiological system also known to be involved in attentional processes.

Supported in part by the Department of Neurology Research Fund.

549.15

CARBACHOL DEPOLARIZES ASTROCYTES AND INDUCES POSITIVE SLOW POTENTIALS AT THE HIPPOCAMPAL FISSURE. J. Brankačk*, Y. Verbny, Y. Yanovsky and H.W. Müller-Gärtner. Dept. of Nuclear Medicine, Heinrich-Heine-University Düsseldorf and Reaserch Center Jülich GmbH, Jülich, Germany

The hippocampal theta rhythm recorded in vivo during urethane anaesthesia and wheel running consists of alternating (4 to 12 Hz) and sustained potentials. Amplitudes of both components are maximal at the hippocampal fissure. The muscarinergic agonist carbachol was shown to induce theta-like oscillations in hippocampal slices. The aim of the current study was to test the hypothesis that acetylcholine acting on astrocytes near the hippocampal fissure induces the sustained negative potential recorded during theta rhythm. Transversal hippocampal slices were perfused with artificial cerebrospinal fluid. Intracellular recordings from 36 astrocytes at the hippocampal fissure revealed a mean membrane potential of 84.75±0.73 mV. Carbachol (CCh, 0.5 to 100 μM) added to the bath, depolarized the membrane of all tested cells. The mean depolarization for 10 μM CCh was 5,44±0.87 mV (N=18, range 1 to 14 mV) and the mean latency 103.78±6.49 s. CCh caused a slow extracellular positive potential shift, increased pyramidal cell firing and induced bursts and oscillations. Tetrodotoxine (TTX, 0.75 μM) abolished bursts and oscillations, induced a negative extracellular potential, hyperpolarized astrocytes but did not abolish the depolarizing effect of CCh. The muscarinergic depolarizing effect on astrocytes is not the cause of the negative sustained potential during theta.

This study was supported by the Research Center Jülich, GmbH.

549.16

C-FOS EXPRESSION IN RATS WHEN LEARNING AN ACTIVE VISUAL AVOIDANCE DISCRIMINATION. E.R. Delay*[1], S.L. Patrick[1] and J.M. Walker[1], [1]Dept. Psychology, Regis University, Denver, CO 80221 and [1]Walter S. Hunter Laboratory of Psychology, Brown University, Providence, RI 02912.

c-Fos-like immunoreactivity (c-FLI), an indicator of certain types of neuronal activity, is thought to be expressed by processes related to learning. For example, Tischmeyer et al. (*Behav. Neural Biol.*, 1990 (54): 165-171) reported increased *Fos* mRNA expression after brightness discrimination learning. The present study examined c-FLI in CNS structures after discrimination training in which rats received 50 trials to learn to avoid shock cued by an increase in light intensity in a 4-way shuttle device. c-FLI expression in this group was compared to c-FLI seen in rats of four stimulus matching conditions: 1) exposure to the test apparatus, 2) shock intensity and duration, 3) light intensity and duration, and 4) the combined but noncontiguous presentation of light and shock stimuli. Generally, c-FLI was lowest in rats in the test exposure condition and the highest in the combined-noncontingent stimulus group. Cortical c-FLI to the light cue was greatest in secondary visual areas. Exposure to shock induced c-FLI in posterior retrosplenial cortex and amygdaloid nuclei. c-FLI appears to occur in structures thought to be involved in visual avoidance learning.

Supported by NINDS 1 F33 NS09885-1

549.17

C/EBP AND C-FOS GENE EXPRESSION IN LONG TERM MEMORY OF *APLYSIA* AND CHICK, **C. Bessho*, K. Morikawa and H. Ageta, Dept. of Physics, Kyoto Sagyo Univ., Kyoto 603 Japan.**

Recent works show that cAMP response binding protein (CREB) is a mediator of long term memory from *aplysia* to mice, that CCAT enhancer binding protein (C/EBP) is required for the consolidation of long term facilitation in *aplysia* sensory-motor neuron synapses and that c-fos gene is induced in LTP. To detect the C/EBP and c-fos gene expression in *aplysia* neurons and chick brain during long term memory, we performed RT-PCR using primers coding a part of C/EBP or c-fos gene and cDNA from reverse transcribed RNA of *aplysia* ganglia treated with serotonin or chick brain after bitter taste avoidence training. *Aplysia kurodai* abdominal and pleural ganglia including sensory neurons were incubated in L15-ASW solution containing 10 μ M serotonin at 18 ° C for an hour and a half and the others as the controll were done in L15-ASW. Chicks were presented with a small chrome bead dipped in water or the bitter aversant methylanthranilate (MEA). At 1 day after training, chicks were tested and their brains were taken. Total RNA was extracted from *aplysia* gangli on and chick brain using RNAzol. The first-strand cDNA was generated following T. Ito's method. PCR amplification was performed under the following condition: 93 ° C for 1 min, 50 ° C for 1.5 min and 72 ° C for 2 min. The process was repeated for a total of forty cycles. We have found that C/EBP gene was induced in *aplysia* pleural ganglion treated with 5-HT and that c-fos like gene was expressed in *aplysia* abdominal gangli on treated with 5-HT. We also found that C/EBP gene was induced in the brains of chicks pecking bead dipped in MEA but that it was expressed in the brain of chick doing bead dipped in water.

549.18

AMNESTIC EFFECT OF MONOCLONAL S-100β and S-100α ANTISERA ON ONE-TRIAL PASSIVE AVOIDANCE MEMORY FORMATION IN DAY-OLD CHICKS. Brona S. O'Dowd, Wei Q. Zhao, Kim T. Ng, Andrew J.P. Francis* and Stephen R. Robinson. VTHRC, University of Queensland, QLD., AUSTRALIA.

The β subunit of the calcium-binding dimeric protein S-100 is upregulated in Alzheimer's disease and Down's syndrome, and has been implicated in memory-related processes. Up to now, the involvement of the α subunit of S-100 in memory has not been examined. In the present study, day-old chicks (*Gallus domesticus*) trained on a one-trial passive avoidance task received intracranial injections (10μl per hemisphere) of either saline or monoclonal antisera to S-100β (1:500, Sigma), S100α (1:50, Sigma) or one of a range of concentrations of carbonic anhydrase antisera (1:50-1:10,000, Sigma). Compared to control chicks that had been injected with either saline or the carbonic anhydrase antisera, those injected with either the S-100β or S-100α antisera experienced significant retention deficits ($p < 0.05$). The S-100β antisera needed to be administered between 5 minutes before and 5 minutes after training and produced a decline in recall from 20 minutes post-training, the start of the A phase of the intermediate memory (ITM) stage of the Gibbs-Ng three-stage memory model[1]. The S-100α antisera, on the other hand, could be administered up to 20 minutes after the training trial, producing amnesia from 30 minutes post-training, the point of transition between the A and B phase of the ITM stage. Our immunohistochemical studies showed that S-100 antisera are rapidly taken up into cells (within 20 minutes). Thus, although the antisera are likely to be acting on extracellular S-100 binding sites, they may also prevent the respective S-100 proteins from binding to their intracellular targets. The finding that memory loss occurred at different times after training depending on the subunit of S-100 antisera injected suggests that the β and α subunits may act through separate memory-related mechanisms operating at different times after the aversive experience.

1. Gibbs, M.E. and Ng, K.T. (1984). *Behavioural Brain Research, 12*. 21-27.

Funded by the NHMRC and the ARC in Australia.

NEURAL PLASTICITY III

550.1

DISSOCIATIONS BETWEEN BEHAVIORAL RECOVERY AND RESTORATION OF ACTIVITY IN THE DEAFFERENTED VESTIBULAR NEURONS AFTER A UNILATERAL LABYRINTHECTOMY IN THE ALERT GUINEA PIG. L. Ris, B. Capron, C. de Waele, P-P. Vidal and E. Godaux*. Lab. of Neuroscience, Univ. of Mons-Hainaut, 7000 Mons, Belgium and LPPA, CNRS, Paris, France.

In the guinea pig, a unilateral labyrinthectomy induces postural disturbances and an ocular nystagmus on the one hand, and a collapse of the spontaneous activity in the neurons of the ipsilateral vestibular nuclei on the other hand. These initial changes are followed by an abatement of the behavioral symptoms and by a restoration of the resting activity in the deafferented vestibular neurons. The aim of this study was to compare the time courses of those two recoveries.

Postural disturbances were measured from videorecordings whereas ocular nystagmus was recorded using the search coil technique. For neuronal-discharge measurements, animals were prepared for chronic extracellular recording of single unit activity. The criterion used to select the 1333 analysed vestibular neurons was their recruitment by an electrical shock on the vestibular nerve.

Although there was a rough parallelism between bevavioral and neuronal recoveries, some discrepancies emerged. (1) During the first 90 min after labyrinthectomy, while vestibular neuronal activity was collapsed, an important step in the postural recovery occurred (the animal which could not stand up began to manage to do it). (2) The major part of the abatement of the nystagmus occurred during the first 10 h after labyrinthectomy while vestibular neuronal activity remained collapsed during that period. (3) Behavioral static vestibular compensation was almost finished 48 h after the lesion while a significant part of the restoration of the neuronal activity still occured after that time.

We conclude that restoration of neuronal activity in the vestibular nuclei in only one of the mechanisms underlying vestibular compensation.

Supported by a grant from the Belgian Fund for Scientific Medical Research

550.2

SENSORIMOTOR ASYMMETRIES FOLLOWING UNILATERAL LESIONS OF THE RAT SOMATIC SENSORIMOTOR CORTEX: NEGLECT, EXTINCTION OR HYPOKINESIA? C.E. Raad* and T.M. Barth, Department of Psychology, Texas Christian University, Ft. Worth, TX 76129.

Previous work has shown that unilateral lesions in several different areas of the rat somatic sensorimotor cortex (SMC) produce behavioral asymmetries on a bilateral-tactile extinction test. Specifically, unilateral lesions of the rostral forelimb area (RFL), the caudal forelimb area (CFL), or the anteromedial cortex (AMC) produce a preference for contacting an adhesive patch placed on the forelimb ipsilateral to the cortical lesion. The present study attempted to distinguish between the asymmetries following unilateral lesions to the AMC or the RFL. The specific question asked was whether the appearance of a sensorimotor asymmetry required the simultaneous presentation of bilateral-tactile stimulation (i.e. extinction) in the form of an ipsilateral bias or if unilateral stimulus presentation alone would reveal a behavioral asymmetry in the form of contralateral neglect (i.e. no response to contralateral stimuli) or long latencies to contact patches placed on the contralateral forelimb (i.e. hypokinesia). Rats were given either unilateral lesions in the RFL or AMC, or a sham surgery. During the first five days after surgery, the rats with RFL lesions showed an asymmetry with both bilateral and unilateral testing procedures. On the bilateral test, RFL-damaged rats showed an ipsilateral bias. However, with unilateral tests the RFL-damaged rats showed longer latencies to contact a patch placed on the contralateral forelimb when compared to ipsilateral latencies and to sham control rats. In contrast AMC-damaged rats failed to show an asymmetry with the unilateral testing procedure and therefore required the presence of bilateral-tactile stimulation. These data suggest the sensorimotor asymmetry following RFL lesions may be characterized as a hypokinesia, whereas lesions in the AMC produce an asymmetry more closely resembling tactile-extinction. Supported by TCURF #5-23745.

550.3

NMDA RECEPTOR BLOCKADE DOES NOT BLOCK THE IMMEDIATE "UNMASKING" THAT FOLLOWS MEDIAN NERVE TRANSECTION IN ADULT SQUIRREL MONKEYS OR REVERSE REORGANIZATION ONCE IT HAS OCCURRED. W.A. Myers[2], J.D. Churchill[1,2], J. Besheer[2], S.E. Voss[2], N. Muja[2], and P.E. Garraghty*[1,2]. [1]Prog. Neur. Sci., [2]Dept. Psych., Indiana University, Bloomington, Indiana, 47405.

We have previously shown that most of the reorganization that typically follows median nerve transection in adult squirrel monkeys is dependent on normally-functioning NMDA receptors. Here, we have evaluated two hypotheses. 1) Is the immediate "unmasking" found after median nerve transection NMDA receptor-dependent? 2) Are NMDA receptors necessary for both the initiation and the maintenance of reorganizational changes, or only the former? We implanted subcutaneous osmotic minipumps to deliver CPP systemically. Drug delivery was initiated either before examining the immediate effects of median nerve transection, or after reorganization had presumably occurred. For the first set of experiments, NMDA receptor blockade was initiated either 1 or 4 weeks prior to multiunit mapping in area 3b followed by transection of the median nerve and a remapping of the cortex. For the second set of experiments, median nerve transection was followed 4 weeks later by either 1 or 4 weeks of NMDA receptor blockade prior to a terminal cortical mapping experiment. We found that the immediate unmasking of new receptive fields after acute nerve injury was not prevented by NMDA receptor blockade; nor were completely reorganized cortical maps dependent upon NMDA receptors for their maintenance. We conclude that the immediate unmasking is likely due to release from tonic inhibition, and that the later phase of reorganization, as for LTP in the hippocampus, is dependent on normally-functioning NMDA receptors for its initiation, but not for its maintenance. (Supported in part by RO3 MH55548-01).

550.4

Norepinephrine Manipulation and Postoperative Practice Influence recovery of Locomotor Placing Following Cortical Lesions in the Rat. S.L. Irish *† & T.M. Barth. †Q.P. Corporation, Tokyo, Japan & Dept. of Psych., Texas Christian University, Ft. Worth, TX 76129.

The NE neurotransmitter system has been of interest for its role in recovery of function following SMC lesions as well as its role in sensorimotor learning. Drugs that enhance NE activity have been shown to facilitate recovery of locomotor placing following SMC lesions in the rat while drugs that deplete NE retard recovery. It has been proposed that drugs that enhance NE activity facilitate recovery by activating distant areas depressed following brain damage. Similar to the effects of NE-acting drugs on recovery, manipulations of NE also influence the rate of motor learning. Therefore, an alternative explanation of the NE effect on recovery might be that NE-acting drugs affect recovery by influencing sensorimotor learning as the rat learns an alt[illegible]ate behavioral strategy to compensate for the lost function. The present study hoped to elucidate the mechanisms of recovery of the foot-fault and beam-walking tests by manipulating postoperative practice and NE levels. Following surgery subjects were divided into either practice or no-practice groups. Postoperative testing began for the practice groups on day 2 and for the no practice groups on day 14. Subjects were given posttrial injections of saline, yohimbine, or clonidine every other day following surgery. It was hypothesized that if NE-acting drugs influence recovery merely through a relief of diaschisis then pharmacological manipulations would influence recovery regardless of postoperative practice. On the other hand, if NE-acting drugs influence recovery through enhancement of learning strategies then no drug effect would be observed until it was given in conjunction with postoperative practice. The findings were that postoperative practice had the strongest influence on recovery of foot-faults and beam-walking ability. NE-acting drugs only influenced recovery on the foot-fault test and this influence appears to be independent of postoperative practice. Therefore, both a release from diaschisis and sensorimotor learning may be acting in the recovery process. NE-acting drugs may affect diaschisis while practice may affect sensorimotor learning.

550.5

SURVIVING TISSUE SURROUNDING CORTICAL DAMAGE IS VULNERABLE TO BEHAVIOR-DEPENDENT GLUTAMATE ELEVATION AND IMMUNE SYSTEM ACTIVITY. J.L. Humm*, S.T. Bland, R.R. Cocke, D.C. James, J.E. Gotts, J.K. Sexton, R. A. Gonzales & T. Schallert. Institute for Neuroscience & Departments of Psychology and Pharmacology, University of Texas at Austin, Austin, TX 78712.

Following injury to the forelimb area of the sensorimotor cortex (FL-SMC), rats were fitted with casts of the non-impaired forelimb, which forced overuse of the contralateral limbs for 2 weeks. This procedure greatly expanded the injury and severely disrupted recovery of contralateral limb function (Kozlowski, et al. *J. Neurosci.*, in press). Primary injury to FL-SMC may render surrounding cortical and subcortical tissue susceptible to overuse-related glutamatergic activity. In casted rats, the NMDA receptor antagonist, MK-801 (1mg/kg daily, a dose that did not reduce forelimb activity), prevented lesion expansion and improved recovery of function. Microdialysis experiments indicated that behavioral activity is associated with increased levels of dialysate glutamate in brain regions surrounding the FL-SMC. The role of the immune system was examined in additional experiments. Animals forced to overuse their impaired limbs after FL-SMC damage, while being simultaneously injected with the T-cell immunosuppressant, Cyclosporin A, exhibited sparing of subcortical tissue and facilitation of behavioral recovery. The bacterial endotoxin, LPS, which stimulates cytokine (IL-1) production, aggravated cortical damage in the absence of forced limb overuse. We propose that a cascade of events triggered by behavioral pressure or immunological processes may be lethal to otherwise-surviving tissue after brain injury. Funded by NS23964, Texas Advanced Research Program AA0747, AA08484, AA00147.

550.6

DOES THE ONSET OF BEHAVIORAL TESTING AFFECT THE EFFICACY OF MAGNESIUM CHLORIDE TO PROMOTE RECOVERY FOLLOWING CORTICAL LESIONS IN THE RAT. M.R. Hoane* and T.M. Barth. Department of Psychology, Texas Christian University, Ft. Worth, TX 76129.

In studies investigating the behavioral effects of neuroprotective drugs the onset of post-operative testing has varied considerably. In some experiments testing has begun 24 hrs post-injury and in other cases, one or two weeks may pass before behavioral testing begins. The purpose of the present study was to examine the effects of delaying post-operative testing following cortical lesions and to determine if delays affect the efficacy of the neuroprotective agent magnesium chloride ($MgCl_2$). Rats received unilateral electrolytic lesions of the sensorimotor cortex (SMC) and a regimen of $MgCl_2$ (1 mmol/kg) starting at specific post-operative intervals (15 min, 8 hr, or 24 hr) or saline. Forelimb placing tests were initiated post-operatively starting on day 2, day 8, or day 14 after the lesion. The results indicated that recovery of forelimb placing seems to occur in the absence of post-operative practice. Moreover, when testing began on day 2 all $MgCl_2$ treated animals showed a facilitation of behavioral recovery. That is, the window of opportunity includes treatment beginning at 24 hours after the injury. However, when testing is begun either on day 8 or 14 there were no significant differences in recovery between the $MgCl_2$ and saline treated rats. These data suggest that onset of testing is an important factor to consider when evaluating the effectiveness of drugs that may be beneficial to recovery of function. This research was funded by TCURF #5-23745.

550.7

RECOVERY FROM LOCOMOTOR PLACING DEFICITS FOLLOWING UNILATERAL CORTICAL LESIONS IN THE RAT: A FLOOR EFFECT. C. L. Hart*, M.R. Hoane and T.M. Barth. Department of Psychology, Texas Christian University, Ft. Worth, TX 76129.

There is considerable evidence that suggests preoperative and/or postoperative experience may affect the severity of lesion-induced deficits and the rate of behavioral recovery after damage to the cortex. In some of these studies rats are placed in either an "enriched" or an "impoverished" environment, with the "enriched" environment condition promoting the restoration of function. Other studies have manipulated the amount of task-specific practice and found that practice may also affect recovery. The present study was designed to investigate whether manipulations of the housing environment could be directed towards promoting restoration of a specific function. The task we chose was the foot-fault test. In this task rats are required to locomote on a grid floor (openings in the grid were 3.5 cm) by placing their limbs on the rungs of the grid. A "foot-fault" occurs when the rat misplaces the forelimb and it falls through an opening in the grid or when the forelimb slips off one of the rungs into the opening. The housing manipulation was the floor of the home cage. Postoperatively, some rats were placed into standard wire mesh cages with a grid floor (1.25 cm openings) and other rats into similar cages, but with a solid floor. All rats received a small unilateral electrolytic lesion in the forelimb area of the somatic sensorimotor cortex (SMC) and were then placed into the individual cages. Behavioral testing began on postoperative day 2. Surprisingly, the results were that rats housed in the cages with solid floors showed a reduction in the number of postoperative foot-faults and an acceleration of behavioral recovery when compared to those housed with the grid floor. These data suggest that the grid floor used in the home cage may have promoted the development of behaviors incompatible with the task. Supported by TCURF #5-23745.

550.8

EVIDENCE FOR ALTERATIONS IN THE FORM OF SYNAPTIC CONNECTIVITY IN THE CORTEX OPPOSITE UNILATERAL SENSORIMOTOR CORTEX LESIONS: TIME-DEPENDENT DEGENERATIVE AND SYNAPTIC STRUCTURAL CHANGES. T.A. Jones* Dept. of Psychology and Behavioral Neuroscience Program, Univ. Washington, Seattle, WA 98195.

Unilateral lesions of the forelimb representation area of the sensorimotor cortex (FLsmc) in adult rats have been found to result in dendritic growth and synaptogenesis in layer V of the motor cortex contralateral and homotopic to the lesions (Jones & Schallert, 1992; 1994; Jones, Kleim & Greenough, 1995). The neuronal growth appears to be influenced by lesion-induced changes in the use of the forelimbs. The present study used electron microscopy and stereological procedures to assess possible structural alterations in axonal processes and their synaptic connections in layer V contralateral to FLsmc lesions. Myelinated axons were reduced in volume fraction, volume per neuron and number per unit area in comparison to sham-operated rats at 10 but not 18 days after the lesion, consistent with lesion-induced degeneration and subsequent growth. Following this, at a time point corresponding to increases in the total number of synapses per neuron (30 days), the lesions were found to result in a marked increase in the proportion and the number per neuron of individual axonal boutons forming synaptic contacts with 2 or more dendritic spines (multiple synaptic boutons). Furthermore, the number of synapses per neuron with perforated subsynaptic plates, which are believed to be associated with enhanced synaptic efficacy, were increased in comparison to shams. These findings are suggestive of alterations in the pattern and possibly the strength of synaptic connections. Together, these changes may represent a lesion-initiated and behavior-influenced major restructuring of synaptic connectivity in the cortex opposite FLsmc lesions. Supported by MH35321 and MH55525.

550.9

LIGHT DEPRIVATION AFTER FRONTAL CORTEX INJURY REDUCES LOCOMOTION, STRIATAL FOS EXPRESSION, AND RECOVERY TIME FROM UNILATERAL NEGLECT. J.M. Vargo[1]*, H.V. Lai[2] and J.F. Marshall[2]. [1]Dept. Of Surgery, Hennepin County Medical Center and Dept. of Neurosurgery, Univ. of Minnesota, Minneapolis, MN 55415, [2]Dept. of Psychobiology, Univ. of California, Irvine, CA 92717-4550.

Light-deprivation (LD) after unilateral aspiration of medial agranular cortex (AGm) produces accelerated recovery from unilateral neglect, but the neural mechanisms mediating this recovery are unknown. Neglect after AGm ablation is associated with hemispheric asymmetries (lesioned < intact) in striatal c-*fos* expression, while spontaneous recovery under normal light cycle conditions is associated with symmetrical c-*fos* expression. This study examined striatal c-*fos* expression 5 days after AGm ablation in rats experiencing 48 h of either LD or 12h light/12h dark cycling (12/12) soon after surgery. All lesioned rats demonstrated severe multimodal neglect before being placed in their respective light conditions. After 48 h, all were maintained under 12/12 conditions. During LD, horizontal motion was reduced in both unlesioned and lesioned rats ($P < .001$). By postoperative day 4, only lesioned rats that had experienced LD showed significant recovery from neglect ($P < .001$). LD reduced the numbers of amphetamine-induced (5 mg/kg, i.p.) Fos-positive nuclei seen in left or right dorsolateral striatum in both lesioned and unlesioned rats ($P \leq .007$). In lesioned rats, those that underwent LD had less hemispheric asymmetry in Fos expression (lesioned/intact = .76± .04) than did those experiencing the 12/12 cycle (.61 ± .05). Less motion during the light-cycle manipulation was associated with greater recovery on postoperative day 4 ($r = -.611$, $P = .007$), and with greater hemispheric symmetry in striatal Fos on postoperative day 5 ($r = -.499$, $P = .041$), suggesting that LD may produce accelerated recovery by reducing asymmetries in subcortical functioning.

Supported by NS-33670 (J.F.M.), NRSD-MH10598 (J.M.V.), UCI PUF (H.V.L.).

550.10

PERCEPTUAL CORRELATES OF NEURAL PLASTICITY IN THE ADULT HUMAN BRAIN: AN UPDATE. D. Rogers-Ramachandran* and V. S. Ramachandran , UCSD, La Jolla, CA 92093-0109.

In 1991, we examined two arm amputees and found that they systematically referred sensations from the ipsilateral face to the phantom (Ramachandran et al., *Science*). We suggested that this was at least partially due to the remapping of sensory input from face into regions in the brain that ordinarily represent the (amputated) hand. We were also able to show cortical remapping as revealed by MEG (Yang, Ramachandran, Galen et al., *Nature*, 1992) -- changes that may be analogous to those reported in monkeys (Pons, 1991). We suggested that, in addition to referral of non-painful sensations, referred pain may also be caused by remapping, an idea that is consistent with the recent observations of Taub et al. (*Nature*, 1996).

We have now studied over 18 patients. Seven systematically refer touch from face to the phantom. In some (but not all), the referral was modality specific, e.g., warmth, cold or vibration on the face was perceived as warmth, cold or vibration on the phantom and the referral was also topographically organized. Thus the emergence of such referred sensations is a robust phenomenon that probably arises due to remapping in the sensory areas of the brain. However, the absence of visual feedback may lead some patients to eventually "ignore" the referred sensations, or the sensory input might be recognized as "abnormal" by higher centers and interpreted as pain instead of touch.

These results demonstrate a surprising amount of plasticity in adult humans and may be clinically important for phantom pain.

Supported by NIMH.

550.11

LIGHT DEPRIVATION INDUCED RECOVERY FROM SEVERE NEGLECT INDUCED BY UNILATERAL DESTRUCTION OF THE POSTERIOR PARIETAL CORTEX IN RATS. J.V. Corwin* and K.J. Burcham. Psych. Dept., Northern Ill Univ., DeKalb, IL 60115.

Light deprivation (LD) has been found to produce dramatic sparing and recovery of function from neglect induced by unilateral lesions of the medial agranular prefrontal cortex in rodents, the rodent analog of area 8. However, the area most often related to severe neglect in humans is the inferior parietal lobule (IPL). In the present study we examined whether LD would produce accelerated behavioral recovery of function from neglect induced by unilateral destruction of the posterior parietal cortex (PPC), the rodent analog of the IPL.

Subjects received unilateral PPC lesions and were tested for the presence of severe neglect of visual, tactile, and auditory stimulation. If severe neglect was obtained, the subjects were randomly placed into one of three groups: a 48 hr LD group, a 48 hr constant light group, or a normal light/dark cycle group. After the environmental manipulation, the subjects were tested for multimodal neglect 3 times per week for 3 weeks.

The LD group demonstrated significantly greater recovery than the controls (p's < 0.01). Significant recovery was present across all sensory modalities on the first test post LD, and lasted for the duration of testing. The results provide further support for the therapeutic effectiveness of LD on neglect induced by cortical lesions. Further, the results of studies of the effects of LD suggest that LD may provide significant therapeutic effects in situations where the behavioral deficits are related to a disruption of dopaminergic mechanisms.

Supported by funds from the NIU Psychology Department

550.12

LACK OF EVIDENCE FOR SUBSTANTIAL SPROUTING IN THE MOLECULAR LAYER OF THE MONKEY DENTATE GYRUS FOLLOWING ENTORHINAL LESIONS. J.R. Alonso[1,2], C. Crespo[1] and D.G. Amaral[2]* [1]Dept. of Cell Biology, Univ. Salamanca, Spain and [2]Dept. of Psychiatry and Center for Neuroscience, University of California, Davis. Davis, CA 95616.

In a previous behavioral study (Leonard et al., 1995; J. Neurosci. 15:5637-5659), five Macaca fascicularis monkeys were subjected to bilateral lesions of the entorhinal cortex. Animals survived for approximately 1-1 1/2 years after surgery during which they were behaviorally tested and demonstrated a transient initial memory impairment followed by normal performance just prior to sacrifice. In order to determine potential morphological substrates of this functional recovery, three neuroanatomical techniques were employed to examine possible plasticity in the cyto- and fiber architecture of the hippocampal formation: NADPH-diaphorase (ND) histochemistry, acetylcholinesterase (AChE) histochemistry, and choline acetyltransferase (ChAT) immunocytochemistry. ND and AChE labeled both neurons and dense fiber plexuses whereas ChAT immunoreactivity demonstrated prominent and regionally selective fiber systems. None of these markers demonstrated substantial alterations in fiber or cell labeling in the deafferented hippocampal formation. Minor changes included a reduction of ND-positive fibers in the dentate gyrus and the relative expansion of the diffuse AChE staining present in the supragranular zone of the dentate molecular layer. But, no clear evidence of collateral sprouting was observed. Since a significant transverse expansion of the perirhinal cortical projection to the hippocampal formation was observed in these same animals (Leonard et al., 1995), it can be concluded that not all fiber systems in the primate hippocampal formation demonstrate morphological plasticity. In particular, and in contrast to what has been demonstrated in the rat, we observed little or no evidence of sprouting in the dentate gyrus following entorhinal lesions.

Supported by the DGICyT (PR95-350), and NIMH grants NS-16980 and MH R37 41479 to D.G.A.

550.13

EFFECTS OF ENTORHINAL LESIONS ON THE DISTRIBUTION OF GLUTAMATE RECEPTORS IN THE MONKEY HIPPOCAMPAL FORMATION. A.L. Jongen-Rêlo* and D.G. Amaral. Dept. of Psychiatry and Center for Neuroscience, University of California, Davis. Davis, CA 95616.

Leonard et al. (J.Neurosci., 1995, 15(4): 5637-5659) demonstrated that *Macaca Fascicularis* monkeys with bilateral entorhinal cortex (EC) lesions were transiently impaired in the performance of the delayed nonmatching to sample task. In addition, a significant transverse expansion of the perirhinal terminal field in the CA1 region of the hippocampus was observed in the lesioned animals. Since the entorhinal and perirhinal inputs to the hippocampal formation are glutamatergic, we have now investigated whether the distributions of NMDA and AMPA receptors were altered in the hippocampal formation of these EC lesioned monkeys. Animals were sacrificed approximately 1-1 1/2 years after their neurosurgical procedure and sections through the hippocampal formation of lesioned and control animals were immunohistochemically processed with antibodies against NMDAR2a/b and AMPA GluR1 and GluR2/3 subunits. Initial qualitative analysis indicates that immunoreactivity for GluR1, GluR2/3 and NMDAR2a/b was increased in the outer one third of the molecular layer of the dentate gyrus and in the stratum lacunosum-moleculare of the hippocampus. While neuropil staining appeared to be slightly increased in the hilar region of the dentate gyrus, cellular labeling in CA3 was similar in both groups of animals. Interestingly, GluR2/3-immunoreactivity was markedly decreased in the layer II pyramidal cells of the presubiculum. We are currently investigating alterations in other neurotransmitter systems.

Supported by NIH grants NS 16980 and R37 MH41479 to DGA and HFSP fellowship LT-315 to A.L.J.-R.

550.14

EFFECTS OF PRENATAL ALCOHOL EXPOSURE ON GAP43/B50, c-MYC AND N-MYC PROTEIN LEVELS IN FETAL RAT HIPPOCAMPUS, CEREBELLUM, STRIATUM AND HYPOTHALAMUS. J.H. Hannigan*, L.M. Treas, M.A. Sperry, D.E. Saunders. Fetal Alcohol Research Center, C.S. Mott Center, Department of Ob/Gyn, Wayne State University, Detroit, Michigan, USA 48201

We reported previously that alcohol exposure in cultured LA-N-5 human neuroblastoma cells (Saunders, et al Dev Brain Res, 86, 16, 1995) and in young (≤ 10 days) postnatal rats decreased growth-associated protein 43 (GAP43/B50) and increased c-myc and N-myc levels. Similar effects were not seen reliably *in vivo* in hippocampus of young offspring of Long-Evans rats given 4 g/kg or 6 g/kg of ethanol by intubation from gestational day 8 (GD 8) through GD 19. In the current study using a similar *in vivo* design and prenatal alcohol exposure, GAP43/B50, c-myc and N-myc protein levels were measured immunohistochemically at GD18 in fetal rat hippocampus, cerebellum, striatum and hypothalamus. Fifty cells per brain were assessed for levels of each protein (ROD-background) in both proliferating (periventricular) and differentiating zones of the 4 brain areas. GAP43/B50 levels in the differentiating zone of hippocampus were 81% to 88% of control levels following 4 g/kg to 6 g/kg of prenatal alcohol, whereas N-myc levels were 117% to 130% of control levels, respectively. There were no significant changes in c-myc levels, and effects in intubation stress control rats (0 g/kg ethanol) were not reliable. Comparisons of these results among brain areas in cells at different developmental stages can indicate critical periods and specific neurotrophic or regulatory mechanisms of alcohol teratogenesis in *in vivo* systems. Results are consistent with effects in the *in vitro* LA-N-5 cultures, and suggest that alcohol-induced alterations in these differentiation-regulating proteins in rat brain contribute to the life-long CNS sequelae that produce neurobehavioral dysfunction after prenatal alcohol exposure.

Supported by NIAAA grants P50-AA07606 and R01-AA06721.

550.15

KAINIC ACID INDUCED LIMBIC SEIZURES, ELECTRICAL KINDLING AND LTP *IN VIVO* INCREASE EXPRESSION OF TENASCIN-C IN RAT HIPPOCAMPAL NEURONS. M. Nakic, N. Mitrovic*, D. Manahan-Vaughan†, L. Butler°, J. McNamara°, G. Sperk#, K. Reymann†, M. Schachner. Department of Neurobiology, Swiss Federal Institute of Technology, Zürich, Switzerland, °Epilepsy Research Laboratory, Duke University Medical Center, Durham, N.C., USA, #Department of Pharmacology, University of Innsbruck, Innsbruck, Austria, †Department of Neurophysiology, Federal Institute of Neurobiology, Magdeburg, Germany.

We have investigated changes in the expression of the extracellular matrix molecule tenascin-C (TN) in different models of synaptic plasticity in the hippocampus of adult rats *in vivo* : kainic acid induced limbic seizures, electrical kindling and long-term potentiation (LTP). Changes in TN mRNA and protein levels were revealed by *in situ* hybridization, immunocytochemistry and Western blot analysis. Under control conditions, TN mRNA was not detectable in all paradigms tested, while protein was detectable in the strata oriens and lacunosum moleculare of CA1 and in the molecular layer and at the inner surface of the granule cell layer in the dentate gyrus. Drastic up-regulation of TN mRNA, seen as early as 4 h after a single intraperitoneal kainic acid injection, was followed by a decrease of message to control levels 24 h later and increase in protein level throughout the whole dentate gyrus. Thirty days after injection, mRNA and protein levels were slightly elevated in CA3 pyramidal cells, when compared to control levels. Twenty four hours after evoking generalized seizures by electrical kindling of the amygdala, TN mRNA was slightly up-regulated throughout the pyramidal cell layer in the stimulated but not contralateral hemisphere. This increase was observed also 5-8 weeks later but confined to the CA3 subregion. Four hours after eliciting LTP via stimulation of the perforant pathway, TN mRNA was up-regulated in granule cells and some pyramidal cells of the hippocampal formation. Twenty four hours after stimulation TN mRNA was no longer detectable. Together with previous observations on the ability of TN antibodies to decrease acoustic imprinting in chicken, our data indicate that TN is involved in changes underlying synaptic plasticity in several paradigms.

550.16

BEHAVIORAL AND NEUROCHEMICAL RECOVERY FROM PARTIAL 6-OHDA LESIONS OF THE SUBSTANTIA NIGRA(SN) IS BLOCKED BY DAILY TREATMENT WITH D1 RECEPTOR ANTAGONIST, SCH 23390. A. Emmi,* H. Rajabi and J. Stewart. Center for Studies in Behavioral Neurobiology, Dept. Psychology, Concordia University, Montreal, Canada, H3G 1M8.

Partial unilateral lesions of the SN produce a transient sensorimotor asymmetry. We reported recently that behavioral and neurochemical recovery from partial 6-OHDA lesions of the SN is blocked by daily treatment with glutamate receptor antagonists, MK-801 and CPP. Here we report that a D1 DA receptor antagonist, SCH 23390, can also prevent recovery following the same lesions. After surgery some animals were treated daily on Days 1-8 with SCH 23390; others were given the inactive isomer, SCH 23388. In behavioral tests on Day 3 before the daily injection, all animals showed rotation ipsilateral to the lesion. On Day 15, animals given the inactive isomer no longer displayed asymmetrical rotation, whereas SCH 23390-treated animals continued to rotate ipsilaterally. Microdialysis on Days 16-17 confirmed that in animals given the inactive isomer, there were no differences in basal DA between the lesioned and nonlesioned side. In contrast, basal levels of DA on the lesioned side of SCH 23390-treated animals were lower compared to those on the nonlesioned side. These findings suggest that D1 DA receptors play a role in the development of the long-lasting compensatory changes in intracellular events that accompany recovery from partial lesions of the nigrostriatal pathway. Supported by Medical Research Council, Canada

550.17

ALTERATIONS IN THE GLYCINERGIC NEUROTRANSMITTER SYSTEM ARE ASSOCIATED WITH STEPPING BEHAVIOR IN NEONATAL SPINAL CORD TRANSECTED RATS. R.J. Talmadge, R.R. Roy, and V.R. Edgerton*. Dept. of Physiological Sci. and Brain Res. Inst., UCLA, Los Angeles, CA 90095.

To elucidate if adaptations in specific neurotransmitter systems are associated with training-induced improvements in hindlimb stepping behavior after spinal cord injury, 12 neonatal (Neo) rats (7 days old) were randomly placed into 3 groups. One group (Neo-ST) was subjected to a mid-thoracic spinal cord transection (ST). A second group (Neo-ST-Tr) was subjected to ST and trained to step on a motorized treadmill 15 min/day, 5 days/week for 12 weeks. A third group served as controls. Locomotor ability was determined by mean step length during 30 sec of bipedal hindlimb stepping at 0.9 m/s. Mean step length was lower in both ST groups compared to control. The Neo-ST-Tr rats had longer step lengths than Neo-ST. Quantitative western blot determinations of two subunits of the glycine receptor macromolecule, i.e., the α_1-subunit (48 kDa) of the receptor and gephyrin (93 kDa), revealed that step training normalized an increase in the total amount of each subunit in the lumbar cord (L3-L5) of Neo-ST rats. Thus, locomotor training resulted in significant improvements in hindlimb stepping ability and normalization of glycine receptor protein subunits. These data suggest that the glycinergic neurotransmitter system is involved in stepping behavior and is modulated by spinal cord injury and subsequent locomotor training. Supported by NIH grant NS 16333 and American Paralysis Association grant TA1-9402-1.

550.18

EFFECTS OF MONOCULAR ENUCLEATON AT BIRTH UPON LEARNING OF A VERTICAL-HORIZONTAL DISCRIMINATION IN HOODED RATS. F. Yagi* and F. Okutani. Neuropsychology Lab., Kochi Med. Sch., Nankoku, Kochi 783, Japan.

Previously we have demonstrated that adult albino rats with one eye removed at birth (OEB) relearn a black-white discrimination faster than those monocularly enucleated at maturity (OET) when relearning is conducted after lesioning of the visual cortex contralateral to the remainig eye (Type A Exp.). This faster relearning phenomenon is considered to be one behavioral expression of the functioning of the expanded uncrossed visual pathways (expanded UXVPs) resulting from monocular enucleation at birth. However, neither OEBs nor OETs were able to master the discrimination when the experiment was conducted without previous learning following the same surgical treatment (Type B Exp.). We hypothesized that this occurs because the cue to discriminate might be close to the threshold of discrimination for either the normal UXVPs or the expanded UXVPs. In order to gain insight into hypothesis, the present study was undertaken using hooded rats as subjects which possess larger and more efficient UXVPs. In the Type A Exp. OEBs relearned discrimination of the 10-mm stripes [0.44 cycles/degree (c/d)] faster than OETs, yet in the Type B Exp. neither OEBs nor OETs were capable of acquring that discrimination. However, they could originally master the discrimination equally well when the width of stripes was broadened to 30 mm (0.15 c/d). And when the width of stripes was systematically reduced thereafter, the width of the smallest stripes for the expanded UXVPs to discriminate was found to be 6 mm (0.73 c/d) and that for the normal UXVPs 10 mm (0.44 c/d). These findings were discussed in relation to the hypothesis advanced on our previous data in albino rats.

BIOLOGICAL RHYTHMS AND SLEEP: CIRCADIAN RHYTHMS III

551.1

FORSKOLIN RESETS THE CIRCADIAN OSCILLATOR IN *XENOPUS* RETINAL PHOTORECEPTORS. M. Hasegawa and G. M. Cahill* Dept. of Biology, Univ. of Houston, TX 77204.

Previous studies indicate that the *Xenopus* retinal photoreceptor layer (PRL) contains a circadian oscillator that drives rhythmic melatonin release in constant darkness. This oscillator can be entrained in vitro by cyclic light or activation of D2-like dopamine receptors. Both light and dopamine can influence cyclic nucleotide pathways in photoreceptors. Therefore, we have begun to investigate the possibility that cAMP regulates the PRL oscillator. The PRL were prepared and cultured individually for 5 days in flow-through culture chambers by methods previously described (Cahill and Besharse, 1993, Neuron; 10: 573-577). We examined the effects of forskolin, a drug that increases cAMP by stimulating adenylate cyclase. A single 6-h pulse of forskolin (1 µM) increased melatonin synthesis and reset the oscillator in a phase-dependent manner. Forskolin caused phase delays when PRLs were treated during the late subjective night or early subjective day, and phase advances when PRLs were treated during the late subjective day, resulting in a "dark-pulse" type of phase response curve. Maximum phase delay (-2.15±0.33 h: n=4) and phase advance (2.97±0.30 h: n=4) were induced by the pulse at zeitgeber time (ZT) 21 and ZT 9, respectively. These phase-shifting effects were blocked or attenuated by light or quinpirole pulse at the same time. A pulse of 1,9-dideoxyforskolin (1 µM), an inactive analog, did not induce phase shifts or affect melatonin synthesis. When PRLs were exposed continuously to 1 µM forskolin, melatonin levels were increased at all phases of the rhythm, but the period of the rhythm was not affected. These results suggest that changes of cAMP level can reset the oscillator in the *Xenopus* retinal PRL and are consistent with, but do not prove, the hypothesis that light and dopamine reset the oscillator through suppression of cAMP. Supported by NIH-R01MH49757 (G.M.C) and Uehara Memorial Foundation (M.H.)

551.2

ROLE OF THE INTERGENICULATE LEAFLET (IGL) AND OLFACTORY BULBS IN PHOTIC AND NONPHOTIC ENTRAINMENT IN DIURNAL OCTODON DEGUS. N. Goel* and T.M. Lee, Dept. of Psychology, 525 East Hall, University of Michigan, Ann Arbor, MI 48109.

Octodon degus is a diurnal rodent species with stable, robust behavioral circadian responses to photic and social (olfactory) entrainers. This experiment determines the role of the intergeniculate leaflet (IGL) and the olfactory bulbs in mediating olfactory and photic information to the biological "clock" in degus. Female degus were housed in entrained conditions for 3 weeks to obtain the phase angle of entrainment for wheel-running and body temperature rhythms. Animals then received bilateral IGL lesions (n=16), sham IGL operations (n=4), bilateral olfactory bulbectomies via aspiration (n=10) or sham bulbectomies (n=5). After surgery, females were rehoused in entrained conditions for 3 weeks to assess phase angle. Next, animals underwent a six-hour phase advance of the LD cycle, first with photic cues present for 3 weeks, and subsequently with both social and light cues present for 3 weeks; in both cases reentrainment rate was measured. Finally, animals were maintained in constant darkness (DD) for 5 weeks to determine tau. Following behavioral testing, the tissue will be processed with cresyl violet staining to determine the accuracy of IGL lesions and olfactory bulbectomies. In the IGL-lesioned animals, immunocytochemistry will be used to assess neuropeptide Y (NPY) fibers, terminals and cells in the suprachiasmatic nucleus (SCN) and IGL. Data collection is still in progress and therefore the results will be presented at the meeting. NIMH grant R03MH49089.

551.3

ApC/EBP mRNA HAS A CIRCADIAN RHYTHM AND IT IS AFFECTED BY LIGHT IN THE EYE OF *APLYSIA*. S. Hattar and A. Eskin*. Dept. Biochem. and Biophys. Sci., Univ. of Houston, Houston, TX 77204.

The CCAAT enhancer-binding protein transcription factors (C/EBP) represent a family involved in terminal differentiation of a variety of cells including adipocytes, enterocytes, and monocytes. These transcription factors also play a role in mediating induction of memory by serotonin (5-HT) in *Aplysia* (Alberini et al., 1994). Because 5-HT also regulates the circadian rhythm in *Aplysia*, we investigated the role of C/EBP in the ocular circadian system. ApC/EBP mRNA in eyes, measured by ribonuclease protection assays, changed rhythmically when animals were kept in 12:12 light-dark cycles. ApC/EBP was lowest during the late day time and highest during the late night time. Changes persisted for at least a few cycles when animals were kept in constant darkness. Some preliminary results indicate that the rhythm of ApC/EBP also persists in vitro when eyes are kept in culture. In another set of experiments, we have begun to investigate the effects of light and 5-HT on the isolated eyes. Light pulses given at CT 18-19:30 or CT 18-24 when light phase advances the rhythm, produced large increases in ApC/EBP. In preliminary experiments the effect of light does not appear to be phase dependent as light at CT 3-9, when light does not affect the phase of the rhythm, also produced large increases in ApC/EBP. ApC/EBP is the first *Aplysia* gene shown to vary rhythmically. ApC/EBP may be involved in activating certain genes that are important in the generation of the rhythm. A better understanding of the role of ApC/EBP will emerge from future studies of the protein changes of this gene. (Supported by MH41979).

551.4

OLOMOUCINE, A CYCLIN DEPENDENT KINASE INHIBITOR, PHASE SHIFTS THE *APLYSIA* EYE RHYTHM POSSIBLY VIA INHIBITION OF TRANSCRIPTION. N. Sankrithi*, Q. Tran, and A. Eskin. Department of Biochemical and Biophysical Sciences, University of Houston, Houston, TX 77204

Cyclin levels oscillate and consequently control the activity of cyclin-dependent kinases. If cyclin dependent kinases were involved in opisthobranch ocular rhythms, then inhibiting them would affect the phase and/or period of the rhythm. Olomoucine, a purine analog , acts as a competitive inhibitor of ATP for cyclin dependent kinases *in vitro* with relative specificity (Vesely, 1994). Isolated eyes in constant darkness were exposed to 100 µM olomoucine at various phases. Single 6h pulses of olomoucine produced phase delays in the *Aplysia* ocular rhythm at all phases tested. Response curves with all delay phase shifts are characteristic of inhibitors of transcription and translation. Therefore, the effect of olomoucine on transcription and translation was examined by investigating ^{3}H uridine and ^{3}H leucine incorporation into TCA precipitable material. Label was given during the last 4h of 6h treatments of olomoucine. Olomoucine inhibited uridine incorporation by 84 ± 4 % (n=4) during CT 7-13. During the same phase, olomoucine did not inhibit leucine incorporation. Also, isoolomoucine (100 µM), an inactive analog of olomoucine, did not inhibit transcription nor did it phase shift the rhythm. These results suggest that olomoucine's action on the circadian rhythm could be mediated through inhibition of transcription (Supported by MH41979).

551.5

AGING ALTERS THE CIRCADIAN RHYTHM OF *APLYSIA* EYES. M. Sloan* and A. Eskin. Dept. of Biochem. & Biophys. Sci., Univ. of Houston, TX 77204.

It has been shown that aging affects the circadian rhythms of mammals (Turek et al., 1995). We have begun to investigate aging changes in the circadian rhythm of spontaneous nerve impulses from the isolated eye of *Aplysia californica*.

Aplysia of various ages, from 4 months to 14 months, were obtained from the University of Miami Aplysia Resource Facility, Miami, Florida. Animals were maintained in artificial sea water at 15°C and entrained to a light/dark cycle (LD 12:12) for at least 3 cycles. Isolated eyes, with their attached optic nerves located inside a polyethylene tube containing an electrode, were placed in recording chambers containing buffered filtered sea water. The circadian rhythms of spontaneous optic nerve impulses were recorded in DD conditions.

The amplitude of the rhythms progressively decreased with age. 4-6 month old animals had robust rhythms averaging a peak value of around 309 spikes per hour on the first day, decreasing to around 119 on the fifth day. 9-10 month old animals had medium rhythms averaging a peak value of around 245 spikes per hour on the first day, decreasing to around 75 on the fifth day.12-14 month old animals had much weaker rhythms, averaging a peak value of around 177 spikes per hour on the first day, decreasing to around 36 on the fifth day. Also, the phase angle of the entrained rhythm of the isolated eyes was more delayed in older animals. On the other hand, the free-running period of the rhythm did not appear to change with age, although a greater percentage of older eyes displayed arrhythmicity.

Another interesting finding was that 63% of a group of 545 animals, ages 12-14 months, had cloudy lenses. Recordings of rhythms from these eyes had starting peak values around 175 spikes per hour, but quickly fell off after the first day to a level below 50 spikes per hour, becomng arrhythmic thereafter. These findings indicate that aging has some pronounced affects on the circadian system in eyes of *Aplysia*. (Supported by MH41979)

551.6

FROM PHOTORECEPTORS TO CIRCADIAN RHYTHMS IN A BLIND MAMMAL (Spalax ehrenbergi) H.M. Cooper *, J. Negroni, R.G. Foster, W. DeGrip, J.K. Bowmaker, A. Szél, E. Nevo *CERVEAU et VISION, INSERM U-371, 69675 Bron, FRANCE; Imperial College, London, UNITED KINGDOM; Department of Biochemistry, University of Nijmegen, THE NETHERLANDS; Inst. of Opthalmology, London, UNITED KINGDOM; Univ. Semmelweis, Budapest, HUNGARY; Inst. of Evolution, Haifa, ISRAEL*

We have used molecular approaches, opsin immunohistochemistry, microspectrophotometry (MSP), activity recordings, retrograde tracing and viral tract tracing methods to dissect out the photic pathway regulating circadian physiology in the blind mole rat, *Spalax*. *Spalax* demonstrates clear entrainment of locomotor activity by light and expresses an endogenous circadian rhythm in constant darkness. As in other mammals, the induction of c-fos expression in the SCN by a light pulse is gated by the phase of the endogenous clock: c-fos immunopositive cells are only observed following photic stimulation administered during the subjective night. The SCN receives is innervated by almost all retina ganglion cells. Their morphology, demonstrated by transneuronal transport of *Alpha-Herpes virus* or by carbocyanine dyes (DiI) suggests the presence of a single morphological class. Ganglion cells appear to receive input from at least two classes of photoreceptors. Using several antibodies directed against different visual photopigments, we find that the majority of photoreceptors contain a rod-like pigment, whereas a small population contains a short wavelength cone-like photopigment. These results differ from previous RT-PCR analysis which revealed the presence of a single opsin closely homologous to the mouse green cone pigment. Further molecular dissection and *in situ* MSP measures of photoreceptors should help to clarify the identity and absorbance properties of these cells.

Research supported by: Human Frontiers (# RG-68B), NATO (#950334), ESF (#185).

551.7

COUPLING BETWEEN TWO PHOTORECEPTOR SYSTEMS THROUGHOUT MONOCHROMATIC LIGHT IN CRAYFISH *PROCAMBARUS*. V. Inclán-Rubio* and A.Borgonio-Aguilar. Departamento de Fisiología. Facultad de Medicina, UNAM. AP. 70-250. CP04510. México, D.F.

The circadian rhythm result from the complexity integration between endogen rhythms and environmental signals. These rhythms could be couple to environmental signals due to their ability to be synchronized. In the crayfish *Procambarus*, there are at least two photoreceptor systems, retinal and caudal. It is known that the white light stimuli upon the caudal photoreceptors of the sixth abdominal ganglion (6th AG) in the crayfish increases the electric activity along the abdominal chain, evoking an inhibitory effect on the amplitude of the electric response to the light of the visual photoreceptors (electroretinogram, ERG). The ERG presents circadian characteristics. The objective of this work is to study the entrainment characteristics of the ERG circadian rhythm when monochromatic light (ML) is applied on the 6th AG. We worked with *Procambarus clarkii* adult. The ERG was obtained with a conventional recorder technique under a L:D (12:12) photoperiod. In the 4th day of recording, a ML stimulus was applied (590 or 632 nm) along one hour and 400 lux intensity, at four different circadian times (6,12,18 and 24 CT). After six days changes were analyzed. Upon the results we can see that: (a) the ML (590 y 632 nm) decreases the amplitud of the ERG always after the light stimulation; (b) the phase response curve shows delays at 6 and 24 CT (590 nm) and 12 CT (632nm) and advances at 12 and 18 CT (590 nm) and 6,18 and 24 CT (632 nm); (c) the presence of a transitory state lasting from 24 to 36 hrs in both ML, and (d) oscillation level change when light was applied during subjective night for both ML. These results show the functional relationship between the caudal and retinal photoreceptor systems and a particular entrainment range for each wave length codified by the 6th AG.(PAPIT-DGAPA: IN204593).

551.8

MODULATORY ACTIONS OF THE CRAYFISH SINUS GLAND IN THE CIRCADIAN RHYTHM OF THE RATE OF CONTRACTION OF THE HEART. J. Hernández-Falcón*(1), J. Serrato (2) and F. Ramón(3). (1) Depto. Fisiología, Fac. Med., UNAM, (2) Depto. Fisiología, Biofísica y Neurociencias, CINVESTAV, IPN, (3) Div. Posgrado, Fac. Med., UNAM, México, D.F. 04510, MEXICO.

Circadian oscillations of numerous animal functions are entrained by light in accordance with day-night cycles. In crayfish the rate of contraction of the neurogenic heart shows a circadian rhythm in which accelerator and inhibitor nerves are involved.

The aim of this work was to assess the role of a neurohemal organ such as the sinus gland in the circadian rhythm of the rate of contraction of the crayfish heart. Thus, heart rate was continuously monitored during 5-7 days in control, blinded, sinus gland and eyestalk excised adult animals subjected to natural photoperiod, constant darkness and constant lightness. Results show that the rate of contraction of the heart oscillates with a period of 22.12 ± 3.6 hours in control and blinded animals. Excision of sinus gland or eyestalks do not change the period of the oscillation, but amplitude, activity level and α/ρ values decrease. These results indicate that the circadian rhythm of the rate of contraction of the crayfish heart is under neural and humoral control.

Supported by: Prog. Div. Posgrado No. 359.

551.9

EFFECT OF THE RED MONOCHROMATIC LIGHT UPON THE SYNCHRONIZING MECHANISMS OF THE MOTOR ACTIVITY RHYTHM OF THE JUVENILE CRAYFISH *P. clarkii*. Miranda-Anaya, M; Barrera-Mera, B. and Fanjul-Moles, M. L*. Lab. de Neurofisiología. Facultad de Ciencias; Depto. de Fisiología. Fac. Medicina, UNAM, México D. F. a. p. 70-371

Red monochromatic light synchronizes locomotor activity rhythm during the ontogeny in crayfish. The objective of this work was to study the putative circadian photoreceptors involved in this phenomena. Three groups of 60 days old crayfish consisting of 1) animals with a restricted lesion in retina, 2) animals with a restricted lesion in supraesophagic ganglion and 3) intact control animals. All the animals were individually recorded with a motor activity recording system; ten days under dark constant conditions, other 10 days under a 24 Hs skeleton photoperiod cycle (SP), consisting of two red light signals (25 $Wm.^{-2}s^{-1}$) and afterwards transferred again to constant darkness conditions during 10 days. Results analyzed by means of actograms and chi square periodograms, indicated that intact animals display a unimodal activity rhythm (τ=23.7±1.8) able to sinchronize to SP (τ=24± 0.5). Retine injured animals showed an evident longer activity rhythm (τ=25.5±1) synchronizing under SP (τ=24.2±0.4). Supraesophagic injured animals showed mostly a lack of rhythmicity under constant conditions and were unabled to sinchronize to SP. These results, although preliminary, could indicate in the crayfish extraretinal synchronizing mechanisms sensible to the monochromatic red light.

This work was supported by PADEP UNAM No. 003001

551.10

ONSET OF PRENATAL ENTRAINMENT BY MELATONIN IN THE SYRIAN HAMSTER. N. Viswanathan* and F.C. Davis, Dept. of Biology, Northeastern Univ., Boston, MA 02115.

Day/night rhythms of the mammalian circadian pacemaker occur during late gestation and are entrained by rhythms of the mother. The maternal signal or signals responsible for causing prenatal maternal entrainment have not yet been identified. However, there is evidence in hamsters that the pineal gland hormone, melatonin, can mediate prenatal entrainment. Melatonin when given daily to pregnant hamsters during the last 4-5 days of gestation or given only once on day 15 of gestation sets the phase of the pups rhythm at weaning. The aim of the present study was to determine when during gestation a single prenatal melatonin injection can set the phase of the offsprings' circadian rhythm.

Pregnant Syrian hamsters were maintained in constant dim light and SCN ablation was done on day 7 of gestation. They received a single intraperitoneal injection of melatonin (25µg) at different stages of gestation (E 11.75,12.25*,12.75*,13.25,13.75,14.25, and 14.75.(* in progress). After the injection the hamsters and their pups born on day 16 of gestation were left undisturbed until the pups were introduced individually into the wheel cage on postnatal day 20 (weaning). The activity rhythms of the pups were recorded for 3-4 weeks and the phases on the day of weaning were determined.

A single melatonin injection when given on E13.25, 13.75, 14.25, 14.75 successfully set the phases of pups' rhythms whereas melatonin on E 11.75 failed to set the phases. Since SCN neurogenesis in Syrian hamster is complete on E12.5 (Davis et al 1990), the present results indicate that the circadian pacemaker (SCN) responds to melatonin only after SCN neurogenesis is completed. It is likely that the mechanisms for the generation of oscillations and/or for entrainment by melatonin require postmitotic differentiation of SCN cells.

Supported by NIH grant HD 18686.

551.11

MODULATION OF TONIC IMMOBILITY BY MELATONIN IN 10-11 DAY OLD CHICKENS (G. GALLUS). Z. A. Rodd,* N. B. McCutcheon, D. J. Shillito, & G. G. Gallup, Jr. Dept. of Psychology, University at Albany: SUNY, Albany, NY 12222.

Tonic immobility (TI) is an innate behavior which is reliably elicited by a brief period of manual restraint. After initial struggling, the animal assumes a motionless, catatonic-like posture which persists in the absence of further restraint. Previous theories pertaining to TI, have linked the behavior to a state of sleep. Additionally, a number of researchers have studied the similarities between TI and rapid eye movement (REM) sleep. A previous study reported that melatonin inhibited REM sleep in young chicks. The intent of the studies was to determine whether melatonin influenced TI in a similar manner as REM in chicks. The present series of studies were conducted to examine the effects of an administration of melatonin on the susceptibility to and duration of TI in young chicks. The results indicated that melatonin exerted an inhibitory influence on the duration of and susceptibility to TI. These results were comparable to the reported inhibitory influence melatonin expressed on REM sleep in young chicks. Therefore, the effects of melatonin on TI parallel the effect of melatonin on sleep parameters in young chicks.

551.12

ELF-EMF EXPOSURE AND NOCTURNAL MELATONIN PRODUCTION IN HUMANS:AN 11-WEEK EXPOSURE STUDY WITH A HEATLESS ELECTRIC BLANKET

Michinori KABUTO[1]*, Yoshika KUROKAWA[1] and Seung Chul HONG[2]
[1]Jpn. Natl. Inst. Environ. Studies, 16-2 Onogawa, Tsukuba City, Ibaraki, 305 JAPAN
[2]Univ of Tokyo Faculty of Med, Bunkyo-ku, Tokyo, 113 JAPAN

With respect to the suggested effects of ELF-EMF (extremely low frequency-electromagnetic fields) in our daily lives on melatonin (MEL) production, especially on its nocturnal rise, and also its rhythm, four healthy young subjects were exposed to ELF-EMF of around 5 μ Tesra at its peaks using a self-made sheet of "heatless blanket" being wired with electric code connected with an usual electric blanket during sleep every night for 11 weeks. Ten urine specimens were collected for 5 time-periods of 1800-2400, 2400-400, 400-800, 800-1200 and 1200-1800 hours during successive three days a week. MEL excretion rate (UMER, ng/hr) was obtained based on malatonin measurements with a RIA method (Bühlmann Lab AG). Amplitude and 24-hr rhythm of the UMERs were estimated with the Cosinor method on the basis of the three-day data per week for one week before, initial and last phase (2 weeks each) during, and two weeks after exposure. Only in one out of the four subjects, a marked reduction of the amplitude of UMER during 2400-400 hours and a delay of the acrophase was observed for the last two weeks during exposure. This result seems to support some reported in vivo animal experiments and also findings for female electric blanket users, suggeting a possible effect among highly sensitive individuals. It should be noticed, however, that there were generally large individual as well as intra-individual variations in UMER values, the reason of which has now been investigated with increasing the number of subjects. (This study was supported by NIES grant T-13 & H-16)

551.13

QUIPAZINE MIMICS THE EFFECTS OF A LIGHT PULSE ON MELATONIN RHYTHMICITY AND INDUCES SUPRACHIASMATIC NUCLEUS FOS EXPRESSION IN THE RAT.

D. J. Kennaway, R. W. Moyer and K. S. Türker*. Departments of Obstetrics and Gynaecology and Physiology, University of Adelaide, Adelaide, South. Australia, 5005.

Serotonergic pathways to the suprachiasmatic nucleus (SCN) are thought to modulate the effects of light on circadian rhythmicity. The evidence comes from *in vitro* studies of the effects of serotonin agonists on SCN neuronal firing rate rhythms in rats and *in vivo* studies in hamsters showing that serotonergic agonists *block* SCN c-fos induction by light. To determine the physiological consequences of activation of serotonergic pathways to the SCN, we have investigated the effect of a serotonin agonist on rhythmic melatonin production and SCN c-fos induction in rats. Wistar male albino rats (100g) were initially kept in a 12L:12D photoperiod and then continuous darkness. Thirty hours after initiation of continuous darkness (CT18), rats were injected with saline vehicle, quipazine (1-10 mg/kg) or exposed to a 2 lux, 15 minute light pulse and urine collected hourly for a further 3 subjective nights. Administration of vehicle had no acute effect on the excretion of the melatonin metabolite 6-sulphatoxymelatonin (aMT.6S) whereas both quipazine and the light pulse resulted in a significant and prolonged suppression of melatonin production. On the next night, the time of onset of the aMT.6S excretion was unaffected by the vehicle treatment (0.2 ± 0.2h), but was significantly delayed by quipazine treatment (1.5 ± 0.2h) and the light pulse (2.7 ± 0.5h). In other experiments at CT18 both quipazine (5-10 mg/kg) and light treated animals (2 lux, 15 minutes) showed intense c-fos protein immunoreactivity of cell nuclei in the ventrolateral SCN, whereas no immunoreactivity was observed in the vehicle treated animals. These results show for the first time that administration of a serotonin agonist *mimics* both the acute suppression and phase delaying effects of light on pineal gland rhythmicity in the rat. The latter response and the induction of fos protein in the ventrolateral SCN suggests that serotonergic pathways are important *mediators* of light effects in the rat. These results contrast with previous data reported for the hamster.

This research was supported by the NHMRC of Australia.

551.14

DIURNAL VARIATIONS IN MELATONIN RECEPTOR AND mRNA EXPRESSION IN CHICK BRAIN. S. Iacob*, M.I. Masana and M.L. Dubocovich, Dept. Molec. Pharmacol. Biol. Chem. and Northwest. Univ. Inst. Neuroscience, Northwestern Univ. Med. School, Chicago, IL 60611.

ML_1 melatonin receptor density in retinorecipient areas of chick brain displays distinct diurnal rhythms, with higher levels during the day (Brooks and Cassone, Endocrinol., 131:1297, 1992). Here we report correlations between diurnal oscillations in specific 2-[^{125}I]-iodomelatonin binding and melatonin receptor (ml_{1a}, ml_{1c}) mRNA expression in chick brain. Chicks (21 days old) maintained on a 14 /10 L/D cycle, were sacrificed every 4 hours: ZT2, ZT6, ZT10, ZT14, ZT18 and ZT22 (ZT0 = onset of light). Coronal brain sections throughout the telencephalon, diencephalon and mesencephalon were processed for quantitative receptor autoradiography with 2-[^{125}I]-iodomelatonin (60 and 240 pM) (specific binding defined with 1 µM melatonin) and *in situ* hybridization with antisense and sense ml_{1a} and ml_{1c} [^{35}S]-riboprobes. Specific 2-[^{125}I]-iodomelatonin binding was higher during daytime. Both primary [optic tectum (OT), DSV] and secondary [rotundus (ROT) and triangularis (T) nuclei] visual brain areas exhibited higher levels of specific binding during the day, peaking at ZT10. Binding density to tertiary visual input recipient areas [ectostriatum (E)] did not oscillate. Expression of ml_{1a} melatonin receptor mRNA in the ROT and E was higher at ZT2, while ml_{1c} melatonin receptor mRNA expression in the pineal gland and the E was higher at ZT6 and ZT10. In summary, these findings suggest that melatonin receptor protein and mRNA expression in chick brain visual areas follow diurnal rhythms. [Supported by MH42922 to MLD]

551.15

MECHANISMS UNDERLYING INCREASES IN MELATONIN RECEPTOR DENSITY FOLLOWING WITHDRAWAL OF MELATONIN. M.I. Masana*, P. Witt-Enderby and M.L. Dubocovich. Dept. Mol. Pharmacol. Biol. Chem. and Northwest. Univ. Inst. Neurosc., Northwestern Univ. Med. School, Chicago, IL 60611.

Mammalian melatonin receptor expression exhibits a diurnal rhythm. In the pars tuberalis this rhythm is generated by fluctuations in plasma melatonin, while in the suprachiasmatic nucleus the rhythm is regulated by light and is suppressed in constant dark (Brain Res., 641:92, 1994). Two mammalian melatonin receptors cDNAs have been cloned; the ml_{1a} expresses in the SCN, pars tuberalis and paraventricular nucleus of the thalamus and the ml_{1b} in the retina (Cell, 83:1059, 1995). Here we report the effects of withdrawal from melatonin exposure on the density and regulation of the human ml_{1a} melatonin receptor. CHO cells stably transfected with the human ml_{1a} melatonin receptor (K_D = 74 pM and B_{max} = 679 fmol/mg protein) were exposed to 400 pM melatonin for 8 hours and 2-[^{125}I] iodomelatonin binding was measured at different times after cessation of melatonin treatment. A time dependent increase in 2-[^{125}I] iodomelatonin binding was observed after melatonin withdrawal, reaching a plateau (~150%) at 8 hours. Melatonin receptor mRNA levels also increased following one hour and reached a value of ~300 % following 16 hours after cessation of melatonin treatment. In addition, pertussis toxin-catalyzed ADP ribosylation of the inhibitory G-protein (heterotrimeric form $G i\alpha\beta\gamma$) increased in a time-dependent manner following melatonin withdrawal. In summary, the increase in 2-[^{125}I] iodomelatonin binding could be due to increases in melatonin receptor protein and, at least in part, to the stabilization of the high affinity complex melatonin/melatonin receptor/G protein. These results suggest that *in vivo*, the nocturnal melatonin exposure may be one of the mechanisms by which diurnal regulation of melatonin receptors occurs. [Supported by USPHS grant MH42922 (MLD) and F32 HL08965 (PWE)]

551.16

IDENTIFICATION OF NOVEL BENZIMIDAZOLES WITH HIGH AFFINITY AND SPECIFICITY FOR THE MELATONIN ML_2 BINDING SITE. K. S. Takaki, B. T. Watson, R. A. Deshpande, G. N. Karageorge, J. P. Yevich*, A. A. Ortiz, A. Johnson, R. McGovern, D. Molstad, N. Sarbin, J. Stanley, C. Mahle. Bristol-Myers Squibb Co., Wallingford, CT 06492.

Melatonin has been found to bind to a number of distinct sites but the significance of all of these has yet to be elucidated. The human ML_{1A} receptor, found in the SCN, has been cloned and is presumed to mediate the chronobiotic effects of melatonin. The human ML_{1B} receptor has also been cloned but its localization and function have yet to be determined. In addition, a binding site for melatonin in hamster hypothalamus has been reported to which no function has been ascribed. This ML_2 site is distinguishable from the ML_{1A} and ML_{1B} receptors because of its high affinity for the α_1 adrenergic antagonist, prazosin. We have identified a series of novel benzimidazole derivatives which are highly selective for the melatonin ML_2 binding site found in hamster hypothalamus. These compounds do not bind to ML_{1A}, ML_{1B} or α_1 receptors. In addition, members of this series have been shown to possess anticonvulsant properties when evaluated in the maximal electroshock or pentylene-tetrazol-induced seizure models. A systematic structure affinity relationship study was performed to optimize ML_2-selectivity and resulted in the identification of a lead compound with better affinity and selectivity for the ML_2-site than melatonin.

Funding: Bristol-Myers Squibb Company

551.17

EVIDENCE THAT THE MEL1A MELATONIN RECEPTOR MEDIATES MELATONIN-INDUCED VASOCONSTRICTION OF RAT CAUDAL AND CEREBRAL VESSELS. A. J. Watson*, P. Agarwal, G. D. Goggins, E. Ryan and C. D. Mahle. C. N. S. Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492.

The effects of the pineal hormone melatonin are mediated through high affinity G protein-coupled receptors. Melatonin binding sites have been described in vascular tissue, notably rat caudal and anterior cerebral arteries. In rat caudal artery, the non-hydrolyzable GTP analog, Gpp(NH)p, induced an attenuation of 2-[^{125}I]iodomelatonin binding, consistent with the interaction of the binding site with a G protein. In rat tail artery, melatonin (10^{-12} to 10^{-4} M) had no direct effect on baseline tension. Melatonin, 2-iodomelatonin and S-20098 induced a dose- and concentration-dependent potentiation of precontracted rat caudal and anterior cerebral arteries. The potentiating effects of melatonin were not altered in the presence of the α-adrenergic antagonists prazosin or phentolamine, or by blockade of sodium-dependent neurotransmitter release with tetrodotoxin. Finally, we show, by reverse transcription-polymerase chain reaction (RT-PCR) that mRNA for the MEL1A melatonin receptor is present in both rat caudal and cerebral arteries. Degenerate RT-PCR of melatonin receptor transcripts from caudal artery demonstrates that these are bona fide MEL1A sequences, and not those of a closely related subtype. Transcripts corresponding to other melatonin receptor subtypes were not detected. These results suggest that the MEL1A receptor is the mediator of melatonin-induced vasoconstriction.

Research supported by Bristol-Myers Squibb Company.

551.18

Effects of a melatonin agonist and antagonist on hamster suprachiasmatic nucleus (SCN) neurons *in vitro*. G. Scott, P. Delagrange*, B. Guardiola-Lemaître, E. Mocaër and B. Rusak. Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4J1.

S-20098 (N-(2-(7-methoxy-1-naphthyl) ethyl) acetamide) acts as a melatonin receptor agonist as demonstrated by its ability to entrain daily rhythms in locomotor activity and its ability to suppress firing of photically responsive cells in the SCN *in vivo*. Neurophysiological effects of S-20098 *in vivo* are partially attenuated by systemic injections of S-20928 (N-(2-(1-naphthyl) ethyl) cyclobutyl carboxamide), a putative melatonin receptor antagonist. Because systemic injections did not identify the target at which these effects occurred, we have investigated the effects of S-20928 on responsiveness of single SCN cells in an *in vitro* brain slice preparation to melatonin and to S-20098.

Ionophoretic application or pressure ejection of S-20098 (1mM in 2% DMSO; 0-80nA; 1-7 psi; 5-120s duration) or melatonin onto single SCN cells potently attenuated spontaneous firing in a current- or pressure-dependent manner. S-20098 or melatonin also attenuated the activational effects on single SCN cells of ionophoretically applied NMDA (0-40nA; 10-30s duration). S-20928 (1 mM in 2% DMSO; 1-7 psi; 35-120s duration) had no effect on cell firing when pressure ejected alone, but its prior application was able to antagonize the suppression of firing rates by application of S-20098. These results indicate that S-20098 potently suppresses spontaneous and NMDA-evoked firing in SCN cells *in vitro*, presumably by acting at melatonin receptors, and that S-20928 acts directly on SCN cells to antagonize these effects.

Supported by Servier, NSERC (Canada) and the U.S. AFOSR.

551.19

ML_{1A} MELATONIN RECEPTORS IN HUMAN AND RAT CEREBRAL AND PERIPHERAL ARTERIES: mRNA EXPRESSION AND FUNCTIONAL PHARMACOLOGY. D.N. Krause*[1], M.L. Dubocovich[2], L. Edvinsson[3], G.G. Geary[1], M.I. Masana[2] and S.P. Duckles[1]. [1]Pharmacol, Col Med, Univ Calif Irvine, CA 92717; [2]Mol Pharmacol & Biol Chem, Northwestern Univ Med Sch, Chicago, IL 60611; [3]Internal Med, Univ Lund, S-221 85 Sweden.

Melatonin, secreted from the pineal during the night, modulates circadian rhythms and sleep/wake cycles. The presence of melatonin receptors in certain vascular beds suggests a role for melatonin in vascular regulation. We now report molecular and pharmacological data indicating that human and rat cerebral and peripheral arteries express functional melatonin receptors of the ML_{1A} subtype. Melatonin receptor fragments were amplified from human cerebral arteries by reverse transcriptase-PCR using degenerate primers for the known ml_{1a} and ml_{1c} receptors, and identity with the ml_{1a} receptor was confirmed by cloning. Functional effects of vascular melatonin receptors were characterized using rat middle cerebral and tail arteries in vitro. Melatonin (1-100 nM) directly constricted cannulated, pressurized cerebral arteries and potentiated constriction of tail arteries, effects antagonized by luzindole with a pK_B value (6.4) that correlates with the binding affinity of luzindole for recombinant ml_{1a} (6.8) but not ml_{1b} (8.0) or ml_{1c} (5.5) receptors. Thus melatonin appears to regulate the cerebral circulation of humans and other mammals via vascular ML_{1A} receptors. Activation of these receptors may contribute to circadian variations in cerebral blood flow and onset of stroke as well as to possible side effects of melatonin administered for sleep disorders. *NIH HL-50775 and MH-42922*

551.20

ML_{1B} MELATONIN HETERORECEPTORS MEDIATE PRESYNAPTIC INHIBITION OF DOPAMINE RELEASE FROM RABBIT RETINA. M. L. Dubocovich*, S. Iacob, K Mistry and M. I. Masana. Dept. Mol. Pharmacol. Biol. Chem. and Northwestern Univ. Institute for Neuroscience, Northwestern Univ. Med. School, Chicago, IL 60611.

Melatonin receptors were originally classified based on kinetic properties and pharmacological profiles into the ML_1 and ML_2 classes (TIPS, *16*: 50, 1995). The ML_1 class contains at least three molecular receptor subtypes, i.e., ml_{1a}, ml_{1b}, ml_{1c}, with about 60 % amino acid homology among themselves (Cell *83*: 1059, 1995). To identify subtype-selective drugs, we compared the pharmacological profiles of the recombinant ml_{1a} (human), ml_{1b} (human) and ml_{1c} (*Xenopus* melanophore) receptors expressed in COS 7 cells with the well characterized functional melatonin heteroreceptor in rabbit retina. The relative order of agonist potencies to inhibit the calcium-dependent release of dopamine from retina was identical to the order of affinities (pKi) of the analogues for the recombinant ml_{1b}: [S20098 (12) > mel (9.8) ≥ 6Clmel (9.7) ≥ 2Imel (9.7) ≥ 6,7 $Cl2CH_3$ mel (9.7)], but not the ml_{1a} [2Imel (10.2) > S20098 (9.1) ≥ mel (9.1) > 6, 7 di $Cl2CH_3$ mel (8.3) > 6Cl mel (7.9)]. Partial agonists [e.g., 5-methoxy-luzindole, N-acetyltryptamine] and competitive melatonin receptor antagonists (e.g., 4-phenyl-2-chloroacetamidotetraline, 4-phenyl-2-propionamidotetraline, 4-phenyl-2-acetamidotetraline, luzindole] distinguished the ml_{1b} (- log Ki= pKi: 9.9, 7.9, 9.1, 8.8, 8.9, 8) from the ml_{1a} (pKi: 7.5, 6.7, 6.5, 6.3, 6.2, 6.8) and ml_{1c} (pKi: 7.4, 6, 6.4, 6.3, 6, 5.6) subtypes. These analogues showed 100 times higher affinity for the ml_{1b} recombinant subtype. The corresponding dissociation affinity constants (K_B) of these analogues (pK_B: 10.2, 9, 9.3, 9.5, 8.8, 7.7) to antagonize the melatonin-induced inhibition of dopamine release correlated with their affinities (pKi) for the ml_{1b}, but not the ml_{1a} or ml_{1c} subtypes. We conclude that the presynaptic melatonin heteroreceptor modulating dopamine release is the ML_{1B} subtype. Supported by MH 42922.

552.1

AGE- AND SEX- RELATED COMPARISONS OF VOCAL CONTROL REGIONS IN FREE-LIVING DARK-EYED JUNCOS (*JUNCO HYEMALIS*). Cynthia C. Gulledge* and Pierre Deviche. Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks AK 99775-7000.

In songbirds, neural control of singing behavior is accomplished via an interconnected set of vocal control regions **(VCRs)**, collectively called the vocal control system. Other studies have shown that VCR volumes are sexually dimorphic in species in which only the males sing. In the spring and early summer, adult male dark-eyed juncos sing a long-distance song from tree-tops, while adult female juncos and juveniles of both sexes do not. We have shown previously that male junco VCR volumes change seasonally, being large during breeding, and smaller afterwards in the fall. We collected adult and juvenile male and female juncos from a wild population near Fairbanks, Alaska, in the fall, when no juncos were singing. A blood sample was collected immediately after capture to be assayed for androgen. Brains were fixed with 4% paraformaldehyde via intracardiac perfusion, sectioned, then stained with thionin. Vocal control regions (Area X, MAN, HVC, RA) and one non-vocal control region (n. rotundus) were measured. VCRs were 3-7 times smaller in females than in males (depending on the region), and some regions were smaller in fall adults than in fall juveniles. Neither the size of the n. rotundus nor plasma androgen concentrations differed among groups. Thus, VCR volumes of juncos are sexually dimorphic, and plasma androgen concentrations do not act as the proximal determinant of VCR size outside of the breeding season.

Supported by NSF award BNS-9121258 to P.D.

552.2

INDIVIDUAL DIFFERENCES IN HVC NEURON NUMBER PREDICT DIFFERENCES IN THE PROPENSITY FOR AVIAN VOCAL IMITATION. B. C. Ward, E.J. Nordeen*, and K.W. Nordeen. Dept. Brain and Cog. Sci. and Neuroscience Program, U. Rochester, NY 14627.

In several species of passerine songbirds, the overall volumes of two different vocal control regions (the HVC and RA) correlate with song repertoire size. It is unlikely that these correlations reflect effects of song learning on neural growth since early deafening does not diminish the size of these regions. In the present study, we explored an alternative hypothesis; that normal variation in the size of song regions affects how much song material can be faithfully imitated.

Within 8 days of hatching, male zebra finches were isolated from adult song and raised to independence by their mother. At 30 days, they were housed permanently in a large cage with two adult tutors and their mates. Songs were recorded at 120 days of age, and birds then were sacrificed to measure neuron number and nuclear volume for HVC, RA and Area X.

The tutors produced 16 distinct syllables, but the subjects copied no more than 6 syllables, and never copied from both tutors. Several measures of brain space correlated significantly with the number of syllables copied from the tutors, but not with total repertoire size. Individual differences in the volume of the HVC and number of HVC neurons strongly predicted the number of syllables copied from the tutor ($r = .93$; $r = .95$, respectively; $p \leq .01$). RA volume and neuron number also correlated with the amount of learned song material, but less strongly ($r=.70$; $r=.66$, respectively). Neither Area X volume nor neuron number correlated well with measures of song learning.

NIMH MH 45096.

552.3

PERCEPTUAL SELECTIVITY CORRELATES WITH l-MAN NUCLEAR VOLUME IN A NON-SINGING FEMALE SONGBIRD. K. Cleal, S. E. Allan*, A.P. King, D.R. Sengelaub, and M.J. West. Departments of Psychology and Biology, Indiana University, Bloomington, IN 47405.

Song production in brown-headed cowbirds (Molothrus ater) is sexually dimorphic. Males use song during courtship, with virtually all copulations preceded by at least one vocalization. During the breeding season, captive females respond to song playbacks with a copulatory posture, the frequency of which can be used to measure the relative potency of individual songs. Females show strong individual differences in song selectivity; while all females respond more frequently to high potency songs, some are less discriminating, responding to low potency songs as well. We have begun to assess the neural basis of song selectivity in female cowbirds, examining the brain nuclei involved in song production, perception, and learning.

Song selectivity, the difference in response frequency to high and low potency song, was evaluated in adult females. Morphology of song nuclei in males and females was examined in frozen, thionin-stained sections (40 µm). Consistent with the sexual dimorphism in song production, volume, neuron number, and neuron size within HVC, RA, and Area X were up to 10 times larger in males than in females. In contrast, no sex differences were observed in l-MAN, a structure previously implicated in song learning and perception. Furthermore, l-MAN volume varied due to individual differences in neuron number, and was strongly correlated ($r=0.96$; $p<.001$) with song selectivity in females. Thus, although female cowbirds do not sing, their l-MAN is as large as that of males, and may be involved in discrimination of male song quality. Supported by NSF 88 21152 (MJW)

552.4

RELATIVE VOLUME OF L-MAN IN FEMALE WARBLER SPECIES VARIES WITH THE NUMBER OF SONGS PRODUCED BY CONSPECIFIC MALES. T.J.DeVoogd[1], J.A.Cardin[1], T. Szekely[2], J. Büki[3] and S.W.Newman[4]*. [1]Dept. Psychology, Cornell Univ., Ithaca, NY; [2]Dept. Zoology, Kossuth Univ., Debrecen,Hungary; [3]Ornith. Soc., Budapest, Hungary; [4]Dept. Anat., Univ. Mich., Ann Arbor, MI.

In some songbird species, the males learn large numbers of songs, a process that appears to have been driven by female choice of males with the most complex songs (Catchpole, 1987; Bensch & Hasselquist, '92). Species differences in a male's capacity for vocal learning are correlated with species differences in the relative volume of HVC (DeVoogd et al., '93; Szekely et al., '96), a nucleus essential for song production (Nottebohm et al., '76). Effective female choice requires not only that a female identify the song of her species but also discriminate songs of greater and lesser complexity. We examined the brains of females of 7 sylviid warbler species, caught in May, perfused with 10% formalin, sectioned and Nissl stained. We find that differences in the relative volume of l-MAN across species are closely related to the complexity of songs produced by males of the species. In males, this nucleus is essential for song learning (Bottjer et al., '84) and contains cells responsive to the bird's own song (Doupe & Konishi, '91). The relative volumes of HVC, RA and n. rotundus in the females do not show this association. Thus, aspects of structure in a nucleus that may be involved in song perception differ between females of species that experience different auditory demands--perhaps providing the acuity that underlies evolutionary selection for enhanced learning ability. Supported by US-Hungary Joint Fund 91-117 and NATO Collab. Res. Award 93-1542.

552.5

SEASONAL PLASTICITY OF THE SONG SYSTEM IN WILD NUTTALL'S WHITE-CROWNED SPARROWS. E. Brenowitz*, L. Baptista, K. Lent, and J. Wingfield. Depts. of Psychol. & Zool., Univ. of WA, Seattle, WA 98195 & Calif. Academy of Science, San Francisco, CA 94118.

Seasonal changes in song behavior and the morphology of song nuclei in the brain occur in nearly all species of seasonally breeding songbirds studied. The only published report of a seasonally breeding bird that *lacks* seasonal plasticity in the song nuclei is for the Nuttall's race of White-crowned sparrow (*Zonotrichia leucophrys*) (Baker et al. '84). Their study used captive sparrows that were exposed to long-day (LD) or short-day (SD) photoperiods in the laboratory. In a study of the conspecific Gambel's race of white-crowns (Smith et al. '95), we found that plasma levels of testosterone (T) in captive males may be lower than those of wild breeding males. Seasonal changes in plasma T serve as the dominant proximate mechanism underlying changes in the song nuclei (Smith et al. '93). It is possible, therefore, that the LD Nuttall's white-crowns in Baker et al.'s study did not experience plasma T levels high enough to stimulate growth of the song nuclei. To test this possibility, we measured seasonal patterns of song, hormones, and song nuclei in **wild** Nuttall's white-crowns.

Adult males were captured in spring (April, n=10) and fall (September, n=9), bled for hormone analysis, and perfused. The volumes of song nuclei and other regions were measured in Nissl stained sections. Song was recorded in the field at both times of year.

We found pronounced seasonal changes in the size of song nuclei and in T levels. Spring:fall ratios were: HVC = 2.23*, RA = 1.80*, Area X = 1.57*, T = 24.2* (* = $P \leq 0.005$, t-test). The thalamic visual nucleus Pt did not change in size (S:F = 0.96). These results indicate that, as in other seasonally breeding species, there is seasonal plasticity in the song system of Nuttall's white-crowned sparrows. (NIH MH53032 & NSF DCB-9005081)

552.6

EFFECTS OF CASTRATION ON THE VOLUME OF SONG CONTROL NUCLEI AND TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN MALE CANARIES G.F. Ball*, T.P. Hahn, E. Edmonds, and J. Balthazart Department of Psychology, Behavioral Neuroendocrinology Group, Johns Hopkins University, Baltimore, MD 21218; Lab of Biochemistry, Univ. of Liège, Belgium

Canaries (*Serinus canaria*) possess a neural circuit that mediates the learning and production of song. Several nuclei in this circuit such as the High Vocal Center (HVC) change seasonally in that the apparent volume of the nucleus is larger in the spring when plasma levels of testosterone are high than in the fall when they are low. The seasonal change in volume is not apparent if one defines the boundaries of HVC based on cells containing estrogen receptors or based on cells that project to the song nucleus, robustus archistriatalis (RA). In this study we investigated the effects of castration on the volume of HVC, RA and another song control region, area X. Nuclear boundaries were defined based on Nissl staining characteristics or based on dense immunoreactivity for the enzyme tyrosine hydroxylase (TH-ir). Photosensitive male canaries (N=12) were held on short days (7L:17D) and 7 males were castrated and 5 were sham castrated. The males were then transferred to long days (16L:8D) and after 6 weeks they were perfused and their brains collected. Sections were collected throughout the forebrain and alternative sections were either Nissl stained or stained for TH with an immunocytochemistry procedure. The volume of HVC, RA and area X as reconstructed based on the Nissl stained sections was smaller in the castrated males than in the intact males. This difference in HVC was also apparent when one defined the boundaries of HVC based on the dense plexus of TH-ir fibers. Castration also induced an apparent anatomical reorganization of the TH innervation of HVC. TH-ir fibers were more or less randomly distributed in the HVC of intact birds but they formed dense basket-like ring structures around a limited number of cells in the HVC of castrates. These data suggest that testosterone is required for the spring increase in song control nucleus volume and that the noradrenergic input to HVC as defined by TH-ir changes seasonally in a T-dependent fashion. Supported by NIH grant MH 50388.

552.7

HIGH VOCAL CENTER AND ENCEPHALIZATION: SPECIALIZATION AND DEVELOPMENTAL CONSTRAINTS.

D.C. Airey, J.K. Niederer, A.L. Nelson, T.J. DeVoogd* & B.L. Finlay. Department of Psychology, Cornell University, Ithaca, NY 14853.

In mammals, developmental timetables of neurogenesis predict brain structure size (Finlay and Darlington, 1995). For example, larger mammalian brains have a proportionally larger neocortex because the period of neurogenesis associated with neocortex is longer for larger-brained mammals. Adaptive specialization of brain function must occur in the context of such developmental constraints. In birds, an analysis of published data suggests the avian neostriatum is also proportionally larger in larger-brained birds. Additionally, the neostriatum occupies a greater proportion of the telencephalon in Passeriformes than in either Columbiformes or Galliformes (Rehkaemper et al., 1988). In songbirds, the High Vocal Center (HVC) is a neostriatal nucleus involved in the production of learned vocalizations. The relative size of HVC predicts song complexity across more than fifty species (DeVoogd et al., 1993; Szekely et al., in press). A larger relative HVC tends to confer increased behavioral complexity.

These findings suggest that differences in HVC size between species may relate to differences in the size of the inclusive neostriatum or overall telencephalon size. We examined the relation between size of HVC and telencephalon in 42 species of songbird from published data. We also measured the size of the neostriatum in 19 songbird species. We find a significant relation between size of HVC, neostriatum, and telencephalon. Interestingly, neostriatum or telencephalon each significantly predict song repertoire size in simple regressions. In a multiple regression predicting song repertoire size from HVC, neostriatum, and telencephalon size, the only significant factor is HVC size. We suggest that evolutionary forces operate at the level of both overall brain size (neostriatum, telencephalon) and at the level of dedicated neural circuits (HVC).

Supported by US Hungary Joint Fund, NATO, and NIH Grant R01 NS 19245.

552.8

COMMON ORGANIZATIONAL FEATURES IN BUDGERIGAR AND OSCINE FOREBRAIN VOCAL CONTROL SYSTEMS.

Sarah E. Durand*, James T. Heaton and Steven E. Brauth. Dept. of Psychology, Univ. of MD, College Park, MD 20742

Connections of vocal control nuclei in budgerigars were examined with pathway tracing techniques. Afferent and efferent pathways associated with the magnicellular nucleus of the dorsomedial thalamus (DMm), the oval nucleus of the ventral hyperstriatum (HVo), and a newly defined region, the medial division of the oval nucleus of the anterior neostriatum (NAom), reveal that important similarities exist between the budgerigar and oscine models.

A specific projection from central DMm innervates NAom. NAom is afferent to 1) the central nucleus of the archistriatum (AAc) which projects to brainstem vocal motor nuclei, and 2) the magnicellular nucleus of the lobus parolfactorius (LPOm). LPOm projects back to DMm. Pathways through DMm and NAom resemble the oscine "recursive loops" through Area X of LPO. Although HVo is clearly not homologous to the oscine "high vocal center" of the caudolateral neostriatum, it may share functional correlates with the latter given the connections of HVo with thalamic, neostriatal and paleostriatal nuclei: HVo is afferent to both the circuit through DMm and NAom, via projections onto LPOm, and to the principal descending motor pathway, via projections onto lateral neostriatum. In addition, HVo may be a source of auditory input to the forebrain vocal control system: biocytin injections into HVo fill neurons in the auditory neostriatum overlying nucleus basalis and scattered cells in hyperstriatum and neostriatum overlying Field L2. Furthermore, HVo lesions severely disrupt vocalization in both nestling and adult budgerigars. In sum, the psittacine "high vocal center" may reside in hyperstriatum. *Support:* DC00105-02 to SED, MH40698 to SEB.

552.9

NEURAL PATHWAYS THEORIZED TO MEDIATE AFFILIATIVE AND AGONISTIC VOCALIZATIONS IN BUDGERIGARS (*MELOPSITTACUS UNDULATUS*).

K.K. Cookson*, S.E. Durand, and K.J. Heaton. Deptartment of Psychology, UMCP, College Park, MD, 20742-4411.

Budgerigars offer a model for investigating the relationship of neural pathways mediating unlearned and socially learned vocalizations. Previous studies have shown that budgerigars incoporate socially learned contact calls into their reproductive warble, and that their reproductive vocalizations stimulate gonadal activity and reproductive behaviors. Preliminary pathway tracing studies reveal several connections between vocal learning pathways, the preoptic area (POA), and ventral hypothalamus (Hy): (1) the POA projects to the magnocellular nucleus of the dorsomedial thalamus (DMm), (2) the DMm projects to the central nucleus of the anterior archistriatum (AAc), the central nucleus of the lateral neostriatum (NLc), the POA, and the Hy, and (3) the ventral Hy projects to the lateral neostriatum (NLs, NLc), to the region of the oval nucleus of the anterior neostriatum (NAo), and to the medial archistriatum (Am). Budgerigars also produce unlearned agonistic calls, which in other birds are mediated by the medial and ventral archistriatum (AM, Av), the hypothalamus, and the intercollicular midbrain (ICo). Preliminary pathway tracing studies in budgerigars show that: (1) the Am and the Av project to the ICo, (2) regions of ICo receiving input from Am and Av project to the hindbrain and to the dorsomedial nucleus of the ICo (DM), which in turn projects to the hindbrain, and (3) the ventral Hy receives projections from the Am and the Av, and the ventral Hy projects to the Am. These pathways have few or no connections with telencephalic vocal learning nuclei. The thalamic DMm may play an important role in coordinating vocal behavior, especially at the level of the archistriatum, since it is connected to pathways mediating both unlearned and socially learned vocalizations. Funding: SED, DC00105-02; W.S.H., Whitehall J91-17; S.E.B., MH40698.

552.10

SEX DIFFERENCES IN VOCAL BEHAVIOR IN BUDGERIGARS ARE ASSOCIATED WITH SEX DIFFERENCES IN THE VOLUME OF SEVERAL VOCAL CONTROL NUCLEI.

A. A. Nespor*, R. J. Dooling, & G. F. Ball. Dept. of Psych., University of MD, College Park, MD 20742 & Dept. of Psych., Behavioral Neuroendocrinology Group, Johns Hopkins Univ., Baltimore, MD 21218.

Budgerigars produce a complex vocal repertoire that develops through learning and includes a long rambling song called warble. Warble is produced almost exclusively by males. In songbirds such differences in vocal behavior are associated with marked sex differences in the volume of several nuclei in the telencephalon that are part of the song control system. Sex differences in the brain that might be related to sex differences in vocal behavior have not been identified in any psittacine (parrot) species such as the budgerigar. We compared the volume of several vocal control nuclei in male (n=5) and female (n=5) budgerigars. We focused on vocal control areas in the budgerigars analogous to areas in the songbird brain known to exhibit marked sex differences in volume. This includes the oval nucleus of the anterior hyperstriatum ventale (HVo most similar ro MAN); the magnicellular nucleus of the parolfactory lobe (LPOm, most similar to area X); the central nucleus of the anterior archistriatum (AAc, most similar to RA) and the central nucleus of the lateral neostriatum (NLc, most similar to HVC). We delineated the boundaries and re-constructed the volume of these nuclei in adult budgerigars with the use of three different methods. Alternate sections throughout the forebrain were either Nissl stained or labeled via autoradiographic methods for muscarinic cholinergic receptors (as defined by N-methyl scopolamine binding) or alpha-two adrenergic receptors (as defined by para-amino clonidine binding). With the use of all three methods to delineate nuclear boundaries it was found that the volume of HVo was larger in males than in females but LPOm was not. No sex differences in receptor density were discerned. A significant difference was also found in the volume of AAc and NLc based on the Nissl defined boundaries. These data suggest that sex differences in song in budgerigars are associated with sex differences in the volume of telencephalic vocal control nuclei. Supported by NIH MH-50388 and MH-00928, and DC-00198.

552.11

TESTOSTERONE ACCUMULATION IN SPINAL MOTONEURONS OF A "WING-SNAPPING" BIRD.

B.A. Schlinger* Dept.of Physiological Science and Lab. of Neuroendocrinology of the Brain Research Institute, UCLA, Los Angeles, CA 90095.

The study of birdsong has contributed substantially to our understanding of the neural and hormonal substrates underlying brain development and adult brain function. However, many birds use a variety of mechanisms to communicate visually and acoustically and we know little about the neural and hormonal bases of these behaviors. The Golden-collared manakin (*Manacus vitellinus*) is a member of a family of sub-oscine birds that are common in neotropical forests. These birds are highly sexually dimorphic in plumage and in behavior. Breeding males engage in vigorous courtship displays that include much physical activity. Males also make sounds like very loud finger-snapping. Remarkably, these sounds are not vocal (syringeal) but are produced by the wings. Since wing-snapping is a sexually dimorphic reproductive behavior, I tested whether spinal motoneurons in these birds are sensitive to sex steroids. Breeding manakins (from Panama) were injected with ^{3}H-testosterone (^{3}H-T). After sacrifice 90 mins later, spinal cords were removed, frozen, shipped to the US, sectioned longitudinally and prepared for autoradiography. Silver grains were counted over cell nuclei and adjacent neuropil (background). Grain densities (3- to 13-times background) were found over medium to large neurons (somas 237-1140 sq. μm) found centrally and ventrally in the spinal brachial enlargement. These data suggest that these cells are sensitive to T. Thus, T may act directly on spinal motoneurons to control wing-snapping in male manakins. Supported by UCLA Academic Senate.

552.12

MYOTOPIC REPRESENTATION OF SYRINGEAL MUSCLES IN THE HYPOGLOSSAL NUCLEUS OF THE COWBIRD.

J. Ruan and R.A. Suthers*. Indiana University School of Medicine, Bloomington, IN 47405.

The vocal organ, or syrinx, of songbirds is controlled by six pairs of muscles which are innervated by motoneurons in the tracheosyringeal portion of the hypoglossal nucleus (nXIIts). We used retrograde tracing techniques to examine the myotopic organization of motoneurons in nXIIts of the brown-headed cowbird (*Molothrus ater ater*). Cholera toxin subunit B conjugated horseradish peroxidase was injected into individual muscles and the location of labeled cells in nXIIts was mapped. Data from 5 syringeal muscles (2 to 5 adult males for each muscle) indicate that a myotopic map exists in this motor nucleus, with the motoneurons for each muscle being distributed in different, though partially overlapping, regions along its rostrocaudal and mediolateral axes. Motoneurons of syringealis ventralis (vS) are mainly located in the rostral portion of the nucleus. Tracheobronchialis dorsalis (dTB) and syringealis dorsalis (dS) are both represented in the caudal portion of the nucleus, where dTB extends laterally and dS extends medially. Motoneurons to tracheolateralis are located along the lateral portion of the nucleus and neurons innervating the sternotrachealis occupy a small caudolateral region. The results suggest a functional organization in nXIIts. Ventral muscles such as vS, which are primarily involved with controlling the frequency or phonetic properties of song, are controlled by the rostral part of nXIIts, whereas the dorsal adductor muscles dS and dTB, which control the timing of ipsilateral phonation by regulating airflow through that side of the syrinx, share its caudal portion. Supported by NIH NS 29467 and NSF IBN 9411191 to R.S.

553.1

CONTRIBUTIONS OF ENHANCED TYPE-B CELL EXCITABILITY AND SYNAPTIC STRENGTH TO INHIBITION OF TYPE-A CELLS IN *HERMISSENDA*: A PHYSIOLOGICAL ANALYSIS. J.W. Fost* and G.A. Clark. Program in Neuroscience, Princeton University, Princeton, NJ 08544.

During conditioned suppression of phototaxis in *Hermissenda*, the inhibitory type-B photoreceptors exhibit both increased excitability and synaptic facilitation at efferent B-A synapses. Both of these changes have been proposed to contribute to the conditioning-dependent decrease in firing of type-A photoreceptors, and, hence, the reduction in phototaxis. To examine the relative contributions of each form of plasticity, we induced baseline firing ($\bar{x}$ = 3.49 Hz) in type-A cells with DC injections (to mimic light-induced activity), and then superimposed artificial inhibitory PSPs of various magnitudes and frequencies. To explore the isolated effect of synaptic facilitation, we increased PSP magnitude (from 1.0 mV to 2.0 mV, as measured in A-cell soma) while maintaining a constant PSP frequency. Conversely, to quantify excitability effects, we increased PSP frequency (from 12.6 Hz, corresponding to three B-cells at 4.2 Hz, to 17.3 Hz = 3 × 5.77 Hz) while maintaining constant PSP size. Results showed that both forms of plasticity, in isolation or in combination, decreased A-cell firing rates. A-cells in the default condition fired at 3.49 Hz, slowing significantly both in the large PSP condition (3.15 Hz, LSD planned comparison $p<0.001$), and in the high excitability condition (3.07 Hz, LSD $p<0.0003$). Effects of large PSP and enhanced excitability together slowed A-cells to 2.97 Hz (LSD $p<0.00005$). These results suggest that each of these forms of plasticity, though modest, can contribute to reduced A-cell output in the real circuit.

Supported by a Princeton Univ. honorific fellowship (JWF), and a Pew Biomedical Scholars Award and a Sloan Fellowship (GAC).

553.2

A PARADOXICAL FORM OF ACTIVITY-DEPENDENT FACILITATION MAY UNDERLIE CONTEXT LEARNING IN *HERMISSENDA*

I.A. Muzzio*, A.C. Talk, & L.D. Matzel
Department of Psychology, Rutgers University, New Brunswick, NJ 08903

Most modern learning theories suggest that contextual stimuli should acquire associative strength in much the same manner as discrete conditioned stimuli (CSs). In the present experiments, *Hermissenda* were trained to discriminate between adjoining light and dark contexts. Thirty unsignalled rotations were presented during each of three 7 min sessions in one of the two contexts. Prior to training, the animals displayed a slight preference for the lit context. After exposure to unsignalled rotation, animal's preferences shifted strongly to the lit side if unsignalled rotations were presented in the dark, or to the dark side if unsignalled rotations were presented in the light. Prior research has indicated that the B photoreceptors of the *Hermissenda* eye undergo several forms of activity (i.e., Ca^{2+})-dependent facilitation (e.g., an increase in neuronal input resistance and evoked spike frequency) following pairings of discrete light and presynaptic vestibular stimulation. Similar facilitation was observed during *in vitro* experiments following presentations of presynaptic stimulation during a persistent 7 min light. Moreover, ratiometric analyses using the fluorescent indicator fura-2AM determined that intracellular Ca^{2+} concentrations increased at least five-fold early during a 7 min light presentation and dissipated to only half that level by the end of the presentation. These results suggest that activity-dependent facilitation might underlie learning about diffuse contextual stimuli as well as discrete stimulus presentations. Paradoxically, such a mechanism cannot account for both context learning and temporal specificity in classical conditioning, which has also been observed behaviorally in *Hermissenda*. These results suggest that modifications of sensory receptors are insufficient to account for the full range of associative processes observed in this animal.

553.3

COOLING INDUCED RETROGRADE AMNESIA IN *C. ELEGANS*. Glenn E. Morrison* and Derek van der Kooy. Neurobiology Research Group, Dept. of Anatomy, University of Toronto, Toronto, ON, Canada, M5S 1A8.

We have previously demonstrated the ability of C. elegans to learn using an associative conditioning assay that paired an ion CS with a food US (E. coli). In this study we have investigated phases of memory formation using brief (15 minute) cold shock presentations at various times before, during or after conditioning. Cold shock was found to block the formation of memory if given either during conditioning, immediately following conditioning (0-15 minutes) or 15 minutes following conditioning (15-30 minutes). Cold shock did not block memory formation if given one hour following conditioning. As well, if the worms were given sufficient time to recover from cold shock (one hour), then treatment before conditioning did not affect the formation of memory. Thus, we have identified at least two phases of memory formation in C. elegans for associative learning, one (short term memory) which is labile and affected by cold shock and a second (long term memory) which is stabile and not affected by cold shock. Interestingly, cold shock given 30 minutes or 15 minutes prior to conditioning did block the presence of memory measured immediately following conditioning. However, if tested three hours following conditioning, worms cold shocked 30 minutes prior to conditioning demonstrated learned preferences. Moreover, worms cold shocked at 15 minutes following conditioning still demonstrate memory deficits when tested three hours following conditioning. These results suggest that the deficits seen in worms cold shocked prior to conditioning are deficits in retrieval (i.e. the deficits are seen immediately following but not three hours after conditioning), whereas the worms cold shocked in the 30 minutes following conditioning suffer from deficits in memory consolidation.

Supported by NSERC Canada.

553.4

SHORT-TERM NOCICEPTIVE SENSITIZATION OF A DEFENSIVE STRIKING RESPONSE IN *MANDUCA SEXTA*. E.T. Walters, P.A. Illich, J.C. Weeks*, P. Le and S. Bell. Dept. Integrative Biol., Univ. TX - Houston, TX 77030; [1] Inst. Neurosci., Univ. Oregon, Eugene, OR 97403.

Sensitization of defensive responses following noxious mechanical stimulation occurs in mammals, snails, and leeches (Walters, *Int. Rev. Neurobiol.* 36:325, 1994) but has not, to our knowledge, been reported in insects. We tested larvae with a soft von Frey hair applied sequentially to the side of each proleg (8 stimuli at 10-s intervals) every 5 or 15 min. This gentle test sequence rarely evoked overt responses. In contrast, hard poke of a proleg using a stiff hair, or sharp pinch of a proleg with forceps, usually evoked 1 or more rapid strikes of the head towards the site of noxious stimulation. A single stiff poke (n=7 animals) or pinch (n=10) to a proleg significantly enhanced the number of strikes evoked by the weak test sequence 5 min, but not 10 or more min, later. Four rapid pinches to a proleg had the same effect (n=5). Eight pinches to a proleg increased the period in which strikes were evoked by weak test stimuli to 30 min, and the effect was similar for intertest intervals of 5 (n=10) and 15 (n=9) min. No significant increase in strike responses was observed 1 or 24 hr after any of the pinch protocols. No difference was seen at any time between responses to test stimuli applied to the pinch site and to other sites, including contralateral sites. These results show that an insect can display robust sensitization of defensive responses for up to 30 min after noxious stimulation. Unlike some types of nociceptive sensitization, this example in *Manduca* appears to be neither persistent nor site specific. It may be a form of defensive arousal (Walters 1994) having parallels to appetitively induced "central excitatory states" reported in insects.

Supported by grants IBN-9210268 (NSF) and MH38726 (NIH) to E.T.W.

553.5

DYNAMIC REGULATION OF THE SIPHON WITHDRAWAL REFLEX OF *APLYSIA* IN RESPONSE TO CHANGING ENVIRONMENTAL CONDITIONS J.W. Yuan, T.M. Fischer*, and T.J. Carew. Yale Univ., Dept. Psych., New Haven, CT 06520

Previous cellular studies examining activity-dependent inhibition in the siphon withdrawal reflex (SWR) of *Aplysia* predicted that inhibition in the SWR network should be activated by weak tactile input (Fischer and Carew, 1995). To directly test this prediction, we examined the effect of weak input (turbulence) on the duration and threshold of SWR. For each animal, the SWR was examined in both calm and turbulent conditions; turbulence was generated by bubbling air into the sea water. The duration of the SWR was significantly reduced in turbulent conditions ($\bar{x}$ %=45) in comparison to calm (baseline) conditions (N=10, $p<0.05$). This adaptation occurred within one min of turbulence onset and was stable over time. Recovery back to baseline levels occurred within one min of restoration to calm conditions. In examining modulation of this adaptive behavior, we found that tail shock (60-80mA AC, 1 sec) did not alter the turbulence-induced reduction of SWR duration, but it did produce a significant *increase* in response duration in shocked animals 10 min after returning to calm conditions (N=10, $p<0.05$); there was no increase in controls (exposed to either turbulence alone or shock alone). Finally, cellular experiments revealed that turbulence also induced a significant reduction in reflex input to siphon motor neurons ($\bar{x}$ %=50; N=5; $p<0.01$).

We also examined the threshold of the SWR by determining the minimum current (through an implanted wire in the siphon) required to elicit the SWR. SWR threshold significantly increased ($\bar{x}$ %=39) within 1 min of turbulence onset (N=10, $p<0.05$). Recovery occurred within 1 min after offset of turbulence. Tail shock had no significant effect on the turbulence-induced increase in threshold. Taken collectively, our results indicate that the SWR network promotes a rapid and dynamic adjustment in response to changing environmental conditions. Supported by NIH grant MH48672 to TJC.

553.6

SENSITIZATION OF THE OLFACTO-CARDIAC RESPONSE IN *PROTOPHORMIA TERRAENOVAE*. A. M. Angioy*, P. Muroni, N. Piroddi, C. Reali and I. Tomassini Barbarossa. Department of Experimental Biology, Section of General Physiology, University of Cagliari, Viale Poetto 1, 09126 Cagliari, Italy.

Olfactory stimulation evokes the "vegetative" response of heart-beat reversal in blowflies. Persistent cardiac responses are induced by repetitive stimulation with behaviorally-repellent odor concentrations. On the other hand, acquisition and retention of response habituation occur, i.e., non-associative learning and memory formation develop, with behaviorally non-repellent odor concentrations. Here we report results on sensitization of the cardiac response in adult *Protophormia* flies. Simultaneous recordings of heart activity and sensory input were performed on intact specimens. Behaviorally-repellent odor concentrations delivered on fly antennae induce a ten-fold decrease of the olfacto-cardiac response threshold. The response to olfactory stimulation can be promptly restored in habituated flies by intensely stimulating mechano-taste receptors on the labellar area. A faster rehabituation results after the antagonistic process of sensitization develops.

Supported with funds from the Italian Ministry for Universities (M.U.R.S.T., 40% and 60%).

553.7

ALTERATIONS IN SYNAPTIC ACTIVITY TO AND WITHIN A CPG MAY UNDERLIE THE OPERANT CONDITIONING OF AERIAL RESPIRATORY BEHAVIOUR IN *LYMNAEA*. G. Spencer*, T. Inoue, R. Cotter, N. Syed and K. Lukowiak. Neuroscience and Respiratory Research Groups. University of Calgary, Calgary, Alberta, T2N 4N1 and Arabian Gulf University/CMMS, Manama, Bahrain.

Lymnaea are bimodal breathers and aerial respiration occurs when the animal becomes hypoxic. Aerial respiratory behaviour is controlled by a central pattern generator (CPG). Three identified interneurons were found to be both necessary and sufficient to produce rhythmicity. Rhythmicity is the result of emergent properties of the network and is normally triggered by activity in RPeD1. This aerial respiratory behaviour can be operantly conditioned by the contingent presentation of a tactile stimulus to the respiratory orifice, the pneumostome, each time the snail attempts to breathe in the hypoxic pond water. A training period of 0.5 h per day, twice daily results in learning which persists for up to 4 weeks. *Yoked Control* animals do not exhibit significant changes in their aerial respiratory behaviour. Isolated nervous systems from both *Operant Conditioned* and *Yoked Control* snails were examined using a blind experimental procedure. We found that the level of spontaneous activity in RPeD1 was reduced in the ganglionic preparations from *Operant Conditioned* animals compared to controls. The inferred activity of another CPG neuron (IP3I) was also different in *Operant* as compared to control preparations. Further, the ability of RPeD1 (when depolarized to produce action potentials) to induce rhythmicity in the circuit was reduced in the *Operant Group* compared to the control group preparations. A higher-order interneuron (RPeD11) which mediates whole animal withdrawal behaviour is excited by tactile stimulation of the pneumostome area and directly inhibits the CPG. Associative learning may, therefore, be mediated by changes in both intrinsic membrane properties and changes in synaptic connectivity within the CPG network, which affect the emergent network properties. These change may be brought about by the activity of RPeD11 onto the CPG network. Supported by MRC (Canada).

553.8

A WHOLE-BRAIN "IN-VITRO" PREPARATION FOR ELECTROPHYSIOLOGICAL RECORDING FROM ADULT DROSOPHILA MUSHROOM BODY NEURONS. N.J.D.Wright* and Y.Zhong. Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, New York 11724.

The paired mushroom body structures (MBs) found in the insect brain are known to be important for olfactory learning and memory. The fruit fly has been extensively utilized as a model for the study of the molecular basis of learning and memory due to its amenability to genetic manipulation. Behavioral mutants may provide a unique tool to dissect the neural basis of learning and memory if the electrophysiology of the MBs can be examined. Previously we have utilized a genetic marker (*lacZ*) in order to study identified mushroom body neurons in culture but were unable to observe any synaptic events. We are therefore developing a whole-brain preparation using the specific expression of "green fluorescent protein" to identify and record intracellularly from these structures. Our initial results have confirmed the viability of the tissue and we have been able to record membrane potentials (-30 to -70mV), action potentials, complex bursting patterns and synaptic events from identified neurons. We are currently studying these MB neurons and their response to olfactory nerve stimulation and how this may be altered in learning and memory mutants.

553.9

MULTIPLE MECHANISMS CONTRIBUTE TO THE INFLUENCE OF INTERTRIAL INTERVALS ON LEARNING IN *HERMISSENDA*
R.R. Ramirez, I.A. Muzzio, A.C. Talk, & L.D. Matzel*
Department of Psychology, Rutgers University, New Brunswick, NJ 08903

It has been universally observed that longer intertrial intervals (ITIs) produce better learning than massed trials. In *Hermissenda*, pairings of light and rotation result in a conditioned foot contraction to light when pairings are spaced 60 or 120 sec apart, but not with shorter ITIs. *In vitro* conditioning procedures have determined that the longer ITIs produce an increase in excitability of B photoreceptors which is correlated with a cumulative depolarization during successive pairings that has been suggested as a potential mechanism underlying learning. To further study the effects of the ITI on facilitation and its relation to cumulative depolarization, animals were trained using an *in vitro* conditioning procedure (light paired with hair cell stimulation) to evaluate changes in excitability (input resistance and evoked spike rate). Animals received pairings at either 30, 120, 240, or 480 sec ITIs. Increasing degrees of facilitation (resistance and evoked spikes) were observed with increasing ITIs. However, cumulative depolarization was greatest in cells trained with the 120 sec ITI whereas none was observed with either 240 or 480 sec ITIs. Since both light transduction and voltage-dependent Ca^{2+} flux across the B cell membrane is impeded by steady-state depolarization, this likely contributes to the weaker facilitation at ITIs less than 120 sec. Since no cumulative depolarization was observed in either the 240 or 480 sec ITI conditions, the enhanced facilitation in the 480 sec condition must depend on a second mechanism, possibly the inactivation of protein phosphatases that are activated during each trial. This later possibility is presently under investigation.

553.10

THE EFFICACY OF EXPOSURE TO COLD AS THE UNCONDITIONAL STIMULUS IN DROSOPHILA MELANOGASTER. L. L. Phelan*, Z. A. Rodd, & R.A. Rosellini. Dept. of Psychology, University at Albany: SUNY, Albany, NY 12222.

Exposure to cold as an Unconditional Stimulus (US) was investigated in D. melanogaster using a Pavlovian odor conditioning paradigm. The initial study employed a delayed conditioning procedure which consisted of a 60 s exposure to an odor as a Conditional Stimulus (CS) in conjunction with the putative US consisting of a 15 s exposure to an environment maintained at 0° C. The CS-US delayed conditioning groups showed greater avoidance of the odor CS than either CS-Only, US-Only, and Unpaired CS / US groups. A second study examined the impact of the duration of exposure to the US (either 0, 5, 10, 15, 20, or 30 sec) on conditioning to the odor CS. The results demonstrated that conditioning increased as a function of increasing US duration in the 0-20 sec range. At the longest duration employed (30 sec), no evidence of conditioning to the CS was observed. An additional study examined the temporal relationship between presentation of the CS and the US. The results demonstrated conditioning to the odor CS at forward trace intervals of 5 and 30 sec but not 60 sec. Additionally, there was evidence for backward excitatory conditioning to the odor CS at a backward trace interval of 5 sec. To our knowledge, the present studies are the first to demonstrate the efficacy of exposure to cold as a US in Pavlovian aversive conditioning in D. melanogaster. (This research was supported by personal funds).

553.11

CONDITIONED INHIBITION IN Hermissenda: LEARNING THAT LIGHT SIGNALS THE ABSENCE OF ROTATION. G. Britton* and J. Farley. Program in Neural Science, Indiana University, Bloomington, IN 47405.

We previously reported (Britton et al., 1995, Soc. Neurosci. Abstr. 21:1266) that Hermissenda exposed to explicitly unpaired presentations of light and rotation show an increase in phototactic behavior, i.e., H.c. learn to approach a light that has, in the past, signaled the absence of rotation. Observations of behavior in a light gradient in an underwater open field indicated that 24 hr following training, animals that had received explicitly unpaired presentations of light and rotation moved closer to the center of the light gradient than they did before conditioning. In contrast, animals that had received random presentations of light and rotation, presentations of light alone, or no training at all failed to show these changes. Prior to conditioning, measurements of animals' trajectories of movement indicated that they were, on average, oriented neither towards nor away from the center of the light gradient, but rather perpendicular to it. Following training, animals that had received explicitly unpaired presentations of light and rotation moved in such a way that they were oriented towards the light. Control animals' movements were oriented either slightly away from the light (random control) or perpendicular to it (light-alone, untrained). Intracellular recordings from synaptically intact Type B photoreceptors obtained 24-48 hr following training revealed that explicitly unpaired training produced a significant decrease in the steady-state light response as well as a decrease in action potential frequency. The latter was observed most reliably during the first of three successive light steps. No training-associated changes were observed in resting membrane potential or dark input resistance. These results further clarify the behavioral characteristics of conditioned inhibition in H.c., and suggest that retention of conditioned inhibition is mediated via persistent reductions in B cell excitability. Supported by grants from NIH.

553.12

TURNING BEHAVIOR IN THE PREDATORY SEA SLUG *PLEUROBRANCHAEA*: FUNCTIONAL ANATOMY AND NEURAL MECHANISMS. J. Jing*, and R. Gillette. Department of Molecular and Integrative Physiology, University of Illinois, Urbana, IL 61801.

Orienting and avoidance turns are major components of *Pleurobranchaea*'s stimulus-directed behaviors, typically the former is accompanied by locomotion (ciliary movement). Turn decisions are made by integrating sensation with motivational state and learning experience. Avoidance turns can be reliably elicited by asymmetric electrical shocks to tentacles and oral veil; such turns are relatively stereotypic actions that are completed within 35 s post-stimulus. The main muscles involved in turning appear to be a prominent muscle band, the lateral body wall muscle (LBWM), which originates from lateral columellar muscle and more distributed large muscle fascicles of longitudinal muscle of the foot (LMF). Anterior portions of LBWM terminate in diverse head organs, like the oral veil, tentacles, and rhinophore as well as the mouth, oral tube and buccal mass, indicative of roles in head movement and feeding. Major nerve innervation of LBWM comes from the posterior lateral body wall nerve (pLBWN) of the pedal ganglion, and the LMF is innervated by the pedal nerves (PNs). Unilateral lesion of both of these nerve sets eliminated ipsilateral turning, while turning was not eliminated by lesion of either LBWM or LMF nerves alone; this suggests that both muscles contribute to turning. Unilateral lesion of PNs did eliminate ciliary movement on the lesioned side of the foot. Differential activity with similar timing as turning behavior in bilateral nerves, in particular the pLBWNs, can be recorded in the hemi-animal preparation, when aversive shocks that can elicit turning in the intact animal are delivered to the tentacle and oral veil on one side. Similar motor patterns in the pLBWNs can be induced by depolarizing the swimming interneurons A3b,c, suggesting their possible role in turning. These observations provide a basis for further investigation of the premotor decision mechanisms for orienting or avoidance turns. Supported by NIH RO1 NS26838.

553.13

LONG-TERM DEPRESSION OF THE CRAYFISH LATERAL GIANT RESPONSE BY A LONG TRAIN OF LOW FREQUENCY STIMULATION OF PRIMARY AFFERENTS. Sunhee C. Lee* Dept of Physiol, College of Medicine; Div of Neurosci, Med Res Ctr, Ewha Womans University, Seoul, Korea

A descending tonic inhibition (TI), observed during restraint or feeding in crayfish, is known to depress selectively the lateral giant (LG), a command neuron for one form of escape reactions, without affecting the sensory processing (Krasne & Wine, 1975). It is also known that TI originates in the rostral parts of the CNS. The present study was aimed at finding a physiological mechanism that is responsible for a long-term depression (LTD) of the LG response. Crayfish preparations whose descending influence was removed by severing the nerve cord between thorax and abdomen were used. The results showed that a long train of low-frequency stimulation (LFS) of the primary afferents (PAs) cause LTD of the LG response without affecting the sensory processing. A 5 min long train of 0.2 ms pulses at 1-5 Hz caused a significant increase in LG firing threshold by a test pulse to sensory roots at once per min (111 ± 18 %, mean ± SEM; n =15, $P < 0.01$) and 30 min after LSF, it was still increased by 66 ± 11 % ($P < 0.01$). The same LFS, however, did not change the threshold for firing of interneuron-A (IN-A), one of the largest sensory interneuron. A strong LFS (2-4 times LG threshold) was more effective at depressing the LG response than those of a weak LFS. Another preliminary result showed that the LG response to PAs from an unstimulated segment is also depressed if that to PAs in another segment has been depressed by the LFS. The present results suggest that the increased firing threshold for LG, but not for IN-A, caused by the LSF may be due to the activation of tonic inhibition system. Experiments using picrotoxin are currently underway to assess the possibility that repetitive LFS of PAs may activate a GABAergic inhibitor (Vu & Krasne, 1993), which may also originate in the abdomen. *(Supported by Korea Science and Engineering Foundation: grant 961-0701-015-2)*

553.14

ODOR AVOIDANCE CONDITIONING IN D. MELANOGASTER: THE EFFECTS OF CONDITIONING PARAMETERS ON AVOIDANCE LEARNING. R.A. Rosellini*, Z. A. Rodd, D. Byers, & L. Phelan, Dept. of Psychology, University at Albany: SUNY, Albany, NY 12222.

The present series of studies investigated associative odor avoidance conditioning in Drosophila melanogaster. The first set of studies investigated whether variables which are known to influence associative learning in mammals would have the expected effect in the Benzer group odor avoidance learning paradigm in D. melanogaster. The variables investigated were: 1. Number of Trials of Conditioning, 2. Inter-Trial-Interval (ITI), and 3. Intensity of the Unconditional Stimulus (US). The results of these studies demonstrated that the strength of an odor avoidance conditioned with electric shock as the reinforcer was a function of the: 1. number of acquisition trials (1, 3, or 6) administered with increasing number of trials producing greater learning, 2. length of the ITI (0, 15, 30, or 90 s), with increasing intervals resulting in greater learning, and 3. intensity of the US (0, 10, 20, 40, or 80 v) with increasing intensities from 10 to 40 volts resulting in progressively increasing avoidance and a decrement in the learning at the 80 volt intensity. A second set of studies investigated the impact of the three conditioning variables in individual D. melanogaster. The individual learning results were consistent with the effects of each of the variables investigated in the group conditioning paradigm. The overall findings of these two sets of studies, whether employing the group or individual learning conditioning paradigm, were consistent with the extant literature on the impact of these variables in mammalian associative learning in general and avoidance learning in particular. (This research was supported by personal funds).

553.15

LONG-TERM FACILITATION IN *APLYSIA* SENSORY NEURONS: EFFECTS OF SPACED VS MASSED TRAINING AND INHIBITION OF SYNAPTIC TRANSMISSION J. Mauelshagen, C.M. Sherff*, & T.J. Carew. Yale Univ., Dept. Psych., New Haven, CT 06520

At *Aplysia* tail sensory neuron (SN) to motor neuron (MN) synapses, 5 spaced applications of serotonin (5HT) induce short-term and long-term synaptic facilitation (STF and LTF). We recently identified a rapid time course of decay of STF (15 - 30 min) after 1 to 4 spaced pulses of 5HT, while 5 pulses resulted in a unique prolonged intermediate phase of facilitation (ITF; 60 to 90 min; Mauelshagen et al, *Neurosci. Abs.* 1995). In the present study, we further investigated the requirements for ITF and LTF induction.

In one experiment, we examined the effect of spaced (5 x 5 min, 15 min interval) or massed (1 x 25 min) 5HT applications on both ITF and LTF. We monitored the monosynaptic EPSP from SN to MN before 5HT (baseline), at 5 min after the start of 5HT (STF), across 1 hr immediately after 5HT (ITF), and at 20 - 24 hrs after 5HT (LTF). With both massed (M) and spaced (SP) 5HT applications we observed significant STF (M and SP: n=14, $p<.001$) and significant ITF at 0, 15, 30 and 45 min after 5HT (M and SP: $p<.05$ in all cases). In a subset of preparations (M: n=5; SP: n=4) in which we could test for LTF, both massed and spaced paradigms were able to induce LTF.

In a second experiment, we asked whether synaptic transmission is necessary for the induction of STF and LTF. First we established that artificial seawater (ASW) with 3x normal Mg^{2+} and no Ca^{2+} (MgASW) blocked both spontaneous and evoked EPSPs in tail MNs. Next, 5HT was presented in 5 SP applications in MgASW. To assess STF, we briefly perfused the ganglia with ASW after the first 5HT pulse. We observed both significant STF (n = 14, $p<.03$), and 24 hrs later, significant LTF (n=6, $p<.02$). These data show that the induction of STF and LTF does not require synaptic transmission either directly from SNs to MNs or indirectly through heterosynaptic pathways. Support by a DFG grant to J.M. and NIH grant R01-MH-14-1083 to TJC.

553.16

SPACED RATHER THAN MASSED CONDITIONING LEADS TO PROTEIN SYNTHESIS DEPENDEND LONG-TERM MEMORY IN HONEYBEES. R. Menzel*, Freie Universität Berlin, Institut für Neurobiologie, Königin-Luise-Str. 28/30, D - 14195 Berlin, Germany

Previous studies lead to the astonishing result that > 95% protein synthesis inhibition in the bee brain by the translation blocker cycloheximid has no effect on long-term olfactory memory (LTM), (Wittstock et al. 1993, J. Neuroscience 13, 1379-86). We now find that different forms of LTM are formed after massed and spaced conditioning.

Bees were conditioned with 5 to 8 trials using various olfactory, mechanosensory or infrared (temperature increase) stimuli as conditioned stimuli, and sucrose as unconditioned stimulus. The conditioned response was proboscis extension (PER). Intervals between conditioning trials varied between 30 sec (extremely massed) and 30 min (extremely spaced). Memory tests were performed at intervals of 1 to 7 days. Spacing of conditioning trials leads to higher and longer lasting memory scores. Furthermore, memory at 3 or more days is more resistant to extinction after spaced conditioning than after massed conditioning. Transcription was blocked to > 50% in the bee brain by injection of 2 or 4 µg actinomycin D per animal into the flight muscle. LTM tested after > 2 days is significantly impaired after spaced conditioning, but unimpaired after massed conditioning. Blockade of translation with cycloheximid leads to similar effects if training is weak, but no effects were found after strong conditioning.

(Supported by DFG grant Me 365/16-1)

INVERTEBRATE LEARNING AND BEHAVIOR IV

554.1

PATTERNED JAW MOVEMENTS DURING REJECTION OF SEAWEEDS IN *APLYSIA KURODAI*. T. Nagahama* and N. Shin. Dept. of Biol., Fac. of Sci., Kobe Univ., Kobe 657, Japan

Japanese species, *Aplysia kurodai* usually feeds *Ulva* and *Undalia* well. In the present experiments we collected several species of seaweeds at the location alive with animals, and explored whether animals were attracted to the test seaweeds against *Ulva* by two choice experiments and whether animals ingest them. Animals were attracted to *Pachydictyon* (*Pac.*) as well as *Ulva*, but they strongly rejected it when it was pushed into their mouth. On the other hand, *Dictyoteris* (*Dic.*) was more repellent than *Ulva*, but animals often ingested it which was pushed into their mouth. These results suggest that *Pac.* and *Dic.* may induce the different preferential behavior of animals in the sense of smell and the sense of taste and/or touch. *Gelidium (Gel.)* was more repellent than *Ulva*, and also induced the rejective response when it was pushed into the mouth.

Recording activity of jaw-closing (mm) and -opening (ma) muscles in free-moving animals showed that the onset of the mm activity, which always started later than the ma activity during each cycle of ingestion of raw *Ulva*, was advanced toward that of the ma activity during each cycle of rejection of raw *Pac.* or *Gel.*. Semi-intact experiments also showed that application of the extract of *Ulva, Pac.* and *Gel.* to the lips produced similar patterns in firing of the jaw-closing motor neurons to the mm activity. Video analysis showed that application of *Ulva* extract to the lips produced maximum jaw-opening in the radula protraction phase and still produced half-opening state in the earlier time of radula-retraction phase while application of *Pac.* or *Gel.* extract did not produce half-opening state and the jaws smoothly closed in the radula-retraction phase. These results are consistent and suggest that the rejection of *Pac.* or *Gel.* may be induced as a result of chemoreception.

554.2

FMRF-AMIDERGIC NATURE OF PLEURO-BUCCAL PROJECTING NEURONS COMMON TO DIVERSE PULMONATE MOLLUSCS. M. Alania and D.A. Sakharov*. Inst. Devel. Biol., 117808 Moscow, Russia

In gastropod molluscs, activity of the buccal central pattern generator (CPG) for feeding is generally arrested during activation of the CPG for defensive withdrawal. We used a nickel lysine axon filling technique for examining a variety of species with the aim to determine if they are provided with projecting neurons comparable with those found earlier in *Helisoma*. In this latter pulmonate snail, two bilaterally symmetric cells in the pleural ganglia were suggested to be responsible for this motor coordination since they project to the buccal ganglia and suppress activity of the CPG for feeding (Murphy, 1990, *Brain Res.* 525, 300). We studied both basommatophoran, fresh-water pulmonates (*Lymnaea stagnalis, L. auricularia, Planorbarius corneus*) and stylommatophoran, terresrtrial ones (*Bradibaena fruticum, Helix pomatia, H. lucorum, Limax cinerea-niger*). In all these molluscs, like in *Helisoma*, a neuron projecting to the ipsilateral cerebro-buccal connective, the PlB (pleural-buccal) cell, was found to occur in the right and left pleural ganglia. Identity of PlB cells of diverse species was established due to a unique route of the axon which traverses the ipsilateral pedal ganglion on its way to the cerebral one. Since FMRF-amide is known to inhibit the gastropod CPG for feeding, we combined a biocytin axon filling technique with FMRF-amide immunostaining in the same CNS preparation. In two species examined with this dual-labelling technique, *L. stagnalis* and *P. corneus*, the experiment has proved unambiguously that the PlB cells are immunoreactive to FMRF-amide. The results thus suggest conservation of the cellular basis, and involvement of FMRF-amide in the mechanism, of the motor coordination in question. Grants INTAS-93-3504 and RFFI-96-04-49181.

554.3

THE CONTRIBUTION OF ENVIRONMENTAL FACTORS IN THE DEVELOPMENT OF THE RESPIRATORY NETWORK IN *LYMNAEA STAGNALIS*. P.M. Hermann*, A.G.M. Bulloch and K. Lukowiak. Neuroscience Research Group, HSC, University of Calgary, Calgary, AB, T2N 4N1, Canada.

Respiration in *Lymnaea* is bi-modal: 1) across the skin and 2) via the lung by opening and closing of the lung orifice (i.e. the pneumostome). This study investigated the relative contribution of genetic and environmental factors in the development of aerial respiratory behavior and its underlying neural network. To this end, animals were raised from eggs without ever being able to experience lung breathing. Behavioral experiments showed that these animals were competent to open and close their pneumostome. The average duration of each opening was not different but, both in normoxic as well as in hypoxic conditions, the total breathing time and the number of pneumostome openings was significantly decreased when compared with control animals. Intracellular recordings in the isolated CNS and Lucifer Yellow stains of respiratory network neurons showed that the electrophysiological properties and projections of these cells are indistinguishable from control animals. We conclude that aerial respiration is not necessary for the normal development of the respiratory circuitry. However, while aerial respiration per se occurs in animals prevented from performing this behavior during the course of the normal development, such rearing has significant effects on major components of aerial respiratory behavior.

Supported by NSERC (Canada)

554.4

NEURAL CONTROL OF THE MALE COPULATORY ORGAN IN THE SNAIL *LYMNAEA STAGNALIS*. P.A.C.M. De Boer, A.W. Pieneman, R.F. Jansen and A. Ter Maat.* Graduate School Neurosciences Amsterdam, Research Institute Neurosciences, Vrije Universiteit, Faculty of Biology, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

Male copulatory behavior of the hermaphroditic snail *Lymnaea stagnalis* is complex. The behavior consists of an appetitive phase that is highly variable in temporal structure, followed by a fixed consummatory phase. The appetitive phase is composed of five elements including locomotion (Mounting and Circling) and movement of the male copulatory organ (Eversion, Probing and Withdrawal). We are studying the neural control of the male copulatory organ.

Almost 400 neurons, concentrated in five different groups in the CNS, have projections to the male copulatory organ. One of these groups is found in the anterior lobe of the right cerebral ganglion. In these cells a number of colocalized peptides has been found, most notably conopressin and APGWamide. We have started to examine the effect of these peptides on the eversion of the male copulatory organ. Injection of conopressin into the body cavity induces a partial eversion whereas injection of APGWamide induces a total eversion. The neurons of the anterior lobe project to the male copulatory organ via the penis nerve. Animals with a lesion of this nerve were also injected with conopressin and APGWamide. It was found that in these animals a total eversion lasts indefinitely. This suggests that an intact penis nerve is necessary for withdrawal of the organ. We are currently investigating the motor control of the male copulatory organ in *Lymnaea*.

554.5

COMPUTATIONAL PRINCIPLES OF A TINY NERVOUS SYSTEM. S.R. Lockery and M.B. Goodman.* Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The anatomical connectivity of the nervous system of the nematode *C. elegans* has been described almost completely. Little is known, however, about the biophysical properties of individual neurons, which may have complex input-output functions to compensate for their small number (302). We tested this idea using whole-cell current clamp recordings from neurons (n = 24) whose processes project into the nerve ring, the major site of synaptic integration in *C. elegans*. In some cases, cells were identified using green fluorescent protein expressed under the control of a neuron-specific promoter. In all cells examined, the response to a negative current pulse was a simple exponential hyperpolarization. The response to a positive current pulse had two components: a fast rising phase that accounted for most of the total depolarization followed by a slow rising phase that began once the membrane potential reached about 0 mV. The rate of depolarization in the slow phase may have been reduced by a voltage- and time-dependent outward current seen in voltage clamp. This current activated between -10 and +40 mV and decayed with a time course that matched the slow rising phase observed in current clamp. Action potentials could not be elicited. Thus, the electrical behavior of *C. elegans* neurons is remarkably simple and homogenous. These findings suggest that the rich behavioral repertoire of *C. elegans* reflects complexity of the connections between neurons rather than complexity of the individual neurons themselves.

Supported by NIMH MH51383, NSF IBN9458102, ONR N00014-94-1-0642, the Sloan Foundation, and The Searle Scholars Program.

554.6

NEURAL NETWORK MODELS OF CHEMOTAXIS IN *C. ELEGANS*. T. C. Ferrée, B. A. Marcotte, J. T. Pierce, S. R. Lockery.* Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Nematodes move up chemical gradients by detecting changes in concentration at the tip of the nose and biasing their normal sinusoidal locomotion toward higher concentrations (klinotaxis). Laser ablations of identified neurons in *C. elegans* have shown the neural circuit for chemotaxis is a highly interconnected network of chemosensory neurons, interneurons, and motor neurons with feedforward and feedback connections (C. Bargmann, unpublished). Moreover, physiological recordings from neurons in *C. elegans* suggest that they are functionally isopotential and that signal propagation is largely graded. To understand how such a nervous system might control chemotaxis we constructed a reduced model and used a neural network optimization algorithm (simulated annealing) to find sets of connections that produce chemotaxis. Many such sets were found. These results suggest that the biological network may be one of many different possible solutions for chemotaxis in graded neural networks.

Supported by NIMH MH11373, NIMH MH51383, NSF IBN 9458102, ONR N00014-94-1-0642, the Sloan Foundation, and The Searle Scholars Program.

554.7

BEHAVIORAL RESPONSES OF CRAYFISH ANTENNULES TO ODORANT AND HYDRODYNAMIC STIMULI. DeF. Mellon*, Dept. Of Biology, Univ. Of Virginia, Charlottesville, VA 22903.

All decapod crustaceans flick their antennules in the presence of sensory stimulation. Antennular flicking is thought to enhance the detection of odorant stimuli; for example, it is known to facilitate the onset of high-frequency impulse trains to odorants in the olfactory receptor neurons of the spiny lobster (Schmitt BC & Ache BW, 1979, Science 205:204-206). In several species, flicking behavior is accelerated in the presence of odorant stimuli and also by water currents. I have studied antennular flicking in the crayfish *Procambarus clarkii* to compare the relative influence of odorant and hydrodynamic stimulation upon both the intensity and the frequency of this behavior. I recorded from single fibers in the phasic portion of the antennular depressor muscle during stimulus presentations to the external filament. Responses to odorant stimuli had long latencies and were characterised by prolonged trains of high-frequency flicks and tonic activity in the slow portion of the depressor muscle. Responses to water pulses had very low stimulus thresholds, short latencies and consisted of single flicks. Responses to brief, single pulses of water persisted even during prolonged washing of the antennules with constant-velocity water currents.This suggests that hydrodynamic receptors on the antennules can detect turbulent eddy features of the imposed currents that may also contain steep concentration gradients of chemical stimuli. Supported by grant IBN-9319406 from NSF.

554.8

A mechanism to regulate sexual proclivity in the snail

J.M. Koene and R. Chase*, Dept. of Biology, McGill Univ., Montreal, Canada.

Sexual proclivity, i.e. the probability that mating will occur, varies with the duration of sexual isolation in the garden snail *Helix aspersa* (Adamo & Chase, 1990). We hypothesized that proclivity is related to the amount of sperm available for mating. The sperm are stored in the hermaphroditic duct (HD) prior to copulation. We estimated the amount of stored sperm by measuring the dry weight of the HD. Snails which were sexually active, but non-copulating, had significantly heavier HDs than either non-active snails or snails that had just copulated. Thus, the change in the amount of the stored sperm corresponds to the variation in sexual proclivity. Previous work in the pond snail, *Lymnaea stagnalis*, has shown that a neural signal provides information about the amount of the sperm available (De Boer, Koene, Jansen and Ter Maat, in prep.). In *Helix aspersa* a branch of the nervus intestinalis (ni) innervates the HD. The activity of the ni-branch was recorded with a suction electrode in a semi-intact preparation while the normal increase in volume of the HD was mimicked by injections of saline. The recordings showed that inflation of the HD increased spiking activity in the ni-branch. This indicates that in *Helix aspersa* a nervous connection exists between the HD and the CNS that is capable of providing information about the amount of sperm available for copulation. We conclude that a general mechanism operates in snails, and possibly in other animal groups, that informs the animal when sufficient sperm are available for the successful fertilization of eggs. (Supported by NSERC Canada)

554.9

HOW DOES THE PARASITIC WASP *COTESIA CONGREGATA* SUPPRESS FEEDING IN ITS HOST THE TOBACCO HORNWORM, *MANDUCA SEXTA*? Shelley A. Adamo[1]*, Charles E. Linn[2], and Nancy E. Beckage[3],[1] Dept. Psychol., Dalhousie Univ., Halifax, NS, CANADA [2] Dept Entomol., N Y State Agri. Sta., Geneva, NY [3] Dept of Entomol., Boyce Hall, Univ. of Calif., Riverside, CA.

The parasitic wasp, *Cotesia congregata*, lays its eggs within the body of its host, the larval form of *Manduca sexta*. Host behaviour appears normal until about 8 h prior to the parasitoids' emergence from its host. Once the wasp larvae emerge, *M. sexta* feeding and locomotion declines dramatically. This change in host behaviour may be to the advantage of the wasp. Unparasitized *M. sexta* eat wasp pupae. Despite the decline in feeding and locomotion, hosts with emerged parasitoids have normal reflexes and show no other signs of debilitation. Concomitant with the change in host behaviour, octopamine concentration increases from 22.2 ± 2.1 pg/μL to 143.7 ± 7.8 pg/μL (measured using HPLC-ED) in the hemolymph of the host. In unparasitized *M. sexta* increased octopamine levels correlate with increased activity. The co-occurrence of high hemolymph octopamine levels and low behavioural arousal in parasitized *M. sexta* is opposite to that observed in unparasitized *M. sexta* and other insects.

Wasp larvae moult to the 3rd instar when cultured *in vitro*. Injections of media from wells containing newly moulted wasp larvae into unparasitized *M. sexta* induces a temporary depression of feeding in the hornworm. Media from cultured 2nd instar wasp larvae has no effect on feeding when injected into unparasitized *M. sexta*. This suggests that factor(s) secreted by the parasitoid may be involved in the suppression of host feeding. The wasps do not appear to secrete octopamine. Supported by an NSERC grant to S.A.A. and a USDA grant (92-37302-7470) and NSF grant (IBN-9420638) to N.E.B.

554.10

THE EFFECT OF OCTOPAMINE ON DIETARY SELF-SELECTION BY THE COCKROACH, *RHYPAROBIA MADERA*. H. Rosen, T.L. Uhlendorf, A.M. Zein, H.D. Can and R.W. Cohen*. Department of Biology, California State University, Northridge, CA 91330.

Insects have the ability to self-select an optimal diet from a choice of two or more incomplete diets that lack an essential nutrient. Previous work has shown that 5-hydroxytryptamine (serotonin) regulates carbohydrate feeding in insects. Our study details the pharmacological effects of the neurotransmitter octopamine on feeding behavior of the Madeira cockroach, *Rhyparobia madera*. Experimental nymphs were offered a choice of lyophilized casein (protein) or sucrose cubes (each cube contained 0.3% of either an octopamine agonist synephrine or antagonist phentolamine), and were compared to controls consisting of untreated cubes. Although not statistically significant ($p>0.05$), the nymphs ate slightly more of the synephrine-treated cubes compared to controls. However, the nymphs fed phentolamine-treated diets ate 80% less ($p<0.001$; ANOVA) of both diets than the controls. These results suggest that octopamine, unlike serotonin, does not affect nutrient self-selection. When *R. madera* nymphs were given a complete diet of 20% casein/80% sucrose cubes which had drugs (synephrine or phentolamine) incorporated, similar results were seen: Nymphs fed phentolamine-diets ate 90% less than controls ($p<0.001$; ANOVA). In order to separate the effects of taste from any neurophysiological changes caused by phentolamine or synephrine, *R. madera* nymphs were injected with a drug or saline (control) and then allowed to feed on 20:80 diets for 24 hours. The nymphs injected with synephrine ate the same as controls, while phentolamine-injected nymphs ate 75% less than controls. Taken together, these results suggest a major regulatory role for octopamine in the onset of feeding in insects.

554.11

OCTOPAMINE MIMICS EFFECTS OF PARASITISM ON THE FRONTAL GANGLION AND FOREGUT OF THE MOTH *MANDUCA SEXTA* C. I. Miles[1]* S. A. Adamo[2], R. Booker,[1] and N.E. Beckage[3] [1]Neuro. and Behav., Cornell University, Ithaca, NY 14853 [2]Dept. Psychol., Dalhousie Univ., Halifax, NS, CANADA, and [3]Dept. of Entomology, Univ. of Calif., Riverside, CA

Larvae of the moth *Manduca*, that are parasitized by the wasp *Cotesia congregata* exhibit a dramatic decline in feeding about 8 hours before the parasite larvae emerge from their host. We examined the function of the foregut and the ganglion which innervates it, the frontal ganglion (FG), in both unparasitized and parasitized *Manduca* larvae. Neurons in the FG of unparasitized larvae are always active, firing in a rhythmic bursting pattern. The foreguts of normal animals typically exhibit posteriorly directed peristalsis, consistent with the FG and foregut playing a role in swallowing. Parasitized *Manduca* examined before they stop feeding show normal activity in the FG and normal peristalsis of the foregut. However, concomitant with the cessation of feeding in parasitized *Manduca*, the foregut stops moving and the FG neurons fire in an arrhythmic pattern. These animals may make chewing movements but they do not appear to swallow food. Similar effects on FG and foregut activities are observed if the blood from non- feeding parasitized *Manduca* is applied to the FG and foregut of an unparasitized control animal, indicating that a factor in the parasitized animals' blood produces these effects. Octopamine levels in the blood of non- feeding parasitized *Manduca* are elevated 10- fold over levels in unparasitized control animals or parasitized animals that are still feeding (S.A. Adamo et al., submitted). Applying this concentration of octopamine (10^{-6}M) to the FG and foregut of control *Manduca* produces the same changes in activity as blood from non- feeding parasitized animals or what is observed for the FG and foregut of such an animal.

These results suggest that one mechanism by which the parasite *Cotesia* may alter the behavior of its host is by increasing the level of octopamine in the host's blood. This increased octopamine level alters the output of neurons in the FG to affect the function of the foregut and the swallowing behavior in the host. There was no source of funding for this work.

INGESTIVE BEHAVIOR: FOREBRAIN MECHANISMS

555.1

EFFECTS OF SODIUM DEPLETION ON THE ACTIVITY AND SENSITIVITY OF NEURONS LOCALIZED IN THE SEPTO-PREOPTIC REGION. F. Liénard , O. Galaverna, S.N. Thornton* and S. Nicolaidis. I.E.S.G.I., CNRS UPR 9054, Groupe - Neurobiologie des Régulations, Collège de France, 75231 Paris, cedex 05, France.

Sodium (Na^+) depletion is known to activate the renin-angiotensin II (AII)-aldosterone system (RAAS). Aldosterone production is increased as well as the renin activity and hence circulating AII levels are enhanced. Furthermore, Na^+ depletion induces a robust intake of hypertonic salt solutions.

This electrophysiological study examined, in the SFO and in the MnPO, the effect of Na^+ depletion on the neuronal responsiveness to iontophoretic application of AII, losartan (AT-1 receptor specific antagonist), PD123319 (AT-2 receptor specific antagonist), and aldosterone. Rats were depleted with the natriuretic, furosemide (0.5 mg/0.5 ml sc, 2 injections separated by 2 hours group LASIX) and had no access to any source of salt. Electrophysiological experiments were performed under urethane anaesthesia on the fifth day following the depletion. The control group was not depleted and was fed with a normal Na^+ diet.

No changes were found in the number of spontaneously active neurons (control : 11.1 ± 1.6 vs LASIX 9.4 ± 1.4, mean ± SEM). Preliminary results in LASIX treated rats revealed an increase in short term inhibitory responses to iontophoretic application of AII (100% of responding cells) that were not reversed by AT-1 or AT-2 receptor specific antagonists (losartan or PD123319 respectively). Whereas in control rats, AII produced both an excitation (75% of responding cells) which could be inhibited by losartan or PD123319, and an inhibition (25% of responding cells).

These results suggest that depletion-induced avidity for salt could be encoded by an inhibitory action of AII on neuronal activity. This is in contrast to mineralocorticoid-induced salt appetite which is accompanied by increased neuronal responsiveness to locally applied AII and decreased long term AII-induced inhibition. Thus, the central mechanism of salt appetite appears to depend on a complex pattern of induction.

Supported by Evian Company and MH43787

555.2

SPECIFIC CONTROL OF FEEDING BY EXCITATORY AND INHIBITORY AMINO ACIDS IN THE SHELL SUBREGION OF THE NUCLEUS ACCUMBENS. T.R. Stratford*, C.J. Swanson and A.E. Kelley. Department of Psychiatry, University of Wisconsin Medical School, Madison, WI 53706

Previous work in this laboratory has demonstrated that blockade of AMPA/kainate receptors in the shell subregion of the nucleus accumbens (AcbSh) with DNQX (250-750 ng) induces pronounced feeding in satiated rats (Maldonado-Irizarry, et al., J. Neurosci., 1995). Expanding on this finding, we have recently discovered that while this treatment increases intake of both solid and liquid foods, it does not alter water intake or time spent gnawing wooden blocks. This strongly suggests that the system is involved specifically in the control of food intake. We also have found that activation of these receptors via bilateral microinjections of AMPA (50 ng) into this region significantly suppresses food intake in food-deprived rats. Blockade of AMPA/kainate receptors most likely increases food intake by disrupting a tonic excitatory input to the AcbSh, thus decreasing the firing rate of a population of local neurons. Since the application of GABA agonists into the AcbSh also decrease neuronal activity, we investigated the effect of microinjections of the $GABA_A$ agonist muscimol (1-100 ng) and the $GABA_B$ agonist baclofen (17-187 ng) on food intake in non-deprived rats. Both treatments dose-dependently increased food intake without altering water intake. At the highest doses, the GABA agonists immediately elicited a period of intense feeding during which the rats injected with baclofen ingested an average of 4.2 g of solid chow (30 min test), and those injected with an equimolar dose of muscimol ingested an average of 7.2 g. Taken together, these results suggest that glutamatergic and GABAergic systems in the medial nucleus accumbens play an important, and possibly specific, role in the modulation of food intake. (Supported by NIDA DA04788)

555.3

EFFECT OF OPIATE AGONISTS MICROINJECTED INTO NUCLEUS ACCUMBENS ON SUCROSE DRINKING IN RATS. Min Zhang and Ann E. Kelley*. Dept. Psychiatry, University of Wisconsin Medical School, Madison, WI 53706

Previous studies have indicated that central opioid peptides play an important role in the modulation of ingestive behaviors. The nucleus accumbens (N.Acc.), a brain region involved in reinforcement, contains high levels of opiate receptors. The present investigation was undertaken to determine the relative involvement of opiate receptor subtypes in sucrose drinking. Morphine (0, 0.5, 5 µg/0.5µl), the mu agonist D-Ala2,NMe-Phe4,Glyol5 (DAMGO; 0, 0.025, 0.25 and 2.5 µg), the delta agonist D-Pen2,5-enkephalin (DPEN; 0, 0.031, 0.31, 3.1 µg), the kappa agonists U50,488H (0, 0.0186, 0.186, 1.86 µg), and dynorphin (0, 0.05, 0.5, 5 µg) were microinfused into N.Acc. Intake (g) of a 5% sucrose solution, drinking duration, locomotion, rearing, and grooming were measured in a 30-min session in rats previously adapted to sucrose. After microinjection into N.Acc, morphine induced a robust increase in both sucrose intake and drinking duration at the low dose, while having a suppressive effect at the higher dose. DAMGO enhanced sucrose drinking at lower doses, and suppressed drinking at the highest dose. DPEN also increased sucrose intake, but only at the highest dose. U50488H and dynorphin had no effect on sucrose drinking behavior. The opioid agonists had little or no effect on spontaneous motor behaviors. These findings demonstrate that both mu and delta receptors within the accumbens, especially mu receptors, may have an important modulatory role in ingestion. Kappa receptors in this region appear not to be involved in this behavior. Further experiments will examine the effects of mu agonists on other ingestive behaviors such as water intake, as well as whether responses within the core and shell subregions can be differentiated. Supported by NIDA grant DA09311.

555.4

MICRODIALYSIS REVEALS THAT BOTH FOOD INGESTION & STRESS ENHANCE CRF RELEASE AT THE CENTRAL AMYGDALOID NUCLEUS. J. McIntosh*[1], P. Kent[1] & Z. Merali[1,2]. [1]School of Psychology & [2]Dept. of Pharmacology, University of Ottawa, Canada.

There is increasing evidence that the central nucleus of the amygdala (Ce), plays a key role in the control of visceral, autonomic and ingestive responses. Recent work in our lab has shown that CRF levels at the Ce change in response to food intake, and that CRF antagonist blocks various endocrine and behavioral effects of bombesin (BN). The 1st objective of this study was to determine, via *in vivo* micro-dialysis, whether the release of CRF and BN is altered during spontaneous food intake. Probes were implanted at the Ce and four 30-min baseline samples were collected during the light phase, followed by 6 more samples during the dark phase. Animal's food consumption was visually observed (under red light). Upon meal initiation, the collection tube was changed and 30-min samples were collected during and after the meal. When compared to baseline, prandial and post-prandial conditions showed a marked elevation in CRF release while there was only a slight elevation in the release of BN. The 2nd objective of the study was to measure changes in CRF and BN release in response to immobilization stress. As before, four 30-min baseline samples were collected, following which rats were exposed to two 20 min periods of manual restraint (spaced 2.5 h apart). Five 30 min samples were collected during and after each restraint episode. Stress increased both CRF and BN release at the Ce. CRF and BN increases became significant at the 3rd and 4th samples, respectively, following 1st stress and continued to rise throughout. These results demonstrate that 1) CRF and BN are released at the Ce in response to both aversive and positively reinforcing situations, and 2) the stress related release of both CRF and BN is sustained and not prone to rapid adaptation, suggesting that these peptides at the Ce may mediate the more long-term effects of stress.
Supported by Medical Research Council of Canada.

555.5

MORPHINE MICROINJECTIONS IN THE SHELL (BUT NOT IN THE CORE) OF THE NUCLEUS ACCUMBENS ENHANCE FEEDING AND PALATABILITY. S. Peciña* and K. C. Berridge. Department of Psychology, The University of Michigan, Ann Arbor, MI 48109.

It is a well established finding that the Nucleus Accumbens (NAcc) plays a major role in the mediation of opioid-induced feeding behavior, an effect which seems to be at least partially mediated by an enhancement in the perceived palatability of tastes. The NAcc is divided into two anatomically and pharmacologically disunct subareas, the shell and the core, which may play different roles in feeding (Kelley, 1995). Do these subareas play different roles in the mediation of opioid-induced feeding? We injected 0.5 µg/0.5 µl/ side of morphine into two groups of rats implanted with bilateral intracranial cannulae aimed at the shell (A=2.0, L=0.9, V=7.5) or the core (A=2.0, L=1.9, V=7.5) of the NAcc. Increases in food intake (i.e. commercial baby cereal) were measured at 15 min, 1 hr, 2 hrs and 3 hrs after morphine microinjections. Increases in taste palatability to a 2% sucrose solution were measured using the taste reactivity technique (Grill and Norgren, 1978) at 15 min, 1 hr and 2 hrs. The results of our preliminary studies showed that morphine selectively enhanced feeding and taste palatability at all times tested in the shell but not in the core of the NAcc. The findings of the present study suggest that the NAcc is a functionally heterogeneous structure with respect to opioid-induced enhancement of feeding and that opioid circuits in the NAcc shell make a special contribution to the mediation of opioid-induced feeding and taste affect.

555.6

CYTOTOXIN TARGETING OF OXYTOCIN (OT) RECEPTIVE CELLS OF CENTRAL AMYGDALA: OT RECEPTORS AND SALT APPETITE. T. Vargas, M.Blake*, W.K. Samson. Physiology and Pharmacology, UND School of Medicine, Grand Forks, ND 58202.

Targeting of OT receptive cells with ricin A chain-oxytocin conjugates revealed a potential role for OT in the control of salt appetite (AJP 69:R245-R251, 1995). Using the same model system (PEG-induced hypovolemia with *ip* mannitol-induced elevation in plasma osmolality) we examined the effect of our cytotoxin treatment on OT-receptor distribution in central amygdala (CA) of the rat, an area of distinct, dense OT binding. Rats received lateral ventricular injection of ricin A chain-OT conjugate (5 µg in 5 µl vehicle) to target for compromise cells bearing OT receptors or a similar injection of unconjugated ricin A chain (toxin control). As expected, rats pretreated with ricin A chain-OT cytotoxin conjugates drank significantly more (72%) saline in response to hypovolemic, hyperosmotic challenge, than controls seven days after *icv* injections. Autoradiographic analysis of I-125 ornithine vasotocin (OVTA) binding in CA was conducted using phosphor imaging (Image Quant Program) in frontal sections of brains from these rats. Total (ratio of CA/cortex) OVTA binding of ricin A chain-OT conjugate treated rats was significantly (29%) less that that present in controls. In addition to this decrease in binding density, a significant spread of OVTA receptor distribution was observed (31% and 32% in volume and area). We hypothesize that this spread in receptive field was due either to compensatory axonal sprouting or glial infiltration since astroglia express OT receptors. (NSF grant #9507751S).

555.7

IMMEDIATE-EARLY GENES IN THE AMYGDALA PLAY AN ESSENTIAL ROLE IN CONDITIONED TASTE AVERSION. R. Lamprecht and Y. Dudai*. Weizmann Institute of Science, Rehovot 76100, Israel .

We study the role of gene expression in rat brain in conditioned taste aversion (CTA). We recently reported that several immediate early genes (IEG's) including c-*fos* are differentially modulated following LiCl i.p., an unconditioned stimulus used in CTA training, in several rat brain areas including the central amygdaloid nucleus (CeA) (NeuroReport 7: 289, 1995). To further elucidate the functional significance of IEG's in CTA, we specifically and locally inhibited translation of *c-fos* and of the cAMP response element binding protein (CREB), which was shown to be involved in transcriptional regulation of *c-fos*. We microinjected 5nmol of phosphorothioate modified oligodeoxynucleotides (PONs) antisense to CREB or to *c-fos* bilaterally into the CeA. Microinjection of CREB antisense 14 hr before CTA training significantly impaired CTA memory tested 3 days later (aversion index (AI) 73+/-5, vs. 96+/-1 in sense micro-injected controls, n=11-17). CREB antisense 7 days before training, or 3 days after training and before testing, was ineffective (95+/-3 vs. 93+/-4, n=5-7). Microinjection of *c-fos* antisense 8 hr before training reduced c-Fos protein in the amygdala to 57% and significantly impaired CTA memory (71+/-6 vs. 92+/-2 in sense micro-injected controls, n=9-11). Injection of *c-fos* antisense 4 days before training, or before testing, or injection 2 mm above the CeA, was ineffective (93+/-2, 95+/-2, and 91+/-3, respectively, n=6 each). Our findings thus indicate that CREB and c-Fos in the amygdala play an essential role in CTA learning. (Supported by the US-Israel Binational Science Foundation, Jerusalem.)

555.8

BODY WATER COMPARTMENTATION IN SODIUM-DEPLETED RATS WITH LESIONS OF THE SUBFORNICAL ORGAN. E. M. Starbuck*, J. R. Lane, and D. A. Fitts. Dept. of Psychology, Univ. of Washington, Seattle, WA 98195

We previously reported that rats with lesions of the subfornical organ (SFOX) expressed salt appetite during sodium depletion only when they were well hydrated with water during the depletion period. Sham-lesioned rats fully expressed a salt appetite under both euhydrated and underhydrated conditions. Since differences in saline intake may have resulted from differences in body fluid content or distribution between sham-lesioned and SFOX rats, we repeated these experiments in order to measure plasma electrolyte levels, osmolality, protein concentration, and hematocrit at the usual time of the salt appetite test in the previous experiments. SFOX and sham-lesioned rats were given 10 ml/kg furosemide (sc) followed during the next 22 hr by 3 intragastric administrations of either 2 ml/kg water (SFOX, n = 10; sham, n = 9) or 10 ml/kg water (SFOX, n = 12; sham, n = 8). Rats were then rapidly anesthetized, and a 10-ml sample of blood was drawn by cardiac puncture. Results of plasma assays revealed higher plasma potassium levels in rats with SFO lesions than in sham-lesioned rats. No significant differences were found in plasma osmolality, protein, sodium concentration, or hemtocrit among the rats in the different lesion and hydration conditions. No interaction was found for sodium excretion or water balance between the lesion and hydration conditions. Thus, the reduced salt intake in underhydrated SFOX rats in previous studies does not appear to result from a gross difference in content or distribution of body water.
Supported by NS22274.

555.9

DOES THE K-ATP CHANNEL ON HYPOTHALAMIC NEURONS AUTO-REGULATE *VIA* PHOSPHORYLATION? V.H. Routh*, J.J. McArdle, and B.E. Levin. Depts Neurosci, and Pharmacol & Physiol, NJ Med Sch (UMDNJ), Newark NJ 07103; Neurology Service, DVA Medical Center, E. Orange NJ, 07018.

ATP-sensitive K^+ channels (K-ATP) exist on glucosensing neurons in the ventromedial hypothalamic nucleus (VMN), a key site for regulating autonomic activity. Inactivation of K-ATP channels by ATP increases neuronal firing rate. Thus, the VMN K-ATP channel may serve as a mechanism whereby metabolic status influences autonomic activity. Neither the mechanism of this inactivation nor the requirement of low levels of ATP for K-ATP channel activity is understood. To explore this issue, we recorded K-ATP channel activity in cell attached and excised inside-out (io) patches of VMN neurons isolated from neonatal rats. When io patches were exposed to symmetrical K^+, single channel currents reversed at 0 mV and had a conductance of approximately 50 pS at a holding potential of 20 mV. While the basal open probability (P_o) fluctuated greatly making it difficult to obtain satisfactory concentration-response relations, both 10 mM ATP/0.1 mM ADP and 100 μM glibenclamide reduced P_o. The non-specific kinase inhibitor H7 (200 μM) decreased P_o and prevented the inhibitory effect of ATP on K-ATP channels of io patches. This observation suggests that the K-ATP channel of VMN neurons may autoregulate *via* intrinsic kinase/phosphatase activity as already suggested for a neuronal Ca^{++}-activated K^+ channel, whose P_o is increased by ATP (Reinhart & Levitan, J. Neurosci. 15(6): 4572-9, 1995). Supported in part by the American Diabetes Association.

555.10

TCDD CANCELS THE EFFECTS ON BODY WEIGHT OF LESIONS OF VENTROMEDIAL HYPOTHALAMIC AND PARAVENTRICULAR NUCLEI. Jouni T. Tuomisto, Mikko Unkila, Raimo Pohjanvirta, Matti Viluksela and Jouko Tuomisto* Laboratory of Toxicology, Division of Environmental Health, National Public Health Institute, P.O.B. 95, FIN-70701 Kuopio, Finland.

Body weight set point (BWSP) is a descriptive term for the level of body weight that will be defended in certain conditions. Very few chemicals change the BWSP as their toxic effect, but TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) and its congeners have this effect (Pohjanvirta & Tuomisto, Pharmacol. Rev. 46:483, 1994). Since some hypothalamic lesions influence BWSP, it is of interest to study their interactions with TCDD effects. Typical lesions causing increased food intake and increased body weight are those of ventromedial hypothalamic and paraventricular nuclei (VMN and PVN, resp.). In Han/Wistar (Kuopio) rats a large dose of TCDD was able to completely abolish the lesion-increased food intake and lowered the body weight to approximately the same lowered level as TCDD in sham-operated controls. After lesions of VMN the effects were more dramatic than after those of PVN, in fact the obese animals stopped eating totally for 10 to 14 days, until their body weight had dropped to the same level or lower as in controls given TCDD. After each type of lesion there was interaction between the lesion and TCDD (ANOVA). This is contrary to the effects of TCDD on diet induced obesity where there was no interaction (ANOVA), i.e. TCDD lowered the body weight in the same manner both in obese and lean animals. In obese Zucker rats leptin receptor is non-functional, and there was also an interaction between the phenotype and TCDD. We predict that the site of action of TCDD on the regulation of body weight is distal from these nuclei and also distal from the leptin receptor, and that TCDD could be used as a tool in obesity studies.

555.11

LATERAL HYPOTHALAMIC AFFERENTS ASSOCIATED WITH NMDA-ELICITED EATING STUDIED BY REVERSE DIALYSIS OF NMDA AND FLUOROGOLD. E.M. Tomkins*, M.A. Duva, C.Y. Kuo, L.M. Moranda, B.G. Stanley. Depts. Neurosci. & Psych., Univ. CA, Riverside, CA 92521.

To reveal the origins of lateral hypothalamic (LH) afferents associated with eating elicited by excitatory amino acid agonists, reverse microdialysis was used to deliver NMDA and the retrograde tracer Fluorogold (FG) into the LH of adult male rats. NMDA (3.3 mM) infused through the probe elicited intense, sustained eating, usually beginning within 3 min and averaging 13.7gm within 45 minutes. Subsequently, 0.2% FG was administered via the dialysis probe at 5 µl/min for 30 min. After 3-4 days with the dialysis probe in place, rats were perfused and 60 µm horizontal sections were collected, dehydrated and observed with a fluorescent microscope. Observations revealed that the FG spread about 0.5 mm around the probe tip. In association with the NMDA-elicited eating responses, retrogradely-labeled cells were consistently found in the infralimbic and perirhinal cortex, core and shell of the accumbens, olfactory tubercle, ventral pallidum, anterior and central amygdala, bed nucleus of the stria terminalis, subfornical organ, median preoptic, lateral preoptic area, posterior hypothalamus, ventral tegmental area, substantia nigra, raphe nuclei, medial and lateral parabrachial, and the caudal part of nucleus tractus solitarius. This study reveals a pattern of LH afferent labeling associated with NMDA feeding, and suggests that reverse dialysis of neuroactive compounds with anatomical tracers may be useful in revealing the neural substrates of some behaviors.

(Funded by NIH NS-24268.)

555.12

FEEDING ELICITED BY *N*-METHYL-D-ASPARTATE (NMDA) IN THE LATERAL HYPOTHALAMUS (LH) IS SUPPRESSED BY A TYROSINE KINASE INHIBITOR. A.M. Khan[1,2], D.S. Welsbie[2], A.M. Khan[2], C.S. Ton[2], J. Nikpur[2], E.R. Gillard[2], P.P. Pir[2], and B.G. Stanley[2]*. Depts. of [1]Biochemistry, and [2]Neuroscience & Psychology, Univ. of California, Riverside, CA 92521.

Increasing evidence suggests that the activity of NMDA receptors can be modulated by intracellular phosphorylation. In particular, protein tyrosine kinase inhibitors have been shown to decrease NMDA receptor-mediated calcium influx *in vitro* (Wang, Y.T. and Salter, M.W., Nature, 369 (1991) 233). We have recently shown that NMDA injected into the LH of satiated rats markedly stimulates eating behavior, and that a NMDA receptor antagonist can suppress natural eating (Stanley, B.G. *et al.*, Brain Res., 613 (1993) 88; Am. J. Physiol., 270 (1996) R443). To investigate whether NMDA-elicited eating is modulated by phosphorylation, adult male Sprague-Dawley rats (n=25) with indwelling LH guide cannulas were injected with the protein tyrosine kinase inhibitor, genistein (240 nmol), followed by NMDA (10 nmol). Compared to vehicle, LH injection of NMDA elicited significant eating (7.9 ± 1.9 g in 30 min, p<.05) and this response was suppressed by as much as 53% with genistein pretreatment (3.7 ± 1.1 g in 30 min, p<.05). In contrast, 240 nmol of dadzein, an inactive analog of genistein, did not suppress NMDA-elicited feeding (7.1 ± 1.8 g in 30 min). These results suggest that tyrosine kinase-mediated signaling may be an important cellular mechanism regulating NMDA-elicited eating in the LH.

(Supported by NIH grant NS 24268)

555.13

ADAPTATION TO AN IMBALANCED AMINO ACID DIET (IMB) IS ATTENUATED FOLLOWING IBOTENIC ACID LESIONS (IBO-L) OF THE DORSOMEDIAL HYPOTHALAMIC NUCLEUS (DMNL) IN RATS. J.F. Evans, L.L. Bellinger* and D. W. Gietzen. Dept. of Biomedical Sci., Baylor College Of Dentistry, Dallas, TX. 75246 and Vet. Med. Anat. Phys. & Cell Biol. Univ. of Calif., Davis, CA 95616.

Rats show food intake (FI) suppression in as little as 3 h after giving an IMB; this decrease in FI can be attenuated by giving Tropisetron (TROP) a $5HT_3$ receptor antagonist. Wang et al., (The FASEB J. 8:A385, 1994) showed c-Fos expression in the DMN 2 h after giving rats an IMB. In the present study male S. D. rats were given IBO DMNL or sham operations (SHAM). The rats were placed on chow for 7 d and then basal diet for 9 d; the DMNL rats were hypophagic ($P<0.01$) compared to SHAM. On basal diet the rats were injected i.p. with saline (SAL) 1 h prior to lights out and FI recorded 3, 6, 12 and 24 h later. Following this the rats were injected with SAL or TROP (9mg/kg): SHAM + SAL (218g BW, n=9); SHAM + TROP (214g BW, n=10); DMNL + SAL (195g BW, n=11); and DMNL + TROP (200g BW, n=12) and 1 h later presented with an isoleucine IMB; the BWs of the DMNL grps were less ($p<0.01$) than SHAMs. For both SAL treated grps their 3, 6, 12, and 24 h FI (expressed as a % of their basal FI) were similar and ranged from 33-49% of their basal FI. The TROP grps FI were similar, but greater ($P<0.01$) than the SAL grps (range 53-78%). On day 2 IMB FI of the SHAM-TROP grps was higher ($p<0.01$) than the other grps whose FI were similar. On day 3 the DMNL-TROP grp's FI was less ($P<0.01$) than all other grps. On days 4-7 the FI of the DMNL grps was less ($P<0.01$) than the SHAMs. BW (expressed as a percentage of last day on basal BW) changes reflected FI and by day 4 both DMNL grps weighed less ($P<0.01$) than the SHAMs. The data show that IBO-L of the DMN doesn't affect the ability of TROP to attenuate the FI suppression caused by IMB. The IBO DMNL attenuates the rats normal adaptation to the IMB. Supported by DK42274 to D.W.G. and BCD Research Funds.

555.14

HYPOTHALAMIC GALANIN AND DIETARY OBESITY. J.T. Alexander*, J.T. Dourmashkin, D. Chan, A. Manitiu, J. Wang, and S.F. Leibowitz. The Rockefeller University, New York, N.Y. 10021

To identify an animal model of dietary obesity, adult male Sprague-Dawley rats (n=100), allowed to select freely from 3 macronutrient diets over a 4-week period, were compared to a group of rats (n=14) given a single balanced nutrient diet (protein 27%, carbohydrate 45% and fat 28%) for the same period. Comparisons between these two groups revealed little difference in total Kcal intake (100-105 Kcals). However, in the nutrient choice group, daily fat intake increased from 31 Kcals (balanced diet subjects) to 42 Kcals ($p<0.05$), enhancing total dietary fat to >41% and reducing dietary carbohydrate to 25%. This rise in fat ingestion was positively correlated with body fat (e.g., inguinal fat pad, $r=+0.61$, $p<0.05$) and body weight ($r=+0.56$, $p<0.05$) and was accompanied by a significant ($p<0.05$) decline in insulin (-25%) and corticosterone (-45%) levels and a rise in glucose (+7%). To characterize associated changes in the brain, additional animals, allowed to select from the 3 nutrients, were separated into 2 groups that consume either high (>45% of total diet) or low (<15%) amounts of fat. Their brains were analyzed for galanin (GAL) mRNA using in situ hybridization with digoxigenin labeled cRNA probe. Results indicate higher number (+45%) of neurons expressing GAL (40±2 vs 24±2 cell number/mm^2, $p<0.05$), specifically in the anterior portion of the paraventricular nucleus (PVN). While a small difference was seen at the middle portion of the PVN, no difference was seen in the medial preoptic area or the arcuate nucleus or in other hypothalamic areas. This high level of GAL gene expression, seen in animals of greater body weight, may be due to the suppression of insulin and corticosterone.

555.15

HYPOTHALAMIC GALANIN GENE EXPRESSION IN RELATION TO A HIGH-FAT DIET IN ADULT MALE RATS. J. Wang*, I. Silva, C. Harris, M. Bhattacharyya and S.F. Leibowitz, Rockefeller Univ., N.Y. 10021

With injections into the hypothalamic paraventricular nucleus (PVN), galanin (GAL) stimulates fat and carbohydrate intake, and GAL antisense oligonucleotides suppress fat intake. Through studies of endogenous GAL, levels of this peptide in the PVN are found to be positively related to the rats' natural daily intake of fat but inversely related to carbohydrate or protein intake. This relationship was examined further in animals that were maintained on a single high-fat (60% fat + 10% carbohydrate, n=5), high-carbohydrate (63% carbohydrate + 10% fat, n=5) or standard lab chow (n=4) diet. After 3 weeks on these diets, the rats were intracardially perfused and their brains examined using in situ hybridization with digoxigenin labeled cRNA probe for mRNA levels and immunocytochemistry for peptide analyses. Comparisons between the high-carbohydrate rats and chow-fed rats showed similar GAL mRNA or peptide measures in the hypothalamic areas examined. However, high-fat diet rats, compared to low-fat diet subjects, showed an increase in neurons expressing GAL mRNA (+36%, 6.97±0.6 vs 4.45±0.7 cell number/mm^2, $p<0.05$) and in peptide immunoreactivity ($p<0.05$) in the anterior PVN, as well as increased peptide immunoreactivity (342.6±81.2 vs 143.6±18.5 objects/mm^2, +238%, $p<0.05$) in the external zone of the median eminence. No significant group differences were seen in other hypothalamic areas (medial preoptic area, arcuate nucleus, or dorsomedial nucleus) where GAL neurons are concentrated. These results show clear, anatomically specific changes in GAL mRNA and peptide immunoreactivity suggesting a positive relationship between this neurochemical and dietary fat.

555.16

NALOXONE DOES NOT BLOCK NEUROPEPTIDE Y-INDUCED *c-fos* EXPRESSION IN THE PARAVENTRICULAR NUCLEUS. J.D. Pomonis*, A.S. Levine, and C.J. Billington. Graduate Program in Neuroscience, Univ. of Minnesota and VA Medical Center, Minneapolis, MN 55417

Neuropeptide Y (NPY) is a powerful orexigenic agent that has its primary site of action in the hypothalamus, especially the paraventricular nucleus (PVN). The effects of NPY on food intake and thermogenesis can be blocked by peripheral or central injection of the opioid antagonist naloxone. This blockade by central administration has been shown to be site-specific. When injected into the PVN, naloxone must be administered at high doses in order to inhibit the effects of NPY injected into the PVN. However, low doses of naloxone injected into the nucleus of the solitary tract (NTS) block the effects of PVN administered NPY. In this study, rats were injected with saline or 1 mg/kg naloxone (i.p.) 30 minutes prior to a PVN injection of either saline or 1 µg NPY. Rats had ad lib access to food except for one group that received peripheral saline and PVN NPY. One hour following the PVN injection rats were sacrificed and tissue was processed for c-Fos immunoreactivity (Fos-IR). Fos-IR in the PVN was significantly increased in all animals receiving PVN NPY compared to those that received PVN saline injections, regardless of food intake {$F(4,14)=5.111$, $p=0.0094$}. Thus, even when naloxone blocked feeding induced by NPY, the NPY-induced Fos-IR in the PVN remained elevated. These data suggest that the effects of naloxone are mediated downstream from the PVN, presumably in the NTS, rather than via efferent projections from the NTS to the PVN. (NIH DKA 42698)

555.17

INDUCTION OF NEUROPEPTIDE Y MRNA IN THE ARCUATE NUCLEUS BY FASTING IS ATTENUATED IN DIET-INDUCED OBESITY. H. T. Bergen*, T. M. Mizuno, and C. V. Mobbs, Dept. of Anatomy, U. of Manitoba, Winnipeg, MB, Canada and Fishberg Center for Neurobiology, Mt. Sinai School of Medicine, New York.

Neuropeptide Y (NPY) in the hypothalamus increases food intake and body weight, and NPY mRNA levels in the arcuate nucleus increase in response to fasting. NPY mRNA levels in the arcuate are also elevated in some models of obesity. This study was performed to determine whether regulation of NPY mRNA is altered in diet-induced obesity. Mice (CBA and C57BL/6J strains) were fed either rodent chow (control) or a high-fat, high-carbohydrate diet for 14 weeks. This diet produced obesity in both strains. The mice were either fasted for 48 hours or fed ad lib before being killed. NPY mRNA levels in the arcuate nucleus were assessed by in situ hybridization. In control CBA mice, fasting increased NPY mRNA. In contrast, in obese CBA mice, fasting did not significantly increase NPY mRNA levels as compared to non-fasted mice. In addition, NPY mRNA levels were lower in obese CBA mice than in controls under both fasted and fed conditions. In control C57BL/6J mice, fasting increased NPY mRNA levels. Although fasting also increased NPY mRNA levels in obese C57BL/6J mice this effect was more modest as NPY mRNA levels were significantly lower in fasted obese than in fasted control mice. These results suggest that diet-induced obesity is not associated with increased NPY activity in the arcuate nucleus and that induction of NPY mRNA by fasting is attenuated in diet-induced obesity. Supported by the Manitoba Medical Services Foundation.

555.18

REDUCTION OF NPY2-36'S OREXIGENIC POTENCY BY ESTRADIOL IN OVARIECTOMIZED RATS IS ASSOCIATED WITH NEUROPEPTIDE Y RECEPTOR MODULATION IN THE PERIFORNICAL HYPOTHALAMUS. T.K. Bhatti, D.B. Conze, N. Geary, R.C. Young* and E.S. Corp. E.W. Bourne Lab, Cornell University Medical College, White Plains, NY 10605.

Ovariectomy (OVX) produces a profound increase in body weight (BW) in rats that is prevented by replacement treatment with estradiol benzoate (EB). Central NPY has been implicated in hyperphagia and obesity, and it is possible that EB's effects on food intake (FI) and BW may arise through a regulatory action of EB on NPY receptors. To investigate this possibility, we tested the potency of NPY2-36 to stimulate FI after lateral icv injection in OVX rats (n=12) receiving either chronic EB treatment (5 µg/rat, 3 days per week) or the oil vehicle. After one month of treatment EB rats weighed less than controls (EB, 270 ± 5g vs. oil, 319 ± 8g, $p<0.01$). NPY2-36 stimulated dose-related increases in FI in both groups; however, analysis by 2-way ANOVA of total FI revealed a significant treatment x dose interaction, $p< 0.02$. This occurred because 1000 pmole NPY2-36 stimulated feeding less potently in EB-treated rats than in oil-treated rats (see Table, *$p<0.05$ Tukey's test).

		NPY2-36 (pmoles)		
Treatment	**Vehicle**	**100**	**300**	**1000**
Oil	0.4 ± 0.2	2.1 ± 0.6	4.8 ± 0.8	7.3 ± 1.0
EB	0.3 ± 0.1	1.4 ± 0.4	3.4 ± 0.5	3.9 ± 0.9*

We next determined parameters of [^{125}I]PYY receptor binding in the perifornical region of the hypothalamus. EB treatment resulted in a 56% downregulation of receptor number; Bmax (fmol/mg tissue) EB, 22 ± 4 vs. oil, 50 ± 12, $p< 0.05$. NPY receptor affinity, however, was increased in the EB-treated; KD's (nM) EB, 0.4 ± 0.1 vs. oil, 1.2 ± 0.4, $p<0.02$. These data indicate that EB decreases the orexigenic potency of NPY2-36 and produces a downregulation of NPY receptor number. However, the upregulation of receptor affinity suggests that further studies are required to clarify the influence of EB treatment on NPY, its receptors and the regulation of FI and BW. *Support: Chairman's Fund CUMC, Whitehall Foundation and MH51135*

555.19

THE DISTRIBUTION OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR (GLP1-R) mRNA IN THE RAT BRAIN. P.J. Shughrue*, M.V. Lane and I. Merchenthaler. Women's Health Research Institute, Wyeth-Ayerst Research, Radnor, PA 19087.

GLP-1 has been shown to dramatically reduce food intake in fasted rats. The effect of GLP-1 on feeding is both dose dependent and blocked by a GLP-1 receptor antagonist, exendin. GLP-1, when injected intracerebroventricularly, has also been found to attenuate the stimulatory effect of neuropeptide Y on appetite and induce the expression of *c-fos* in brain regions associated with appetite and feeding. While great strides have been made in understanding the role of GLP-1 in the brain, little is known about the distribution of GLP1-R and its mRNA. The purpose of the present study was to utilize *in situ* hybridization histochemistry to determine the anatomical distribution of GLP1-R mRNA in the rat brain. The brains of female rats were frozen and 20µm cryostat sections collected on microscope slides. The section-mounted slides were hybridized with a ^{35}S-labeled antisense riboprobe complimentary to GLP1-R mRNA, stringently washed and appossed to emulsion. The results of these studies revealed an extensive distribution of GLP1-R mRNA throughout the rostral-caudal extent of the brain; with the majority of the perikarya concentrated in the hypothalamus, pons and medulla. A dense accumulation of GLP1-R mRNA-containing cells was observed in the supraoptic, paraventricular, arcuate and dorsomedial nuclei of the hypothalamus; raphe nuclei; nucleus of the solitary tract and spinal trigeminal nucleus. In addition, GLP1-R mRNA was detected in the lateral septum; diagonal band of Broca; bed nucleus of the stria terminalis; periventricular nucleus; amygdala; posterior thalamic nuclei; and central gray. The results of these *in situ* hybridization histochemical studies have provided detailed and novel information about the distribution of GLP1-R mRNA in the rat brain. In addition, this morphological data provides important information about the neuronal systems modulated by GLP-1 and their potential role in feeding behavior.

Supported by Wyeth-Ayerst Research

555.20

DECREASED INTAKE OF A LIQUID DIET FOLLOWING INTRA-PVN INJECTIONS OF GLUCAGON-LIKE PEPTIDE-1. L.R. McMahon* and P.J. Wellman. Dept. of Psychology, Texas A&M University, College Station, TX 77843-4235.

ICV administration of glucagon-like peptide-1 (7-36) amide (GLP-1) dose-dependently suppresses food intake in rats (Turton et al., *Nature*, 1996). Although the locus of action is unknown in rat brain, ICV administration of GLP-1 induces c-*fos* activation within rat paraventricular hypothalamus (PVN). The present study sought to determine whether intra-PVN administration of GLP-1 acts to suppress food intake in rats. Adult male rats (n=14) were prepared with indwelling guide cannulae aimed at the PVN. Rats were allowed access to a palatable liquid diet (Ensure) during a daily 60 minute test period with intakes measured every fifteen minutes. Intra-PVN administration of GLP-1 (0, 0.01, 0.05, 0.1, and 0.2 ug) did not alter latency to feed, but did induce a significant dose-dependent suppression of liquid diet intake during the first 15 minutes of the 60 minute test periods. These results suggest that a portion of the central action of GLP-1 on feeding may result from interactions with cells within the PVN.

Support Source - Texas A&M University

556.1

CENTRAL INFUSION OF LEPTIN (OB PROTEIN) AND GLP-1-(7-36) AMIDE DIFFERENTIALLY STIMULATE C-FOS EXPRESSION IN RAT BRAIN. **T.E. Thiele, G. van Dijk, J.C.K. Donahey, L. Arthur Campfield, F. J. Smith, I.L. Bernstein, S.C. Woods*, and R. J. Seeley.** **Dept. of Psychology, U. of Washington, Seattle, WA 98195.**

Considerable evidence suggests that leptin and glucagon-like peptide-1-(7-36) amide (GLP-1) are regulators of food intake. Given the different physiological characteristics of these peptides, it was hypothesized that reduction in food intake following central infusion of leptin or GLP-1 is mediated by different neural substrates. To examine this question, male Long-Evans rats were infused with either synthetic CSF (s-CSF), leptin (3.5 μg dissolved in s-CSF), or GLP-1 (10.0 μg dissolved in s-CSF) into the third ventricle. Relative to s-CSF, both leptin and GLP-1 reduced food intake by approximately 60% during the first 4 hr-period of the dark phase. As revealed by c-Fos immunohistochemistry, central infusion of leptin and GLP-1 produced c-Fos expression in the region of the paraventricular hypothalamus and the central amygdala. On the other hand, leptin selectively induced c-Fos expression in the dorsomedial hypothalamus, whereas GLP-1 selectively produced c-Fos in nucleus of the solitary tract, area postrema, lateral parabrachial nucleus, and the arcuate hypothalamic nucleus. Because c-Fos expression following leptin and GLP-1 infusion were observed mainly in separate regions of the brain, results suggest that these peptides modulate food intake and body weight via different central mechanisms.

This research was supported by grants from the Dutch Diabetes Association, AA07455, DC-00248, DK-35816, and DK-17844. Leptin was generously provided by Hoffmann- LaRoche, Nutley, NJ.

556.2

DISTRIBUTION OF LEPTIN BINDING AND RECEPTOR MRNA IN THE OBESE MOUSE BRAIN. M.E. Goldstein*[1], J. Wang[1], R.A. Parker[2], T.G. Kirchgessner[2], H.K. Chan, R.N. Wright[1], A.S. Eison[1]. CNS[1] and Metabolic Diseases[2] Depts, Bristol-Myers Squibb Co., Wallingford, CT 06492 and Princeton, NJ, 08543.

Leptin, a circulating protein synthesized by adipose tissue, regulates feeding in response to changes in adipose mass. To investigate the mechanism by which the brain responds to leptin, we have examined the distribution of ^{125}I-leptin binding and leptin receptor mRNA using receptor autoradiography and *in situ* hybridization. Leptin binding is observed in the choroid plexus, meninges and major blood vessels of ob/ob, ob/+, db/db mouse brains and in rat brain. Though no binding is detected in the arcuate nucleus, we see high levels of leptin receptor mRNA in this region of ob/ob and db/db mice, in addition to other hypothalamic nuclei, choroid plexus, and meninges. Lower levels of receptor mRNA are seen in the arcuate of lean animals (ob/+ mouse and rat) suggesting that the obese animals may upregulate leptin receptor message in the arcuate in response to increased adiposity and circulating leptin. Acute injections of leptin into the region of the arcuate nucleus of ob/ob mice result in marked decreases in feeding and body weight supporting a role for this region in the regulation of food intake. (Research supported by Bristol-Myers Squibb Company).

556.3

CENTRAL LEPTIN INJECTION INDUCES NEURONAL ACTIVATION IN RAT HYPOTHALAMUS. A.E. Willing*, D. Hwang, D.A. York, and H.-R. Berthoud, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana, 70808.

With the cloning of the mouse ob gene, it recently became possible to directly test the role of the ob gene product, leptin, which is produced in adipose tissue, on the regulation of body weight and feeding. Central and peripheral leptin administration decrease both food intake and body weight in ob and diet-induced obese mice (Campfield et al, Science, 269,1995). More recently, the leptin receptor was cloned and found to be present in the lung, kidney, choriod plexus, and hypothalamus of the mouse (Tartaglia et al, Cell, 83, 1995). The aim of this study was to determine the central site of leptin action by measuring c-fos expression in response to leptin, vehicle, or artificial CSF injection. Guide cannuli were aimed at the lateral ventricle in male Sprague-Dawley rats and correct injector placement was verified by the drinking response to angiotensisn II (15 ng/ 3 μl). One week later, the animals were injected with either recombinant mouse leptin (1 or 10 μg in 5 μl which we produced, or as a gift from Ciba, Switzerland), the leptin fusion protein (5 μl) or artificial CSF(5 μl) and then perfused 90 min later. c-Fos immunocytochemistry on 50μm frontal sections taken through the entire brain demonstrated that central leptin injection increased c-fos expression in the hypothalamus (paraventricular, dorsomedial, lateral, ventromedial nuclei), amygdala, and paraventricular thalamus. This suggests either that leptin receptors are present on neurons in these nuclei or that these neurons are part of a neural network that is activated by leptin. It supports the hypothesis that leptin acts directly at brain sites to control body weight and food intake. Supported by NIH grants DK47348, DK41868 and USDA 93-37200-8961.

556.4

EXPRESSION OF THE LEPTIN RECEPTOR DURING DEVELOPMENT AND IN THE ADULT MICE. S.-C. Chen[1], M. Nettleton[2], T. Wang*[1], J. P. Kochan[2] and R. J. Smeyne[1]. [1]Neurogenetics Group and [2]Department of Metabolic Diseases, Hoffmann-La Roche Inc., Nutley, NJ 07110.

The cloning of the mouse obesity (*ob*) gene has made it possible to study the mechanisms of obesity at the molecular level. *ob* gene appears to encode a signaling factor, leptin, for body weight homeostasis. Interestingly, studies have suggested that decreased sensitivity to leptin may be a more important problem in human obesity. Thus, it is crucial to understand the molecular mechanism involved in leptin signal transduction. Recently, a high affinity receptor (OB-R) for leptin has been identified and cloned by expression cloning from mouse choroid plexus. As a result of alternative splicing, at least two forms of protein with different lengths of intracellular domain were identified. The gene encoding OB-R was subsequently identified and confirmed as the *db* gene. A single nucleotide mutation causes the inability of the *db/db* mice to produce the long form OB-R.

We have carried out *in situ* hybridization to investigate the expression and developmental regulation of OB-R gene. Riboprobes derived from different regions of OB-R gene were used in the experiments. With a probe specific to the extracellular domain of OB-R, its message was detected in many adult tissues. These include hypothalamus and choroid plexus in CNS and several peripheral tissues, e.g., kidney, intestine and stomach. In contrast, a much more restricted tissue distribution was observed with a probe that is specific to the long alternative splice variant of OB-R gene. Developmentally, the OB-R gene transcript could be detected in mouse embryo as early as e9.5. Similar tissue distribution patterns were observed throughout development. These results will further our understanding of the sites and molecular mechanisms of leptin in regulating body weight.

556.5

ACTIVATION OF FOS-LACZ BY PERIPHERAL AND CENTRAL INJECTION OF OB PROTEIN (LEPTIN): IMPLICATIONS FOR DOWNSTREAM SIGNALING PATHWAYS. R.J. Smeyne*[1], M. Renzetti[2], F. Smith[2]. [1]Neurogenetics Program and [2]Department of Metabolic Diseases. Hoffmann-La Roche, Nutley, NJ 07110.

The product of the recently cloned *ob* gene, Leptin, has been shown to have remarkable biological import in the regulation of body weight in rodents. This protein has been demonstrated to reduce food intake as well as increase energy expenditure. Little is known about the central and peripheral signaling pathways which underlie these biological properties. To investigate this question, we injected Fos-lacZ (FLZ) transgenic mice with purified recombinant mouse Leptin through IV or ICV administrative routes in order to map out the anatomical pathways used in *ob* gene signaling. Two hours following IV Leptin injection, we find FLZ labeling in both CNS and peripheral tissue. Sites of expression following peripheral injection include the liver, kidney, skeletal muscle and areas of brown and white fat. In the nervous system, expression of FLZis seen in a cerebral cortices and a vast number of diencephalic and hindbrain nuclei. In order to see if the central and peripheral activation was a direct CNS effect, we injected Leptin via ICV. When administered by this route, we found that the peripheral expression, except in brown fat, disappeared. In addition, far fewer areas of the CNS demonstrated FLZ expression. One exception was the arcuate, DM and VPM nuclei of the hypothalamus. This work suggests that there must be both peripheral and central receptors that can activate OB signaling. Further work should define this matter.

556.6

METABOLIC ACTIVITY IN THE MIDBRAIN AND FOREBRAIN ASSOCIATED WITH INTESTINAL INFUSIONS OF GLUCOSE VERSUS SALINE IN RAT PUPS. **C.B. Phifer* and A.M. Nance.** Louisiana Scholars' College, Northwestern State University, Natchitoches LA 71457.

The nutritive value of diet begins to affect ingestion in rat pups during the second week of life, and pup's first response appears due to preabsorptive signalling in the intestine. Direct duodenal infusions of glucose decrease intake relative to saline infusions, and this inhibition is attenuated by topical anesthesia of the intestinal mucosa. In the present study, [^{14}C]2-deoxyglucose (2-DG) autoradiography was used to study potential CNS correlates of a response to intestinal nutritive signals.

Polyethylene cannulas were implanted in the anterior duodenum. Following 2-DG injections, pups were allowed to ingest milk for 30min. During the first 8min of the test, pups received duodenal infusions of 0.6M glucose or isotonic saline at 0.25%BW/min. Average autoradiograms were made from brain slices of eight pups in each treatment group. Differences in activity were obtained by subtracting one average image from the other average image. Previous analyses revealed effects in the brainstem; the present analysis covered the anterior midbrain andn forebrain areas through the diencephalon.

Absolute differences between brains of glucose- and saline-infused pups were seen in numerous midbrain and forebrain regions, including entorhinal, occipital, parietal and frontal ctx, retrosplenial granular and agranular ctx, medial forebrain bundle, horizontal limb of diagonal band, lateral and medial lemniscus, deep mesencephalic nu, various amygdaloid nu, zona incerta. lateral hypothalamus, caudate putamen and various thalamic nu. These results will be discussed in relation to pathways and regions known or postulated to subserve ingestive behavior and sensory pathways, particularly audition and olfaction.

(Support: Louisiana Education Quality Support Fund and Whitehall Foundation.)

556.7

SEROTONERGIC MECHANISM OF THE LATERAL PARABRACHIAL NUCLEUS ON NaCl INTAKE INDUCED BY VOLUME DEPLETION. J. V. Menani*, L. A. De Luca Jr and A. K. Johnson. Dept. of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242, USA, Dept. Ciências Fisiológicas, Fac. Odontologia, UNESP, Araraquara, SP, Brazil.

In this study we investigated the effects of injections of serotonergic (5HT) drugs into the lateral parabrachial nucleus (LPBN) on the intake of NaCl and water induced by 24 h water deprivation or 24 h sodium depletion (injection of the diuretic furosemide + 24 h of sodium deficient diet). Rats had stainless steel cannulas implanted into the LPBN. Bilateral LPBN injections of the 5HT antagonist methysergide (4 μg/0.2 μl) increased 0.5 M NaCl intake induced by sodium depletion and 0.3 M NaCl intake induced by water deprivation. Bilateral injections of the serotonergic $5HT_{2A2C}$ agonist DOI (5 μg/0.2 μl) reduced 0.5 M NaCl intake induced by sodium depletion. Water intake increased after methysergide into LPBN and was reduced after DOI. The specificity of LPBN-5HT blockade on fluid intake was also tested in rats on *ad lib* food and water by determining effects on the intake of 2% sucrose. Methysergide in the LPBN produced no change in 2% sucrose intake. The results suggest a specific increase in experimentally-induced sodium and water intake by serotonergic antagonism in the LPBN and this may be important for the control of blood volume.

Supported by FAPESP, CAPES-AEX and NHLBI HL.

556.8

COOLING BLOCKADE OF THE PARABRACHIAL NUCLEUS ATTENUATES CONDITIONED TASTE AVERSION IN FREELY MOVING RATS. Yuan Wang, David G. Lavond, Kathleen C. Chambers*. Psychology Department at University of Southern California, Los Angeles, CA 90089-1061

Permanent or temporary inactivation of the area postrema (AP) causes a malaise which can induce conditioned taste aversions (CTAs). It has been suggested that this malaise interferes with the capability of rats to acquire CTAs induced by LiCl. However, by using cooling to temporarily inactivate the AP, we have demonstrated that a LiCl-induced CTA is attenuated when the AP-lesion-induced malaise is dissociated from a novel food by preexposure to the malaise prior to conditioning. We have found recently that temporary inactivation of the parabrachial nucleus (PBN) also can induce a CTA. The following experiment was designed to determine whether cooling the PBN could attenuate a LiCl-induced CTA if rats are preexposed to cooling prior to conditioning. Twenty-three rats were divided into three groups: Cooling-PBN (n=7), Cooling-Above-PBN (n=8), and No-Cooling (n=8). The PBN of all three groups were preexposed to cooling daily for one week prior to conditioning. On acquisition day, all rats were given access to a 10% sucrose solution for 1 hour. Then the No-Cooling rats were injected with LiCl. Cooling was initiated in those rats assigned to the cooling condition and 5 minutes later LiCl (0.15M, 2ml/kg, i.p) was injected. The PBN was cooled for 1 hour. Two days later, daily extinction trials were initiated. When sucrose solution consumption on acquisition day and extinction day 1 were compared, rats in the Cooling-Above-PBN and No-Cooling groups showed a decrease in the amount consumed whereas rats in the Cooling-PBN group showed no change. These results support the hypothesis that the PBN is necessary for acquisition of LiCl-induced CTAs. Supported by USC-FRIF and NIMH 1 R01 MH51197.

556.9

CNS NEURONS REGULATING THE SYMPATHETIC OUTFLOW TO WHITE ADIPOSE TISSUE AS REVEALED BY TRANSNEURONAL LABELING WITH PSEUDORABIES VIRUS. W. S. Warren, M. G. Adkison and T. J. Bartness*. Depts. of Psychology and of Biology, Georgia State Univ., Atlanta, GA 30303.

Siberian hamsters exhibit a decrease in body mass with short photoperiod exposure, an effect due almost exclusively to a decrease in body fat. This decrease in lipid stores is not uniform and seems to be due to a differential drive by sympathetic nervous system (SNS) on white adipose tissue (WAT) pads and consequential differential rate of lipolysis. This effect is achieved via relatively separate innervation of WAT pads by neurons in the SNS chain. We explored the SNS innervation of WAT further by using a viral transneuronal retrograde tract tracer, pseudorabies virus (PRV). PRV was injected into the inguinal WAT (IWAT) pads (10^8 pfu/μl, 3-5 100nl injections throughout the pad) and hamsters were killed 4-6 days later. Brains, spinal cords and sympathetic chains were removed and processed for immunocytochemical (ICC) identification of virally-infected neurons. Specific labeling was found in neurons of the sympathetic chain, intermediolateral (IML) horn of the spinal cord, rostral ventrolateral and ventromedial medulla, and the paraventricular and suprachiasmatic nuclei of the hypothalamus. Labeling of the sympathetic chain and IML was unilateral to the injection side, with more uni- than contralateral labeling in the higher CNS areas. These results provide an initial identification of the SNS outflow to WAT.

Funding: NIH RO1 DK35254 & NIMH MH00841

556.10

HYPOVOLEMIA STIMULATES DRINKING IN BARORECEPTOR-DENERVATED RATS. E.M. Stricker*, A.M. Schreihofer, and A.F. Sved. Department of Neuroscience, University of Pittsburgh, Pittsburgh PA 15260.

Hypovolemia is an established stimulus of thirst and vasopressin secretion. Both responses are thought to be mediated by changes in afferent input to the brain from cardiopulmonary baroreceptors. However, we recently observed that hypovolemia still elicits vasopressin secretion in rats after cardiopulmonary and arterial baroreceptor input to the brain had been eliminated chronically by electrolytic destruction of the nucleus tractus solitarius (NTSX), their brain stem site of termination. Therefore, the present experiments sought to determine whether hypovolemia still elicits drinking in rats with NTSX. Complete loss of cardiac afferent input was confirmed in all NTSX rats by an absence of cardiovascular reflex responses evoked by injection of phenylbiguanide, and by the absence of carotid and aortic baroreceptor reflex responses. Then hypovolemia was produced by injection of 30% polyethylene glycol solution (5 ml, sc); food was not available during the 8-hour test. Control and NTSX rats consumed comparable amounts of water (6.1 ± 2.0 ml, n=13; 7.4 ± 1.1 ml, n=10, respectively), with comparable latencies to drink. Because water intake during hypovolemia underestimates stimulus-evoked drinking due to the inhibitory effects of osmotic dilution, we also examined the intakes of 0.15 M NaCl during hypovolemia in these animals. Again, control and NTSX rats drank comparable amounts of saline in 8 hours (33 ± 6 ml, 30 ± 4 ml, respectively). In neither of these tests did urine volume differ between the two groups. These results indicate that baroreceptor afferent signals arising from the heart and major blood vessels are not necessary for drinking induced by hypovolemia in rats. (HL-38786, MH-25140)

556.11

EVIDENCE FOR VAGAL INVOLVEMENT IN THE PATHOPHYSIOLOGY OF BULIMIA NERVOSA. B.K. Hartman*, P.L. Faris, S.W. Kim, W.H. Meller, N.C. Raymond, and E.D. Eckert. Div. of Neuroscience Research and the Eating Disorders Program, Dept. Psychiatry, Univ. of Minnesota, Mpls, MN 55455

The present study was designed to determine the involvement of the afferent vagus (see Aicher and Randich; Pain 42:103,1990) in mediating both the altered pain detection (PDT) in bulimia nervosa (Biol.Psych 32:462,1992) and the frequency of binge eating/vomiting. Subjects were thirteen females meeting DSM IV criteria for bulimia nervosa plus the more stringent criteria of at least 7 binge/vomit episodes/week. First, the effect of bulimic behaviors on PDT was examined by statistical correlation of weekly measurements of PDT with the time interval since the subject last engaged in a binge/vomit episode. PDT were found to be positively correlated with the time interval since the last binge/vomit episode ($z=4.85$; $p < 0.00001$). This finding suggests that PDT (and presumably afferent gastric vagal activity) are dynamically changing across the binge/vomit cycle and are lowest (low vagal activity) temporally near to the binge/vomit episode and rise (increase in vagal activity) as the next episode is approached. The second experiment determined vagal involvement in mediating both the bulimic behaviors and the dynamic changes in PDT across the binge/vomit cycle. In ten subjects vagal activity was pharmacologically reduced by chronic ondansetron (OND) administration (24 mg/day). During OND treatment, PDT no longer changed as a function of the time interval since the last binge/vomit episode ($z=-1.5$; $p=.14$). In a double blind study, subjects treated with placebo (n=7) exhibited no significant improvement during a six week study period (ANOVA repeated measures $F(6,5)=1.102$; $p=.38$). In the OND group (n=4) there was a significant decrease in binge/vomit frequency during the double-blind phase (ANOVA repeated measures; $F(3,5)=5.9$; $p<0.01$). These results support the hypothesis that afferent vagal hyperactivity is involved in the pathophysiology of bulimia nervosa. Our findings suggest (1) that an increase in spontaneous vagal activity, as indexed by increasing PDT, drives the binge/vomit episodes and (2) not engaging in the pathologic behaviors is associated with a worsening of the physiological abnormalities. Supported by MH 49385.

556.12

PERIVAGAL CAPSAICIN TREATMENT BLOCKS INCREASED FOOD INTAKE AND BRAIN FOS-LIKE IMMUNOREACTIVITY (FOS-LI) AFTER TREATMENT WITH 2,5-ANHYDRO-D-MANNITOL (2,5-AM). C.C. Horn* and M.I. Friedman. Monell Chemical Senses Center, Philadelphia, PA, 19104, USA.

2,5-AM, a fructose analogue, increases food intake and brain Fos-li in vagal afferent pathways. We investigated whether these effects of 2,5-AM are mediated by afferent c-fibers by applying capsaicin to the cervical vagus nerve. Rats were given either perivagal capsaicin treatment (n=9)or sham surgery (n=7)at least 2 weeks before testing. Capsaicin-treated rats did not increase food intake after injection of 2,5-AM (200 mg/kg, i.p.) whereas sham-operated rats did. Furthermore, both groups of rats increased food intake in response to 2-deoxy-D-glucose, which stimulates food intake by a different neural pathway than 2,5-AM. To investigate the effects of capsaicin treatment on brain Fos-li after 2,5-AM, rats in each surgical group were reinjected with either saline or 2,5-AM. Preliminary data suggest that capsaicin treatment reduces Fos-li in vagal afferent pathways, but in a limited fashion. These results indicate that not all brain Fos-li expression after 2,5-AM is related to the effect of 2,5-AM on food intake, and that vagal afferents of the c-fiber variety, mediate the eating response to 2,5-AM.
(This research was supported by NIH grants DK36339 and DC00014)

556.13

LESIONS OF THE COMMISSURAL REGION OF THE NUCLEUS TRACTUS SOLITARII REDUCE FOOD INTAKE AND BODY WEIGHT GAIN IN RATS. W.T. Talman*[1], E. Colombari[2], J.V. Menani[3], and A.K.Johnson[4]. Depts. of Psychology[4], Pharmacology[4], and Neurology[1], and the Cardiovascular Center, Univ. of Iowa & VAMC, Iowa City, IA 52242; Dept. of Physiology[3], School of Dentistry, Paulista State Univ., Araraquara, SP 14800 and Dept. of Physiology[2], UNIFESP-EPM, São Paulo, SP 0423-900, Brazil.

Previous studies have shown that lesions of the area postrema and adjacent nucleus tractus solitarii (NTS) alter food intake. We investigated the effects of an electrolytic lesion of the commissural subnucleus of the NTS (commNTS) on body weight, daily food and water intake, and plasma glucose and insulin in adult male Sprague Dawley rats. In the first 6 days after surgery, daily food intake was significantly ($p<0.05$) reduced in rats with commNTS lesions (7.1 ± 2.2 g/24h; n=6) when compared with rats with sham lesions (18.1 ± 1.8 g/24h; n=5). Subsequently, rats with lesions increased food intake, but their intake remained significantly reduced until the 12th postoperative day (11.7 ± 2.9 vs 21.7 ± 0.9 g/24h). A significant reduction in body weight was observed 4 days after commNTS lesion and reached a maximum on the 12th day (283 ± 14 vs 378 ± 12 g). After the 12th postoperative day partial recovery of body weight was observed, but weight remained significantly less in rats with lesions when compared with sham operated controls. Food intake and body weight gain in other rats with partial lesions (n=8) of the commNTS or with lesions outside the commNTS did not differ from sham rats. Daily water intake and plasma glucose and insulin were not changed by commNTS lesions. These results suggest that commNTS is involved with mechanisms that control food intake and body weight in rats. Support: E.C.-CNPq (200 006/93-2); J.V.M.-FAPESP (93/0167-7); W.T.T.- VAMC Merit Review and Career Award, NIH HL32205 and HL14388, and Amer. Heart Grant in Aid; A.K.J.- HL44546.

556.14

UNILATERAL VAGOTOMY ATTENUATES C-FOS INDUCTION BY CHOLECYSTOKININ AND BOMBESIN IN THE CONTRALATERAL NUCLEUS OF THE SOLITARY TRACT. T.A. Houpt*, S.P. Frankmann, and R.A. Berlin. E.W.Bourne Behav.Res.Lab., Cornell Univ. Med.Coll., White Plains, NY, 10605 and Dept. Psychology, Univ. South. Colo., Pueblo, CO 81001.

The subdiaphragmatic vagus mediates the satiety effects of peripheral cholecystokinin (CCK) at low doses (< 8μg/kg), and of bombesin (BN) in the absence of spinal afferents. Spinal afferents can also mediate the satiety effects of CCK at high doses, and of BN in the absence of the vagus. The nucleus of the solitary tract (NTS) receives vagal and spinal afferent input, as well as descending forebrain input. To determine the relative contributions of the vagus and other inputs in activating the NTS after CCK or BN injections, we examined c-Fos induction in the NTS of unilaterally subdiaphragmatic vagotomized rats (uniVgx).

Male rats (200g) underwent right or left uniVgx just below the diaphragm and above the hepatic and celiac branches (n=16), or sham vagotomy (shamVgx;n=8). Five days later, rats received 1 mg Fluorogold (FG) i.p. Ten days after uniVgx, rats received CCK (8μg/kg), BN (8μg/kg) or saline (1ml/kg) i.p. and were perfused after 1 h. Sections through the NTS were processed for c-Fos and FG immunoreactivity.

UniVgx rats showed unilateral FG staining only in the dorsal motor nucleus ipsilateral to uniVgx, verifying axotomy of fibers projecting to the contralateral NTS. Saline induced litte or no c-Fos in the NTS of any rats. In shamVgx rats, CCK and BN induced numerous c-Fos cells in the NTS, and CCK induced more c-Fos in the area postrema (AP) than BN. In uniVgx rats, the c-Fos induced by both CCK and BN was reduced (but not eliminated) in the NTS contralateral to uniVgx; c-Fos in the AP and ipsilateral NTS was not reduced. This confirms the contribution of the vagus in mediating NTS activation by peripheral CCK and BN. The remaining activation of the contralateral NTS may be due to contralateral vagal input, spinal afferent input, or bilateral inputs descending from the forebrain.

Supported by the Whitehall Foundation.

556.15

THE ROLE OF THE NUCLEUS OF THE SOLITARY TRACT IN TASTE AVERSION AND SODIUM APPETITE USING BRIEF INTAKE TESTS. R. Norgren*, P. Lyuboslavsky, K. Smith, and P.S. Grigson. College of Medicine, Penn State Univ., Hershey, PA 17033.

Six rats received bilateral electrophysiologically-guided electrolytic lesions (30 μA/30 sec) of the gustatory zone of the solitary nucleus (NST), 3 rats received SHAM lesions, and 3 rats served as non-surgical controls. Experiment 1: Rats were given repeated 10-sec access periods (total=30 min) to water plus 6 concentrations of citric acid and QHCl (dehydrated) and water plus 6 concentrations of sucrose and NaCl (rehydrated). Experiment 2: Taste aversion learning (CTA). Water deprived rats were given repeated 10-sec access periods (total=15 min) to 0.3 M alanine, and 15 min later, injected ip with LiCl (0.15 M, 1.33 ml/100g bw). Three taste-LiCl pairings occurred at 72 h intervals. Experiment 3: For NA^+ appetite, intake of water and 6 concentrations of NaCl was evaluated across repeated 10-sec trials (total=30 min) 24 h following 2 sc injections of furosemide (7.0 mg/0.7 ml). The results showed that NST lesions (1) disrupted responsiveness to increasing concentrations of sucrose and QHCl, (2) suppressed the expression of a NA^+ appetite, but (3) left the CTA intact. Previously, using a consumption test, similar NST lesions permitted expression of an attenuated NA^+ appetite. Thus, NST damage may increase the access time required for some taste-guided behaviors. Supported by DC 00240, MH 00653, and DC 02016.

556.16

NUCLEUS TRACTUS SOLITARIUS LESIONS BLOCK GASTRIC AFFERENT SIGNALS. J.C. Graham*, A.M. Schreihofer, E.M. Stricker, and A.F. Sved. Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Disruption of vagal afferent transmission from the gut either by gastric vagotomy or capsaicin treatment is known to block oxytocin (OT) release and the inhibition of food intake elicited by cholecystokinin (CCK) injection in rats. The present experiments examined whether these responses would be blocked by lesions of the nucleus tractus solitarius (NTS), the brain stem site to which vagal afferent nerves project. At least 10 days after placement of bilateral electrolytic lesions in the NTS, rats were deprived of food overnight and then were injected with isotonic saline (5ml/kg) or CCK (10μg/kg). Five min later, they were given access to pelleted rat chow for 30 min. Intact rats (n=10) given CCK decreased their food intake by 56±4% of control values, whereas in NTS-lesioned rats (n=10) food intake was decreased only by 16±6%. Similarly, CCK-evoked OT release was markedly blunted in NTS-lesioned rats. Together, these results indicate that CCK-sensitive gastric signals are disrupted by NTS lesions in rats. One possible role of these afferents is to signal gastric filling during an ingestive episode, in which case rats with NTS lesions may not inhibit ongoing ingestion appropriately. To test this hypothesis, rats were maintained on a schedule of 30 min access to water per day and then were given a novel, sweet-tasting solution (10% pancake syrup) to drink in place of water. Control rats drank 25±1 ml whereas NTS-lesioned rats consumed significantly more fluid (43±4 ml). Collectively, these results suggest that NTS lesions disrupt afferent signals related to gastric distention that mediate food intake and neurohypophyseal secretion. (Supported by NIH grants HL-38786 and MH-25140, and by a fellowship to JCG from the American Heart Association, Pennsylvania Affiliate.)

556.17

cFOS ACTIVATION OF CATECHOLAMINERGIC NEURONS IN THE DORSAL VAGAL COMPLEX AFTER LiCl ADMINISTRATION IN RATS. R.C. Brown, E.A. Baker, L. Rinaman, E.M. Stricker, G.E. Hoffman, and J.G. Verbalis*. Interdisciplinary Program in Neuroscience and Department of Medicine, Georgetown University, Washington, DC 20007, and Departments of Neuroscience and Neurobiology, University of Pittsburgh, Pittsburgh, PA 15260.

Expression of the immediate early gene product cFos in catecholaminergic neurons was evaluated in the medulla and hypothalamus of rats using double immunocytochemical labeling for cFos and tyrosine hydroxylase (TH) following administration of LiCl (3.0 mmol/kg) given ip as an isotonic (150 mM) solution. One hour after LiCl administration, 61±2% of TH-positive cells in the nucleus of the solitary tract (NTS) were activated as reflected by cFos expression versus 13±4% in controls given an equal volume of 150 mM NaCl ($p<0.01$). Similarly, in the ventrolateral medulla (VLM) 56±4% of TH-positive cells were activated to express cFos versus 17±6% in the controls ($p<0.01$). The results in the NTS are analogous to those of previous studies from our laboratories using CCK and food-induced gastric distension, while those in the VLM resemble those produced by CCK but not gastric distension. These data demonstrate relatively similar phenotypic and anatomical profiles of cells in the dorsal vagal complex that are activated to express cFos by LiCl, gastric distension, and CCK administration. Our results therefore suggest that both LiCl and CCK administration may mimic some of the anorexigenic effects of a large gastric distension by activating a common subset of catecholaminergic neurons in the NTS (supported by NIH grants NS28744 and MH25140).

556.18

AREA POSTREMA AND MEDIAL NUCLEUS OF THE SOLITARY TRACT LESIONS ATTENUATE THE EFFECTS OF MAMMALIAN BOMBESIN-LIKE PEPTIDE ON FEEDING. F.W. Flynn*. Dept. of Psychology and Graduate Neuroscience Program, Univ. of Wyoming, Laramie, WY. 82071

Caudal brainstem systems play an important role in mediating the effects of bombesin (BN) on feeding. Within this area, lesions of the area postrema and adjacent nucleus of the solitary tract (AP/cmNST) attenuate the ability of BN to inhibit feeding. BN binds to the gastrin releasing peptide (GRP) and neuromedin B (NMB)-preferring receptors, both of which are found in the AP/cmNST region and would be damaged by lesions. In the following experiments the effects of AP/cmNST and medial NST lesions, on the ability of BN, GRP, and NMB to suppress feeding was tested. Controls, and rats with either AP/cmNST or medial NST lesions were administered 4, 8, or 16 μg/kg BN, GRP, or NMB and milk intake by non deprived rats, and pellet chow by food deprived rats were measured. Injections of BN and GRP reliably suppressed milk intake in control rats. GRP had no effect on pellet intake in any of the food deprived groups. None of the doses of GRP and only the highest dose of BN suppressed milk intake in rats with AP/cmNST lesions. Medial NST damage produced deficits like those following AP/cmNST lesions and blocked the ability of GRP to suppress milk intake and prevented all but the highest dose of BN to suppress milk intake. None of the doses of NMB tested (up to 128 μg/kg) suppressed milk intake in control rats. The results suggest that damage to GRP-preferring receptors is largely responsible for the inability of BN and GRP to suppress feeding following AP/cmNST and medial NST lesions. (Supported by NIH NS24879 to F.W.F.)

556.19

ABLATING THE AREA POSTREMA (AP) ATTENUATES THE DECREASE IN FOOD INTAKE PRODUCED BY 5-CARBOXAMIDOTRYPTAMINE (5-CT) BUT NOT BY α-METHYL-5-HYDROXYTRYPTAMINE (α-ME-5-HT). K. J. Simansky* and V. Adipudi. Department of Pharmacology, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19129 USA

Peripherally administered 5-hydroxytryptamine (5-HT) reduces food intake by two mechanisms in rats. One involves the 5-HT_1-like class of serotonergic receptor, for which the 5-HT analog, 5-CT, is an agonist probe; the other mechanism is mediated by 5-HT_2-like receptors, which are probed by α-Me-5-HT. Recently, we reported that lesions in the area postrema (APX) blunted the action of high but not low doses of peripheral 5-HT to reduce food intake (Adipudi and Simansky, AJP, 1995, 269:1314-1320). As a follow-up, we tested the actions of 5-CT (vehicle, 0.015, 0.03, 0.06, 0.12 and 0.24 μmol/kg, i.p.) and α-Me-5-HT (2.0 and 5.0 μmol/kg, i.p.) to decrease 30-min intake of sweetened mash 4 months after APX. All doses of 5-CT larger than 0.015 μmol/kg reduced intakes in controls (APC); only the highest dose did so in the lesioned rats. Both doses of α-Me-5-HT decreased intake in control and lesioned rats; the groups did not differ. These data demonstrate that the AP, or areas modulated by connections with the AP, mediate inhibitory effects of peripheral 5-HT_1-like stimulation on feeding. In contrast, peripheral 5-HT_2-like stimulation does not recruit AP-related activity during satiation. Supported by MH 41987 to KJS.

556.20

DOES AREA POSTREMA ABLATION (APX) AFFECT ACQUISITION OF CONDITIONED FLAVOR PREFERENCE? E. M. Knight, J. D. German and N. J. Kenney* Dept. of Psychology, University of Washington, Seattle, WA 98195.

Hungry rats develop preferences for cue flavors that are associated with the postingestive actions of nutrients during conditioning. It is known that vagal afferents from the digestive tract terminate within the area postrema and subjacent caudal-medial aspect of the nucleus of the solitary tract (AP/cmNTS) and that this brain region is rich in receptors for hormonally-mediated satiety signals originating in the gut. Further, ingestion of a CS+ for a conditioned taste preference elicits Fos expression in this hindbrain region (Emond et al., SSIB. 1995). Taken together, this evidence suggests that the AP/cmNTS may play a role in the learning or display of conditioned flavor preferences.

The current study assessed the role of the AP/cmNTS in the acquisition of conditioned flavor preference. Days 30-39 post-surgery, AP/cmNTS-lesioned (APX) and sham-lesioned (SHMX) rats were trained to drink a 0.2% saccharin solution for 30 min/day and chow-restricted to 85% their free-fed post-surgery weight. Rats then received the saccharin solution mixed with either one cue flavor plus 16% Polycose (CS+US) or with another cue flavor with no Polycose (CS-) on alternate days over a 4-day training cycle. On each of the following two days, rats were offered both the CS+ and CS- flavors in a 15-min, 2-bottle choice test. Data for the two test days did not differ and were averaged. SHMX rats consumed 77.3±6.1% of their total test-day intake from the CS+ flavor. APX rats showed a nearly-identical preference for the CS+ flavor (76.1±6.0%). These data suggest that the AP/cmNTS region is not necessary for the acquisition of polysaccharide-based conditioned flavor preferences using a simultaneous conditioning procedure.

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR V

557.1

Estrogen regulation of mu- and delta- but not kappa-opioid receptor messenger RNA in the forebrain of female rodents. **Vanya Quiñones-Jenab*, Shirzad Jenab, Sonoko Ogawa, Charles E. Inturrisi, and Donald W. Pfaff.** The Rockefeller University and Cornell University Medical College, NY, NY, 10021.

Previous studies have suggested that opioids play a role in the regulation of hormone-dependent reproductive behaviors in the female rat. The present study examined if estrogen treatment alters the μ-opioid receptor mRNA levels in certain areas of the forebrain of ovariectomized (OVX) female rats and the δ-and κ-opioid receptors mRNA levels in the forebrain of OVX female mice using the *in situ* hybridization technique. We observed an increase in the μ-opioid receptor mRNA levels in the ventromedial nucleus of the hypothalamus (VMH) and arcuate nucleus (ARN) after 48 hr of 10 μg of 17-β-estradiol-3-benzoate (EB) treatment, when compared to OVX rats. No effects of estrogen were observed on μ-opioid receptor mRNA levels in the posterior medial nucleus of the amygdala (MeAmyg), hippocampus, and caudate-putamen (CPu). In OVX mice, 48hr-EB treatment increased the δ- but not κ-opioid receptor mRNA levels in the VMH, MeAmyg, and ARN, but not in the CPu . Our results suggest that the estrogenic regulation of μ- and δ-opioid receptor in the CNS may in part be mediated by *de novo* synthesis of the receptors' mRNA and/or stability of the messages. It is provocative to postulate that the regulation of opioid receptors' mRNA levels may be an important point in the regulation of opioid sensitivity during reproductive behaviors and/or pain control in the rodent CNS(supported by NIH Grant HO05751; NIDA: DA05130, DA00198, DA01457).

557.2

THE EFFECTS OF TESTOSTERONE ON TYROSINE HYDROXYLASE IMMUNOREACTIVE CELLS IN THE CNS OF MALE LEOPARD FROGS, *RANA PIPIENS*. J. Chu* and W. Wilczynski. Dept. of Psychology, University of Texas, Austin, TX 78712.

While gonadal steroids are known to control reproduction in vertebrates, and dopaminergic activity is believed to be one of the major neurochemical systems modulating sexual behavior in several taxa, little is known as to how these systems may act upon each other. Tyrosine hydroxylase (TH) is the rate limiting enzyme for the production of dopamine and other catecholamines in the CNS. We investigated how long term testosterone treatment influenced TH immunoreactivity (THir) in cells in the diencephalon of male frogs. Previous neuroanatomical studies indicate that the preoptic area (POA), suprachiasmatic nucleus (SCN), and dorsal and ventral hypothalamus (HYP) are areas that show strong THir labeling. These areas are also believed to be dopaminergic in nature. Adult male leopard frogs were gonadectomized and implanted with either a testosterone filled (N=7) or blank capsule (N=9). After 4 weeks, brains were removed and simultaneously processed for whole mount immunohistochemistry. All THir cells in the POA, SCN, HYP were counted and compared across treatments. Analysis of variance showed T implanted males had significantly more THir cell bodies in all three brain areas compared to castrates ($p < 0.01$). Planned comparisons showed treatment effect was significant for each brain region: POA ($p<0.02$), SCN ($p<0.05$), HYP ($p<0.02$). Given that the POA and HYP have been implicated as areas directly controlling reproductive behavior, and the SCN controls circadian rhythms, these results give indirect evidence that steroid induced TH expression may increase the production of dopamine in these areas and thus play a role in modulating sexual and seasonal behaviors. Supported by NIMH T32MH18837 to J.C., and NIMH RO1 MH45696 to W.W.

557.3

PROJECTIONS FROM FOREBRAIN AND MIDBRAIN AREAS TO THE MEDIAN EMINENCE AND NEUROHYPOPHYSIS IN THE FROG *RANA PIPIENS*. S. Burmeister* and W. Wilczynski. Department of Psychology and Institute for Neuroscience, Univ. of Texas, Austin, TX 78712.

Previous studies utilizing immunocytochemistry have identified several populations of cells that contain neuromodulators that are important in controlling reproductive behavior, such as arginine vasotocin (AVT), gonadotropin releasing hormone (GnRH), as well as tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine synthesis. We injected HRP into the caudal-most part of the hypothalamus at the median eminence and neurohypophysis of male frogs in order to determine which such populations of cells project to this area and therefore, may be neurosecretory. HRP filled cells were seen in the anterior preoptic area (aPOA), the magnocellular population of the preoptic area (mgPOA), the suprachiasmatic nucleus (SCN), the dorsal hypothalamus (DH), and more caudally in the ventral tegmental reticular formation. Fibers were seen in the POA, medially ventral to the hypothalamus throughout its extent, as well as in the dorsal and ventral hypothalamus. HRP cells in the DH and POA are similar to populations of TH containing cells, and HRP cells in the mgPOA may be similar to AVT containing cell populations. With these most caudal injections, no cells were seen rostral to the aPOA in regions known to contain LHRH-GnRH in frogs, such as the septal region, or AVT populations in the ventral striatum-accumbens and amygdalar areas. However, the ventral tegmental cells may be similar to chicken II GnRH containing cells. Supported by NIMH grant R01 MH 45696.

557.4

EFFECTS OF CASTRATION AND HORMONE REPLACEMENT ON NITRIC OXIDE SYNTHASE IN THE MEDIAL PREOPTIC AREA OF MALE RATS. J. Du, L. A. Lumley and E. M. Hull*. Psychology Department, State University of New York at Buffalo, Amherst, NY 14260.

We have shown that the nitric oxide (NO) precursor, L-arginine, enhances dopamine (DA) release in the MPOA and that this enhancement is blocked by a NOS inhibitor (Lorrain & Hull, 1993). The NOS inhibitor also blocked DA release during copulation (Lorrain et al., in preparation), suggesting NO may be the permissive or enhancing factor for DA release in response to a female. Ovariectomy dramatically decreases NOS in female rats' MPOA (Okamura et al., 1994), and castration decreases apparent NOS staining in the penis of male rats (Baba et al., 1994).

Since NADPH diaphorase histochemistry (NADPH-d) is the most used marker for NOS in the brain (Fang et al., 1994; Matsumoto et al., 1993; Wolf et al., 1992), we examined effects of castration and hormone replacement on NADPH-d in the MPOA of male rats. Two weeks following castration, there was no significant difference in NADPH-d stained neurons in the MPOA among castrated, sham castrated and hormone replaced rats. However, there were fewer NADPH-d stained neurons in the MPOA in one month castrates, compared to sham castrated and hormone replaced rats. Furthermore, no significant difference of NADPH-d activity was found in the paraventricular nuclei of castrated, sham castrated and hormone replaced rats.

We have shown that DA is released in the MPOA of male rats before and during copulation, and is critical for copulation, genital reflexes and sexual motivation. Recent testosterone is permissive for DA release and copulation. Findings of this study suggest that the permissive effects of gonadal hormones on copulatory behavior may be achieved in part by regulating the activity of NOS in the MPOA. NO may be one factor controlled by gonadal hormones that permits or enhances DA release in response to the estrous female. (Supported by NIMH grant, NH-40826 to EMH)

557.5

OVEREXPRESSION OF *APLYSIA* BAG CELL AND ATRIAL GLAND EGG-LAYING HORMONE PRECURSORS. A. Kurosky, E.L. Gorham, Z.-C. Yang, and J.E. Blankenship*. Depts. of Human Biological Chemistry and Genetics and of Anatomy and Neurosciences, Univ. Texas Medical Branch, Galveston, TX 77555.

The egg-laying hormone (ELH) of *Aplysia* is a 36-residue neuropeptide synthesized within a precursor (preproELH) of 271 residues (region 206-241) in the bag cells. A related family of genes express an ELH-related precursor of 173 residues (preproA and preproB) in the atrial gland, an exocrine organ. We have expressed all three precursors including proELH, proA-ELH, and proB-ELH in a pRSET-related bacterial expression system. The prohormones were purified to homogeneity by HPLC. The proteins were characterized by compositional analysis, sequence analysis, and electrospray mass spectrometry. Results from protein analyses were in exact agreement with the predicted sequences obtained from nucleotide sequence analysis of the cDNA clones. Injection of 1 to 10 nmol of proELH into mature *Aplysia* did not cause egg-laying. ProELH injection was followed by ELH injection (5 nmol) as a control, and in all cases (n=8) egg-laying ensued. Processing of proELH with small aliquots of bag cell extracts demonstrated a predominant cleavage at the tetrabasic site RRKR[184] and also yielded free ELH. Strikingly, the processing of proELH with atrial gland extracts showed a different pattern of processing when compared with processing by bag cell extracts. (Supported by NIH grant NS 29261).

557.6

GONADOTROPIN RELEASING HORMONE MODULATES EXCITABILITY OF SALAMANDER OLFACTORY RECEPTOR NEURONS. H.L. Eisthen*, R.J. Delay, and V.E. Dionne. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

The bipolar cells of the terminal nerve extend their fibers into the nasal epithelium and hypothalamic/preoptic area, and contain gonadotropin releasing hormone (GnRH). Although the terminal nerve has generally been considered to serve a sensory function, recent evidence indicates that it may play a role in modulating neuronal activity. In teleost fishes, a branch of the terminal nerve projects to the retina, and the excitability of goldfish retinal ganglion cells has been shown to be modulated by GnRH and other terminal nerve-derived compounds. Although the olfactory and vomeronasal epithelia contain receptors for GnRH, the physiological effect of GnRH on these sensory neurons has received little attention. We are investigating the possibility that excitability of olfactory receptor neurons in an aquatic salamander, the mudpuppy *Necturus maculosus*, is modulated by mammalian GnRH (mGnRH), the form of GnRH that is present in the terminal nerve of amphibians. Using whole-cell patch recordings from olfactory epithelial slices, we find that a substantial increase in the magnitude of the inward, voltage-activated Na^+ current is observed within 10 min of application of 10 μM mGnRH. The magnitude of the Na^+ current continues to increase for at least 30 min in mGnRH, and the effect is reversible. Under different recording conditions, we have observed that application of mGnRH causes a reversible shift in activation of an outward current. Substitution of Cs^+ for K^+ in the intracellular solution eliminates this effect, indicating that GnRH may affect a K^+ current. The activation profile indicates that this current may be Ca^{2+} dependent. We are presently conducting experiments to determine whether mGnRH affects this current directly, or through an indirect effect on Ca^{2+} conductances. Taken together, these data indicate that GnRH from the terminal nerve modulates excitability of salamander olfactory receptor neurons.

Supported by NIH DC02879.

557.7

RECIPROCAL CONNECTIONS BETWEEN NEURONS IN THE MEDIAL AMYGDALA OF THE MALE SYRIAN HAMSTER THAT PROCESS CHEMOSENSORY OR HORMONAL SIGNALS. L.M. Coolen* and R.I. Wood Dept OB/GYN, Yale Univ Med School, Box 208063, New Haven, CT 06520.

In the male Syrian hamster, mating is dependent on chemosensory and hormonal stimuli and interruption of either input prevents copulation. The medial amygdaloid nucleus (Me) is a key nodal point in the neural circuitry controlling male sexual behavior because it relays both odor and steroid cues. Me is comprised of two major subdivisions. The anterior subdivision (MeA) receives the majority of chemosensory input from the olfactory bulbs, whereas the posterior subdivision (MeP) contains the majority of steroid receptor-containing neurons. The aim of the present study was to elaborate on the connections linking the two subdivisions of Me. Specifically we tested the hypothesis that reciprocal connections exist between steroid-responsive neurons in the MeP and neurons in the MeA that relay chemosensory information.

To test this hypothesis anterograde (Pha-L) and retrograde (Fluoro-gold; FG) tract tracers were injected in Me through a single glass micropipette by iontophoresis. Following injections into MeA, sections were stained for Pha-L, FG and androgen receptor-immunoreactivity (AR-IR), to determine if steroid-responsive neurons in MeP have reciprocal connections with MeA. For injections in MeP, sections were stained for Pha-L, FG and Fos-IR induced by chemosensory stimulation, to identify neurons activated by chemosensory cues which are connected with MeP. Following tracer injections in MeA, AR-IR neurons in MeP retrogradely labelled with FG were in close apposition to fibers and terminals labelled with Pha-L. Moreover, neurons expressing Fos in MeA after chemosensory stimulation projected and appeared to receive projections from the injection site in MeP.

These results demonstrate reciprocal connections between neurons in the chemosensory and the hormonal circuits within Me. They provide anatomical evidence for neural coordination of chemosensory and hormonal cues in the Syrian hamster brain. (Supported by NIH HD-32669).

557.8

INCREASED STAINING FOR NPY IN THE ARCUATE NUCLEUS OF FOOD RESTRICTED, LACTATING RATS. PERSISTS AFTER REFEEDING A. Abizaid*[1], C.-D. Walker[2], and B.C. Woodside[1]. [1]Center for Studies in Behavioural Neurobiology, Concordia University and [2]Douglas Hospital Research Center, McGill University, Montreal, Canada.

In rats, food restriction from days 8-14 of lactation prolongs lactational diestrus . Neuropeptide-Y (NPY) may play an important role in the central mechanisms prolonging lactational acyclicity since it is known that NPY inhibits LH release in the absence of steroid hormones. Using immunocytochemistry we determined whether 1) food restricted (FR) lactating rats would show an increased number of cells in the ARC showing dense staining for NPY at the end of the food restriction period and 2) if so, how long this effect would persist after refeeding. On Day 2 pp all rats were implanted with cannulae in the right lateral ventricle. Rats were sacrificed on either Day 15 or Day 20 pp, 24hrs after colchicine treatment. To determine whether any differences in NPY staining reflected an influence of nursing underfed pups, in other groups of dams, litters were switched daily between ad lib fed (AL) and FR females between Days15 and 20 pp. After perfusion with saline and paraformaldehyde brains were processed for NPY immunocytochemistry. More cells stained for NPY in FR than AL rats on Day 15 and Day 20 pp($p < .05$) in both switched and unswitched litter conditions. These findings suggest that cell bodies within the ARC of food restricted lactating rats continue to show evidence of high levels of NPY five days after the food restriction is terminated. Elevated levels of NPY may play a role in inhibiting reproductive function in food restricted lactating rats.(Supported by Grant #MT 10158 from MRC to B.W.)

557.9

EFFECT OF CHRONIC ICV INFUSION OF NPY IN LACTATING AND NONLACTATING RATS. B. Woodside* and C. Beaulé . Center for Studies in Behavioral Neurobiology, and Department of Psychology Concordia University, Montréal, Canada.

Neuropeptide Y (NPY) has been implicated in the control of both food intake and the hypothalamic-pituitary-gonadal axis and chronic NPY infusions have been shown to suppress cyclicity in female rats. We investigated the possibility that the increased levels of NPY observed in food restricted lactating rats might contribute to their prolonged period of lactational diestrus by determining the effects of chronic icv NPY infusions on the length of lactational diestrus in ad lib fed lactating rats. NPY or vehicle was infused into the right lateral ventricle via an indwelling cannula attached to an osmotic minipump from Day 12 to Day 19 postpartum at a daily dose of NPY of 14.4μg/rat. To compare the effects of NPY infusions between lactating and cycling animals, virgin females received similar treatments. Consistent with previous reports, NPY infusion resulted in an increase in food intake and bodyweight in virgin females together with a suppression of vaginal cyclicity. Lactating females receiving NPY infusions showed a shorter period of lactational diestrus than vehicle-treated controls. NPY infusions also caused a suppression of milk production and/or letdown as reflected in a reduction in litter growth. It is possible that both of these effects result from the ability of NPY to suppress prolactin release . (Supported by MRC Grant #MA10158 to B.W.)

557.10

STRESS UP-REGULATES PREPROENKEPHALIN (PPE) mRNA LEVELS IN THE LIMBIC SYSTEM AND HYPOTHALAMUS OF THE FEMALE RAT. **C. B. Eckersell*, P. Popper AND P. E Micevych** Dept. of Neurobiology and Lab. of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90095.

Estrogen injection causes an early rise in PPE gene expression (peak at 1 hour) in the ventromedial hypothalamic nucleus (VMH) and the posterior dorsal medial amygdala (MeApd) followed by a nadir at 4 hours and a second rise in PPE mRNA which reaches maximal levels at 24 hours. Acute stress has also been shown to increase enkephalin gene expression in the hypothalamus. To investigate the source of PPE mRNA induction in the VMH and MeApd, ovariectomized female rats were given estradiol benzoate (EB; 50 μg in 0.2 ml safflower oil) or the estrogen receptor blocker tamoxifen (TMX; 20 mm Silastic capsule) followed by either an injection of EB or safflower oil vehicle. Animals were sacrificed at the time of injection or 1, 4 or 24 hours later. Brain sections were processed by in situ hybridization for PPE mRNA. Serum glucocorticoid levels were higher at 1 hour in both oil and EB injection groups than at any other time point. EB alone induced a significant increase in PPE mRNA levels in the VMH and MeApd at 1 and 24 hours. In the oil and EB plus TMX treated animals PPE mRNA levels were elevated at the 1 hour time point only. These findings indicate that early increases observed in PPE mRNA levels within the VMH and MeApd may be due to stress which increases glucocorticoid levels rather the direct effect of estrogen. Since it was blocked by TMX, the 24 hour peak in PPE mRNA levels appears to be induced by EB. Supported by NS-21220 and HD-07228.

557.11

ACUTE ESTROGEN STIMULATES CHOLECYSTOKININ (CCK) mRNA EXPRESSION IN THE LIMBIC-HYPOTHALAMIC CIRCUIT OF THE FEMALE RAT. **P. E Micevych*, C. B. Eckersell and K. Sarrafzadeh.** Department of Neurobiology and Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90095.

Reproductive behavior in the female rat has been correlated with the estrogenic regulation of CCK in the hypothalamus and the limbic system. Estrogen induces an increase of CCK mRNA in the central part of the medial preoptic nucleus (MPNc) principal portion of the bed nucleus of the stria terminalis (BSTp) and the posterodorsal medial amygdala (MeApd). To determine if a brief exposure to estrogen was sufficient to elevate CCK mRNA levels, ovariectomized rats were treated with nothing; estradiol benzoate (EB; 50 μg in 0.2 ml safflower oil injected s.c.); EB preceded by the antiestrogen TMX (20 mm Silastic capsule implanted s.c.) 48 hours prior to EB injection; or EB followed by TMX (20 mm Silastic capsule and 300μg in 0.15 ml safflower oil injected s.c.) 2 hours after EB. All animals were sacrificed 24 hours after EB injection and tissue was processed for CCK mRNA *in situ* hybridization histochemistry. Estrogen treatment caused a significant increase in the CCK mRNA levels within the MPNc, MeApd and BSTp as previously reported. This increase was blocked by TMX administration prior to estrogen treatment but was not blocked by TMX treatment 2 hours after estrogen administration. These findings indicate that continuous estrogen exposure is not necessary to stimulate CCK mRNA in the MPNc, BSTp and MeApd. A brief exposure to estrogen initiates a cascade of events that up-regulate CCK mRNA expression and Supported by grant NS-21220 and HD-07228.

557.12

MEDIAL PREOPTIC AREA LESIONS DO NOT INCREASE HYPOTHALAMIC ESTROGEN RECEPTOR-IMMUNOREACTIVITY IN JUVENILE FEMALE GUINEA PIGS. Deborah H. Olster*, Psychology Department, University of California, Santa Barbara, CA 93106.

Ovariectomized (OVX), juvenile guinea pigs rarely display lordosis following injections of estradiol and progesterone that are behaviorally effective in adults. A previous study has documented a smaller population of estrogen receptor-immunoreactive (ER-IR) cells in the rostral, ventrolateral hypothalamus (VLH) of OVX juvenile, as compared to adult guinea pigs (Olster, 1994). Electrolytic lesions of the medial preoptic area (MPOA) accelerate the maturation of adult-typical lordosis responses to estradiol and progesterone in OVX guinea pigs (Olster, 1995). This study was designed to test the hypothesis that MPOA lesions result in enhanced behavioral responsiveness to estradiol and progesterone in juvenile females, at least in part, by increasing the concentration of ERs in the VLH. Hartley guinea pigs were OVX at 10-11 days of age and received bilateral, electrolytic (1.5 mAMP X 10 sec) or sham lesions aimed at the MPOA 4 days later. At 21 days of age, all animals were transcardially perfused and hypothalamic tissue was processed for ER-immunostaining, using Abbott Laboratories' H222 anti-ER antibody. No significant differences in the number or staining intensity of ER-IR cells in the paraventricular nucleus, arcuate nucleus or VLH were observed in MPOA-lesioned vs. sham-lesioned animals. These data do not support the hypothesis that MPOA lesions enhance behavioral responsiveness to estradiol and progesterone in prepubertal guinea pigs by increasing the concentrations of ERs in the VLH; the facilitation of sexual receptivity following lesion of the MPOA appears to result from other, as yet unknown, mechanisms.

Supported by NIH HD 28636.

557.13

ESTROGEN INDUCES AXONAL OUTGROWTH IN THE NUCLEUS RETROAMBIGUUS-LUMBOSACRAL MOTONEURONAL PATHWAY IN THE ADULT FEMALE CAT. V.G.J.M. VanderHorst* and G. Holstege. Department of Anatomy, University of Groningen, The Netherlands.

Recently, a motor pathway has been discovered in the cat which has been postulated to form the final pathway for male mounting (VanderHorst and Holstege, 1996) and female receptive or lordosis behavior (VanderHorst and Holstege, 1995). The pathway originates from the nucleus retroambiguus (NRA), a compact group of interneurons in the caudal medulla, and terminates directly in distinct lumbosacral motoneuronal cellgroups. The question is whether in female cats estrogen has an effect on this NRA-lumbosacral pathway, because only females in estrous display lordosis behavior.

Light microscopically, the effect of estrogen on the NRA-lumbosacral and the rubrospinal tracts was studied in 20 cats using wheat germ agglutinin-horseradish peroxidase (WGA-HRP) as a tracer. The rubrospinal pathway served as control. The density of labeled NRA fibers in their target motoneuronal cell groups appeared abundant in estrous and very weak in non-estrous cats, while such differences were not found in the rubrospinal pathway. Electromicroscopical examination of the NRA-semimembranosus motoneuronal projection in 8 cats revealed that labeled terminals in estrous cats were larger and were almost 10 times as numerous than in non-estrous cats. Moreover, in the semimembranosus motoneuronal cell group WGA-HRP labeled **growth cones** were found in estrous but not in non-estrous cats. Such growth cones appeared to contain growth associated protein (GAP-43). These observations demonstrate that estrogen induces axonal outgrowth of NRA fibers to distinct somatic motoneuronal cell groups, which lends further support to the hypothesis that the NRA is involved in lordosis behavior. The possible mechanisms underlying this outgrowth are discussed.

557.14

CLONING OF MULTIPLE GENES FOR GONADOTROPIN RELEASING HORMONE (GnRH) IN A SINGLE SPECIES. Richard B. White and R. D. Fernald*. Program in Neuroscience, Stanford University, Stanford, CA 94305-2130.

Recent experiments suggest that multiple GnRH peptides and thus multiple GnRH genes exist in all vertebrate classes. The African cichlid fish *Haplochromis burtoni* is a unique model for understanding possible functions of alternate GnRHs because it expresses three different forms, each in a distinct brain region. As a first step in studying the possible coordinate regulation of the genes encoding GnRH, we have isolated genomic clones by screening a *H. burtoni* genomic library using DNA probes transcribed from the three distinct GnRH cDNAs previously isolated from this species (White et al. 1995). The clones were then used for Southern blot analysis, which revealed that each gene is present as a single copy. Upstream regulatory sequences of the genes were compared to existing data from salmon, chicken, rat, mouse, and human GnRH genes. The complete sequences of multiple GnRH genes in a single species should provide key information for decoding the phylogenetic relationships between the various GnRH forms that exist in vertebrates.

Supported by a Hitchings-Elion Fellowship to R.B.W. and NIH HD 23799 to R.D.F.

557.15

THE ROLE OF THE CORTICO-MEDIAL AMYGDALA IN HAMSTER SOCIAL ODOR RECOGNITION. A. Petrulis*, M. Schiller, M. Peng and R. E. Johnston. Department of Psychology, Cornell University, NY 14853.

Hamsters, like many mammals, are capable of discriminating a conspecific's sex, species and individual identity on the basis of their odors. Although these abilities appear to be common-place, the neural mechanisms underlying individual discrimination or other forms of recognition have not been well characterized. To investigate this topic further, we have focused on the vomeronasal organ and its projections to the medial and cortical amygdala as a possible neuroanatomical substrate for individual as well as other types of social odor discrimination. Males with complete removal of the vomeronasal organ did not discriminate between individual scents (using a habituation paradigm) on the basis of three of the four odors used. Sham males were able to discriminate using all four odors; males with partial vomeronasal organ damage showed intermediate results. Thus, the vomeronasal organ plays an important but not exclusive role in individual odor discrimination. In contrast, females with lesions to the cortico-medial amygdala were able to individually discriminate two of the same odors used with males. However, females showed impairments in sex odor discrimination / attraction and had decreased levels of scent marking. This apparent discrepancy in individual discrimination ability after lesions may be a result of a true sex difference in vomeronasal usage or may indicate that other vomeronasal system target structures are involved. This research was funded by NIH and NSF grants to R.E.J and by a NRSA to A.P.

558.1

MOLECULAR CHARACTERIZATION OF A NOVEL EPILEPSY GENE. G.P. Donovan,* L. Ho, and M. Toth. Department of Pharmacology, Cornell University Medical College, New York, NY 10021.

We have identified a mouse gene named *jerky* that, when disrupted by insertional mutation, causes epileptic seizures. Using a multifaceted approach, we examined the function of *jerky* and how its deletion can lead to epileptic seizures. First, we studied the spatial distribution of *jerky* in order to correlate the site of its expression with the seizure focus. We have utilized quantitative RT/PCR and *in situ* hybridization to achieve this. While *jerky* is predominantly expressed in the brain, it can be detected in many other organs. However, the highest expression within the brain was found in the neocortex and especially the hippocampus, which correlates well with the site of seizure initiation. As a secondary approach, we studied the developmental expression of *jerky* and found that expression is highest at postnatal day 5, corresponding to the formation of neuronal synapses. Therefore, *jerky* could play a critical role in maintaining proper neuronal function and suppressing hyperexcitibility. Finally, we examined the molecular functions that jerky protein plays in neurons. Affinity chromatography studies have shown the jerky protein preferentially binds single stranded DNA. In order to delineate the specific sequence(s) to which the jerky protein binds, we have used PCR-based site selection to identify a series of specific single stranded sequences. These sequences were then verified to bind jerky protein by testing in electrophoretic mobility shift assays. Taken together, we suggest that expression of *jerky* is involved in the development and maintenance of neuronal function, especially in the hippocampus, by binding single stranded DNA. Presently, we are focused on the identification of cellular processes (such as DNA replication, repair, and recombination) that may be linked to *jerky* function. Supported by NIH RO1NS34151-01A1.

558.2

TRANSPOSON *MARINER* IN THE HUMAN BRAIN: ITS POSSIBLE IMPLICATION IN HUMAN EPILEPTOGENESIS. H. Xie*, M.L. Brines, and N.C. de Lanerolle, Sections of Neurosurgery and Neuroendocrine Program, Yale Univ. Sch. of Med., New Haven, CT.06520

mRNA differential display methodology was used to identify genes which are differentially expressed in epileptogenic tissue. We applied this methodology to human hippocampal tissue surgically removed from temporal lobe epilepsy patients and normal autopsy controls. Among the many differentially displayed bands, one band of 320 base pairs was found to be 70% similar in its DNA sequence to the invertebrate transposon *mariner*. The *mariner* was first discovered in *Drosophila mauritiana* due to its ability to mutate the eye color gene. Conservative estimates suggest that transposons are responsible for 0.5-0.1% of mutations within the human genome. We cloned both the genomic copy and the full length mRNA of the human *mariner* gene. This gene has an open reading frame coding for a transposase of 345 amino acids, contains the characteristic 32 base pair inverted terminal repeats (ITR), and the insertion site TA dinucleotide duplication. There are ~60 copies of this gene in the human genome. Northern blot analyses demonstrated an elevated level of *mariner* transcription in human epileptogenic tissue compared to tissue from autopsy controls. Neither the *mariner* gene nor its transcripts were detectable in rat, pig or chicken. At the nucleotide sequence level, *mariner* transposons isolated from human epileptogenic tissue share more than 95% of their nucleotide sequence, while those from normal human genomic DNA share less than 80%. Database search showed that a copy of *mariner* exists in the T cell receptor gene (TCRB) as well as in the Abelson leukemia oncogene intron 1b. Interestingly, one ITR "signature" of the *mariner* transposon is located in the human HLA locus in chromosome 6p21.3, which has previously been linked to juvenile myoclonic epilepsy (JME). Further suggestion that *mariner* may be relevant for neurologic disease is the recent report of its presence within a recombination "hotspot" on chromosome 17p11.2-p12 responsible for Charcot-Marie-Tooth disease type 1A (CMT1A). This study is the first to associate the *mariner* transposon with human epilepsy. [Supported by NS 27081 & 30619 from NIH]

558.3

INDUCTION OF FRA-2 BUT NOT FRA-1 mRNA IN HIPPOCAMPAL NEURONS FOLLOWING EPILEPTIC SEIZURES IN RATS. J. Beer*, M. Zimmermann and T. Herdegen. II. Physiologisches Institut, Universität Heidelberg, 69120 Heidelberg, Germany.

Inducible transcription factors (ITFs) are among the first genes expressed in neurons after stimulation. ITFs are thought to play a specific role in coupling neuronal stimulation by regulating the expression of target genes. Dependent on the stimulus (i.e. axotomy or transsynaptic stimulation), different sets of ITFs are transiently or persistently expressed. We investigated the spatiotemporal expression of fos-related antigens 1 and 2 (fra-1 and fra-2) after epileptic seizures with kainic acid, a model for human temporal lobe epilepsy.

Kainic acid was injected i.p. (10 mg/kg in 0.9% saline) into adult male Sprague-Dawley rats. The expression of fra-1 and fra-2-specific mRNA was investigated by *in situ* hybridization (ISH) after recovery periods of 0.5, 1, 3 and 6 h. Fra-specific oligonucleotides and cRNA were labelled with ^{35}S using terminal deoxynucleotide transferase or the T3/T7 transcription system (Promega, Heidelberg, Germany), respectively. ISH with both types of radiolabelled probes revealed strong expression of fra-2 in the dentate gyrus and moderate expression in the CA-regions of the hippocampus 6 h postinjection. Only minor expression could be seen in the amygdala and piriform cortex. Virtually no expression was detected of fra-1-specific mRNA at all times investigated. fra-1 and fra-2 contain the same regulatory elements in their promoter region but not in analogous positions (Foletta et al. 1994, Oncogene 9:3305-3311). We suggest that this may cause differential regulation and consequently different expression patterns of these ITFs.

This work was supported by the Deutsche Forschungsgemeinschaft.

558.4

INDUCTION OF TRANSCRIPTION FACTORS IN ABSENCE SEIZURE MODEL MICE Y. Ito*, K. Ishige, M. Aizawa, and H. Fukuda. Department of Pharmacology, College of Pharmacy, Nihon University, Funabashi 274, Japan

The cerebral cortical and thalamic circuitry has been shown to play an important role in the genesis and spreading of absence seizures. We have shown that $GABA_B$ receptors play an important role in γ-butyrolactone-induced absence-like seizures (GHB model) and that the increase in nuclear cyclic AMP responsive element (CRE)- and activator protein 1 (AP-1) DNA-binding activities in the cerebral cortex and thalamus is correlated with these seizures. In this study, we compared nuclear CRE- and AP-1 DNA-binding activities in lethargic (*lh/lh*) mice, a genetic model of absence seizures, with those in nonepileptic control mice (+/+). Electroencephalogram recording showed spike and wave discharges spontaneously accompanied by seizure behavior in lethargic mice at 3 weeks of age and older. Nuclear extracts were prepared from various brain regions of age-matched (3 to 6 weeks of age) lethargic and control mice. Gel-shift assay showed that nuclear CRE- and AP-1 DNA-binding activities in the cerebral cortex and thalamus in lethargic mice were higher than those in control mice. In contrast, in the hippocampus, no difference of these DNA-binding activities was observed between lethargic and control mice. These results suggest that the increase in nuclear CRE- and AP-1 DNA-binding activities in the cerebral cortex and thalamus is correlated with absence seizures in both lethargic mice and the GHB model. Supported by Uehara Memorial Foundation.

558.5

ACUTE EFFECTS OF C-FOS ANTISENSE OLIGO-NUCLEOTIDE ON HIPPOCAMPAL PARTIAL SEIZURES ELICITED BY ELECTRICAL STIMULATION IN RATS. H. Katsumori[1,2]*, K. Hashimoto[1], S. Tomitaka[1], N. Narita[1] and Y. Minabe[1]. [1] Div. of Cortical Function Disorder, Natl. Inst. of Neurosci., NCNP, Tokyo, 187, Japan; [2] Dept. of Pediatr., Tokyo Women's Med. Collage, Tokyo, 162, Japan.

To investigate the role of c-fos in the brain during seizures, we examined the effects of phosphorothioate antisense oligodeoxy-nucleotides (ODNs) to c-fos mRNA on hippocampus-generated partial seizure model of epilepsy in Wistar male rats. As control, sense and scrambled control ODNs were also tested. Each ODN (10 nmol/10 μl /2 min) was injected into lateral ventricles for consecutive 2 days. 15 hrs after the last injection, electrical stimulations were delivered to assess the effects of each ODN on the two seizure parameters; seizure threshold and afterdischarge duration (ADD). 1 hr after seizures, c-Fos and Jun-B immunohistochemistries of the brain were also performed. The results were obtained as follows: 1) Antisense ODNs decreased ADD selectively and control ODNs failed to change any parameters at tested doses. 2) In antisense ODNs group, ipsilateral c-Fos expression occurred to the stimulation site of dentate gyrus, while Jun-B expression bilaterally. In the major cases of control ODNs groups, c-Fos and Jun-B expression occurred bilaterally. These results indicate that the injection of antisense ODNs selectively inhibit c-Fos expression and c-fos plays some roles in seizure phenomenon. This research was supported by the research grant from the Ministry of Health and Welfare, Japan.

558.6

SEIZURE-INDUCED EPILEPTOGENESIS AND THE GENERALIZED SEIZURE PHENOTYPE DETERMINE THE PATTERN OF FOS INDUCTION IN MOUSE BRAIN. G.M. Samoriski*, D.T. Piekut and C.D. Applegate. Program in Neuroscience, University of Rochester School of Medicine, Rochester, NY 14642.

We have shown that 8 daily consecutive generalized clonic seizures promote reorganizational processes that require time to develop. The evolution of these processes occurs in the absence of continued seizure induction and is manifest predominantly as a change in the behavioral seizure response. The behavioral shift is significantly altered by 2 wks and is characterized by an increase in the probability that the animal will express a "brainstem" seizure. Because the modified seizure susceptibility is defined by a change in the type of motor convulsion that is expressed, the activation of structures selectively involved in the expression of generalized seizure behaviors may be altered in response to subsequent ictal events. Thus, Fos immunohistochemistry was used to evaluate the degree of neuronal activation on the basis of the seizure phenotype and the seizure history. Generalized seizures were elicited in C57BL/6J mice using flurothyl and classified as either "forebrain" or "brainstem". Fos immunoreactivity was then examined 1.5 hrs following either a single generalized seizure, the last of 8 consecutive daily seizures or a retest seizure evoked 2 wks after the last of 8 seizures. The predominant differences in Fos labeling were related to the type of seizure expressed regardless of the seizure history. Furthermore, the different motor components that make up a "brainstem" seizure could not be distinguished by the pattern of Fos labeling suggesting that multiple convulsive behaviors are mediated by one anatomical system. While Fos induction in the ventromedial (VMH) and paraventricular hypothalamic nuclei and hippocampal dentate gyrus was influenced by the seizure history, changes in the degree of Fos labeling in the VMH most closely paralleled alterations in the behavioral response. These data support the concept that separate anatomical systems mediate the expression of the two generalized seizure phenotypes. Also, the VMH may be a point of interaction between the systems and may play a role in seizure-induced neural reorganization. (Supported by NIH grant NS26865).

558.7

BEHAVIOR AND FOS ACTIVITY IN AUDIOGENIC SEIZURE-PRONE LONG-EVANS RATS WITH TECTAL GRAFTS. K.C. Ross[1*] and J.R. Coleman[1,2]. Depts. of Psychology[1] and Physiology[2], Univ. of South Carolina, Columbia, SC 29208.

The goal of this research is to examine a novel model for audiogenic seizure activity with grafting in Long-Evans (L-E) rats. Previous AGS research has utilized mainly albino rat strains. Subjects (n=8) primed for AGS at PND 14 by exposure to 10 KHz tone bursts for 8 m. at 120 dB p.e. SPL were tested for AGS at PND 24 using 120 dB white noise. Subjects were observed as displaying no seizure activity, wild running only, or wild running followed by clonus.

Subjects were lesioned in the right IC and grafts of whole fetal caudal tecta were implanted in the lesion site following the method of Zrull and Coleman (1991). Subsequent behavioral testing show that the lesion and graft reduced the incidence of clonus, increased observations of no seizure activity, and increased the incidence of wild running alone ($p<.000$). Onset latency before lesion and graft (M=33.95 s, SD=12.57) was significantly shorter than after lesion and graft (M=38.4 s, SD=12.29; $p<.03$). Onset duration before lesion and graft (M=22.16 s, SD=9.01) was significantly longer than after lesion and graft (M=18.65 s, SD=12.21; $p<.05$).

Alternate sections processed for Fos immunochemistry and Nissl stains reveal 14-month graft survival. Host animals display higher stimulus-driven Fos activity in the dorsomedial IC than in other IC sectors and graft.

These data suggest that unilateral lesion and graft alleviate some aspects of seizure activity and identify a focus of Fos expression. The exact role of the graft-host interaction is under further investigation.

(Supported by the Deafness Research Foundation and NSF SBR-00285)

558.8

ATTENUATION OF KINDLING-INDUCED DECREASES IN NT-3 mRNA BY THYROID HORMONE DEPLETION. J.B. Rosen*, S.Y. Kim, M.A. Smith, and R.M. Post. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

The expression of neurotrophins are known to be altered by amygdala kindling. Because thyroid hormone can regulate the transcription of some neurotrophins, we asked whether thyroid hormone regulates neurotrophin mRNA expression following amygdala kindling.

Rat with electrodes in the basolateral amygdala received PTU in their water, thyroidectomy, T3 in their water, thyroidectomy plus T3 in their water or water only. Ten days after the beginning of the treatments half the rats were kindled daily until they reached one stage five seizure. They were sacrificed 4 hours after this seizure and the brains processed for in situ hybridization for NGF, BDNF and NT-3 mRNA.

In non-kindled rats, thyroid hormone manipulations affected BDNF mRNA in the PVN and pituitary only. There were no effects on basal levels of either NGF or NT-3. The thyroid hormone manipulations had no effect on kindling or on kindling-induced BDNF and NGF mRNA expression. However, the kindling-induced decrease in NT-3 mRNA expression in the dentate gyrus granule cell layer was partially blocked by PTU or thyroidectomy. These effects could be reversed by T3 treatment.

The results indicate that thyroid hormone plays a specific but limited role in the seizure-induced changes of NT-3 mRNA expression found in the dentate gyrus. Additionally, because T3 manipulations did not affect basal levels of NT-3 mRNA, other transcription factors must also regulate the basal levels and kindling-induced decreases of NT-3 gene expression in the dentate gyrus. Supported by NIMH.

558.9

LONG-TERM INHIBITION OF CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II ACTIVITY IN A MODEL OF PARTIAL COMPLEX (LIMBIC) EPILEPSY. L.D. Kochan[1*], S.B. Churn[2], A.C. Rice[2] and R.J. DeLorenzo[1,2]. Departments of Pharmacology and Toxicology[1] and Neurology[2], Medical College of Virginia, Richmond, Virginia 23298.

Status epilepticus (SE) is a severe seizure disorder associated with significant morbidity and mortality and can induce spontaneous recurrent seizures (SRSs). The pilocarpine-induced SE model of limbic epilepsy is a major *in vivo* model used to investigate spontaneous recurrent seizure discharges and has many of the clinical and pathological features of limbic epilepsy. Ca^{2+}/Calmodulin-dependent protein kinase II (CaM kinase II) plays an important role in signal transduction. Alteration in the activity of this enzyme has been reported in pathological states including stroke and several models of epileptiform activity. Thus, this study was initiated to investigate changes in CaM kinase II activity associated with persistent plasticity changes, utilizing the well-characterized pilocarpine model of limbic epilepsy. CaM kinase II activity was determined by a measurement of ^{32}P incorporation into α and β-CaM kinase II subunits. We determined that there is a statistically significant reduction in the α(44%) and β (19%) CaM kinase II subunit autophosphorylation, measured in whole brain homogenates, isolated from epileptic compared to sham control animals 4-6 weeks after pilocarpine-induced SE. To evaluate hippocampal CaM kinase II activity, we prepared hippocampal homogenates from control and epileptic animals at 4-6 weeks after pilocarpine-induced SE. Prolonged seizure activity resulted in a long-term decrease in hippocampal α- and β- CaM kinase II activity by 49 and 35%, respectively. These results indicate that long-lasting decreases in CaM kinase II activity are associated with the persistent seizures observed in the pilocarpine model of limbic epilepsy and suggests that long-term decreases in CaM kinase II activity may contribute to the long-lasting plasticity changes in brain that accompany epileptogenesis. This work was supported by RO1-NS23350.

558.10

CONNEXIN 43 mRNA EXPRESSION IN TWO EXPERIMENTAL MODELS OF EPILEPSY. K. Elisevich*, S. Rempel, K. Jenrow and B. Smith. Epilepsy Research Laboratory, Henry Ford Health Sciences Center, Detroit, MI 48202.

The expression of mRNA for connexin 43, a gap junction protein constituent found in astrocytes, was studied in two experimental models of epilepsy, the electrically kindled rat and the tetanus toxin injected rat. Animals were kindled by electrical stimulation of the amygdala to Racine class 5 seizures and divided into cohorts of three to undergo 3, 6, and 10 such events in total, respectively. Two cohorts of rats received injections of tetanus toxin into the amygdala at strengths of 3 and 9 MLD_{50}, respectively. Features of epileptogenicity were identified electrographically in both cohorts during the first 4 weeks following injection with spontaneous ictal events recorded frequently in the latter cohort. All rats were sacrificed 4, 8, or 10 weeks after electrode or cannula implantation and the epileptogenic area in the region of the amygdala was harvested and pooled by cohort for Northern blot analysis. These were compared with control nonimplanted and implanted tissues. At time points of 4, 8 and 10 weeks, connexin 43 mRNA expression in epileptogenic tissues was found to be decreased or unchanged relative to control cases.

Both experimental models of epilepsy therefore show no connexin 43 mRNA upregulation despite varying degrees of epileptogenicity. Increased gap junction assembly as a substrate underlying epileptogenesis or as a reaction to increased neuronal excitability is unlikely to occur.

[Supported by the American Epilepsy Society and the Henry Ford Hospital Research Fund].

558.11

DIFFERENTIAL EXPRESSION OF THE INDUCTABLE 72-kDa HEAT SHOCK PROTEIN FOLLOWING KAINIC ACID-INDUCED SEIZURES IN IMMATURE AND ADULT RATS. U. Branner[1], E.F. Sperber[2], S.L. Moshé*[2] and M-T. Romero[1]. [1]Department of Psychology, State University of New York at Binghamton, Binghamton, NY 13902; [2]Albert Einstein College of Medicine, NYC, NY 10461.

The inductible form of heat shock protein (HSP72) is expressed in various areas of the brain of adult rats following kainic acid (KA) seizures (Gass et al., 1995; Armstrong et al., 1995). The level of expression of HSP72 is correlated with the intensity of the seizures and, to some extent, localized to areas susceptible to seizure-induced neurodegeneration.

We have previously reported that the immature brain (5-25 days) appears to be resistant to damage following KA-induced seizures (Sperber et al., 1991). In the present study we compared the immunocytochemical expression of HSP72 in the adult and immature brain (postnatal day 14) following doses of KA which produce status epilepticus in both groups (15mg/kg and 4mg/kg, respectively).

Preliminary data indicate that HSP72 is extensively induced in the adult brain throughout the hippocampus and other limbic structures following status epilepticus in comparison to adult controls. In contrast, in the immature brain there is little or no expression of HSP72 immunoreactivity following KA-induced seizures or saline injections. These differences between the adult and immature brains are found at 12, 24, and 36 hrs following KA injection. Further studies are necessary to determine whether the immature brain may have an earlier induction of HSP72 which is not detected at the times used in this study. These results further demonstrate the immature rat are resistant to seizure induced damage..

Supported by the Research Foundation of SUNY (MTR) and NIH grant NS-30387 (EFS)

558.12

ABERRANT EXPRESSION OF $GABA_A$ RECEPTOR SUBUNITS IN TOTTERING MICE. S.-C. Liu, B.J. Baumgartner, E.M. Barnes, Jr.,* and M.H. Jalilian Tehrani. Depts. of Biochem. and Mol. Physiol. and Biophys., Baylor Col. of Med., Houston, TX 77030.

The single-locus mutant mouse tottering (*tg/tg*) displays spike-wave seizures that resemble those in human petit-mal epilepsy. The molecular defect responsible for this abnormality in *tg/tg* brain is not known. We have recently reported that in comparison to the isogenic background strain (+/+), $GABA_A$ receptor function is reduced in *tg/tg* mouse brain [Tehrani and Barnes (1995) *Epilepsy Res.* **22**, 13-21]. In order to determine whether these alterations can be defined at the level of receptor isoforms, we have examined the expression of $GABA_A$ subunit mRNAs by quantitative RT-PCR and compared the data with that obtained by radioligand binding. Paired RT-PCR experiments revealed an elevation of α2- and β1-subunit mRNA levels in *tg/tg* cortex, relative to +/+, with no difference in amounts of γ2-subunit transcripts. Biphasic displacement of ^3H-flunitrazepam binding by 2-oxoquazepam showed that low affinity sites account for 36 ± 7.6 % of the total in +/+ cortical membranes, but 51 ± 7.5% in *tg/tg*. This is consistent with evidence that recombinant $GABA_A$ receptors with α2 subunits exhibit low affinity for 2-oxoquazepam. Furthermore, the level of ^{35}S-TBPS binding to membranes from *tg/tg* cortex represented 73.6 ± 5.8% of that in +/+, concordant with reports that receptor isoforms containing β1 subunits show low binding of ^{35}S-TBPS. Thus, our data indicate that an aberrant pattern of $GABA_A$ receptor subunit gene expression may contribute to the tottering phenotype.

Supported by NIH grants NS34253, NS11535, and HL07676.

558.13

CEREBELLAR OUTPUT IS REQUIRED TO GENERATE INTERMITTENT FOCAL SEIZURES IN THE MUTANT MOUSE TOTTERING (*tg/tg*). D.B. Campbell* and E.J. Hess. Department of Neuroscience & Anatomy, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

The neurological mouse mutation tottering (gene symbol: *tg*) causes ataxia, spike and wave discharges, and intermittent focal seizures. Previous reports from our laboratory have demonstrated dramatically increased c-*fos* mRNA expression in cerebellar granule and Purkinje cells during *tg/tg* intermittent focal seizures, suggesting that cerebellar output via Purkinje cells is involved in the generation of these seizures. We have tested this hypothesis by examining the effect of lesioning tottering mouse Purkinje cells, the only efferents of cerebellar cortex, on the expression of these seizures. The mouse mutation Purkinje cell degeneration (*pcd*) was used to genetically lesion tottering mouse Purkinje cells by breeding *pcd* onto the tottering mouse line. Although the *pcd* mutation has not yet been identified, chimera analysis has demonstrated that the mutant gene acts specifically within Purkinje cells to cause the death of all Purkinje cells between postnatal days 15 and 29. Double mutants (*tg/tg; pcd/pcd*) were generated by +/*tg*; +/*pcd* x +/*tg*; +/*pcd* matings and the genotypes of all offspring were unequivocally identified by direct molecular analysis. Controls and double mutants were then tested as adults for expression of focal seizures by mild restraint stress, an effective method of inducing seizures in tottering mice. Thirty attempts to induce seizures in each *tg/tg; pcd/pcd* double mutant have failed; in contrast, *tg/tg* controls were induced to seize in approximately 50% of trials. Further, c-*fos* expression in the double mutants after restraint stress was completely abolished, suggesting that the lesion not only eliminated the overt behavior associated with the seizure but also prevented activation of the brain circuitry associated with the seizures. Thus, removing the cerebellar output eliminated the expression of focal seizures in tottering mice, indicating that the cerebellum is a critical link in the neural network involved in the generation of *tg/tg* focal seizures. Supported by a Klingenstein Fellowship and PHS NS33592.

558.14

ABNORMALITIES IN BENZODIAZEPINE RECEPTORS IN BRAIN ASSOCIATED WITH SEIZURES IN Fyn-DEFICIENT MICE AND EL MICE. Y. Nakamoto*[1], T. Miyakawa[2], T. Yagi[3], H. Niki[2] and M. Yoshii[1]. [1]Dept. of Neurophysiol., Tokyo Inst. of Psychiat., Tokyo 156, [2]Dept. of Psychol., Fac. of Lett., Univ. of Tokyo, Tokyo 113, and [3]Dept. of Neurobiol. Behav. Genet., Natl. Inst. of Physiol. Sci., Okazaki 444, Japan.

Fyn tyrosine kinase deficient mice increase the susceptibility to audiogenic seizures by unknown mechanisms (Miyakawa et al., 1995). In the present study, we have investigated whether benzodiazepine receptors in brain are involved in seizures in Fyn-kinase deficient mice as compared with those in EL mice in which peripheral-type benzodiazepine receptors (PBR) are altered (Nakamoto et al., 1996). Fyn-deficient mice were significantly less susceptible to convulsions induced by β-CCM (2-5 mg/kg, i.p.), an inverse agonist for central-type benzodiazepine receptors (CBR), than control heterozygous mutant. The drug action did not differentiate EL mice and their control DDY mice. β-CCM in low nM order inhibited [^{3}H]flunitrazepam binding to CBR in brain tissues less potently (40% higher in IC_{50}) in Fyn-deficient mice. The B_{max} of CBR was slightly decreased in Fyn-deficient mice whereas that of PBR, as measured by [^{3}H]Ro 5-4864 binding, showed no significant difference between Fyn-deficient and control mutant mice. The results indicate that abnormalities in $GABA_A$-benzodiazepine receptors are present in Fyn-deficient mice but not in EL mice.

558.15

EFFECT OF SEIZURE TEST ON QUANTITATIVE TRAIT LOCI (QTL) MAPPING IN THE EPILEPTIC EL MOUSE. M.J. Poderycki, J.M. Simoes, M.T. Todorova, and T.N. Seyfried*. Dept. of Biology, Boston College, Chestnut Hill, MA 02167-3811.

The epileptic EL mouse has been studied extensively as a genetic model for complex partial seizures in humans. Seizures in EL mice begin at around 90 days of age through routine handling, but can be induced to occur earlier by repeated rhythmic vestibular stimulation, i.e., tossing or shaking. Several seizure frequency QTL were mapped previously in crosses between EL and non-epileptic strains using various rhythmic stimulation procedures. The genetics of EL is complex, and the presence of these QTL was dependant upon the strain and type of cross used (Rise et al., 1991, Science, 253:669-673; Frankel et al., 1995, Mamm. Genome, 6:830-838). In a backcross to ABP, *El1* was mapped to Chromosome 9 near the *se* locus and *El2* was mapped to Chromosome 2 near the *Mpmv-28* locus. In the present study we evaluated seizure frequencies at 200 days of age in the EL (n=16) and ABP (n=15) strains, their F1 hybrids (n=20) and in the F1 x ABP backcross (n=123). The mice were tested for seizures every other day for 6 tests and were then tested twice per week for an additional 9 tests. The test involved tossing the mice in a mechanical shaker (4 cm/toss at 5 tosses/sec for up to 30 sec). Over 50% of the EL and F1 hybrid mice seized at least once by tests 2 and 6, respectively, and 100% seized by tests 7 and 10, respectively. ABP mice were largely unresponsive to the testing procedure. Mice in the backcross population seized for the first time at test 3, with 50% and 76% seizing by tests 10 and 15, respectively. The strength of the associations between seizure frequency and *El1* (near *se*) and *El2* (near *D2Mit30*) was strongly dependant upon the number of tests administered. For example, the association with *El1* weakened as the number of tests increased. Maximum LOD scores as determined by Mapmaker/QTL were 2.9, 1.6, and 1.3 at tests 6, 11, and 15, respectively. In contrast, LOD scores for *El2* were 1.0, 4.7, and 4.4 at tests 6, 11, and 15, respectively. These findings suggest that some of the genetic complexity of the EL phenotype may be due in part to genotype-environmental interactions. Supported by the PHS grant (NS 23355) and a grant from the Boston College Research Expense Fund.

ALZHEIMER'S DISEASE: TAU AND NEUROFIBRILLARY DEGENERATION

559.1

CHARACTERIZATION OF TAU IN IMR-32 CELLS. R.D. Williams, S.K. Hall, D. Auperin, and M.K. Ahlijanian*, Divisions of Neuroscience and Medicinal Chemistry, Pfizer Central Research, Groton, CT 06340.

Neurofibrillary tangles (NFTs) are one of the classic pathologies associated with Alzheimer's Disease (AD) and are comprised of highly phosphorylated tau protein. Hyperphosphorylation limits the association of tau with microtubules and may result in cytoskeletal destabilization and neurodegeneration. PCR experiments demonstrate that IMR32 cells (human neuroblastoma) predominantly express the 3-microtubule binding domain repeat, 0 amino terminal insert, isoform of tau. Treatment of cells with okadaic acid, a protein phosphatase 2A inhibitor (1 μM for 30 min), results in increased immunoreactivity of the antibody AT8, which specifically recognizes phosphorylated Ser202 of tau, in western blots. A corresponding decrease in the immunoreactivity of Tau-1, an antibody which specifically recognizes non-phosphorylated residue 198 in tau, is also observed with okadaic acid treatment. Okadaic acid treatment does not alter the mobility of tau in SDS-PAGE or the total content of tau, monitored with the antibody HT-7, which recognizes both phosphorylated and non-phosphorylated tau. Concomitant treatment with 25 μM staurosporine, a non-selective protein kinase inhibitor, does not alter the phosphorylation pattern of tau. These data demonstrate that IMR32 cells may represent an acceptable whole cell model for assessing the modulation of the phosphorylation state of tau by pharmacological probes. Supported by Pfizer Central Research, Groton.

559.2

INCREASED TAU PROTEIN IN PRIMARY CORTICAL NEURONS EXPRESSING HUMAN $A\beta_{1-42}$ and APP751. Robert K.K. Lee*[1], Richard J. Wurtman[1], Benson P.S. Yang[1] and Rachael Neve[2]. [1]Dept. Brain & Cognitive Sci., MIT, Cambridge, MA 02139 and [2]Mol. Genetics Lab., McLean Hospital, Harvard Med. Sch., Belmont, MA 02178.

Neurofibrillary tangles are aberrant forms of tau whereas amyloid is derived from the amyloid precursor protein (APP). To determine if APP can regulate tau expression, we infected rat cortical neurons (7-10DIV;~10^6 cells/dish) with replication-defective herpes simplex virus type-1 vectors (HSV; ~10^6 particles /dish) expressing (*1*) wild-type (WT) or familial AD mutations (FADs) in human APP751 or APP695 (*2*) $A\beta_{1-42}$ or (*3*) carboxy-terminal 100 residues of APP (C-100). FADs in APP751 or APP695 were: 717 (G,F or I substituted for V), 692 (G substituted for A) or 670/671 (N,L substituted for K,M). After 36h, cell-associated APP and phosphorylation-independent tau were detected by mAb 22C11 and 5E2 (from Dr. K. Kosik, Harvard Med. Sch.) respectively on immunoblots. APP and total tau levels in control neurons (Ctrl) infected with HSV expressing *E.coli* β-galactosidase did not differ from uninfected neurons.

APP holoprotein in neurons overexpressing APP751 or APP695 was 1000% that of Ctrl. FADs in APP751 or APP695 had no significant effect on APP or tau levels relative to their WT counterparts. However, tau was significantly increased (150% of Ctrl) by WT and FADs in APP751, but not in APP695, suggesting that the Kunitz protease inhibitor domain contained in APP751 increases neurite growth.

C-100 resulted in neurite dystrophy and decreased total tau levels to 50% of Ctrl after 3 days. In comparison, overexpression of $A\beta_{1-42}$ seemed less toxic and neurons appeared healthy after 5 days of infection. Increased tau and APP (150% and 350% of Ctrl) were also detected in $A\beta_{1-42}$ infectants. Hence, accumulation of intracellular $A\beta_{1-42}$ may contribute to aberrant tau and APP expression. Tau increases caused by APP751 and $A\beta_{1-42}$ will be examined with phosphorylation-dependent antibodies. Our data show that APP751 can regulate tau expression in primary neurons, and also suggest a potential link between amyloid and PHF formation in Alzheimer's disease. *(Supported by NIH =MH-28783 & Center for Brain Science and Metabolism Charitable Trust)*

559.3

THE RISE IN THE CONCENTRATION OF FREE CYTOPLASMIC CALCIUM LEADS TO DEPHOSPHORYLATION OF THE MICROTUBULE-ASSOCIATED PROTEIN TAU. E. Adamec*, M. Mercken, M. L. Beermann, M. Didier and R. A. Nixon. Laboratories for Molecular Neuroscience, McLean Hospital, Belmont, MA 02178.

The effect of a rise in the cytoplasmic concentration of free calcium ($[Ca^{2+}]_i$) on the state of tau protein phosphorylation was studied in rat cortical neurons and mouse cerebellar granule cells in culture. In rat cortical neurons, 1-hour treatment with glutamate, N-methyl-D-aspartate (NMDA), KCl, or a Ca^{2+} ionophore, ionomycin, caused a permanent elevation of $[Ca^{2+}]_i$. On Western immunoblots probed with the phosphorylation-independent antibody LK, the treatment with any of the above mentioned drugs resulted in an appearance of an additional faster moving band. On immunoblots probed with the phosphorylation-dependent antibody tau-1, which recognizes a dephosphorylated epitope, the drug treatments resulted in an increase in the tau-1 immunoreactivity and an appearance of an additional faster moving band. These findings indicate that $[Ca^{2+}]_i$ elevation is associated with dephosphorylation of the tau protein. Administration of 100 nM okadaic acid alone (an inhibitor of phosphatases 1 and 2A) increased the level of tau protein phosphorylation. Administration of okadaic acid together with the above mentioned drugs decreased the drug-mediated dephosphorylation. Pre-incubation with okadaic acid fully prevented the dephosphorylation. Treatment with cypermethrin (an inhibitor of phosphatase 2B) was without effect when administered either alone, or together with the drugs, or pre-incubated. Similarly, in mouse cerebellar granule cells, glutamate or ionomycin also induced okadaic acid-sensitive tau protein dephosphorylation. These findings indicate that, independently of the influx pathway, $[Ca^{2+}]_i$ elevation leads to tau protein dephosphorylation and implicate phosphatase 1 and/or 2A in the process.

Supported by grants from the National Institute on Aging (AG05604 and AG10916).

559.4

A DISSOCIATION OF MITOGEN ACTIVATED PROTEIN KINASE AND TAU HYPERPHOSPHORYLATION IN CORTICAL NEURONS. H. Shayan*, D.T. Ho and T.H. Murphy. Kinsmen Lab, Dept. of Psychiatry, University of British Columbia, Vancouver, Canada V6T 1Z3.

The paired helical filaments (PHFs) of neurofibrillary tangles observed in Alzheimer's disease are produced by the aggregation of the microtubule associated protein tau. PHF-tau is characterized by extensive phosphorylation at specific sites and loss of microtubule binding capacity. Several studies have implicated proline directed kinases such as mitogen activated protein kinase (MAPK) in the *in vitro* phosphorylation of tau. We examined the requirement of activated MAPK for tau phosphorylation in rat cortical cultures using the okadaic acid induced tau hyperphosphorylation model (Harris et al, 1993). Okadaic acid treatment (0.25 μM) reduced Tau-1 immunoreactivity by 80% as assayed using Western blotting, indicative of tau hyperphosphorylation (Tau-1 only reacts with dephosphorylated tau). Treatment of the blots with alkaline phosphatase restored Tau-1 immunoreactivity in okadaic acid treated samples, indicating that loss of immunoreactivity was due to hyperphosphorylation of tau at Tau-1 specific sites. Okadaic acid was also shown to double the activity of MAPK based on the electrophoretic mobility shift and phospho-epitope analysis. The pretreatment of cells with 30 μM PD098059 (2-(2'-amino-3'-methoxyphenyl)-oxanaphthalen-4-one), which is a specific inhibitor of MEK-1, the upstream activator of MAPK, suppressed MAPK activation. Under conditions in which MAPK activation was completely blocked, okadaic acid treatment still produced a robust reduction in Tau-1 immunoreactivity. These results indicate that activated MAPK is not required for tau hyperphosphorylation in intact neurons. Furthermore, in agreement with previous studies demonstrating that okadaic acid results in disruption of presynaptic vesicle clusters, a profound reduction of synaptic transmission was observed in response to okadaic acid treatment. Supported by Alzheimer Soc. of B.C..

559.5

PHOSPHORYLATION OF TAU IN ALUMINUM-INDUCED NEUROFIBRILLARY TANGLES: TAU-1 AND TAU-5 ARE DIFFERENTIALLY DISTRIBUTED S.M. Singer*, M.A. Norlund and N.A. Muma. Dept. of Pharmacology, Loyola Univ. Chicago Stritch Sch. of Med., Maywood, IL. 60153

In susceptible species, aluminum salts induce neurofibrillary pathology in cortex, several brainstem nuclei and spinal cord neurons. We, and others, have demonstrated the presence of tau and neurofilaments in these aluminum-induced neurofibrillary tangles (Al-NFTs) in rabbits. Using double-label immunocytochemistry and quantitative image analysis we demonstrate phosphorylation-state dependent differences in the presence of tau in Al-NFTs. The area of Al-NFTs is identified based on labeling with an antibody against phosphorylated neurofilaments (SMI31). We compared the colocalization of antibodies directed against tau at phosphorylation state-dependent (tau-1) or independent (tau-5) epitopes with SMI31 to examine tau in the Al-NFTs. The proportion of cell area labeled with non-phosphorylated tau in Al-NFTs was significantly different from total tau in NFTs (N=366 cells, 5 animals, $p<0.0001$). Multiple regression analyses demonstrated that the amount of tau in the Al-NFT were highly correlated (R=0.91 for tau-1, R=0.93 for tau-5) to tangle size ($p<0.0001$), area of tau labeling in the cell ($p=0.0005$ for tau-1, $p=0.07$ for tau-5) and the area of Al-NFT without tau labeling ($p<0.0001$). Furthermore, we have found that the amount of tau in the Al-NFT can be predicted by the contributions of (1) tangle without tau labeling (~55%), (2) tangle size (~35%), and (3) area of tau labeling in the cell (~7%). This model suggests that as the area of the cell occupied by the tangle increases, more tau in the tangle is phosphorylated. Our findings suggest that the phosphorylation state of tau is altered during Al-NFT development. A role for aluminum in the pathophysiology of AD remains controversial, however, studies of aluminum-induced neurocytoskeletal degeneration may offer insight to understanding the mechanisms involved in tau and neurofilament perturbations occurring in several neurodegenerative diseases. (Supported by NIH grant NS30460)

559.6

HUMAN STRESS-ACTIVATED PROTEIN KINASE β (hSAPKβ) PHOSPHORYLATES TAU *IN VITRO* AND *IN VIVO*. Y. Kumagae, O.J. Kim, Y. Zhang and C.A. Miller*. Department of Pathology and Neurology. USC School of Medicine, Los Angeles, CA 90033.

Phosphorylation of tau plays a role in the formation of paired helical filaments (PHF) in Alzheimer's disease (AD). Several kinases are known to phosphorylate tau. hSAPKβ is a neuron-specific, stress-activated kinase of 45kD belonging to SAPK/JNK kinase family. It co-localizes in neurons containing PHF. We tested whether hSAPKβ phosphorylates tau *in vitro* and *in vivo*.

Recombinant human tau-40 was incubated with hSAPKβ and analyzed on SDS-PAGE. A time-dependent decrease in mobility of tau was maximal by 6 hours suggesting tau phosphorylation by hSAPKβ. *In vivo* phosphorylation of tau was demonstrated by transiently co-transfecting into CHO cells, human tau-40 cDNA, and a construct of hSAPKβ. Expression of tau alone in CHO cells produced two to three bands on Western blots using anti-tau antibody (T14). When hSAPKβ was co-expressed with tau-40, there was an accumulation of tau with decreased mobility.

These data suggest hSAPKβ phosphorylates tau *in vitro* and *in vivo*. Because its expression is in neurons selectively vulnerable in AD, hSAPKβ may be relevant to the pathogenesis of PHF in AD. (Supported in part, by the SANKYO Co., NIMH and NIA).

559.7

PHOSPHOPEPTIDE MAPPING OF cAMP-DEPENDENT PROTEIN KINASE AND PAIRED HELICAL FILAMENT KINASE PHOSPHORYLATION SITES IN RECOMBINANT TAU G. A. Jicha[1]*, A. O'Donnel[2], R. Angeletti[2], and P. Davies[1] Departments of Pathology[1] and Developmental and Molecular Biology[2] Albert Einstein College of Medicine, Bronx, NY 10461

We have previously demonstrated that a serine/threonine protein kinase (PHF kinase) that co-purifies with paired helical filaments (PHF) from Alzheimer's disease (AD) brain tissue phosphorylates recombinant tau only after prior phosphorylation with cAMP-dependent protein kinase (PKA). In addition, we have shown that this phosphorylation occurs in the C-terminus of tau. However the precise PHF kinase phosphorylation site(s) and the PKA phosphorylation site(s) that direct this phosphorylation were not identified. Recombinant tau was reacted with either PKA alone or PKA followed by PHF kinase in the presence of [γ^{32}P]-ATP. Phosphopeptide mapping of both the PKA phosphorylation sites and the PHF kinase sequential phosphorylation sites was performed using HPLC separations of AP-1, elastase, and trypsin digestions of the phosphorylated recombinant tau. Phosphorylated fragments were identified by scintillation counting, mass spectrometry, and peptide sequencing. Analysis of these phosphorylated fragments provide further support for the novel characteristics of the PHF kinase, allow intepretation of the minimal concensus sequence phosphorylated by the PHF kinase, and further support the role the PHF kinase plays in inducing pathological alterations in tau that contribute to the formation of PHF in AD.

This research was supported by NIH training grant T32GM07288 and NIMH 38623.

559.8

PHOSPHORYLATION OF TAU ON SER 262 BY A MICROTUBULE-ASSOCIATED PROTEIN KINASE. S.M. Jenkins*, and G.V.W. Johnson. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama at Birmingham, Birmingham, AL 35294-0017.

Site-specific phosphorylation of microtubule-associated proteins (MAPs) plays an important role in modulating the function of these proteins. Recently a protein kinase from embryonic chicken brain with a molecular mass of ~100 kD was found to co-purify with MAP-2. Phosphorylation of MAP2 by this kinase completely inhibited microtubule binding (Lopez and Sheetz, JBC 270:12511, 1995). In the present study, the mammalian form of this protein kinase was isolated from a microtubule preparation derived from embryonic and adult rat brain. The 100 kD kinase from rat brain readily phosphorylated both MAP2 and tau as determined by an in-gel kinase assay. In addition, the rat brain protein kinase extensively phosphorylated tau on Ser 262. This was determined by phosphorylation of a peptide encompassing amino acids 257-271 of human tau, and by use of an antibody that selectively recognizes tau when it is phosphorylated at Ser 262 and/or Ser 356 (12E8). The 100 kD rat brain protein kinase was cAMP-independent and inhibited by β-glycerol phosphate, as was the kinase from chick brain. Significantly higher levels of kinase activity were present in embryonic compared to adult rat brain, indicating that the kinase is developmentally regulated. This is intriguing because it has been suggested that inappropriate re-expression of embryonic or juvenile proteins may contribute to Alzheimer's disease pathology. In addition, the phosphorylation state of the kinase apparently modulates its activity. Drewes et al. (1995) recently purified a protein kinase of ~110 kD from bovine brain (MARK). MARK phosphorylated tau on Ser 262, as well as other KXGS sites, and maybe homologous or identical to the rat brain protein kinase.

Supported by NIH grant AG06569.

559.9

INSOLUBLE NFTs FROM ALZHEIMER DISEASE CONTAIN TRANSGLUTAMINASE-CATALYZED GLU-LYS BONDS AS REVEALED BY THEIR SENSITIVITY TO A NOVEL ISOPEPTIDASE. D.M. Appelt[1], M.P. Howard[2], A.G. Loewy[2], B.J. Balin*[1]. Dept. of Pathology and Lab Med[1]., Med. Coll. of Penn. and Hahnemann Univ., Phila., PA 19102, and Dept. of Biology[2], Haverford College, Haverford, PA 19041.

A characteristic in the pathology of Alzheimer's Disease is the accumulation of insoluble protein structures such as neurofibrillary tangles (NFTs) formed from paired helical filaments within dystrophic neurons of the central nervous system. One mechanism by which these tangles may be rendered insoluble is through an enzymatic reaction catalyzed by tissue transglutaminase. Demonstration of the N^{ε}-γ(glutamic)lysine crosslinks in these insoluble structures is important to verify the role transglutaminase(s) may play in the conversion of soluble proteins into insoluble neuronal matrices. We have identified an isopeptidase from the culture fluid of the bacterium Stenotrophomonas multophila and have demonstrated that it releases crosslinked protein chains from a ^{3}H-labeled acetyl lysine methylamide (ALMA)-casein substrate in which the glu-lys bond has been formed with liver transglutaminase. We have used this isopeptidase to release the crosslinked protein chains from NFTs, resulting in solubilization of these structures previously shown to resist proteolysis and unfolding reagents such as urea, SDS and DTT. Using the ALMA-casein substrate, we have demonstrated the presence of isopeptidase activity in human brain. We are presently testing the hypothesis that the steady state level of glu-lys crosslinked cell matrices is regulated by transglutaminase and isopeptidase activities in neuronal tissue. Supported by PHS/NIH AG10160 grant to BJB.

559.10

TRANSIENT STRUCTURES FORMED DURING THE DEVELOPMENT OF ALZHEIMER-RELATED Aß-AMYLOID DEPOSITS AND NEUROFIBRILLARY CHANGES.
E. Braak* and H. Braak. Department of Anatomy, J.W.Goethe University, D-60590 Frankfurt/Main. Germany

Major pathologic hallmarks of Alzheimer`s disease include extracellular and intraneuronal deposits of Aß-amyloid protein and abnormal tau-protein (demonstrated by advanced silver techniques [1]). The pathologic changes appear gradually at specific predilection sites.

Inconspicuous though extensive cloud-like Aß-amyloid deposits with ill-defined boundaries initially develop in anteromedial portions of the temporal lobe. With advance of the disease these precipitations disappear.

Transient forms of neurofibrillary tangles and neuropil threads develop in a subpopulation of CA1 pyramidal cells. These neurons develop conspicuous tangles extending into the tip of the apical dendrite. The change can be recognized first at stage II, is best developed at stage III, and rapidly deteriorates at stage IV [2]. When piercing the stratum lacunosum-moleculare, the altered dendrites develop spindle-shaped dilations filled with argyrophilic material. The varicose segments disappear, leaving no extracellular residues that can be detected at the light microscopic level.

Supported by the Deutsche Forschungsgemeinschaft.

[1] Iqbal et al. J. Histotechnol. 16: 335-342, 1993
[2] Braak and Braak, Acta Neuropathol. 82: 239-259, 1991

559.11

GRADUAL DEVELOPMENT OF Aß-AMYLOID DEPOSITS AND NEUROFIBRILLARY CHANGES: INCIDENCE AND PREVALENCE RATES IN DIFFERENT AGE CATEGORIES.
H. Braak* and E. Braak. Department of Anatomy, J.W.Goethe University, D-60590 Frankfurt/Main. Germany

Alzheimer`s disease is a relentlessly progressing dementing disorder. Major pathologic hallmarks include extracellular Aß-amyloid deposits and intraneuronal neurofibrillary changes (advanced silver techniques according to Gallyas and Campbell et al. [1]). Initial deposits of both changes develop in anteromedial portions of the temporal lobe. From there, the changes spread in a predictable manner across other cortical areas reflected by stages A-C of Aß-amyloid deposits and I-VI of neurofibrillary changes [2]. Staging reveals that the two changes are by no means normal concomitants of aging. No remissions occur in the course of Alzheimer`s disease. Initial neurofibrillary changes develop preferentially in the absence of Aß-amyloid deposits. Stage I cases show the incidence, and stage V-VI cases the prevalence rates of Alzheimer`s disease. Advanced age is not a prerequisite for the evolution of the lesions. The arithmetic means of the assigned stages of both the Aß-deposits and the neurofibrillary pathology increase with age. Alzheimer`s disease is an age-related, but not an age-dependent disease.

Supported by the Deutsche Forschungsgemeinschaft.

[1] Iqbal et al., J.Histotechnol. 16, 335-342, 1993
[2] Braak and Braak, Acta Neuropathol. 82, 239-259, 1991

559.12

CONFORMATIONAL AND PHOSPHORYLATED EPITOPES OF TAU IN EARLY ALZHEIMER'S DISEASE. C. L. Weaver, G. A. Jicha, I.J. Vincent*, and P. Davies. Depts. of Pathology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461.

It has been suggested that phosphorylation of tau occurs early in the course of the neurodegeneration of Alzheimer's Disease (AD). We have previously reported that the phosphoepitope recognized by the monoclonal antibody TG3 can be detected in hippocampal pyramidal cells in early cases of AD, prior to the formation of paired helical filaments. It has recently been determined that Alz-50 and a new monoclonal antibody, MC1, require two sequences in tau for immunoreactivity, indicating that tau assumes a folded conformation for recognition by these antibodies. MC1 appears superior to Alz-50 in that it does not stain neurons in the normal brain. We have examined a group of early AD cases (Braak stages 1 and 2) with TG3 and MC1 to attempt to determine the temporal relationship between phosphorylation and conformational changes of tau.

Serial sections were cut from formalin fixed tissues on a vibratome. A sequence of consecutive sections were stained with PHF1, Alz-50, TG3, MC1 and MC5. In these early cases, many more neurons are labeled with TG3 and MC1 than with the other antibodies. Many of the CA2 pyramidal neurons stained by MC1 or TG3 did not have obvious neurofibrillary tangles. The less numerous neurons visualized with PHF1 and MC5 all appeared to contain a neurofibrillary tangle. Additional pairs of adjacent sections were taken along the length of the hippocampus for more detailed studies with TG3 and MC1. Numbers, locations and types of stained neurons were recorded. In general, MC1 appeared to label more neurons than TG3, especially in the CA2, CA4 and dentate gyrus. In some cases, whole fields of neurons were detectable only with MC1. We have tentatively concluded that conformational changes in tau may precede many of the disease related phosphorylations of this protein. Supported by NIMH 38623.

559.13

ANALYSIS OF MULTIPLE GENE EXPRESSION IN SINGLE TANGLE BEARING AND NON TANGLE BEARING NEURONS IN ALZHEIMER'S DISEASE.
Janet E. Cheetham, Nienwen Chow and Paul D. Coleman*. University of Rochester School of Medicine and Dentistry, Dept. of Neurobiology and Anatomy, Rochester, NY, 14642.

In a diseased brain (such as one suffering from Alzheimer's Disease), one cell may clearly be affected whilst an adjacent cell appears healthy or unaffected. Previous technology has allowed us to examine one message at a time at the level of a single cell (*in situ* hybridization), or multiple messages in a heterogeneous population of cells (northern analysis).
We have developed a methodology, to allow us to characterize individual cells by ICC using antibodies to identify their neurofibrillary tangle (NFT) bearing status and using a modification of Eberwine *et al* (1992), to build up a profile of multiple mRNA expression in single, whole, post mortem cells. Fresh postmortem tissue is spread into a layer 1 cell thick and fixed. Tangle bearing neurons are identified using an antibody to a tangle marker and isolated using a micropipette. The mRNA is reverse transcribed and PCR carried out to confirm material is present. A radioactively labeled antisense RNA probe, which is representative of the messages contained in the cell is then amplified. This aRNA is used as a probe for a reverse northern blot, allowing us to profile many genes from one cell at the same time. Different expression profiles between NFT-bearing and NFT-free neurons will be presented. This technology has the potential to be applied to a wide variety of diseases encompassing many different cell types.
Supported by grants from the NIA (LEAD AG09016, ADC AG08665, R01 AG01121), AHAF and the Markey Foundation (91-24).
Reference : Eberwine *et.al.*, (1992)Proc. Natl. Acad. Sci. USA 89:3010-3014.

559.14

BASAL FOREBRAIN RNA CHANGES IN RELATION TO TAU PROTEIN IN ALZHEIMER'S DISEASE. R. K. Kan, D. S. Morin., L. A. Shaubach., J. N. Finger, R. B. Mitchell, J. R. Day * and A. Anthony. Department of Biology, Pennsylvania State University, University Park, PA 16802.

Degeneration of the basal forebrain cholinergic system and accumulation of tau are characteristics of Alzheimer's disease (AD). These changes are accompanied by alterations in nucleic acid metabolism and protein synthesis. The present study was designed to examine changes in basal forebrain neuronal RNA metabolism in relation to tau immunoreactivity. Nucleus basalis of Meynert (nbM), medial septal (ms) nuclei and diagonal band of Broca (dbB) from 9 AD patients and 9 age-matched, nondemented controls were removed at autopsy, formalin-fixed, paraffin-processed, serially sectioned at 5µm. Adjacent sections were stained with tau antibody or with Shea's (1970) azure B-RNA procedure. Extent of neuronal loss was quantified at magnification of 50x by averaging the number of cells counted in five fields. All three nuclei from AD subjects exhibited a profound cell loss ($p \leq 0.05$) as compared to controls. The amount of neuronal RNA was measured cytophotometrically using a Vickers M85a scanning-integrating microdensitometer. In AD specimens, tau-containing neurons were found to have less RNA than tau-free neurons ($p \leq 0.05$). In addition, the mean RNA values of both neuronal population (tau-free and tau-containing neurons) in AD fell well below the control mean ($p \leq 0.05$). The severity of RNA depletion (in order of percent decline) was nbM (30%) = ms (30%) > dbB(25%). The data indicate that RNA loss occurs in the AD basal forebrain brain and RNA depletion is more pronounced in tau-positive neurons than in tau-negative neurons. These findings suggests that alterations in transcriptional aspects of neuronal metabolism may play a critical role in the pathogenesis of AD. However, whether RNA depletion is primary or secondary to AD pathology, i.e., neurofibrillary degeneration, remains to be established. This research was funded by The Biology Department at Pennsylvania State University.

559.15

A SENSITIVE AND HIGHLY SPECIFIC ASSAY FOR PHF PROTEINS. P. Davies*, R. Zinkowski, R. Kohnken, C. Bevona, G. A. Jicha, E. Lane, I.J. Vincent, D. Kerkman and J. DeBernardis. Depts. of Pathology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461 and Molecular Geriatrics Corp, Lake Bluff, IL 60044.

The assay of the concentration of tau protein in cerebrospinal fluid (CSF) has recently been reported as helpful in the diagnosis of Alzheimer's Disease, despite the presence of elevated concentrations of this neuronal protein in CSF from patients with a variety of other diseases. A much more specific diagnostic assay would be the measurement of the abnormal tau of paired helical filaments (PHF). We have identified several antibodies that might be useful for such an assay.

Antibodies selected for these assays fall into two categories: those detecting phosphoepitopes present on tau in PHF, and those that react with the specific conformation of tau in PHF. Among the antibodies to phosphoepitopes, several can be rejected because these epitopes are present on tau in normal human brain although unstable in the post mortem period. TG3 is a strong candidate because it recognizes a phosphoepitope which does not appear to be phosphory'ated on tau in either autopsy or biopsy brain tissue from normal humans. The monoclonal antibody MC1, like Alz-50, recognizes a conformational epitope formed by bringing together two tau sequences separated by about 300 amino acids: tau in this conformation does not appear to be present at detectable concentrations in the normal brain. Unlike Alz-50, MC1 does not appear to recognize non-tau proteins in the normal brain. Sandwich ELISA formats using a combination of MC1 and TG3 appear to provide very highly specific detection of tau in PHF. Neither recombinant tau, nor tau prepared from biopsy or autopsy brain tissue are not detectable in these formats in concentrations more than 1000 times that of purified PHF. Currently, tau from PHF can be detected in concentrations in the pg/ml range. The utility of these assays in CSF is currently being evaluated. Supported by NIMH 38623

559.16

ALZHEIMER'S DISEASE; PHOSPHORYLATED NEUROFILAMENT AND PAIRED HELICAL FILAMENT IMMUNOREACTIVITIES IN CSF. J. M. Yuan* and P. Xu. Department of Neurology, First Hospital, Beijing Medical University, Beijing, 100034, China

The definite diagnosis of Alzheimer's disease (AD) requires histological examination of brain tissue, it is difficult to accept by patients. To establish a specific testing to help the diagnosis is expected. Using monoclonal antibody as the probes, with purified phosphorylated neurofilaments (PNF) and paired helical filaments (PHF) as coated antigens respectively, we established a competitive enzyme-linked immunosorbent assay (ELISA) for PNF and PHF immunoreactive in the CSF. Using this assay, we measured 21 patients with AD, 17 patients with multi-infact dementia (MID), 33 with other neurological disease (ODC), 33 patients with schizophrenia (SCH), and 40 healthy controls (HC). It was found that the levels of PNF immunoreactivity in CSF were no difference in AD, MID, SCH, and HC groups ($P>0.05$). The levers of PHF immunoreactivity were significantly higher in CSF in AD than in the control groups ($P<0.05$). The values of PNF/PHF ratio in CSF were significantly lower in AD than controls ($P<0.01$). Conclusion: (1) There is considerable significance in detection of PHF in CSF to diagnosis and differentiation of the AD. (2) The value of PNF/PHF ratio is also considerable significance to that. (3) Finally, while combinating the levels of PHF immunoreactivity in CSF with the value of PNF/PHF ratio might be developed as a specific diagnosis method of AD. (Supported by Ministry of Health of People's Republic of China)

559.17

CSF TAU IS INCREASED IN VERY EARLY STAGES OF ALZHEIMER'S DISEASE. D. Galasko*[1], J. Brown[1], C. Clark[2], L. Chang[3], B. Miller[3], R.C. Green[4], R. Motter[5], P. Seubert.[5] [1]Dept. of Neurosciences, UCSD, San Diego, CA 92161; [2]Dept. of Neurology, Univ. of Penn., Philadelphia, PA 19104, [3]Dept. of Neurology, UCLA, Torrance, CA 90509, [4]Dept. of Neurology, Emory Univ. Med. Cntr, Atlanta GA 30329, and [5]Athena Neurosciences, South San Francisco, CA 94080.

In 9 studies over the past 3 years, CSF tau was shown to be increased in patients with Alzheimer's disease (AD) compared to controls. Two studies found a direct correlation between CSF tau and dementia severity, i.e. tau increased cross-sectionally as dementia severity increased. It is not clear how early in AD elevated CSF tau can be found, since relatively few mild AD patients have been studied. Whether CSF tau increases with normal aging also has not been explored.

We therefore measured CSF tau in patients with mild AD (MMSE ≥ 20/30), and in controls (cognitively normal elderly and patients with neurological disorders), and in a group of younger subjects aged 20 - 25. CSF samples were stored frozen until assayed. CSF tau was measured using a sensitive sandwich ELISA.

Tau levels were 509 ± 255 pg/ml in AD and 167 ± 67 in controls. 29 of 36 patients with AD had increased CSF tau levels, exceeding a previously determined cut-off point. Increased tau was present even in patients with very mild dementia (MMSE ≥ 25). The young normal subjects had CSF tau of 160 ± 78 pg/ml. Among all of the normal subjects, CSF tau did not correlate with age. CSF tau therefore is a sensitive early marker in AD. Tau levels in CSF do not change with normal aging.

Supported by NIH Grant AG05131 and a VA MERIT award.

559.18

CEREBROSPINAL FLUID TAU AND Aβ PROTEIN LEVELS AS BIOLOGICAL MARKERS FOR CLINICAL DIAGNOSIS OF ALZHEIMER'S DISEASE

H. Arai[1], M.Terajima[1], T. Nakagawa[1], Y. Kosaka[1], T. Matsui[1], S. Higuchi[2], T. Muramatsu[2], S. Matsushita[2], T. Iwatsubo[3], T.Kosaka[3], M. Miura[4], T. Machida[4], C.Clark[5], V.M.-Y.Lee[5], J.Q.Trojanowski[5]*, H.Sasaki[1]

1)Department of Geriatric Medicine, Tohoku University School of Medicine,Sendai 980, JAPAN 2)Department of Psychiatry, National Institute on Alcoholism, Kurihama National Hospital, Kanagawa 239, JAPAN 3)Department of Neuropathology and Neuroscience, University of Tokyo, Tokyo113, JAPAN 4)Research and Development Department, Mitsubishi Kagaku Bio-clinical Laboratories, Tokyo 174, JAPAN 5)The University of Pennsylvania, Philadelphia PA 19104, U.S.A.

Several independent groups have demonstrated that cerebrospinal fluid (CSF) tau protein (CSF-tau) is increased in Alzheimer's disease (AD) and that measurement of levels of CSF-tau may be aid in the clinical diagnosis of AD. In the present study, CSF-tau levels were examined in 62 AD patients carrying different genetic risk factors for AD including the apolipoprotein E (Apo E) ε4 allele, the α_1-antichymotrypsin (ACT) A* allele and the presenilin-1 (PS-1)1* intronic allele. Further, temporal changes in CSF-tau levels during the progression of AD were surveyed in 17 selected AD cases. The CSF-tau levels were not significantly different between Apo E ε4 carriers and non-carriers, ACT A* carriers and non-carriers as well as PS-11* carriers and non-carriers. The CSF-tau levels were quite stable or slightly increased during the progression of AD. None of the genetic risk factors affected the temporal changes in CSF-tau levels. CSF-Aβ protein consisting of 1-42 amino acids (CSF-$A\beta_{1-42}$) was significantly reduced in AD patients as compared with that in normal control subjects. The CSF-$A\beta_{1-42}$ levels were decreased as the disease progressed. The presence or absence of Apo E ε4 allele did not affect the CSF-Aβ levels.

559.19

STAGING NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASED BRAIN. D.R. Brady*, C.M. Fleming and K. Hatanpää. Lab. of Neuroscience, NIA/NIH, Bethesda, MD 20892.

Alzheimer's disease (AD) is characterized neuropathologically by neurofibrillary tangles (NFTs) and senile plaques in association cortices of the cerebral cortex. Hyperphosphorylated tau, a microtubule associated protein found normally in the axonal compartment of neurons, is a major constituent of somal NFTs. NFTs are mixed in the cortical mantle with apparently normal neurons devoid of NFTs. However, it has been suggested that the complete tau molecule, including the NH_2-terminus, is only found in early NFTs. Neurons bearing NFTs are mixed in the cortical mantle with apparently normal neurons devoid of NFTs. In order to understand how and why NFTs develop, we must first stage the neurons and then focus our attention on that neuronal population that exhibits the earliest signs of NFT formation. The brains of 16 neuropathologically confirmed AD (x age=80.25 ± 1.3 yrs) and 5 neurologically normal patients (x age=50.4 ± 8.6 yrs) were obtained postmortem from the LNS Brain Bank (x post mortem delay: AD-8.17 ± 1.4 hr, normals-17.5 ± 9.3 hr). Double-labeling with Alz-50 (recognizing the NH_2-terminus of tau) and PHF-1 (recognizing the ser-396 phosphorylation site on tau) antibodies was used to differentiate "early" (Alz-50+) and "mature" (PHF-1+ only) NFTs in temporal lobe. The greatest density of "mature" NFTs were observed in middle and inferior temporal cortices, with the fewest in superior temporal cortex (STC). Conversely, the greatest density of "early" NFTs reside in STC. These results form the basis for in situ hybridization analysis focusing on neurons bearing early NFTs.

559.20

ULTRASTRUCTURAL OBSERVATIONS ON PAIRED HELICAL FILAMENTS FORMATION AND THEIR EVOLUTION TO STRAIGHT FILAMENTS IN AGED AND ALZHEIMER'S BRAINS. P. Gómez-Ramos*and M.A. Morán. Dpt. Morfología, School of. Medicine, Autónoma University of Madrid. Spain.

Cytoskeleton changes were studied at both light microscopy (LM) and electron microscopy (EM), in vibratome sections from 2 Alzheimer's and 2 non-demented brains, using the AT8 antibody, which recognizes tau phosphorylated in serines 199 and 202 but does not cross-react with normal tau. Areas containing stained elements were selected at LM, photographed, resectioned and then observed under EM. Neurons which at LM were labeled with granular reaction product loosely distributed throughout the neuron, at the EM showed reaction product not only associated to the nuclear envelope, rough endoplasmic reticulum, poliribosomes and lipofucsin granules but also dispersed throughout the cytoplasm. Neurons which at LM had both granular reaction product and rod-like positive structures, at EM showed, in addition to the above mentioned cytoplasmic staining, bundles of strongly immunostained paired helical filaments. Neurons which at LM showed typical intraneuronal neurofibrillary tangles, at EM exhibited not only cytoplasmic immunostaining but also light or absent immunostaining in straight filaments surrounded by strongly positive bundles of paired helical filaments. These results are discussed in relation to the evolution of the neurofibrillary degeneration. Supported by FISss n° 93/0189, Spain.

560.1

GLIA PROTECTS BRAIN TISSUE BY OPPOSING SPREADING DEPRESSION-LIKE DC POTENTIALS. O. Herreras*, J.M. Ibarz, and C. Largo. Dept. Invest., Hospital Ramón y Cajal, Madrid-28034, Spain.

The participation of glia and potassium in spreading depression (SD) and DC potentials have been studied in a model of glial function deprivation *in situ* by perfusing the gliotoxin fluorocitrate (FC) and/or potassium through a microdialysis fiber in the CA1 area. Extracellular DC potential (V_o), potassium concentration $[K^+]_o$, and evoked field activity were monitored. FC caused a rapid impairment of glial function followed by gradual loss of neuronal electrogenic activity and death. SD waves run faster and lasted longer during FC, and the resistance of the st. pyramidale to develop SD was overridden. Cortical waves, that in untreated animals never spread to the CA1, readily reached this area via entorhinal cortex. Membrane potential of glial cells gradually depolarized during FC. When neuronal function decayed, SD waves were smaller and slower, and eventually failed to enter the region of dead neurons around the FC source. A moderate buildup of $[K^+]_o$ occurred that was accompanied by a sustained negative ΔV_o, even after both glia and neurons were disabled. At this time, exogenous potassium still caused negative V_o shifts as in intact tissue. The $[K^+]_o$ buildup was related to slow DC potentials. Notable differences were found, however, between K^+ and V_o shifts in SD. These results indicate that glial cells are not necessary for SD and constitute a functional barrier for SD to spread within the tissue. This may be a major factor of neuroprotection against brain insults causing SD-like potentials. Supp. DGICYT PB1257

560.2

CONTRIBUTION OF GLIAL METABOLISM DURING A MILD METABOLIC STRESS. W.J. Nicklas* and G.D. Zeevalk. Neurology Dept., UMDNJ-R.W.Johnson Med. Sch., Piscataway, N.J. 08854

Glial cells are relatively resistant to energy impairment requiring prolonged episodes of substrate and O_2 deprivation to cause damage. Little is known of the extent to which glial metabolism is affected during energy impairment and how this might influence events in the neuronal compartment. Our past studies have used an isolated chick retinal preparation to investigate mechanisms associated with energy impairment. Mild energy stress, produced by inhibiting glycolysis with iodoacetate (IOA) for periods up to 45 min, causes an acute toxicity that is predominately NMDA receptor mediated and occurs in the absence of a net efflux of excitatory amino acids (EAAs). Acute toxicity is characterized histologically by selective damage to amacrine and ganglion cells and quantitatively, by GABA release into the medium. In this study, glial metabolism was inhibited with 20μM fluorocitrate (Fl-Cit). The selective effect of this compound on metabolism in retinal glial (Muller) cells was verified by following incorporation of radiolabel from ^{3}H-acetate and 2-^{14}-C-glucose into glutamate (Glu) and glutamine (Gln). As predicted, Fl-Cit decreased incorporation of radiolabel from acetate into both Glu (by 75%) and Gln (by 85%). Incorporation of ^{14}C label from glucose into Gln was decreased by 96% (Gln synthesis occurs only in glia), but was only slightly decreased (by 22%) into Glu, (bulk of glucose metabolism to Glu is in the neuronal compartment). These studies suggest that retinal glial cells have properties similar to astrocytes i.e., 1) they preferentially take up and metabolize acetate and 2) glial metabolism is selectively inhibited by Fl-Cit. To examine the contribution of glial metabolism to acute toxicity caused by a mild metabolic stress, retina were incubated with IOA plus or minus 20μM Fl-Cit for 15, 30 or 45 min. Incubation with Fl-Cit or IOA alone did not cause a net increase in extracellular Glu or aspartate at any time point. Only IOA caused acute toxicity. When Fl-Cit was incubated with IOA, this occurred earlier (at 15 rather than 30 min) and significant increases in EAAs were now found at 30 and 45 min. The NMDA antagonist, MK-801, blocked this toxicity and the rise in EAAs suggesting that these events were subsequent to NMDA receptor overstimulation. The results suggest that under conditions of mild metabolic stress, neuronal metabolism is affected to a greater extent than is glial metabolism. Furthermore, glial cells may play a protective role in this situation, by delaying the onset of acute neuronal damage and maintaining basal levels of extracellular EAAs.
Supported by PHS Grants EY11140 and NS17360

560.3

GLUT5 MICROGLIAL RESPONSE IN ADULT AND IMMATURE RAT BRAIN FOLLOWING STROKE. K. Li, S. J. Vannucci, F. Maher, F. C. Barone, P. G. Lysko*, and I. A. Simpson. NIDDK, Bethesda, MD 20892, SmithKline Beecham, King of Prussia, PA, 19406, Department of Pediatrics, Hershey Med. Ctr.-Penn State Univ. 17033.

The GLUT5 glucose transporter is expressed in testes, sperm, and epithelial cells of the small intestine where it serves as a fructose transporter. Recently we have found GLUT5 to be expressed in macrophages, neutrophils and microglia where its function has yet to be determined. Microglial expression of GLUT5 was monitored by in situ hybridization and immunohistochemistry at various times of recovery following middle cerebral artery occlusion in the spontaneously hypertensive adult rat (MCAO) and hypoxia/ischemia in the 7 day neonatal rat (unilateral carotid artery ligation, 2.5 hrs: 8% O_2, 37°C). GLUT5, although more prominent in the neonatal rat brain, is diffusely distributed throughout the normal brains, and is located exclusively in microglia. At 1 hr of recovery following the respective insults, no differences in GLUT5 expression were detected between the ipsilateral (ILH) and contralateral hemispheres (CLH) whereas at 3 and 5 hrs a global decrease in GLUT5 expression in the ILH was detected in both models. In the MCAO animals at 48 hrs, the number of microglia and the level of GLUT5 protein and gene expression are dramatically increased throughout the ILH whereas by 5 days GLUT5 is maximally expressed at the borders of the infarct where it remains significantly elevated for at least 15 days. In the neonatal animal, enhanced GLUT5 expression around the infarct begins at 24 hrs and at 3 days is intense in the necrotic hippocampus, the thalamus and cortical infarct. The more rapid GLUT5 response is consistent with accelerated necrosis in the immature brains. The changing expression GLUT5 appears to provide a readily detectable index of microglial migration and activation which should be applicable to other brain injuries. Supported by HD31521(SJV), NIDDK, SmithKline Beecham.

560.4

ASTROCYTIC CLASMATODENDROSIS OCCURS IN HIPPOCAMPAL ORGAN CULTURE AFTER SEVERE ACIDOSIS & ENERGY FAILURE. Richard P. Kraig', P.E. Kunkler, & C.D. Lascola. Department of Neurology, The University of Chicago, Chicago, Il 60637.

Excessive acidosis may be a necessary concomitant of brain infarction. During complete ischemia under hyperglycemic conditions, astrocytes become profoundly acidotic (pH_i 4.3-5.3) while their extraglial space remains at 6.2 pH. Under these conditions, astrocytes undergo ***clasmatodendrosis*** (i.e., lose their distal processes) and yet maintain plasma membrane integrity long after loss of high energy phosphates. Although clasmatodendrosis was defined in astrocytes almost 90 years ago and its relation to acidosis (and energy failure) was studied 35 years ago, these phenomena remain largely heretical. To begin examining the mechanisms by which this form of astrocytic death develops, we sought to determine if clasmatodendrosis could be seen in hippocampal organ cultures (HOTCs), where identified cells can be followed in space and time. HOTCs were prepared from early postnatal rats and placed on Millicell™ platforms. After more than 21 days *in vitro*, cultures were loaded with the fluorescent cytoplasmic marker Calcein™. Photographs were taken of HOTCs and their surrounding mixed glial cell population (under "normal" Ringer super-fusion) every 5-10 min using computer-based digital imaging strategies. Then, Ringer was changed to an "ischemic" solution consisting of 46 mM Na lactate, 75 mM KCl, 1.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.2 mM KH_2PO_4, 1 μg/ml antimycin without glucose at pH 5.3. Process-bearing astrocytes at the edge of the HOTC showed a progressive shortening of distal processes and enlargement of cell bodies consistent with clasmatodendrosis. The latter was also evident in a subpopulation of GFAP-positive astrocytes within HOTCs subsequently immunostained for GFAP.

These studies show that astrocytic clasmatodendrosis from acidosis and energy failure can be modeled *in vitro* using an intact tissue. Thus, the mechanisms responsible for this form of astrocytic death should be amenable to direct examination. (Supported by NS-19108.)

560.5

CEREBRAL HYPOXIA-ISCHEMIA IN NEWBORNS RESULTS IN RAPID DEATH OF ASTROCYTES IN STRIATUM: A MECHANISM FOR ISCHEMIC NEURODEGENERATION. LJ Martin*, A Brambrink, C Portera-Cailhau, RC Koehler, JD Rothstein and RJ Traystman. Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The neonatal striatum degenerates following transient hypoxia-ischemia (H-I) by unclear mechanisms. Here, a newborn piglet model of H-I was used to test the hypothesis that damage to astrocytes and abnormalities in the localization of glutamate transporter subtypes accompany striatal neurodegeneration. One-week-old piglets were subjected to 30 min hypoxia (arterial O_2 saturation 30%) and then 7 min of airway occlusion (O_2 saturation 5%), producing cardiac arrest, followed by cardiopulmonary resuscitation and return of spontaneous circulation. H-I and sham piglets recovered for 24, 48, or 96 hours (h). Neuronal damage in putamen was 65.7% ± 36.3% (M ± SD) at 24 h, 63.5% ± 32.9% at 48 h, and 78.7% ± 19.2% at 96 h; mean neuronal densities were reduced 21%, 32%, and 44% at 24, 48, and 96 h, respectively. By the TUNEL method, dying putaminal cells (TUNEL-positive nuclei/mm^2) were increased postischemia from a control value of 4.2 ± 4.2 (M ± SD) to 483.3 ± 350.6 at 24 h, 508.3 ± 205.3 at 48 h, and 147.9 ± 66.7 at 96 h. Glial fibrillary acidic protein (GFAP)-positive cell body densities in the H-I putamen were reduced 48%-55% at 24-48 h, but were greater than controls by 96 h. Early postischemia, GFAP-positive astrocytes were TUNEL-positive, confirming astrocyte death; later postischemia, mitotic figures were GFAP-positive, confirming astrocyte proliferation. By immunocytochemistry, astroglial glutamate transporter protein GLT1 was reduced in the putaminal neuropil at 24 h postischemia, and as GLT1 was depleted in astrocytes later postischemia, neurons became GLT1-positive. In contrast, neuronal glutamate transporter EAAC1 immunoreactivity was maintained early postischemia, but subsequent neuronal loss resulted in lower immunoreactivity. We conclude that rapid astrocytic degeneration is an important consequence of neonatal H-I that could contribute to striatal neurodegeneration through loss of astroglial glutamate transport and resulting excitotoxicity. Supported by NS 20020.

560.6

TRANSFERRIN PROTEIN AND mRNA EXPRESSION IN POST HYPOXIC-ISCHEMIC NEONATAL RAT BRAINS. J.R. Connor, S.L. Menzies and C. Palmer* Depts. of Neuroscience & Anatomy and Pediatrics*, M.S. Hershey Medical Center, Penn State University, Hershey PA 17033

Within 4 hours after hypoxic-ischemic (HI) brain injury in the 7-day old rat (PND7), there is increased stainable iron in the region of tissue injury. Iron when not protein bound is capable of generating free radicals. The response of the iron transport protein, transferrin (Tf), may play a vital role in protecting the brain from damage by binding iron and removing it from the damaged area. In this study, HI brain injury was produced by ligating the R common carotid artery of PND7 rats then subjecting them to 2.25h hypoxia in 8% oxygen. The brains were examined at different time periods post insult. In control animals, Tf mRNA is expressed in choroid plexus and diffusely throughout the rest of the brain at PND 7. By PND 10, Tf mRNA is predominantly in white matter. The normal pattern for Tf mRNA labeling is severely altered by insult. Within 72 hrs of the insult, there are columns of Tf mRNA expressing cells in the cortex on the ischemic side. At the protein level, there is an increase in Tf in astrocytes by 4hrs post insult in those cells with processes surrounding neurons in gray matter. Tf positive astrocytes in columns were still present 3 weeks after the insult. These data indicate the astrocytes are involved in the hypoxic/ischemic response and that involvement includes immediate expression and perhaps uptake of Tf, presumably for removal of iron. Supported by HD30704 (NIH)

560.7

RAPID INDUCTION OF PREPROENDOTHELIN-1 mRNA IN BOTH ASTROCYTES AND CEREBRAL ENDOTHELIAL CELLS OF HYPOXIC-ISCHEMIC MOUSE BRAIN. M. C. S. TSANG[1], K. T. Lam[2], P. T. CHEUNG[2] and S. K. CHUNG[1]. [1] Institute of Molecular Biology, [2] Dept. of Pediatric, The University of Hong Kong, Hong Kong. (Spon HKSN)

The endothelin(ET)-1, a potent vasoconstrictor, has been regarded as a neurotransmitter or neuromodulator since ET-1 and its binding sites can be detected in neurons of the central nervous system (CNS) and intra-cerebroventricular infusion of ET-1 can modulate CNS function. Also, it was shown that immunoreactive ET-1 can be detected in the ischemic rat brain, suggesting its possible role in ischemic-related pathogenesis. Here, we study the expression pattern of preproendothelin-1 (PPET-1) mRNA in hypoxic-ischemic young mouse brain which was induced by permanent occlusion of right carotid artery under isoflurane. The animals were then exposed to 8% O_2/ 92% N_2 for 30 mins. The appropriate lesion sites were obtained at 2 hours, 1 and 2 days. At the level of hippocampus of all normal mouse brain, PPET-1 mRNA was detected only in the thalamic neurons and endothelial cells of vessels and capillaries. On the other hand, PPET-1 mRNA was strongly expressed by both the endothelial cells and astrocytes, not only on the ligated side, but also on the non-ligated side of ischemic mouse brain at 2 hour post-insult. The strongest expression was seen in hypothalamus, thalamus and hippocampus with moderate levels in the cortex. One day after insult, the PPET-1 mRNA expression was limited to the CA1 and parietal cortex ipsilaterally. By 2-day post-insult, the PPET-1 mRNA expression level in both astrocytes and endothelial cells was much less than that of 2 hours post-insult. Degenerated neurons did not show any PPET-1 mRNA expression. These up-regulation of PPET-1 mRNA by the endothelial cells and astrocytes and their cell-cell interaction may play an important role in gliosis and /or changes in blood-brain barrier function associated with post-asphyxial response.

[Grant support: RGC of HKU 220/95M]

560.8

HYPOXIA SENSING MECHANISMS IN HUMAN GLIOMA CELLS

Robert A. Forbes, Paul W. Harris, Stephen L. Facchina, Sandip R. Vaidya and Ajay Verma*Depts. of Neurology and Neuroscience,Uniformed Services Univ. of the Health Sciences.Bethesda, Maryland 20814

Hypoxic gliomas induce neovascularization through the elaboration of vascular endothelial growth factor (VEGF). Hypoxia stimulates VEGF gene expression via transcriptional activation of the VEGF gene as well as an increase in the stability of VEGF mRNA. Sequence specific protein-nucleic acid binding activities figure prominantly in these hypoxia regulated events. The 5' enhancer region of the VEGF gene has recently been shown to contain an octamer homologous to the hypoxia-inducible-factor-1 (HIF-1) consensus recognition element found in other hypoxia sensitive genes such as erythropoeitin. The VEGF mRNA 3'UTR region contains several consensus sequences (AUUUA) known to influence mRNA half-life of other growth factors and cytokines. We have examined the ability of human U87 glioma cells to respond to hypoxia by elaboration of VEGF immunoreactivity, activation of HIF-1 consensus element binding activity in nuclear extracts, and by the activation of -AUUUA- sequence specific RNA binding proteins in cytoplasmic extracts using gelshift assays. Treatment of glioma cells with 1% oxygen for 12 hrs induces HIF-1 consensus sequence binding in nuclear extracts and stimulates -AUUUA- binding activity in cytoplasmic extracts. Hypoxic cells also show a prominant increase in immunoreactive VEGF. Stimulation of VEGF staining and HIF-1 activation was also elicited by cobalt chloride, an agent which has been shown to mimic hypoxic transcriptional activation in other models. Cobaltous ions were not, however, able to stimulate cytoplasmic RNA binding activity. These results suggest that in human gliomas the hypoxic mechanisms regulating transcription and mRNA stability may use distinct signalling pathways.

560.9

OPTICAL IMAGING OF ACUTE ISCHEMIC INJURY IN HIPPOCAMPAL SLICE CULTURES. A. Barth, L. B. Nguyen, L. Barth, J. T. Ho, D. W. Hochman, D. W. Newell*. Dept. of Neurological Surgery, Univ. of Washington Sch. of Med., Seattle, WA 98195.

To investigate acute cellular events during ischemic insult, we measured light transmission (LT) changes through organotypic hippocampal slice cultures exposed to increasing periods of anoxia/hypoglycemia (Ax/Hg). Resulting cell death was assessed by propidium iodide fluorescence 48 hours later. During the initial 15 min of ischemic exposure, only a slight LT decrease of 3-5% was observed and all cultures survived. Further ischemia produced marked darkening of the cultures and a sharp LT decrease of 20±4% was measured after 20 min of Ax/Hg, which plateaued then until maximal Ax/Hg exposure of 60 min. This sharp decrease of LT heralded irreversible cellular damage which resulted in complete dying of the hippocampal cultures after 40-50 min of Ax/Hg. Histological examination showed that LT changes during ischemic exposure corresponded to an increase in cell volume suggesting neuronal-glial swelling. These acute changes were completely blocked when the experiments were performed in a low-chloride medium, indicating that transmembrane movements of chloride and sodium ions were involved in cell swelling and LT decrease. We conclude that this optical imaging method is suitable to study and quantify early neuronal and glial swelling associated with ionic fluxes from ischemia and to screen substances which may influence these acute events. Supported by the Swiss Foundation for Biomedical Grants and Ciba-Geigy Jubilee-Foundation (A.B.), and NIH Grant 1P50NS30305-1 and CIDA 1K08NS01596901 (DWN).

560.10

CORRELATION OF FIELD RECORDINGS AND INTRINSIC OPTICAL SIGNALS IN HYPOXIC HIPPOCAMPAL SLICES. A.S. Obeidat* and R.D. Andrew, Dept. of Anatomy and Cell Biology, Queen's University, Kingston, Ontario K7L 3N6

Particularly in the cerebral cortex, cell swelling is a serious consequence of ischemia arising from stroke or trauma. Cell swelling can be indirectly assessed by measuring elevated light transmittance (LT) through the brain slice. Conversely, a reduction in LT indicates reduced cell volume (*Neuroscience 62, 371-383,* 1994). Intrinsic optical signals (a large component representing LT) were measured using Axon Imaging Workbench (Axon Instruments). We studied superfused hippocampal slices where O_2 gassing the saline was replaced with N_2 and/or glucose was reduced from 11 to 2 mM for a period of 10 min. LT in the CA1 region and the evoked CA1 field potential were simultaneously recorded. Neither hypoxia (n = 12 slices) nor lowered glucose alone (n = 4 slices) evoked obvious change in LT or in the evoked field at 37.5°C. However in 11 slices deprived of both oxygen *and* glucose, a dynamic sequence of LT changes and field potential changes occurred across the CA1 region. Between 2 to 4 min of stress, the evoked field potential was lost, immediately prior to the onset of LT elevation in CA1 dendritic regions. LT continued to increase by 50-70% (cell swelling) but by 9-10 min the signal reverted, returning to baseline and then *decreasing* by 40-60% (shrinkage). At the same time LT *increased* by 50-68% in the CA1 cell body region. This sequence of dendritic shrinkage/cell body swelling was associated with an irretrievable loss of the evoked field potential in 4 of 5 slices tested. Lowering the temperature to 30°C during the recovery period following the hypoxia/low glucose insult resulted in a small, partial recovery of the field potential but no obvious changes in LT.

We conclude that during acute ischemic stress, CA1 dendrites first swell then rapidly shrink with concurrent cell body swelling that is irreversible. This sequence represents neuronal death as indicated by a permanent loss of the evoked CA1 field potential. Induction of the sequence requires *both* hypoxia and low glucose and once initiated is not significantly affected by lowered temperature.

Supported by the Heart & Stroke Foundation of Ontario.

560.11

PLASMA BUT NOT PACKED RED BLOOD CELLS INFUSED INTO PIG CEREBRAL WHITE MATTER INDUCES RAPIDLY DEVELOPING, MARKED EDEMA. G. Xi*, K.R. Wagner, Y. Hua, G. de Courten-Myers, R.E. Myers. Depts. of Neurology and Pathology, Univ. Cincinnati College of Medicine; Dept. of Veterans Affairs Medical Center, Cincinnati, OH 45220.

The "toxicity" of blood is hypothesized to be responsible for edema formation and tissue injury following intracerebral hemorrhage (ICH). Recently, we reported that autologous blood infused into cerebral white matter in pigs produced rapidly developing (1 h), marked edema (>10% increases in water contents) adjacent to hematomas (*Stroke* 34:450-459, 1996). This edema co-localized with interstitial fibrinogen and other plasma protein accumulations despite an intact blood-brain-barrier. Presently, we compared the ability of the blood's plasma and red cell components to induce early edema development.

We infused (2.5 ml) plasma, washed packed red cells (87% hematocrit) or whole blood into the hemispheric white matter of pentobarbital-anesthetized pigs (9-11 kg) over 15 minutes. We monitored blood and cerebral tissue pressures and controlled core temperature, blood gases and pH. We froze brains *in situ* at 1 to 24 h and determined edema volumes and water contents.

Infused plasma from low (but not high)-speed centrifugations of whole blood coagulated and induced rapid (1 h), marked edema in white matter adjacent to and distant from the plasma clots. This edematous white matter was physically changed ("translucent") in appearance due to >10% increases in water contents. No edema was observed at any time point following red blood cell infusions (N=3). In contrast, edema volumes were comparable (at 24 h) with plasma (N=3) and whole blood (N=6) infusions (6.89±1.63 vs. 5.67±1.02 cm^3).

These results which demonstrate that the plasma and not the red cell component of the blood is responsible for early edema development, provide a new direction for therapeutic approaches to prevent white matter injury following ICH. *Supported by NIH NS-30652.*

560.12

NEUROPATHOLOGIC OUTCOMES ARE SIMILAR FOLLOWING INFUSION OF PLASMA OR WHOLE BLOOD INTO PIG CEREBRAL WHITE MATTER. K.R. Wagner*, G. Xi, Y. Hua, G. de Courten-Myers, R.E. Myers. Depts. of Neurology and Pathology, Univ. Cincinnati College of Medicine; Dept. of Veterans Affairs Medical Center, Cincinnati, OH 45220.

Several pathophysiologic mechanisms are proposed to be responsible for brain tissue injury following intracerebral hemorrhage (ICH) including the "toxicity" of blood. Previously, we demonstrated marked perihematomal edema and interstitial plasma protein accumulation following whole blood infusions into white matter in pigs (*Stroke* 34:450-459, 1996). Based on these results, we hypothesized that the blood's plasma component plays an important role in the brain pathologic outcome following ICH.

To test this hypothesis, we infused (2.5 ml) autologous whole blood (N=6) or plasma (N=6) separated by low-speed centrifugation into the frontal hemispheric white matter of pentobarbital-anesthetized pigs (9-11 kg) over 15 min. We monitored blood and cerebral tissue pressures and controlled core temperature, arterial blood gases and pH. We perfusion-fixed brains at 7 or 14 days *in situ* with formalin and stained cut sections with Luxol fast blue/H&E.

In plasma-infused brains fibrin clots comparable to hematomas in whole blood-infused brains occupied the white matter. At 7 days, marked edema continued to be present both adjacent to the mass and in adjoining gyral white matter. Neovascularization was present near the masses' border and macrophage infiltration was present at the masses' edge. Astrogliosis and myelin pallor were prominent. After 14 days, resorbed masses left fluid-filled cysts surrounded by a large rim of gliotic and atrophic demyelinated white matter.

These results demonstrate that plasma alone is able to produce a neuropathologic outcome similar to whole blood suggesting a new therapeutic target to prevent white matter injury following ICH. *Supported by NIH NS-30652.*

560.13

OXYGEN AND GLUCOSE DEPRIVATION-INDUCED SWELLING IN RAT AND HUMAN NEOCORTICAL SLICES. J.L. Werth* and S.M. Rothman. Department of Neurology, Washington University School of Medicine, St. Louis, MO 63110.

Considerable experimental evidence implicates excessive glutamate receptor activation in ischemic neuronal death. We sought to determine if glutamate overload contributes to neurotoxicity in human neocortex. We developed a technique that is amenable to studying human brain tissue obtained from neurosurgical procedures. 400 μm thick brain slices were cut from rat brain and human neocortical biopsies. Tissue impedance was monitored by passing current (4μA, biphasic square wave) between two electrodes and recording the voltage drop across the tissue differentially (Traynelis & Dingledine, J. Neurophys. 61: 927-38). The magnitude of the voltage drop is a function of extracellular space and therefore indicative of cellular swelling. While swelling is not the cause of excitotoxic neuronal death, it is indicative of excessive activation of glutamate receptors. In experiments conducted on brain slices from rat, NMDA induced a 2-5 fold increase in tissue impedance that was blocked by MK801. Oxygen and glucose deprivation (OGD) also increased impedance. Treatment with 5μM MK801 but not non-NMDA receptor antagonist NBQX delayed and lessened the rise in impedance following OGD. Swelling was due largely to Na^+ influx as lowering extracellular Na^+ from 155 mM to 55 mM diminished but did not delay the increase in impedance. In slices from human neocortex, NMDA triggered a rise in impedance that was attenuated by MK801. OGD also led to increased impedance that was delayed by 5μM MK801. The data support the hypothesis that overactivation of NMDA-type glutamate receptors follows OGD in human and rat brain. Supported by grant RO1NS19988 from NINDS.

560.14

A Photothrombotic Stroke Model with Penumbra - Evolution of Perfusion Deficit, Vasogenic Edema and Ischemic Volume
WeiGang Gu and Per Wester*, Dept Medicine, Univ. Umeå, Sweden

This study aimed at further characterization of a laser-induced photochemical thrombotic stroke model with a large area-at-risk (Stroke 1995: 26; 444-450), now slightly modified to achieve spontaneous reperfusion. Halothane-anesthetized temperature and blood gas controlled adult male Wistar rats (270-350g) were subjected to a ring-shaped (outer Ø 5.0 mm and 0.35 mm thick) laser-irradiation beam (514.5 nm; 0.9 W/cm^2) focused on the somatosensory cortex for 2 min simultaneously with iv. infusion of the photosensitizing dye erythrosin B [17 mg/kg] for 30 s. At different times post-irradiation (p.i.), perfusion deficits were visualised by transcardial carbon black perfusion; cortical water content (wet/dry weight) was measured; and infarction volumes were calculated after transcardial FAM-perfusion and evaluation of multiple coronal sections. A ring-shaped cortical perfusion deficit was observed at 4 hr p.i. which progressively increased at 10 - 24 hr's to reach its maximum at 48 hr's with an almost complete perfusion deficit. At 72 hr's p.i., a spontaneous reperfusion was observed in the area-at risk, which was even more pronounced at 5 and 28 days p.i. Brain water content increased steadily from 4 - 24 - 48 hr's p.i. vs. sham ($p<0.01$ by ANOVA and post-hoc); at 72 hr's it levelled off and returned to control values at 7 days p.i. Ischemic volume steadily increased from 4 - 10 - 24 hr's to reach its maximum at 48 hr p.i. ($p< 0.01$). At 3 - 7 - 28 days p.i., infarction volume was gradually reduced to less than 50% compared with 48 hr's p.i. ($p<0.01$). This novel stroke model, now modified by use of a thinner laser ring beam (0.35 instead of 0.50 mm) displays a more delayed ischemic progress in the area-at-risk (penumbra) with late spontaneous reperfusion and a remarkable late tissue recovery.

560.15

TIME COURSE OF EDEMA DEVELOPMENT IN THE RABBIT THREE VESSEL OCCLUSION MODEL. M.A. Widmayer,* J.L. Browning, J.M. Keck and D.S. Baskin, Dept. of Neurosurgery, VAMC and Baylor College of Medicine, Houston, TX 77030

In the cat, we have demonstrated that κ-opioid agonists and an AVP V_2 antagonist reduce ischemia-induced edema and improve survival, tissue damage, and neurologic function. Before evaluating the efficacy of these drugs in the rabbit model, we evaluated the time course of development of edema.

Thirty-six SPF, male, NZW rabbits underwent occlusion of 2mm of the right internal carotid, and middle and anterior cerebral arteries (3VO). Six animals were assigned to be sacrificed at either 6, 12, 18, 24, 36 or 48 h post-occlusion. Four mm sections of the brains were immersed in a percoll density gradient to determine specific gravity, and then stained with TTC to assess infarct volume.

Edema and tissue damage were apparent in the right hemisphere by 6h and increased until 24h ($p < 0.05$, each). Mortality was substantial after 30h. Small sample size resulted in unreliable edema and infarct measurements at the 36h and 48h time points, although there was no additional increase in either measure at the later time points. Therefore, in this 3VO model, sacrifice at 24h is preferable. This research was supported by a VA Merit Review Grant.

ISCHEMIA: GLUCOSE, pH, AND TEMPERATURE

561.1

EFFECT OF INCREASING INTERVALS OF HYPOXIA-ISCHEMIA ON GLUT1 AND GLUT3 GLUCOSE TRANSPORTERS AND INFARCTION IN PERINATAL RAT BRAIN. Susan J. Vannucci, Lisa B. Seaman, Robert M. Brucklacher and Robert C. Vannucci*, Dept. of Pediatrics, Hershey Med Ctr-Penn State Univ., Hershey, PA 17033.

Glucose is the primary fuel for the immature brain and the only fuel able to sustain anaerobic glycolysis. Glucose is transported into brain by the glucose transporter proteins: GLUT1 (55 kDa), blood-brain barrier; GLUT3, neurons; 45 kDa GLUT1, glia. GLUT1 & 3 are low in the 7 day old (P7) rat and limit cerebral glucose utilization. Prolonged (150 min) cerebral hypoxia-ischemia (H/I) in the P7 rat has profound effects on GLUT1 and GLUT3 expression during the early recovery period and produces severe neuronal necrosis/ cerebral atrophy by 30 days. The purpose of this study was to assess the effects of shorter intervals of H/I on GLUT1 & 3 expression and long-term outcome. P7 rats were subjected to right carotid artery ligation followed by 8% O_2 for 60, 90, 120 and 150 min. Brains were analyzed for GLUT1& 3 mRNA expression at 1, 24, 48 & 7 hrs of recovery. Littermates were analyzed at 30 days for neuropathology. 60 min of H/I produces neuronal damage in 25% of pups; 50% were damaged by 90 min. Both intervals produced increases in GLUT1 mRNA in both hemispheres by 1 hr which normalized by 24 h of recovery, followed by mild reductions in the infarcted. The loss of GLUT3 expression, related to neuronal death, was confined to cortical and hippocampal areas in the affected pups. H/I intervals of 120 and 150 min produced more severe damage in 75-90% of pups, with significant atrophy and cystic cavitation. Neuronal injury was apparent in widespread GLUT3 loss in both groups, whereas GLUT1 loss was not as severe following 120 min. Cerebral H/I at P7 produces increasing neuronal injury with increased duration. The early GLUT1&3 responses following shorter intervals of H/I may provide insight into neuroprotection. **Supported by HD-30704 & HD-31521, NICHD.**

561.2

GLUCOSE ANALOGUES IN ISCHEMIC BRAIN SLICES: 3-O-METHYL-GLUCOSE DIFFUSION AND EFFECTS OF LOW GLUCOSE ON THE LUMPED CONSTANT. **G.C.Newman*, F.E.Hospod, C.S.Patlak. Depts. of Neurology and Surgery, SUNY at Stony Brook and Northport VAMC, NY.**

In an effort to understand glucose metabolism in the ischemic penumbra, we have studied 1) ^{14}C-3-O-methylglucose (3OMG) kinetics and 2) the lumped constant (LC) for ^{14}C-2-deoxyglucose (2DG), in 450μm and in 1050μm thick hippocampal slices which spontaneously express features of the ischemic penumbra. 3OMG kinetics were analyzed at 5 mM glucose using a model of extracellular (ECS) diffusion with two serial compartments. The lumped constant was calculated in 450μm slices using the ratio of volumes of distribution for ^{14}C-glucose (V_d) and 2DG (V_d^*) after exposure to buffer with glucose between 0.25 and 10 mM.

3OMG kinetics differ dramatically in 450μm and 1050μm slices. As with ^{14}C-PEG, ^3H_2O and ^{45}Ca, the ECS diffusion coefficient for 3OMG in thick slices is similar to that of bulk solution (5.9±2.1 cm^2/sec) while that in thin slices is much smaller (1.8±0.2 cm^2/sec), consistent with our hypothesis that brain ECS is non-homogeneous. In addition, the second serial compartment is much larger in 1050μm slices (0.14±.08 ml/g) than in 450μm slices (0.045±.001 ml/g). Anion exchange chromatography reveals no 3OMG phosphorylation, suggesting that glucose transport into an unidentified compartment is selectively increased during ischemia.

V_d for ^{14}C-glucose falls much more than V_d^* for 2DG below 2 mM glucose so that LC increases from 0.3 at 10 mM to 1.2 at 0.25 mM, similar to observations *in vivo*. SGU decreases from 40 to 5 μmole/100g/min as glucose is lowered from 10 to 0.25 mM. Thus, brain slices provide an excellent model of cerebral metabolism under conditions of limiting glucose. Support: VA Merit Review and NIH #NS28429.

561.3

CONCENTRATION EFFECTS OF ISCHEMIC FACTORS ON HISTOLOGY, ADENYLATES, GLUCOSE UTILIZATION AND $^{45}Ca^{+2}$ IN HIPPOCAMPAL BRAIN SLICES. F.E.Hospod*, H.Qi, S. Motwani, S.Trowbridge, G.C.Newman. Dept. of Neurology, SUNY, Stony Brook, NY and VAMC at Northport, NY.

Our goal is to understand the neurochemistry of the ischemic penumbra. Our model employs 450μm thick rat hippocampal slices exposed to buffer with varying hypoxia, pH, K^+ and glutamate. Brain slices are studied by measurement of histologic injury, adenylates with HPLC, slice glucose utilization (SGU) with ^{14}C-2DG or $^{45}Ca^{+2}$ spaces.

Hypoxia produces a predictable injury that is dependent upon the degree and duration of O_2 deprivation. Hippocampal regions are sensitive in the sequence CA1>CA2>dentate>CA3>CA4. Increasing histologic injury correlates with reduced adenylates and irreversible accumulation of $^{45}Ca^{+2}$ in a small compartment. Glutamate, up to 100μM, produces minimal histologic injury or reduction of adenylates despite increased SGU and reversible entry of $^{45}Ca^{+2}$ into larger compartments. There is no tissue injury with 8 mM K^+ despite pronounced uptake of $^{45}Ca^{+2}$, while 20 mM K^+ injures CA1 in a pattern similar to hypoxia. At K^+ of 40 or 70 mM there is severe tissue destruction with patterns never seen with other ischemic insults, in association with reduced adenylates and irreversible binding of $^{45}Ca^{+2}$. Varying pH from 7.4 to 6.2 yields histology similar to hypoxia but with less change in adenylates, inhibited SGU and reduced entry of $^{45}Ca^{+2}$ into either the large or small spaces.

Thus each factor produces a unique pattern of tissue response with a complex concentration dependence. Their interactions during ischemia *in vivo* are likely to be complex and dynamic. We gratefully acknowledge support of grants from VA Merit Review and NIH #NS28429.

561.4

CNS AXONS FROM VERY YOUNG RATS ARE HIGHLY RESISTANT TO ENERGY DEPRIVATION. R. FERN* AND B. R. RANSOM Department of Neurology, University of Washington, Seattle, WA 98195.

The neonatal CNS is subject to a variety of metabolic insults including anoxia, ischemia, and hypoglycemia. Although the neonatal CNS is relatively tolerant of these conditions, prolonged CNS ischemia results in severe neurological damage that is typified by lesions of CNS white matter. We have found that white matter from very young rats (P0-P4) is highly tolerant to energy deprivation; in particular to joint withdrawal of oxygen/glucose (ie. ischemia), as assessed by changes in the evoked compound action potential recorded from the isolated rat optic nerve. CNS axons from >P4 rats were less tolerant to joint anoxia/aglycemia than they were to aglycemia alone. This may indicate that metabolism of lactate, possible only in the presence of oxygen, may assist the survival of CNS axons during glucose withdrawal after P4. It has been shown previously than astrocytes convert their intracellular glycogen store into extracellular lactate following the withdrawal of glucose and lactate can support action potential conduction in CNS white matter.

2-deoxyglucose (DOG), which will prevent the conversion of glycogen to lactate, reduced the tolerance of CNS axons to aglycemia at all ages. Recovery from aglycemia in P0-P4 axons was significantly less in DOG, suggesting that mobilization of glycogen was important for the high tolerance to energy deprivation at this age. The observations that P0-P4 axons can tolerate joint anoxia/aglycemia (where lactate produced from astrocytic glycogen can not be metabolized) but not aglycemia in the presence of DOG (where no glycogen can be utilized) suggests that axons contain glycogen at this age point, which contributes significantly to a hightened tolerance to energy deprivation. Supported by the NIH.

561.5

THE EFFECT OF GLUCOSE ON BRAIN ATP, LACTATE, AND GLUTAMINERGIC SYSTEM IN THE NEWBORN PIG. M.H. LeBlanc*, X.B. Qian, Z.Cai. Dept.of Pediatrics, University of Mississippi, Jackson, MS 39216.

We have shown that glucose worsens hypoxic ischemic brain injury in the 0-3 day old piglet. Using this same protocol 35 piglets were randomly assigned to have their glucose increased to 300 ± 30 SE mg/dl with glucose infusion G, (n=12) or decreased to 67 ± 16 mg/dl with insulin I (n=13) or a sham group S (n=10). In the I and G groups at time 0, both carotid arteries were clamped and blood was withdrawn to reduce the blood pressure to two-thirds normal. At time 15 minutes F_iO_2 was reduced to 6%. At time 30 minutes, the brains were frozen in situ in liquid nitrogen, then removed and stored in liquid N_2. The shams were anesthetized and their brains were frozen in liquid N_2 using an identical procedure but without inducing hypoxia or ischemia. Brains were homogenized and lactate and ATP were measured enzymatically, CSF amino acids by HPLC and H{3} MK801 binding by Scatchard Analysis. Brain ATP was 1.7 ± .09 mmoles/kg wet wt. in the S, 0.98 ± 0.09 in the G, and 0.52 ± 0.10 in the I, (all differences statistically significant.p<.01). Brain lactate levels were 4.3 ± 1.0 mmoles/kg wet wt. in the S, 29.4 ± 2.6 in the G, and 18.3 ± 1.9 in the I, (all results statistically different p<.01). CSF glutamate was 9.3 ± 3.6 micro-M in G, 9.6 ± 3.5 in I, and 2.2 ± 0.9 in S (G+I>S, p<.05). Glycine was 65 ± 13 micro-M in G,80 ± 15 in I and 84 ± 17 in S (p=ns). MK801 binding showed a K_d of 6.3 ± 0.4 nM in 6, 6.2 ± 0.3 in I, and 6.0 ± 0.6 in S (p=ns), and a B_max of 2.3 ± 0.2 pMol/mg protein in G (p=ns), 2.6 ± 0.1 in I (p<.05 vs S), and 2.0 ± 0.2 in S. Although providing additional glucose preserves cellular ATP during hypoxic ischemic brain injury in the newborn pig, it does not change the glutaminergic system, and it worsens outcome by elevating cellular lactate levels above the threshold for cellular injury.

561.6

THE ROLE OF HYPERGLYCEMIA IN CEREBRAL ISCHEMIC DAMAGE: AN *IN VIVO - IN VITRO* STUDY

M.M. Li, R.S. Payne, K.H. Reid[a], J.J. Miller[b], V.G. Iyer[c], M.T. Tseng[a], B.M. Rigor* and A. Schurr. Depts. of Anesthesiol, Anat Sci & Neurobiol[a], Pathol[b], and Neurol[c], University of Louisville, School of Medicine, Louisville, KY 40292

Numerous studies have demonstrated a deleterious effect of hyperglycemia on the ischemic brain. In a rat model of chest compression-induced global ischemia, glucose administered 15 min prior to compression induced audiogenic seizures and neuronal damage in surviving rats (*Epilepsy Res.* in press). This study evaluated the relationship between timing of glucose administration, its blood level just before induction of ischemia and the incidence of audiogenic seizures and neuronal damage.

Fasted (24 h) Long-Evans rats, were divided into two groups based on time of glucose injection (15 min or 120 min prior to chest compression) with 3 glucose doses (0, 2, 4 g/kg, i.p.) per group. Procedures for chest compression and testing for audiogenic seizures were as described elsewhere (*Brain Res.* **689**:159, 1995). Each rat was tested for the presence of audiogenic seizures 24 h post-ischemia and hippocampal slices were prepared 7 days after chest compression (*Life Sci.*57:2425, 1995). Slices in which a CA1 population spike of >10 mV (normal PS) was evoked were considered undamaged; those with a PS < 10 mV or no PS were considered damaged. Blood was sampled for glucose determination 3 min pre-chest compression.

CA1 neuronal damage varied with both glucose dose and time of administration

	15-min Pre-ischemia		*120-min Pre-ischemia*		
	glucose	*normal*	*glucose*	*normal*	
Treatment	*(mg/dl)*	*PS (%)*	*(mg/dl)*	*PS (%)*	X^2
Saline	58±0.9	36	57±0.6	38	N.S.
2g/kg gluc	77±0.8	20	93±1.8	66	p < .001
4g/kg gluc	102±1.0	17	130±1.2	35	p < .05

Seizure incidence was similar for both 2 and 4 g/kg glucose, but was significantly higher for the 15-min group (12/14) than for the 120-min group (1/9). Thus, elevated blood glucose alone cannot account for hyperglycemia-induced increase in ischemic damage.

Funded in part by University of Louisville, School of Medicine Research Committee.

561.7

CHANGES IN LABILE ENERGY METABOLITES, REDOX STATE AND INTRACELLULAR PH IN POSTISCHEMIC BRAIN OF NORMO- AND HYPERGLYCEMIC RATS. K.Katsura*, J.Folbergrová, and B.K. Siesjö. Lab. for Exp. Brain Res., Lund Univ. Hosp., S 221 85 Lund, Sweden and Sec. Dep. of Int. Med., Nippon Medical School, 102 Tokyo, Japan

The present experiments were undertaken to study whether preischemic hyperglycemia, which is known to exaggerate ischemic damage and to trigger delayed postischemic seizures, affects the bioenergetic state of brain tissue at early (6h) and late (18h) recirculation times. To that end, normo- and hyperglycemic rats were subjected to 10 min of forebrain ischemia, and neocortical tissue was frozen in situ for analyses of labile energy metabolites.

Animals with preischemic hyperglycemia, subjected to 10 min of ischemia and allowed recirculation periods of 6 or 18 hrs, failed to show a reduction of the phosphorylation state of the adenine nucleotide pool, or a rise in tissue lactate content, nor did they show a change in tissue redox state. However, the hyperglycemia led to a rise in phosphocreatine (PCr) content at 6h. Calculations of intracellular pH (pH_i) from the creatine kinase (CK) equilibrium showed a rise in pH_i above normal, a finding which was verified by a limited number of 5,5-dimethyl[2-^{14}C]oxazoline-2,4-dione (DMO) measurements. The preischemic hyperglycemia also blunted the postischemic rise in tissue glycogen content, the results suggesting an inverse correlation between pH and glycogen content.

The results thus fail to reveal that the hyperglycemia-triggered, massive exaggeration of ischemic brain damage, which is heralded by generalized seizures after 18-24 hrs of recirculation, is preceded by mitochondrial dysfunction of a degree which affects the bioenergetic state or the redox potential of the tissue. However, the results suggest that the hyperglycemia enhances and/or prolongs the postischemic alkalosis.

561.8

Modulation of NMDA toxicity and in vitro ischemic damage by pH and free radical scavengers. K.M. Raley-Susman*, D. Do, M. Monje and S. Cladis, Dept. of Biology, Vassar College, Poughkeepsie NY 12601

NMDA receptor activation contributes to the damage seen following ischemic insults, via elevations in calcium and exacerbation of free radical production. The receptor function can be modulated by magnesium ions, pH and oxidation state. Thus, manipulation of these parameters may alter the outcome of an ischemic or excitotoxic insult. In this study, we explored the effects of in vitro ischemia or NMDA receptor activation on protein synthesis, morphology and cytoskeletal protein expression in the rat hippocampal slice preparation. We exposed slices from adult rats to either 5 min in vitro ischemia or to 5, 15, 30 min exposure to NMDA (30, 50, 100, 500 μM). In some experiments, exposures occurred in media of different pH (6.0, 6.5, 8.0). In other experiments, exposures occurred in the presence of ascorbic acid (400, 800 μM, 1.2 mM). Slices were allowed to recover for two hours and then protein synthesis was assayed autoradiographically (^{3}H-leucine, 4.5 μCi/ml) and neurons from 5 μm methacrylate sections were scored on a morphology scale. In some experiments, CA1 regions were lyophilized, microdissected, homogenized, and extracted proteins were immunoblotted with antibodies against MAP-2 or tubulin. As in our previous work, in vitro ischemia caused a persistent inhibition of new protein synthesis (Raley-Susman and Lipton, Br. Res. 515: 27. 1990), a severe morphological impairment and a specific depletion of MAP2 and tubulin (Raley-Susman and Murata, Br. Res. 694: 92. 1995). Manipulation of pH during this insult did not alleviate the damage (Kaja et al, NS Ab. 21. 1995). In contrast, ascorbic acid attenuated the damage (15-33%) to morphology. Ascorbic acid treatment also alleviated the ischemic depletion of tubulin and MAP-2. NMDA exposure caused a dose-dependent, magnesium-sensitive inhibition of protein synthesis and impairment of morphology.

IBN-93-19433

561.9

Acidosis induces necrosis and apoptosis in cultured hippocampal slices P.J. Bergold*[1,2], S.I. Moskowitz[2], R. Li[2], T.-S. Liu[1], S.B. Lee[3], M. Esteban[3], H.J. Federoff[4], K. Tomaselli[5], I. Chan[3], and D. Ding[1] Depts. of Pharmacology[1], Neuroscience[2], Biochemistry[3], and Neurology[3], SUNY-HSCB, Brooklyn, NY 11203; [4]Dept. of Neurology, U. of Rochester Medical School, Rochester, NY 14642; [5]IDUN Pharmaceuticals, Inc., San Diego, CA 92121

Acidosis, hypoxia and hypoglycemia rapidly and transiently appear after reduction of cerebral blood flow. These metabolic insults, individually or in combination, result in necrotic and apoptotic neuronal loss. We previously demonstrated that transient acidification of intracellular pH from 7.3 to 6.6 induces delayed neuronal loss in cultured hippocampal slices. We now report that a 30 minute acidosis treatment induced both necrosis and apoptosis that was temporally, morphologically, and pharmacologically distinct. Neuronal loss 8 hours after a 30 minute acidosis treatment was largely necrotic while neuronal loss at 16 hours was largely apoptotic. Apoptotic neuronal loss was accompanied by DNA fragmentation and was blocked by inhibitors of protein and RNA synthesis, ectopic expression of the anti-apoptotic gene bcl-2, or an inhibitor of proteases related to interleukin 1B converting enzyme (ICE). Necrotic neuronal loss was unaffected by these treatments. In contrast to the apoptosis and necrosis induced by the 30 minute treatment, a 15 minute acidosis treatment predominantly produced apoptosis. These data suggest that: (1) induction of apoptosis did not depend upon prior induction of necrosis and (2) necrosis has a higher threshold for induction than apoptosis. These data also suggest that acidosis contributes to both necrotic and apoptotic neuronal loss after stroke. Supported by HD31300 to H.J.F and P.J.B.

561.10

HYPOTHERMIC NEUROPROTECTION IN AGED GERBILS. D. Corbett*, S. Nurse and F. Colbourne. Basic Med. Sciences, Fac. Med., Memorial Univ., St. John's, NF, Canada A1B 3V6.

Previous studies from this laboratory (Nurse & Corbett, J.Neurosci., 1994, 14 : 7726, Colbourne & Corbett, J. Neurosci., 1995, 15: 7250) have shown that mild intra-or prolonged (i.e. 12-24 hr) post-ischemic hypothermia conveys long lasting (1-6 months) protection against CA1 ischemic injury. However, all hypothermia studies have used very young animals (~ 3-6 months old). Since stroke incidence rises sharply in late middle age and neuronal plasticity and many other factors change with aging, we decided to evaluate the efficacy of hypothermia in 18-20 month old gerbils.

Gerbils were exposed to a 5 min episode of global ischemia or sham occlusion. One group was occluded at a brain temperature of ~32°C. A second ischemic group was maintained at normothermia during occlusion and the first hour of reperfusion. Beginning 1.0 hour after occlusion, these gerbils were gradually cooled to 32°C and maintained at this level for 24 hours before gradual rewarming to 37°C. The third ischemic group was maintained at normothermia during surgery and the first hour of reperfusion.

Both intra and post-ischemic hypothermia provided robust protection (91.6% and 78.2% respectively; $p<.001$) of hippocampal CA1 neurons when assessed 30 days after ischemia. These data suggest that mild hypothermia may be as effective in old as in young gerbils.

Supported by the Heart and Stroke Foundation of Newfoundland

561.11

DNA FRAGMENTATION AND ITS TIMECOURSE IN NORMO VS. HYPOTHERMIC TRANSIENT FOREBRAIN ISCHEMIA IN THE RAT. M. Drake* and T. Wieloch. Lab. for Experimental Brain Research, University Hospital, Lund 221 85, Sweden.

This study was performed in order to investigate the differences in DNA fragmentation after normothermic- and hypothermic global ischemia, in the hippocampus and the striatum. To study this phenomenon, we used conventional gel electrophoresis after incorporation of ^{32}P labeled nucleotide at 3'-OH ends on DNA fragments, pulsed-field-gel-electrophoresis and the end-labeling of DNA-fragments *in situ* (TUNEL). As previously shown by others (MacManus *et al,* J Cereb Blood Flow Metab, 15, 728-737 (1995)), we observed a similar oligonucleosomal fragmentation pattern of the DNA which peaked at around 48 hours in the striatum and at 72 hours in the hippocampus. Hypothermia completely prevented DNA fragmentation in the CA1 region. The early fragmentation of DNA seen at 24 hours following normothermic ischemia and the residual fragmentation at 24 hours after hypothermic ischemia, emenating from the whole hippocampus is thought to be derived from the hilar neurons which start to degenerate early, and are not protected after hypothermic ischemia. We also observed high-molecular-fragmentation at approximately 50 kb, 300 kb and >600 kb using pulsed-field-gel -electrophoresis.

We conclude that DNA fragmentation at the oligonucleosomal level is prevented in the CA1 neurons and the striatum after an episode of 15 minutes of hypothermic transient global ischemia in the rat. This work was supported by fundings from the Swedish Medical Research Council (grant no. 08644) and NIH (NSO7838).

561.12

THE COMBINATION OF POSTISCHEMIC HYPOTHERMIA AND DELAYED PBN TREATMENT LEADS TO CHRONIC NEUROPATHOLOGICAL PROTECTION AFTER GLOBAL ISCHEMIA. M.Y.-T. Globus*, W.D. Dietrich, I. Valdes, S. Kraydieh, M.D. Ginsberg, R. Busto. Cerebral Vascular Dis. Research Center, Dept. of Neurology, Univ. of Miami Sch. of Med., Miami, FL

We recently provided evidence for a delayed excitotoxic process occurring within the CA1 hippocampus with postischemic hypothermia (J Cereb Blood Flow Metab 15:969-968, 1995). We have now tested the hypothesis that delayed free radical reactions also contribute to the lack of chronic protection in this experimental setting. Wistar rats underwent 10 min of normothermic (37°C) ischemia (2-vessel). Three ischemic groups were studied: no treatment (n = 4); 3 hrs immediate postischemic hypothermia (30°C)(n = 8); and immediate postischemic hypothermia combined with treatment with the spin-trapping agent N-tert-butyl-alpha-phenylnitrone (PBN)(100 mg/kg, i.p.) on postischemic days 3, 5 and 7 (n = 6). At 2 months, normothermic ischemia led to a complete dropout of CA1 neurons; and postischemic hypothermia alone failed significantly to protect the CA1 hippocampus. However, postischemic hypothermia combined with delayed PBN treatment led to significant increases ($p < 0.01$, ANOVA) in normal CA1 neuronal counts compared to normothermic ischemia. Neuronal counts within the lateral, middle and medial subsectors of the hippocampus in the hypothermic and hypothermic + PBN treatment groups were 23 ± 7, 18 ± 7 and 17 ± 7 (mean ± SEM) vs. 53 ± 9, 46 ± 9 and 32 ± 13, respectively. The lack of chronic CA1 protection with postischemic hypothermia appears to involve both secondary excitotoxic and radical-mediated processes.

561.13

NEURONAL DAMAGE EVOKED BY GLOBAL HEMISPHERIC HYPOXIA-ISCHEMIA WORSENED BY PROSTAGLANDIN E_2 FEVERS. J.A. Thornhill*, E. West and M. Smith, Dept. of Physiology & Sask. Stroke Research Center, Univ. of Saskatchewan, Saskatoon, Sask. Canada.

Groups of male, halothane anesthetized Long Evans rats were given intracerebroventricular (icv) injections of prostaglandin E_2 (PGE_2, 200, 400 or 4000 ng) or sterile saline in 4 μl volumes which caused dose-related increases in colonic temperature but no neural damage. Other groups were given icv PGE_2 (400 or 4000 ng) or sterile saline followed by the induction of hemispheric hypoxic ischemia (HHI) at the time of the peak febrile response (ligation of the right common carotid artery plus 35 min breathing 12% O_2). Animals given PGE_2 icv plus HHI showed dose related increases in colonic but especially temporalis muscle (brain) temperatures (also evident in their enhanced fever indices, °C x min). In addition, the 4000 ng PGE_2 treated group given HHI, showed significantly greater ipsilateral hemispheric neural damage to the HHI insult, especially due to increased hippocampal damage, than the non-febrile, icv saline-treated group given the same HHI insult. The results suggest that the pyrogenic effects of icv PGE_2 administration significantly increased the neural damage to HHI from damage observed in the non-pyrogenic saline treated group given the same HHI insult. Supported by the Canadian Heart & Stroke Foundation.

562.1

ALKOXYL RADICAL AND ARACHIDONIC ACID DAMAGE IN NEUROBLASTOMA CELLS. B. J. O'Neil*, T. R. McKeown, B. C. White
Dept.of Emerg. Med.,Wayne State Univ., School of Medicine, Detroit MI,48201

Arachidonic acid (ARA) release during cerebral ischemia, and its oxidation during reperfusion produces superoxide. Superoxide is a weak oxidant but can reduce storage protein iron, thereby releasing Fe^{2+}, which can act as a transition metal catalyst for lipid peroxidation. This accepted model suggests that arachidonate toxicity occurs by iron-requiring radical mediated damage. We examined this hypothess in a neuronal cell culture model.

Neuroblastoma B104 cells (NB104) were differentiated into neurons and then exposed for 2 hours either to an alkoxyl radical generated from cumene hydroperoxide (CumOOH) or to ARA in 4 trials. Neuronal damage was assesed by LDH release as a percentage of total LDH (after detergent lysis). A radical scavenger (BHA 100 μM) and iron chelators (DTPA 880 μM) and EHMP 300 μM) were studied for damage inhibition.

GROUPS	Untreated	BHA	DTPA	EMHP
Controls	26 ± 5%	28 ± 7%	10 ± 1%	35 ± 12%
CumOOH 5μM	56 ± 8%	43 ± 21%	30 ± 2%	19 ± 1%
CumOOH 20μM	79 ± 9%	45 ± 29%	60 ± 2%	25 ± 5%
ARA 10μM	50 ± 5%	37 ± 6%	17 ± 2%	17 ± 4%
ARA 40μM	79 ± 8%	81 ± 2%	76 ± 2%	58 ± 4%

CumOOH and ARA caused similar dose-dependent damage. BHA, DTPA, and EMHP decreased damage in CumOOH and in 10 μM ARA., however all were ineffective at ARA 40 μM. ARA reaches concentrations near 140 μM during brain ischemia. Our results suggest that higher concentrations of ARA induce membrane damage by unknown mechanisms in addition to iron-requiring radical injury.

Supported by NIH Grant NS01585-05 and EMF Center of Excellence Award

562.2

MAINTENANCE OF TURTLE BRAIN ASCORBATE AND GSH LEVELS DURING HYPOXIA-REOXYGENATION. R. Forman, J. Kume-Kick* and M. E. Rice. Depts. Physiology & Neuroscience and Neurosurgery, NYU Medical Center, 550 First Avenue, New York, NY 10016.

Anoxia-tolerant turtle brain has exceptionally high levels of the intracellular antioxidant ascorbate, while levels of GSH are similar to those in mammalian brain. Both antioxidants are seasonally regulated in turtle brain, with higher levels in warm- compared to cold-adapted animals. In the present experiments, we addressed how ascorbate and GSH are affected by hypoxia and hypoxia-reoxygenation in warm- and cold-adapted turtles. Ascorbate and GSH levels in turtle cerebral cortex, CSF and plasma were determined using HPLC. Warm-adapted turtles maintained at room temperature (20° C) were hypoxic for 3 h, with 30 min. reoxygenation in some animals. Cold-adapted turtles maintained at 2° C, were hypoxic for 7 days, with 30 min. reoxygenation in some animals. Normoxic control animals were air-breathing for a period equal to the duration of hypoxia in each group. Brain ascorbate and GSH were unaltered by hypoxia or reoxygenation in both cold and warm-adapted groups. Plasma ascorbate levels were higher in cold-adapted (25-30 μM) compared to warm-adapted groups (15-20 μM), which was opposite from the warm-/cold-dependence of brain ascorbate content. CSF levels in normoxic warm- and cold-adapted animals differed more markedly, with 400-600 μM ascorbate in cold-adapted normoxic controls but only 100-150 μM in warm controls. After 7 days of hypoxia, however, CSF ascorbate levels fell to ~150 μM in cold-adapted animals. Levels of GSH in CSF were relatively constant (~100 μM) under all conditions. Maintenance of ascorbate and GSH levels in turtle brain during hypoxia-reoxygenation suggests that antioxidant mobilization and recycling are important factors in preventing oxidative damage in turtle brain.

Supported by NS-28480 (MER) and MH-18882 (JK-K).

562.3

EVIDENCE FOR AN ASCORBATE-COPPER-PEROXIDE COMPLEX IN LIPID PEROXIDATION. M. R. Emerson, F. E. Samson, and T. L. Pazdernik*. Smith Research Center, Dept. of Pharm., Tox., & Ther., Univ. of Kansas Med. Center, Kansas City, KS 66160.

The generation of reactive oxygen species and subsequent free radical damage is implicated in a variety of neuropathologies including ischemia-reperfusion, seizures, and brain trauma. Following these acute brain insults, the delocalization of transition metals (Fe, Cu) into the extracellular fluid may occur. In the presence of H_2O_2 and ascorbate, a radical generating system may be created and induce the formation of oxygen radicals by Fenton-like chemistry leading to a lipid peroxidation cascade and a loss of cellular integrity and function. Using a liposome preparation as a membrane model, we studied the aqueous vs. lipid reactivity of an aqueous, metal-catalyzed radical generating system consisting of ascorbate (330 μM), copper (1.0 μM) and H_2O_2 (10 mM). Cis-parinaric acid (6 μM) oxidation was measured to assess lipid peroxidation while salicylate (30 μM) hydroxylation to 2, 5-dihydroxybenzoic acid (2,5-DHBA) was used to assess the aqueous reaction. Both reactions were followed fluorometrically. 2,5-DHBA formation began immediately, was rapid, and stopped within 5 minutes at about 2.75 μM. Cis-parinaric acid oxidation began after a lag of 4-5 minutes and continued until nearly 40% was oxidized at 30 minutes. Maximal salicylate hydroxylation required O_2, while cis-parinaric acid oxidation was more complete in an O_2 depleted environment. This data suggests that salicylate hydroxylation occurs through the hydroxyl radical in solution, whereas an ascorbate-copper-peroxide complex mediates lipid peroxidation. This complex may be more important than the hydroxyl radical in the oxidation of biological lipid targets. Furthermore, aqueous antioxidants may not prevent biomembrane damage, while the effectiveness of lipid soluble antioxidants will depend critically on their partition and location in the biomembrane. (Supported in part by DAAH04-95-1-0217 and DAMD17-94C-4045).

562.4

ROLE OF OXIDATIVE STRESS IN ENHANCED VULNERABILITY OF DOPAMINE NEURONS TO ENERGY IMPAIRMENT. G.D. Zeevalk* and Laura Bernard. Neurology Dept., UMDNJ-R.W.Johnson Med. Sch., Piscataway, N.J. 08854

In a previous study we showed that dopamine (DA) neurons in mesencephalic cultures were more sensitive than GABA neurons to a mild energy impairment produced by inhibiting the Krebs cycle enzyme, SDH with malonate (JPET 275, 1995). NMDA receptors played a role in this damage since blocking them during malonate exposure was protective. To further investigate the cellular mediators of damage and potential reason for the greater susceptibility of DA vs. GABA neurons to mild energy stress, the role played by oxidative stress was examined. DA neurons may be more susceptible because of an intrinsic oxidative stress already placed on them due to DA metabolism and oxidation. Several spin trap agents that chelate reactive oxygen species and inhibit lipid peroxidation (PBN, MDL101,002 & MDL, 102,832; gifts of Hoechst Marion Roussel Research Institute, Hoechst Marion Roussel, Inc) were tested for their ability to attenuate damage due to malonate. Cultures were exposed to 50mM malonate on day 6 in vitro for 24 hr in the presence or absence of the spin trap agents, allowed to recover for 48hr, then assessed for toxicity by simultaneous measurement of the uptake of ^{3}H-DA & ^{14}C-GABA. Consistent with previous findings, malonate produced a greater loss of DA (68%) than GABA uptake (30% loss). PBN, or either MDL compound, present during malonate exposure, significantly protected both neuronal populations. The MDL compounds were more potent than PBN in providing protection, (maximal protection ~ 1mM for the MDL compounds vs 30mM for PBN). Because of the involvement of glutamate receptors in malonate-induced damage, the spin trap agents were tested vs. a 1 hr challenge to 50μM glutamate. Protection vs. glutamate was found with the spin trap agents with an order of potency of MDL102,832 >MDL101,002>PBN. To further study the role of oxidative stress in damage caused by energy impairment, cultures were treated with buthionine sulfoxamine (BSO) to prevent glutathione (glut) synthesis. A 24 hr exposure to 10μM BSO on day 5 in vitro reduced glut levels by 65%. Glut depletion per se was not toxic. However, it greatly potentiated malonate-induced toxicity to both neuronal populations and eliminated the differential sensitivity. These findings indicate that oxidative stress plays an important role in damage to mesencephalic neurons due to mild energy impairment. Glut depletion unmasked toxicity due to malonate in the GABA neurons suggesting that they may be more resistant to mild energy impairment because they are more capable of withstanding an oxidative stress. Supported by a Grant from the National Parkinson Foundation and UPS Grant NS17360.

562.5

NEUROPROTECTIVE EFFICACY OF EBSELEN IN THE RODENT MODEL OF PERMANENT MIDDLE CEREBRAL ARTERY (MCA) OCCLUSION. [1]T. Takasago, [2]D.I. Graham, [3]H. Masayasu and [1]I.M. Macrae*. [1]Wellcome Surgical Institute and [2]Dept. of Neuropathology, University of Glasgow, Glasgow G61 1QH, U.K., [3]Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan

The neuroprotective efficacy of post-insult treatment with the glutathione peroxidase mimic and free radical scavenger, ebselen, has been investigated in a reproducible model of maintained focal cerebral ischaemia in the rat.

Adult male Fischer 344 rats (277-340 g,) were anaesthetised (0.8-1.5% halothane in 70% N_2O: 30% O_2) and ventilated with physiological monitoring throughout. The left MCA was permanently occluded by diathermy, transected, the wound sewn up and the anaesthetic withdrawn. Thirty minutes post occlusion ebselen (10 or 30 mg/kg) or placebo (5mls/kg) was administered by gavage (n=15 per group). A second dose of drug or placebo was administered at 12 hours post-occlusion. At 24 hours, following physiological monitoring, the animal was reanaesthetised and perfusion fixed with FAM for subsequent determination of infarct volume by quantitative histopathology.

Post-treatment with ebselen significantly reduced the volume of tissue infarction induced by MCA occlusion. Infarct volume in the cerebral hemisphere was 142±9 mm^3 for placebo, compared to 97±8** mm^3 for low dose and 108±7* mm^3 for high dose ebselen (*p<0.02, **p<0.01 , two-tailed t-test with Bonferroni correction). Ebselen had no therapeutic efficacy on the caudate nucleus at either dose. Tissue salvage was confined to the cortex where the volume of infarct was reduced by 36.7% (p<0.01) with the low dose and 27.5% (p<0.01) with the high dose.

These data demonstrate that the potent inhibitory influence of ebselen on free radical damage results in the salvage of a significant volume of cortex, even with delayed oral administration (30 minutes post-insult).

562.6

PHENIDONE INHIBITS COMBINED OXYGEN-GLUCOSE DEPRIVATION- AND HYDROGEN PEROXIDE-INDUCED NEURONAL INJURY IN MIXED CORTICAL CULTURES. M.B.Wie[1], H.C.Kim[2], K.H.Lee[1], J.H.Shin[1], Y.J.Cho[2], and J.C.Lee.[1] [1]Research and Development Center, Samchundang Pharmaceuticals Inc., Chunchon 200-160, [2]College of Pharmacy, Kangwon National Univ., Chunchon 200-701, Korea.

It has been proposed that free radicals derived from arachidonic acid metabolites stimulate excessive release of excitatory amino acid and neurodegenerative cascade during cerebral ischemia/reperfusion insults.

We examined whether inhibitors of arachidonic acid metabolism exhibit protective effect on combined oxygen-glucose deprivation- and hydrogen peroxide-induced neuronal injury in murine cortical cultures. Mixed cortical cells (14-21 DIV) exposed to oxygen-glucose-deprived media, then placed into oxygenated normal media (Glodberg and Choi,1993). Neuronal injury assessed by measuring LDH release for 20-24 h following oxygen-glucose deprivation. Pretreatment with phenidone (300 μM), both inhibitor of cyclooxygenase/lipoxygenase attenuated oxygen-glucose deprivation-induced neuronal death. However, acetylsalicylic acid (50-500 μM), cyclooxygenase inhibitor and esculetin (10-100 μM), lipoxygenase inhibitor had no effect. Phenidone (30-300 μM) also inhibited hydrogen peroxide (500 μM)-induced acute neuronal injury dose-dependently. Blockade of neuronal injury evoked by phenidone disappeared according to the prolongation of oxygen-glucose deprivation time and the saturation extent of anaerobic gas mixture in media. These results suggest that strategy for both inhibition of cyclooxygenase and lipoxygenase may beneficial to the neuroprotection from oxygen-glucose deprivation - or oxidative radical - induced neuronal injury(Supported by KOSEF 95-0403-19-01-3).

562.7

IN ISCHEMIC GERBIL BRAIN AND RETINA, THE INCREASE OF HYDROXYL FREE RADICAL (°OH) IS TIME-RELATED. B. Delbarre*, G. Delbarre and F. Calinon, Faculté de Médecine, 37032 Tours, France.

A study was undertaken to show the relation between time of reperfusion and release of °OH. 6 groups of 5 adult gerbils were used : 3 sham operated (SO), and 3 subjected to unilateral left carotid occlusion (Isc), 60 min followed by 30 min, 4h or 24h of reperfusion. °OH (2,5+2,3DHBA) was determined in left hemisphere and retina by HPLC/Electrochemical (salicylate as scavenger) according to Floyd method (Free Rad. Biol. Med., 2:13-18, 1986). Salicylate (100 mg/kg, i.p.) was administered 10 min before occlusion or SO. Results were given in difference of mean Isc versus SO (Area °OH/Salicylate).

	30 min	4h	24h
Retina	1.15***	1.20***	1.41***
Brain	1.29*	1.37**	1.57**

Bonferroni test. (n=5). p≤0.05*, p≤0.01**, p≤0.001***.

It is not possible to determine level of °OH 48h after IRI because 40 to 60% of gerbils were dead. °OH is significantly increased in IRI. This increase is more important 24h after IRI. These results would suggest that antioxidant drugs could be administered curatively during a long time after IRI. We have shown that the level of glutamate is more important 4h after IRI. The fact that °OH is more important later, 24h after reperfusion, would suggest that glutamate could be responsible for the high level of °OH later after IRI. Bitherapeutic treatment could be administered before and/or after reperfusion.

562.8

EFFECT OF SALICYLATE, AN HYDROXYL RADICAL (°OH) SCAVENGER, ON GLUTAMATE RELEASE IN ISCHEMIC BRAIN AND RETINA OF GERBIL. INFLUENCE OF TIME AFTER REPERFUSION. G. Delbarre*, B. Delbarre and F. Calinon, Faculté de Médecine, 37032 Tours, France.

Salicylate is used as °OH scavenger by R.A. Floyd to determine °OH. What is the effect of salicylate on level of glutamate ? Level of glutamate was determined in left hippocampus and retina of gerbil, by HPLC/Electrochemical according to Xu et al. method (Xu, X. et al., J. Liq. Chrom., 9:10, 1986). 12 groups of 5 adult gerbils were used : 6 sham operated (SO), and 6 subjected to unilateral left carotid occlusion (Isc), 60 min followed by 30 min, 4h or 24h reperfusion. Salicylate (100 mg/kg, i.p.) or saline was administered 10 min before occlusion or SO. Results are given in difference of mean Isc/SO (µg/mg of protein).

		30 min	4h	24h
Retina	Saline	6.86	8.53	5.01
	Salicylate	2.20***	5.33*	2.60*
Brain	Saline	6.48	14.05	11.31
	Salicylate	6.13ns	7.02***	4.58***

Bonferroni test. (n=5). Salicylate/Saline. p≤0.05*, p≤0.01**, p≤0.001***. Results show that glutamate is increased in IRI. Salicylate significantly reverses this increase. The level of glutamate is more important 4h after reperfusion. Since salicylate prevents the release of glutamate in IRI, it is not possible to determine, on the same ischemic animal, glutamate and °OH when Floyd method is used.

562.9

Increased Hydroxyl Radical Production During Reversible Focal Cerebral Ischemia N. J. Solenski, A. L. Kwan, H. Yanamoto, N. F. Kassell*, J. P. Bennett, K. S. Lee. Departments of Neurology & Neurological Surgery, University of Virginia, Charlottesville, Virginia 22908

Cytotoxic oxygen free radicals may be produced during cerebral ischemia/reperfusion by several mechanisms. Using a reproducible rat model of focal ischemia/reperfusion, we have measured the *in vivo* time course of cerebral hydroxyl radical production and excitatory amino acid levels.

Anesthetized male Sprague-Dawley rats underwent temporary occlusion of the left middle cerebral artery and reversible bilateral ligation of the carotid arteries. A microdialysis probe was stereotactically placed in either the ischemic core or the perifocal area of the left frontoparietal cortex. Artificial cerebral spinal fluid with 5 mM salicylate was continuously perfused and dialysate samples were collected during 1 hour of baseline, 3 hours of ischemia, and 6 hours of reperfusion time. Samples were assayed by HPLC for 2,3- and 2,5-dihydroxybenzoic acid, and for glutamic and aspartic acids. Cerebral reperfusion was confirmed visually and measured by iodo-[^{14}C]antipyrine autoradiography. Infarction area was measured histologically.

A statistically significant increase of hydroxyl radical adduct was observed within the perifocal ischemic area. Hydroxyl radical concentration rose in the perifocal area during 3 hours of ischemia and further increased during 6 hours of reperfusion when compared to the ischemic core and to sham controls. (Two-way ANOVA $p < 0.05$). Preliminary data on the temporal correlation of hydroxyl radical production to excitatory amino acid levels, suggest a high amount of variability during both ischemia and reperfusion in the perifocal area. This *in vivo* study confirms the generation of neurotoxic free radicals during focal ischemia and reperfusion and indicates that this event is most prominent in the perifocal rather than the core of a focal ischemic event. Supported by NS Training Grant # NS 07199-14

562.10

A MICRODIALYSIS STUDY COMPARING HYDROXYL RADICAL PRODUCTION AND DOPAMINE METABOLISM IN VARIOUS RAT MODELS OF CEREBRAL ISCHEMIA. L. Ste-Marie, P. Vachon, L. Vachon* and J. Montgomery. Research Centre, Notre-Dame Hospital, Montréal, Québec, Canada.

The goal of our project is to find a model of unilateral global ischemia in the rat brain (Sprague Dawley, n=10) which is highly reproducible with minimal effect on the contralateral side of the brain (control side). A small subtemporal craniectomy was made under anaesthesia and the middle cerebral artery (MCA) was cauterized. Three models of ischemia were compared 1) cauterization of the MCA only 2) MCA cauterized and permanent occlusion of the ipsilateral common carotid artery (CCA) 3) MCA cauterization and both CCA's occluded: permanent occlusion on the ipsilateral side and temporary (30 min) occlusion on the contralateral side. For each of the three models, microdialysis probes were implanted bilaterally in the striatum and infused with Ringer's solution (2µl/min). To investigate °OH formation during ischemia, 4-hydroxybenzoate (4HBZ) (400 mg/kg) was injected intraperitoneally at the beginning of the microdialysis. Microdialysate samples were analysed for dopamine (DA), dihydroxyphenylacetate (DOPAC), norepinephrine (NE), homovanillate (HVA) and 3,4-dihydroxybenzoate (34DHBZ) (formed by 4HBZ hydroxylation) by HPLC with electrochemical detection. Preliminary results show that MCA occlusion alone did not augment liberation of DA and metabolites. The third model showed that both sides are affected in different ways: DA and NE increased more on the ipsilateral side, but DOPAC and HVA decreased in the same way. Only the second model showed a non affected response on the control side. At the end of the experiments, the brains were perfused with saline-formaldehyde (10%) and prepared for histological staining (hematoxylin and eosin, cresyl violet). Histological damage will be compared with biochemicals results. (Financial support from the Heart and Stroke Foundation of Canada.)

562.11

A FREE RADICAL SCAVENGER, MDL 101,002, IS NEUROPROTECTIVE IN SEVERAL MODELS OF FOCAL AND GLOBAL ISCHEMIA. D.R. McCarty, M.P. Johnson, N.L. Velayo, C.G. Markgraf, P.A. Chmielewski, J.V. Ficorilli, H.C. Cheng, S.F. Chaney, T.C. McCloskey, J.H. Kehne, C.E. Thomas and G. Metcalf*. Hoechst Marion Roussel Inc., CNS Research, Cincinnati, OH 45215.

Reactive oxygen species generated during brain ischemia are believed to cause free radical chain reactions resulting in cell damage and neurodegeneration. The nitrone spin trap, α-phenyl-*tert*-butyl nitrone (PBN), has been shown to be efficacious in focal and global ischemia models. A more potent compound, one of a novel class of cyclic nitrone analogs of PBN, MDL 101,002 was investigated. Permanent distal middle cerebral artery (MCA) occlusion in the Spontaneously Hypertensive (SH) rat was achieved by ligating the ipsilateral common carotid artery (CCA), exposing the MCA via craniectomy, and cutting the vessel by electrocautery. In this model, 100 mg/kg MDL 101,002 given i.v. 30 mins postischemia reduced infarct volume by 40%. In Wistar rats, the contralateral CCA was also occluded for 2 or 3 hrs to achieve more consistant infarcts. A 75 mg/kg bolus + 45 mg/kg*h infusion for 6 hrs i.v. beginning at 5 mins postischemia decreased infarct volume 90% with 2 hrs CCA occlusion and 60% with 3 hrs. Proximal MCA occlusion was also performed in Wistar rats by introducing a 3.0 nylon monofilament into the internal carotid artery and advancing it to the origin of the MCA. This monofilament was removed after 3 hrs to allow reperfusion. Doses of 75 mg/kg bolus + 45 mg/kg*h infusion or 40 mg/kg bolus + 26 mg/kg*h infusion for 6 hrs i.v. beginning 30 mins postischemia decreased infarct volumes by 70 or 50%, respectively. Gerbils treated with 100 or 64 mg/kg i.p. 30 mins prior to 5 mins of occlusion of both CCAs exhibited spontaneous locomotor activity at 24 hrs which was comparable to shams. Partial protection was also achieved with a dose of 32 mg/kg. MDL 101,002 is efficacious in animal models of focal ischemia showing histological decreases in damaged tissue, and also producing behavioural improvement in a model of global ischemia. This suggests that neurological damage caused by stroke could be minimized by treatment with free radical scavengers.

562.12

FREE RADICALS ARE RELEASED IN THE STRIATUM AFTER REOXYGENATION IN A MODEL OF MILD AND SEVERE PERINATAL ASPHYXIA C Loidl[1], F Capani[1], F Aguirre[2], L Piehl[2], G Facorro[2], T.De Paoli[2], A Hager[2] and J Pecci Saavedra *[1] [1] Inst. de Biologia Celular y Neurociencias and LANAIS-MIE. Fac. de Medicina and [2] Cátedra de Fisica and LANAIS-RLBM. Fac.de Farmacia y Bioquimica. Univ. de Buenos Aires. Argentina.

Biochemical and morphological differences between mild and severe perinatal asphyxia (PA) were demonstrated in the rat striatum and cerebral cortex. Using a model that induces PA at different times of oxygen lack, reactive oxygen species (ROS) were measured in an electron paramagnetic resonance (EPR) spectrometer. In term pregnant rats uteri still containing foetuses were taken out by hysterectomy under deep anaesthesia and were placed in a 37°C water bath. The following groups of asphyctic pups were used: slight (10 min of PA), mild (19 min of PA) and severe PA (≥20 min of PA). Controls were obtained by caesarea. Following PA, animals were left to recover at different times of reoxygenation (0, 5, 15 and 30 min). Pups were then sacrificed and in samples of striatum and cortex ROS were trapped by N-t-Butyl-α-Phenylnitrone and analysed with a Bruker ECS 106 EPR spectrometer. Striatal samples showed ROS release in 19 min of PA with 5 and 15 min of reoxygenation groups and the higher detection was observed in the ≥20 min of PA with 5 min of reoxygenation group. ROS release was neither detected in striatal and cortical controls nor in any cortical samples subjected to PA. These results show that free radicals are released in striatum during the short period following mild and severe PA reoxygenation. The quantitative differences detected in both groups could explain the long-term alterations previously observed. Further studies are needed in order to extend the observations to other areas of the CNS.

562.13

DELAYED TREATMENT WITH THE SPIN TRAP α-PHENYL-N-TERT-BUTYL NITRONE REDUCES INFARCT SIZE FOLLOWING TRANSIENT FOCAL ISCHEMIA IN RATS. Q. Zhao*, M.L. Smith and B.K. Siesjö. Lab. for Experimental Brain Research, University of Lund, Lund, Sweden.

In exploring whether or not α-phenyl-N-tert-butyl nitrone (PBN) can ameliorate ischemic brain damage, 2 h of transient MCA occlusion was induced in Wistar rats by insertion of an intraluminal filament under halothane anesthesia. PBN (Sigma Chemical Co., St Louis, MO, USA) was dissolved in saline and administered *i.p.* in a dose of 100 mg/kg, repeated 4 times with 12 h interval. Rectal temperature was kept at normal levels during ischemia and for the first 4 h of recirculation. The infarct volume was measured with the 2,3,5-triphenyltetrazolium chloride (TTC) staining technique after 48 h of recirculation. The five groups were: only saline injection (n=16), PBN treatment starting 15 min before MCA occlusion (n=8), or at 1 h (n=10), 3 h (n=11), and 6 h (n=8) after recirculation. In two additional groups: saline treated (n=7) and PBN treatment starting after 1 h of recirculation (n=7), a histopathological analysis of infarct size was made after 7 days of recirculation.

Pretreatment with PBN reduced infarct volume to < 50 % of control. A similarly beneficial effect was obtained when the drug was given at 1 or 3 h following recirculation, but not at 6 h.

Microscopical evaluation of infarct volume after 7 days of recovery revealed a significant reduction in infarct volume by 30 %. This reduction was less than that observed after 48 h in TTC-stained brains, suggesting progression of infarct development in the period of 2 to 7 days.

These results suggest that free radicals play an important role in the pathogenesis of ischemic cell damage. It may be speculated that continous infusion of PBN, or prolongation of the treatment, might further improve the outcome.

This study was supported by funds from the Swedish Medical Research Council and the National Institutes of Health of the United States Public Health Service.

562.14

Selective vulnerability of the CA1 sector to oxidative injury in organotypic hippocampal culture J.J. Vornov,* A.G. Thomas and J. Park Depts. of Neurology and Neuroscience Johns Hopkins School of Medicine, Baltimore, MD 21287

We have previously reported that the selective regional vulnerability of the CA1 sector of the hippocampus to global ischemia can be reproduced in organotypic culture. Hippocampal slices from 7 day old rat pups are maintained for weeks. Regional injury is quantified in the living culture with propidium iodide.

Paraquat is an herbicide which produces superoxide by redox cycling within cells. We confirmed the paraquat exposure causes superoxide formation by the reduction of nitroblue tetrazolium to a colored formazan product. This was partially blocked by simultaneous incubation with catalase and superoxide dismutase.

Paraquat injury was time and dose dependent. Concentrations that caused submaximal injury consistently produced selective injury in the CA1 region of cultures. The same injury was produced by 3 mM for 5 min, 30 μM for 24 hrs or 3 μM for 5 days. The iron chelator desferoximine significantly decreased injury; the vitamin E derivative Trolox did not. The NMDA receptor antagonist MK-801 protected against brief, but not prolonged exposures to paraquat. Total cellular glutathione was almost completely depleted prior to loss of membrane integrity. There is selective vulnerability of the CA1 region to acute or chronic oxidative stress produced by paraquat.

This research supported by an NIH grant NS-01310

562.15

POLYAMINE-MODIFIED SUPEROXIDE DISMUTASE WITH INCREASED BLOOD-BRAIN BARRIER PERMEABILITY REDUCES ISCHEMIC NEURODEGENERATION IN RAT HIPPOCAMPUS. Thomas M. Wengenack*, Geoffry L. Curran, and Joseph F. Poduslo. Molec. Neurobiol. Lab., Mayo Clinic and Foundation, Rochester, MN 55905.

Free radicals are believed to play an important role in cerebral ischemia. Antioxidant enzymes such as superoxide dismutase (SOD) have shown neuroprotective effects in animal models of cerebral ischemia. Experiments using native enzyme have had limited success. Various modifications to increase the half-life or permeability of SOD have also resulted in limited neuroprotective effects. Previous studies in this laboratory have shown that covalent modification of SOD with the polyamine, putrescine, increases its permeability at the BBB. The specific aim of this study was to systemically administer polyamine-modified SOD (pSOD) following global cerebral ischemia in the rat and investigate the effects on the vulnerable CA1 hippocampal pyramidal cells. Transient, global cerebral ischemia was produced in male Wistar rats for 12 min using the 4-VO method. Animals were dosed (i.v.) 1.5 min after reperfusion with either saline, native SOD (5,000 U/kg), pSOD (5,000 U/kg), or enzymatically inactive pSOD (2.1 mg/kg) twice daily for 3 days. Neuroprotective effects were then assessed by counting the number of remaining pyramidal cells in anatomically defined regions of CA1. Rats that received saline had only 1.4 ± 0.6 neurons/reticle ($\bar{x}$ ± SEM). Native SOD (10.4 ± 3.0) or inactive pSOD (7.3 ± 2.7) resulted in slightly, but not significantly, more CA1 neurons. Polyamine-modified SOD, however, resulted in significantly more CA1 neurons (24.6 ± 5.9), nearly half that of sham controls (58.6 ± 0.7). Polyamine modification of SOD and other antioxidant enzymes, as well as growth factors, resulting in increased permeability across the BBB with retained biological activity, may provide a useful tool for the systemic delivery of therapeutic proteins in neurodegenerative disorders. (Mayo Foundation)

562.16

HIPPOCAMPAL INJURY FOLLOWING TRANSIENT GLOBAL ISCHEMIA IS REDUCED IN CuZn-SUPEROXIDE DISMUTASE TRANSGENIC MICE. K. Murakami[1], T. Kondo[1], S. Chen*[1], E. Carlson[2], C. J. Epstein[2], P. H. Chan[1]. CNS Injury and Edema Research Center, Departments of Neurosurgery and Neurology[1], and Pediatrics[2], University of California, San Francisco, CA 94143

We previously demonstrated that CuZn-superoxide dismutase (CuZn-SOD) transgenic (Tg) mice are highly resistant to cerebral infarction and edema formation following transient focal ischemia and reperfusion. It is, however, unclear whether oxygen derived radicals are involved in the pathogenesis of hippocampal injury following transient global ischemia. In order to clarify the role of CuZn-SOD in hippocampal injury, Tg and littermate non-transgenic (nTg) mice were subjected to 5 or 10 min of global ischemia induced by bilateral common carotid artery occlusion (BCCAO) under controlled ventilation. In a preliminary study, we determined that the severity of the hippocampal lesion depends upon the plasticity of posterior communicating artery (PcomA) in transient global ischemia induced by BCCAO. In the present study, we employed the animals with hypoplastic PcomA in the ipsilateral hemisphere. At 1 or 3 d after ischemia, the plasticity of PcomA was assessed followed by evaluation of the hippocampal lesion in the hemisphere with hypoplastic PcomA using qualitative grading. Plasticity of PcomA was not significantly different between nTg and Tg mice. In the 5 min ischemia group, there was no significant difference in the hippocampal lesion between nTg and Tg mice at 1 d after ischemia, but nTg mice had significantly more severe lesions than Tg at 3 d ($p<0.05$). In the 10 min ischemia group, while nTg mice had significantly more severe lesions than Tg at 1 d after ischemia ($p<0.05$), hippocampal lesions in both nTg and Tg mice were nearly maximized at 3 d. These data suggest that CuZn-SOD may play a protective role in the pathogenesis of hippocampal lesions after transient global ischemia.

(Supported by NIH grants NS14543, NS25372, and AG08938)

562.17

CATALASE ACTIVITY AFTER PERINATAL HYPOXIA-ISCHEMIA IN CU/ZN SOD TRANSGENIC MICE. H. F. Chetkovich, C. Epstein and D. M. Ferriero*. Neonatal Brain Disorders Lab, Dept of Pediatrics and Neurology, Univ. CA San Fran, San Francisco, CA 94143

Wheras Cu Zn superoxide dismutase (CuZn-SOD) has been shown to be neuroprotective in adult animal models of cerebral ischemic injury, in a model of hypoxic-ischemic injury in neonatal transgenic mice overexpressing CuZn-SOD, there was increased tissue damage. This suggests that there is no concomitant increase in the activity of catalase or glutathione peroxidase in the neonatal brain, resulting in increased hydrogen peroxide concentrations leading to increased tissue damage. To test this hypothesis, we first compared catalase activities in noninjured neonatal CuZn-SOD transgenic mice (TgC) and their nontransgenic CD-1 littermates (WtC). Using a standard colorimetric assay, no significant differences in catalase activity in the cortices (44±2 v. 47±3 U/mg)or hippocampi (62±3 v 58 ±2) were detected. To address whether enzyme activity changes after hypoxic-ischemic injury, we measured catalase activity in similar groups of animals immediately after hypoxia-ischemia. In this model, the hemisphere ipsilateral to the carotid ligation is injured. Again, no significant differences in catalase activity in the cortices or hippocampi were seen when comparing ipsilateral to contralateral regions or when comparing ipsilateral damaged regions to controls. Taken together, these findings support the hypothesis that increased hypoxic-ischemic injury in neonatal mice transgenic for CuZn-SOD is due, in part, to increased SOD activity without concomitant increase in catalase activity. Intracellular hydrogen peroxide concentrations in the transgenic neonatal mice may thus reach toxic levels, leading to the increased cerebral tissue injury.

Supported by NS 35902 , NS32553 and AG08938

562.18

Production of extracellular superoxide anion during reperfusion following cerebral ischemia correlates with cerebral infarction. R.H. Fabian*, T.A. Kent. Dept. Neurology, and Pharmacol. Toxicol., of the Sch. Med., and the Marine Biomed. Inst., U. Tex. Med. Branch, Galveston, TX 77555-0539.

Superoxide anion production in the brain during and following cerebral ischemia may be important in the pathogenesis of cerebral infarction. Reactive oxygen species concentrations in the brain have been measured during and following cerebral ischemia using various techniques. However, the relationship between reactive oxygen species production and subsequent cerebral injury or infarction has not been determined. In this study, we attempted for the first time to correlate superoxide production during reperfusion as monitored electrochemically using a cytochrome c coated electrode following cerebral ischemia with the appearance of cerebral infarction resulting from the ischemia.

Extracellular superoxide anion levels were monitored over the surface of the cerebral cortex in Halothane inhalation anesthetized rats subjected to middle cerebral artery occlusion of various durations followed by monitoring during a period of reperfusion. This was compared to the presence of cerebral infarction after ischemia of various durations as determined by TTC staining of brain slices following sacrifice of the animals with a 24 hour post infarction survival. Currents of cytochrome c coated electrodes were compared to currents of uncoated electrodes as a control. Electrode signal specificity was determined by measuring electrode current during systemic injections of superoxide dismutase.

The results indicate that the average period of ischemia resulting in an increase in superoxide anion concentrations is approximately the same as the average period resulting in significant infarction -- 43 ± 7.0 and 49 ± 9.1 min., respectively. These results set the stage for studies to determine whether there is a cause and effect relationship between superoxide anion production and the appearance of cerebral infarction.

Supported by a grant-in-aid from the American Heart Association Texas Affiliate, Inc.

562.19

VITAMIN E ENHANCES Ca^{2+}-MEDIATED VULNERABILITY OF CEREBELLAR GRANULE CELLS TO ISCHEMIA. V. A. Dyatlov, D. A. Lawrence and D. O. Carpenter*. Wadsworth Laboratories, NYS Dept. of Health, and School of Public Health, Albany, NY 12201.

Oxidative damage caused by ischemia/reperfusion-induced lipid peroxidation and free radicals might be prevented by vitamin E, an endogenous antioxidant. Suprisingly, our experiments with immature cerebellar granule cells showed that vitamin E (1-10 μM α-tocopherol phosphate) killed postischemic cells. Exposing freshly dissociated cells from the rat cerebellum (2-week-old male pups) to transient ischemia (10-min oxygen and glucose deprivation) resulted in recovery-induced consumption of cellular anti-oxidants (ascorbic acid, glutathione and α-tocopherol) and development of membrane lipid peroxidation measured by the thiobarbituric acid method. The rate of lipid peroxidation of postischemic cells was stimulated by treatment of cells with α-tocopherol. α-Tocopherol dramatically increased the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which preceded cell death. Hydrogen peroxide mimicked the effect of vitamin E but was less toxic than α-tocopherol phosphate. Dead cells and $[Ca^{2+}]_i$ of viable cells were determined by a two-color fluorescence (7-aminoactinomycin D and fluo-3) from 10,000 cells using a FACScan cytometer. Pre- or cotreatment of the cells with vitamin C and ubiquinol-10 reduced both the α-tocopherol-induced increase in $[Ca^{2+}]_i$ and cell death. The increase in $[Ca^{2+}]_i$ and cell death were also inhibited in the postischemic cells pretreated with the intracellular Ca^{2+} chelator, 1,2-bis-(2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid acetoxymethyl ester. We believe that after consumption of co-antioxidants, α-tocopherol is converted to an α-tocopheroxyl radical, which acts as a toxic prooxidant as cellular bioenergetics deteriorate. This finding has particular importance, because low or physiological concentrations of vitamin E in postischemic cells were sufficient for a potentiation of lipid peroxidation and Ca^{2+}-mediated cell killing. Supported by NS 23807 (DOC).

ISCHEMIA: NEUROTRANSMITTERS

563.1

ADRENERGIC RECEPTOR mRNA EXPRESSION IN THE RAT CNS AFTER FOCAL ISCHEMIA. S. K. McCune*, J. M. Hill, and P. D. Hurn Divisions of Neonatology and Anesthesia and Critical Care Medicine, Johns Hopkins University Hospital, Baltimore, MD and Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD, 21287.

Adrenergic receptors mediate tissue responses to catecholamines. Multiple subtypes of adrenergic receptors are expressed in the CNS, each with a unique pattern of distribution. Little is known of the functional significance of these multiple subtypes. Pharmacologic studies after brain injury have suggested that alpha-1 adrenergic receptors may be involved in improved recovery of function. In order to evaluate the subtype of adrenergic receptors that may be involved, alpha-1D, alpha-1B, alpha-2C4, alpha-2C10, beta-1 and beta-2 mRNA expression was evaluated by in situ hybridization after CNS focal ischemia in the adult rat.

Right-sided ischemia was produced by permanent middle cerebral artery intravascular occlusion via insertion of an intravascular suture. The animals were sacrificed 24 hours after injury and receptor mRNA expression was examined by in situ hybridization using oligonucleotide probes specific for each subtype. A diminution of receptor expression was observed in all subtypes in the region of cellular loss. However, a significant increase in alpha-1B receptor mRNA expression was observed in the rim of healthy tissue medially bordering the ischemic lesion.

These results suggest that the alpha-1B adrenergic receptor subtype may be involved in functional recovery after ischemic lesions. Thus, the routine catecholamine surge at birth may be important in protecting against potential perinatal ischemic injury.

Supported by NIH grant # NS33668 and # P-30-HD27799

563.2

RAPID CAESAREAN SECTION DELIVERY IS ASSOCIATED WITH BRAIN HYPOXIA IN THE RAT. B. El-Khodor*, A. Gratton and P. Boksa. Departments of Psychiatry and of Neurology & Neurosurgery, McGill University, Douglas Hospital Research Center, Montreal, Canada, H4H 1R3.

Alterations in brain dopaminergic activity has been linked to the pathophysiology of several mental disorders such as schizophrenia. We have previously shown that young adult male rats born by rapid Caesarean section (CS) have increased steady state levels of dopamine (DA) in the nucleus accumbens and striatum and decreased DA in pre-frontal cortex, compared to vaginally born animals. Our group has also shown that birth by CS leads to increased release of mesolimbic DA in response to repeated stress in the adult rat. It is unknown which factors in the CS birth procedure are responsible for these long-term changes in CNS DA transmission. To investigate the possibility that rats delivered by CS may experience early postnatal episodes of hypoxia, we measured levels of brain lactate, a widely accepted measure of CNS hypoxia, at 1, 5 and 30 h after birth in rats born vaginally or by CS. Rats born by CS breathed immediately and spontaneously and exhibited excellent color, muscular tone and activity levels at birth and at all time intervals studied. Rats born vaginally showed elevated levels of brain lactate at 1 h after birth (2313.8±420.7 nmole/g) compared to 5 h (564.6± 144.2 nmole/g) and 30 h (748.0±128.5 nmole/g). Similar to vaginally born controls, rats born by CS showed high levels of brain lactate at 1h after birth (1919.4±302.2 nmole/g). However CS rats maintained significantly higher levels of brain lactate at 5 h (1917.0±195.6 nmole/g) compared to the vaginally born group. By 30 h after delivery, lactate levels in the CS group (631.0±62.5 nmole/g) were similar to values for vaginally born controls. Vaginal birth in humans is accompanied by large increases in plasma catecholamines, which is believed to protect the newborn against hypoxia and promote independent breathing. This increase is absent following CS birth. In our model, rats born by CS showed significantly lower plasma epinephrine (-60%) and no change in plasma norepinephrine, in comparison to vaginally born controls. Our results indicate that biochemical evidence of brain hypoxia can be detected at early times after an apparently innocuous CS birth. This transient hypoxia may contribute to long-term alterations in DA transmission observed following CS birth. Supported by MRCC.

563.3

SELEGILINE POST-TREATMENT PROTECTS AGAINST DELAYED NEURONAL DEATH IN GERBIL GLOBAL ISCHEMIA. H. Lahtinen*[1,2], J. Koistinaho[1], R. Kauppinen[1], A. Haapalinna[3] and J. Sivenius[2]. A.I. Virtanen Institute[1] and Dept. of Neurology[2], University of Kuopio, 70211 Kuopio, and Orion Corp., Orion-Farmos[3] Turku, Finland

Occlusion of both common carotic arteries (5 min) of Mongolian gerbil leads to delayed neuronal death of the hippocampal pyramidal cells. This ischemia -induced cell degeneration is first observed in 2-3 days and shares similarities with epileptic cell damage. Selegiline (*l*-deprenyl), an anti-parkinsonian agent, is a selective MAO-B inhibitor. However, low doses of selegiline have shown to posses rescue-like effects - independent of MAO-B inhibition - in different degenerative models. To investigate whether selegiline at low doses could alleviate hippocampal cell death after an ischemic insult, normothermic gerbils underwent a 5-minutes carotic occlusion under halothane anesthesia. Selegiline, 250 μg/kg (s.c.), was administered two hours after ischemia and once a day after that. The animals were decapitated three or seven days later for histological analyses. Pyramidal cell degeneration in the CA1 region of the hippocampus was scored (0-3) using Nissl stained sections. The animals treated with selegiline for seven days showed a significantly lower damage score compared with the saline treated animals (1.73 vs. 2.41, p=0.0133). Animals treated for three days showed also milder damage than the saline treated animals (1.67 vs. 2.05, n.s.). In our preliminary experiments, selegiline showed no effect on the cell damage when the treatment started before or immediately after the ischemic insult. mRNA for hsp70 was strongly expressed in the CA1 pyramidal cell layer still three days after ischemia but had vanished after seven days. The intensity of the hsp70 signal in the CA1a region correlated negatively to the cell damage score ($r=-0.76$, $p<0.001$) showing, that hsp70 does not serve as a marker for neuronal degeneration in this model. Thus, low doses of selegiline administered after the insult seem to protect from or, at least, retard the process of delayed ischemic neuronal death. The neuroprotective mechanism of selegiline remains to be determined.

536.4

ASYMMETRICAL PROTECTION OF STRIATAL NEURONS FROM ISCHEMIA BY DOPAMINE DEPLETION. Y. B. Ren*, X. D. Li and Z. C. Xu Dept. of Neurology, Univ. of Tennessee, Memphis, Memphis TN 38163, U.S.A.

Spiny neurons in neostriatum are vulnerable to transient cerebral ischemia. It has been suggested that dopamine may play a role in postischemic neuronal damage in neostriatum. The present study investigated the temporal threshold for maximal neuronal damage in rat neostriatum after transient ischemia and the impact of dopamine depletion on ischemic outcome in neostriatum using 4-vessel occlusion method with some modification.

Male Wistar rats were anesthetized with halothane and fasted overnight before ischemia. The brain temperature was maintained at 37° C during ischemia. Ischemic depolarization (ID) was used as an indication of severe ischemia. Dopamine neurons were depleted with two injections of 6-OHDA (4 μg/2 μl X 2) into the substantia nigra (SN) of either side by random selection. Apomorphine was injected 11 days after SN lesion. Only the rats with contralateral rotation for at least 20 min were selected for transient ischemia 2 weeks after lesion. The animals were sacrificed 24 h after reperfusion. The neuronal damage was evaluated by counting the survived neurons in both side of neostriatum on HE staining sections.

Under our experimental conditions, the ischemia that induced ~22 min ID produced about 90% of cell lost in dorsolateral neostriatum. No difference was detected between the left and right striatum. Decrease of ischemic duration (~16 min ID) significantly increased the number of survived neurons (~70% cell lost). In animals after left SN lesioning, no significant difference was found between the number of survived neurons in either side of dorsolateral neostriatum following ischemia of 22 min ID. In contrast, after right SN lesioning, the number of survived neurons in right striatum significantly increased comparing with the left striatum after ischemic insult of the same extent.

Despite the mechanism of this asymmetric protection is not clear at this point, the present study suggests that dopamine may play an important role in postischemic cell injury in neostriatum. Supported by NIH NS33103 and AHA 9400813

563.5

POSTISCHEMIC CHANGES OF MEMBRANE PROPERTIES IN CA1 PYRAMIDAL NEURONS OF RAT HIPPOCAMPUS **IN VIVO**. X. Z. Xi*, T. M. Gao and Z. C. Xu Dept. of Neurology, University of Tennessee Memphis, Memphis, TN 38163, U.S.A.

Neuronal hyperactivity caused by excessive glutamate release during ischemia has been postulated to trigger the process of cell death. It is not clear whether the alterations of synaptic transmission or changes in membrane properties are the major contributor to the pathogenesis of postischemic cell injury. We have found an ischemia-induced postsynaptic potential (i-PSP) evoked from CA1 pyramidal neurons preceding cell death after ischemia. The present study investigated the changes of membrane properties in CA1 neurons following reperfusion.

Male Wistar rats were fasted overnight and anesthetized with 1-2% halothane. The brain temperature was maintained at 37° C during ischemia. Four-vessel occlusion was performed to induce ischemic depolarization for approximately 13 min, which consistently produced selective cell death in CA1 region. Membrane properties were compared between morphologically identified CA1 pyramidal neurons before and after ischemia with *in vivo* intracellular recording and staining.

In comparison with those before ischemia, spontaneous firing of CA1 neurons significantly reduced within two days after reperfusion. Hyperactivity as indicated by increasing firing rate was never observed. The rheobase and spike threshold of CA1 neurons were significantly increased after reperfusion. No significant difference was detected in resting membrane potential, spike height and duration, membrane input resistance and time constant after reperfusion.

These results indicate that the spontaneous activity and the excitability of CA1 neurons are suppressed after transient forebrain ischemia. The i-PSP recorded in CA1 neurons is not associated with a significant modification of membrane properties, suggesting that i-PSP may be due to an increased efficacy of synaptic transmission but not the changes in excitability of the neuron.

Supported by grants from NIH NS33103 and AHA 9400813

563.6

NEUROPROTECTIVE ROLE OF THE SEROTONIN 1A RECEPTOR. P. Banerjee*, T. Adayev, and J.K. Singh. Biol. Doc. Prog. of Grad. School & Dept. Chem., CUNY at Staten Island, NY.

A trophic role of the neural serotonin 1A receptor (5-HT_{1A}-R) was suggested in earlier studies, however, a direct demonstration of neuroprotective action of the 5-HT_{1A}-R has not been reported. Our studies demonstrate that the selective 5-HT_{1A} agonist, 8-OH-DPAT, causes inhibition of cell death under stress in the engineered neuronal cells, HN2-5 (hippocampal), as well as in 16-DIV mouse hippocampal neurons. Cell death is initiated by conditions of stress, such as hypoxia or a state of prolonged stress created by continuous culture without feeding. This protection of neuronal cells in each case is eliminated upon pertussis toxin (15 ng/mL) pretreatment and also in the presence of the 5-HT_{1A} antagonist, UH-301 (1 μM), confirming the involvement of the 5-HT_{1A}-R and a pertussis toxin-sensitive G-protein. Since the HN2-5 cells are completely devoid of Ca^{2+} current, the observed protection could not be solely through a 5-HT_{1A}-R mediated direct or indirect inhibition of Ca^{2+} channels. Possible involvement of the mitogen activated kinase (MAPK) pathway was investigated. Agonist stimulation of the 5-HT_{1A}-R was observed to cause an activation of ERK-1 in both hippocampal neurons as well as HN2-5 cells. Inhibition of the upstream regulator ras by transient expression of dominant negative N17ras caused elimination of the 5-HT_{1A}-R mediated protection of HN2-5 cells during hypoxia. This is the first demonstration of direct neuroprotective action of the serotonin 1A receptor, and our experiments demonstrate that the MAPK pathway, channeled to the nucleus through the kinase ERK-1, is responsible for neuroprotection triggered by the serotonin 1A receptor in neuronal cells.

563.7

ENHANCED RELEASE OF GABA AND TAURINE IN THE MOUSE HIPPOCAMPUS IN ISCHEMIC CONDITIONS. P. Saransaari* and S.S. Oja. Tampere Brain Res. Ctr, Univ. Tampere Medical School, Finland.

The massive release of excitatory amino acids from neural structures during ischemia has been thought to contribute to excitotoxicity and cell death. We studied the releases of both endogenous and preloaded labeled GABA and taurine from mouse hippocampal slices in ischemic conditions in vitro, using a superfusion system. The basal release of both inhibitory amino acids was greatly enhanced in hypoxic, hypoglycemic and ischemic conditions applied from the beginning of the superfusion, ischemia being the most effectual. Potassium stimulation (50 mM K^+) was still able to potentiate GABA release in hypoglycemia and ischemia. In Ca-free media, the basal release of GABA was slightly increased and potassium stimulation considerably decreased. The basal release was further enhanced in ischemia but potassium stimulation was not significantly different in Ca-free media in normoxic and ischemic conditions. The basal release of taurine was already so substantial in ischemia that subsequent potassium stimulation failed to have an effect. Glutamate receptor agonists did not affect GABA release in standard or ischemic conditions. The NMDA and kainate stimulations of taurine release were not discernible in ischemic conditions but kainate and AMPA still potentiated taurine release in hypoxia. These effects were apparently not receptor-mediated, however. The enhanced release of both GABA and taurine during ischemia could counteract the harmful effects of excitatory amino acids, forestalling perturbation of homeostasis in the hippocampus upon impending hyperexcitation. Supported by the Medical Research Fund of Tampere University Hospital, Finland.

563.8

IMIDAZENIL, A BENZODIAZEPINE PARTIAL AGONIST, PROTECTS GERBIL HIPPOCAMPAL NEURONS FOLLOWING TRANSIENT CEREBRAL ISCHEMIA. Laura E. Chadwick, Kelly J. McDonough, and Rochelle D. Schwartz-Bloom*. Dept. Pharmacology, Duke University Medical Center, Durham, NC 27710

Benzodiazepines, which enhance GABA-mediated neuronal inhibition, protect hippocampal pyramidal neurons from ischemic injury. We determined whether the benzodiazepine partial agonist, imidazenil (IZ), at non-sedating doses, could produce long-term neuroprotection and prevent working memory deficits induced by ischemia. Gerbils were subjected to 5 min of forebrain ischemia by occlusion of the carotid arteries; IZ (1-6 mg/kg, i.p.) was injected 30 and 90 min later. In a separate experiment, gerbils were trained for 20 sessions on an 8-arm radial maze prior to induction of ischemia. 30 and 90 min later, they were injected with IZ (3 mg/kg, i.p.). Unlike other benzodiazepines, IZ did not produce significant hypothermia or sedation. IZ prevented pyramidal cell death in the CA1 hippocampus 7 days after ischemia in a dose-dependent manner. In the long-term experiment, ischemia produced significant deficits in working memory 1 month later. IZ prevented working memory deficits in those gerbils that exhibited complete protection in area CA1 hippocampal pyramidal neurons 35 days following ischemia. There was a significant negative association between the extent of hippocampal area CA1 cell survival and the number of working errors. The neuroprotective efficacy of IZ 7 and 35 days post-ischemia was below that produced by sedating doses of diazepam. Although the lack of sedation produced by IZ may be a clinical advantage in the prevention of ischemia-induced memory deficits and hippocampal cell death long term, diazepam appears to have greater efficacy, possibly due to hypothermia. Supported by NIH grant NS 28791.

563.9

ATP LEVELS AND ADENOSINE EFFLUX FROM RAT HIPPOCAMPAL SLICES DURING HYPOXIA/HYPOGLYCEMIA. J.C. Fowler* and Y. Li. Department of Physiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Cellular ATP levels decline and adenosine efflux increases during cerebral ischemia *in vivo* as well as during exposure of rat hippocampal slices to hypoxia/hypoglycemia. It is generally thought that adenosine efflux is proportional to ATP degradation. In addition, ATP depletion is generally inversely related to the degree of recovery of evoked synaptic transmission. We examined recovery of evoked synaptic transmission, cellular ATP levels and adenosine efflux under a number of conditions that alter these parameters.

Evoked population spikes from slices superfused for 10 minutes in hypoxic/hypoglycemic artificial cerebrospinal fluid (ACSF) that was either Ca^{+2}-free or contained Co^{+2} (2 mM), MK-801 (10 μM) or TTX (1 μM) exhibited significantly improved recovery. Ca^{2+}-free ACSF, Co^{2+}-and MK-801 block Ca^{2+} influx. TTX blocks voltage-dependent Na^+ influx. Hypoxic-hypoglycemic ATP levels were significantly spared in the presence of Co^{+2}, MK-801 or TTX but not in hypoxic/hypoglycemic ACSF lacking Ca^{2+}. Hypoxic/hypoglycemic adenosine efflux was unchanged in MK-801, but was reduced in Ca^{+2}-free ACSF or ACSF containing Co^{+2}.

In summary, recovery of evoked synaptic transmission and adenosine efflux can be dissociated from changes in hypoxic/hypoglycemic ATP levels. Supported by NINDS NS28027.

563.10

ADENOSINE A_1 RECEPTOR MODULATION OF VESICULAR GLUTAMATE RELEASE DURING ANOXIA IN CA1 NEURONS OF THE RAT HIPPOCAMPUS. A. N. Katchman* and N. Hershkowitz. Department of Neurology, Georgetown University School of Medicine, Washington, DC 20007.

Adenosine release following hypometabolic stresses such as stroke and anoxia may serve a neurprotective function. One potential mechanism is through a presynaptic suppression of glutamate release. Adenosine has been shown to effect two sources of synaptic glutamate release, action potential dependent- (excitatory postsynaptic potentials, EPSCs) and independent-release (miniature EPSCs, mEPSCs). We used whole cell patch clamp recording in CA1 pyramidal neurons in the *in vitro* rat hippocampal slices to examine the role of adenosine in the suppression of both forms of synaptic glutamate release during anoxia . Anoxia was induced by switching perfusion and ambient gas of an interface chamber from a 95%O_2/5%CO_2 to a 95%N_2/5%CO_2 mixture. As we previously demonstrated anoxia leads to a significant increase in the frequency (280 ± 70%, n=17) of spontaneous inward currents which have been identified as glutamate-mediated mEPSCs. When slices are preincubated in the adenosine A_1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) a second larger population of spontaneous inward currents was observed. Their suppression by glutamate antagonists, tetrodotoxin or by the cutting of the Schaffer collateral pathway indicates that they represent action-potential dependent, glutamatergic EPSCs likely originating from CA3 pyramidal neurons. Although DPCPX caused the appearance of EPSCs it did not influence the anoxia-induced increase in mEPSC frequency. We have therefore demonstrated that during anoxia increases in extracellular adenosine may contribute to neuroprotection by suppressing action potential-dependent synaptic glutamate release (EPSCs) but not action potential-independent spontaneous synaptic glutamate release (mEPSCs). (Supported by NIH grant NS32426 and an Epilepsy Foundation of America Research Grant)

563.11

PURINE NUCLEOSIDE CONCENTRATION DURING RETINAL ISCHEMIA IN RATS S. Roth*, J.V. Osinski, A. Toledano, S.S. Park Dept of Anesthesia and Critical Care, University of Chicago, Chicago, Illinois 60637

Adenosine, produced from the decomposition of adenosine triphosphate, is believed to provide protective effects during ischemia. On the other hand, adenosine metabolites may serve as precursors for oxygen free radical formation. The role of adenosine in retinal ischemic injury has not yet been completely elucidated. We sought to determine the time course of formation of adenosine and its purine metabolites during retinal ischemia in rats. Concentrations of adenosine and its purine nucleoside metabolites inosine, hypoxanthine, and xanthine in the retina of ketamine/xylazine-anesthetized rats were measured during retinal ischemia using HPLC. Quantitative measurements were made possible in the small tissue mass through the use of internal standards. Ischemia was induced by ligation of the central retinal artery, and its severity was examined using electroretinography (ERG). In each rat, one retina was ischemic while the other served as a non-ischemic control. Eyes were frozen *in situ* using liquid nitrogen at 1, 5, 10, 20, 30, 60, and 120 min of ischemia. The retina was then removed from the frozen eyes and analyzed. Significant increases in the concentrations of adenosine, inosine, and hypoxanthine in ischemic compared to control retinae were detectable within 1 to 5 min of the onset of ischemia, and within 10 min for xanthine. Increase in adenosine concentration in ischemic relative to control retinae was describable by an exponential function for the first 30 min of ischemia, followed by a plateau. Inosine and hypoxanthine concentrations increased linearly, and xanthine exponentially throughout the measurement period. There was no ERG recovery after 120 min ischemia, nearly complete recovery after 5 min, and intermediate degrees of recovery in the other groups. This study confirmed a possible role of adenosine in retinal ischemia, which may be important during short-term oxygen or blood flow deprivation. However, prolonged ischemia results in ongoing production of xanthine, which by serving as a precursor for oxygen free radical formation, potentially may overwhelm any protective effect of adenosine, and lead to worsening of ischemic damage.

Supported by NIH EY10343

563.12

SATURATION OF A1 RECEPTOR-MEDIATED NEUROPROTECTION BY ENDOGENOUS ADENOSINE IN CORTICAL CULTURES EXPOSED TO OXYGEN-GLUCOSE DEPRIVATION. D. Lobner* and D.W. Choi, Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Experiments in several brain ischemia models have demonstrated that administration of adenosine A1 agonists can reduce neuronal loss. However, contemplation of adenosine agonist administration as a neuroprotective treatment for brain ischemia needs to consider the degree to which adenosine A1 receptors are already activated by release of endogenous adenosine.

In murine cortical cell culture we found that the addition of the A1 receptor agonist cyclohexyladenosine (10 μM) did not affect cell death caused by 50 min oxygen and glucose deprivation, while it did attenuate cell death caused by 5 hr glucose deprivation alone (40% decrease) or 24 hr exposure to NMDA (80% decrease). Adenosine levels endogenously present in the bathing medium at the end of oxygen-glucose deprivation were higher than those found, either after 50 min or at the end, of the glucose deprivation or NMDA exposure. Furthermore, addition of the A1 receptor antagonist 8-cyclopentyltheophylline (10 μM) did potentiate neuronal death induced by oxygen-glucose deprivation, but did not potentiate the death induced by either glucose deprivation or NMDA.

These data suggest that endogenous adenosine release is able to saturate the A1 receptor-mediated protective effect in our cell culture model of ischemic brain injury. If such saturation occurs in portions of the ischemic brain in vivo, it could act to limit the neuroprotective gains to be had through administration of exogenous adenosine A1 agonists. Supported by NIH NINDS NS 30337 (DWC).

563.13

ADENOSINE A1 RECEPTOR AGONISTS PREVENT DELAYED NEURONAL DEATH FOLLOWING ISCHEMIA. M.I. Sweeney*, D.S. Arneson and A. Murton. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, SK, Canada.

Adenosine A1 receptor agonists given acutely protect neurons against ischemic death. Recent evidence suggests that endogenous adenosine mediates the cerebroprotective effect of ischemic pre-conditioning. The aim of this study was to determine whether selective A1 agonists also pre-condition the brain to withstand subsequent lethal ischemia. This was tested in *in vitro* (primary cultures of cerebellar neurons exposed to substrate deprivation plus hypoxia) and *in vivo* models (unilateral global ischemia in rats). Cell death was quantitated by measuring extracellular lactate dehydrogenase (LDH) *in vitro* or by counting live cells stained with hematoxylin and eosin *in vivo*.

Incubation of cultured neurons with the A1 agonist cyclopentyladenosine (CPA, 0.1-1μM) during 1 and 3hr of simulated ischemia, or for 1hr at least 24hr prior to simulated ischemia, prevented subsequent cell death. Thus, LDH release, occurring 24hr later, was reduced by 38±6%, 53±4%, 76±9% respectively. I.p. injections of CPA (1mg/kg) given 1 and 24hr before lethal ischemia provided good protection against hippocampal damage. The relative brain damage scores were 3.3±0.5 in control brains, and 1.8±0.6 and 1.0±0.2 after respective CPA injections given 1 and 24hr previously. In all cases, the neuroprotective effects of CPA were antagonized by the selective A1 receptor antagonist dipropylcyclopentylxanthine. These data indicate that activation of an adenosine A1 receptor may be a useful and effective way of preventing the brain against subsequent ischemic damage. Funded by HSF (Sask).

563.14

UPREGULATION OF TYPE I NOS AFTER TRANSIENT FOCAL CEREBRAL ISCHEMIA USING [^{3}H]L-N^G-NITRO-ARGININE AUTORADIOGRAPHY. H. Hara*[1], C. Waeber[1], P. L. Huang[2], M. C. Fishman[2] and M. A. Moskowitz[1], [1]Stroke and Neurovascular Regulation Laboratory and [2]Cardiovascular Research Center, MGH, Harvard Medical School, Charlestown, MA 02129.

Changes in regional distribution of nitric oxide synthase (NOS) after transient focal cerebral ischemia in the mouse brain were assessed by quantitative autoradiography using [^{3}H]L-N^G-nitro-arginine ([^{3}H]L-NA) binding in wild-type mice (SV-129) and in mice lacking expression of the neuronal (type I) and endothelial (type III) NOS gene.

[^{3}H]L-NA binding did not change during 3 hr middle cerebral artery (MCA) occlusion in any region. In the early phase after reperfusion (1 - 3 hr), the binding increased by 50 -130% in ipsilateral striatum, amygdala and the MCA region of cortex in SV-129 mice, but was not altered in the ipsilateral anterior cerebral artery (ACA) teritory. No changes were observed in the contralateral striatum, amygdala and cortex. The density of [^{3}H]L-NA binding peaked 3 hr after reperfusion. The dissociation constant (Kd) values in ischemic ipsilateral side did not change 3 hr after reperfusion when compared to contralateral side. The density of binding sites (Bmax) values in ipsilateral side increased 3 to 5 times higher than in contralateral side. The binding in amygdala and MCA area decreased 7 days after reperfusion. In type III mutant mice, but not in type I mutant mice, [^{3}H]L-NA binding also increased 3 hr after reperfusion in the same areas as in wild-type mice.

Our data indicate that the rapid up-regulation of neuronal NOS in the ischemic region may play an important role in the early phase after focal cerebral ischemia.

563.15

STRIATAL NITRIC OXIDE (NO) PRODUCTION IS ENHANCED IN FOCAL CEREBRAL ISCHEMIA: AN *IN VIVO* MICRODIALYSIS STUDY. A. Bhardwaj*, F.J. Northington, D.F. Hanley, R.J. Traystman, R.C. Koehler. Johns Hopkins University School of Medicine, Baltimore, MD 21287.

We have previously demonstrated that NMDA, AMPA and metabotropic glutamate receptor stimulation enhances hippocampal NO production *in vivo*. We adapted the microdialysis technique in the rat striatum to measure NO production in the string model of permanent MCA occlusion (n=8). Under controlled conditions of normoxia, normocarbia and normothermia bilateral microdialysis probes were placed in the striatum of halothane anesthetized adult male Wistar rats (250-300 g) and perfused with artificial CSF containing 3μM [^{14}C]-L-arginine. Effluent [^{14}C]-L-citrulline, which was previously shown to be inhibited by nitroarginine was collected every 30 minutes and used as a marker of tissue NO production. In the ischemic striatum, [^{14}C]-L-citrulline recovery (femtomoles/minute ± S.E.M.) was 537 ± 30 at 3 hours post-ischemia as compared to 356 ± 26 in the non-ischemic striatum. In surgical shams (n=5), [^{14}C]-L-citrulline recovery was not different in the left (350 ± 11) and right (363 ± 27) striatum. Probe placement in the striatum and brain infarcts were confirmed by TTC staining and dissection at 24 hours. These data demonstrate that there is increased conversion of arginine to citrulline indicating increased NO production during permanent focal ischemia.

Supported by the National Stroke Association and USPHS NINDS P01-20020.

563.16

LOSS OF NITRIC OXIDE SYNTHASE (NOS) POSITIVE NEURONS IN THE NEWBORN BRAIN AFTER HYPOXIA-ISCHEMIA F.J.Northington*, A.Brambrink, R.J. Traystman, R.C.Koehler, and L. J. Martin. Dept. of Pediatrics, Anes./Critical Care Medicine, Pathology, and Neuroscience. The Johns Hopkins Medical Institutions, Baltimore, MD 21287.

In adult models of ischemia and excitotoxicity, NOS+ neurons may be spared. In a newborn model of hypoxic-ischemic brain injury, we tested the hypothesis that neurons expressing NOS protein or NADPH-diaphorase (NADPHd) activity would survive in the selectively damaged areas of putamen and somatosensory cortex. 13, 1-2 week old piglets were subjected to 30 minutes (m) of hypoxia followed by 7m of respiratory arrest. After resuscitation, they were survived for 24 (n=4), 48 (n=4), or 96 (n=5) hours (h). 40μ sections of striatum and somatosensory cortex were processed histochemically or immunocyto-chemically to detect NADPHd or the neuronal isoform of NOS. Positive neurons in 8 fields in the putamen and 4 fields in the cortex were counted in 3 sections from each animal. In both regions, the two methods gave similar results. NOS+ neurons in the putamen (#/mm^2 ±SEM, *p<0.01) (Sham(n=5) 30.6±1.5, 24h 11.4*±6.8, 48h 6.6*±3.7, 96h 3.1*±.8) were vulnerable to injury, with only a subset surviving past 24 hours. There were no differences after 24h. NOS neurons in the cortex, were not reduced in number after injury (Sham 4.3±0.4, 24h 4.9±0.4, 48h 1.9±0.9, 96h 3.1±1.1). In this model, NOS+ neurons in putamen, a primary site of damage, are vulnerable to hypoxia-ischemia. The loss of the NOS protein/diaphorase phenotype occurs within 24h of the insult and is not progressive. If neuronally derived nitric oxide is a mediator in delayed cell death following neonatal hypoxia-ischemia, only a subset of NOS+ neurons are responsible. Supported by NINDS 20020 & KO81742, & AHA 95009060

563.17

INDUCTION OF NEURONAL NITRIC OXIDE SYNTHASE EXPRESSION, BUT NOT INDUCIBLE OR ENDOTHELIAL ISOFORMS, FOLLOWING SPREADING DEPRESSION. A.O. Caggiano* and R. P. Kraig. Dept. of Neurology, The University of Chicago, Chicago, IL 60637

Nitric Oxide Synthases (NOS) produce NO, which has important roles in brain function ranging from glutamate cytotoxicity to LTP. Enhanced expression of the inducible (iNOS) isoform occurs with a variety of stimuli, while enhanced expression of the neuronal (nNOS) and endothelial (eNOS) forms have only been shown to be induced by conditions resulting in cell injury or death. Here we show that nNOS expression can be rapidly and significantly induced following the non-injurious stimulation of spreading depression (SD). Unilateral SD was elicited in 44 Wistar male rats by micro-injection of 0.5M KCl into the left parietal cortex every 9 minutes for 3 hours. After 6 hours or 3 days of recovery brains were processed immunohistochemically with antibodies against nNOS, iNOS and eNOS. Computer based object counting for nNOS and densitometry analysis for iNOS and eNOS quantitated expression. nNOS expression was consistent with previous reports showing punctate and scattered expression filling large processed neurons. Expression was seen in the caudate, putamen, amygdala, lateral hypothalamic group, ventromedial nucleus of the hypothalamus, the median and magnocellular preoptic nucleus, the paraventricular nucleus of the thalamus, the subthalamic nucleus and the dorsal premammillary nucleus. nNOS expression was rapidly (by 6 hours) and significantly ($p<0.001$) induced in the cortex experiencing SD with a new distribution pattern showing strong expression in small neurons in layers II and III of the primary and supplemental somatosensory cortex, visceral, perirhinal and entorhinal areas. Expression was not induced rostral to -1.0 mm from bregma. Treatment of animals with a single dose of dexamethasone, N_G-nitro-l-arginine methyl ester, phenylephrine or indomethacin significantly reduced the expression of nNOS following SD. Nordihydroguaiaretic acid required longer treatment to reduce expression and mepacrine had no effect. The iNOS and eNOS expression did not change following SD.

These results demonstrate that nNOS expression can be induced by non-injurious stimuli and this new expression may be one mechanism by which plastic changes in brain function and structure are manifest. (Supported by NS-19108)

563.18

INHIBITION OF NITRIC OXIDE SYNTHASE (NOS) BY 7-NITROINDAZOLE (7-NI) AMELIORATES HYPOXIA-INDUCED DEPLETION OF ATP IN BRAIN SLICES M.T. Espanol, L. Litt*, K. Hasegawa, L.-H. Chang, T.L. James, and P.-H. Chan. University of California, San Francisco, California 94143

Certain central nervous system neurons contain neuronal nitric oxide synthase (nNOS), an enzyme that catalyzes formation of nitric oxide (NO) from L-arginine. Studies by others have shown that nNOS mediates glutamate toxicity presumably because it is calcium-dependent and NMDA-receptor activation causes intracellular calcium entry. We have used an nNOS inhibitor, 7-NI, to ameliorate hypoxia-induced depletion of high energy phosphorus metabolites (PCr and ATP), and reduce adverse histological changes. $^{31}P/^{1}H$ NMR spectroscopy studies at 4.7 Tesla were conducted in cerebrocortical slices from 7-day old neonatal Sprague-Dawley rats, pretreated for 60 minutes with 1.0-5.0 mM L-Nitro-arginine methyl ester (L-NAME), or 3-10 μM 7-nitroindazole (7-NI). Data were obtained during NOS inhibitor pretreatment, hypoxia, and reperfusion-recovery. During hyperoxia, both L-NAME and 7-NI did not affect levels of ^{31}P metabolites. During and after hypoxia (PO_2=0) only brains slices pretreated with ≥9.2 μM 7-NI showed sustained ATP levels and demonstrated full metabolic recovery. Histological scores were significantly higher in the 7-NI studies. In perfused respiring cortical slices, as in the cortex in vivo, NOS-containing neurons modulate hypoxia-induced neuronal injury. Supported by UCSF Academic Senate, NIH Grants GM34767, NS22022, NS14543, NS25372, and RR03841.

563.19

ENHANCED NO RELEASE FROM CEREBRAL CORTEX AFTER MIDDLE CEREBRAL ARTERY LIGATION IN THE SPONTANEOUSLY HYPERTENSIVE RATS. S. Z. Lin*, A. L. Chiou and Y. Wang. Depts. of Neurosurgery and Pharmacology, National Defense Medical Center, Taipei, Taiwan, 100

We and others previously demonstrated that nitric oxide (NO) can be released from ischemic cerebral cortex after shorterm cerebral ischemia. It has been shown that the NO synthetase is differentially regulated in the spontaneously hypertensive rats (SHR) and in their normal controls (Wistar-Kyoto rats or WKYs). In this study, we compared the NO release in these two strains during ischemic insults. Animals were anethesthetized by urethane. Temporary cerebral ischemia was induced by middle cerebral artery ligation while clamping bilateral common carotids for 40 minutes. Extracellular NO concentration was recorded through a Nafion and porphyrine -coated carbon fiber electrodes and *in-vivo* chronoamperometry. We found that the peak of NO release from the ischemic cerebral cortex was much higher in the SHRs than in the WKYs. Triphenyltetrazolium chloride (TTC) staining was used to evalulate the volume of infarctions. Similar to the NO release, SHRs had a much greater cortical infarction after shorterm cerebral ischemia than that in WKYs. Taken together, our data suggest that SHRs, compared to WKYs, are more sensitive to the ischemic insults. (This research is supported by a grant from the National Science Council of R.O.C.).

563.20

ROLE OF NITRIC OXIDE IN ISCHEMIA- EVOKED RELEASE OF [^{3}H] NOREPINEPHRINE IN RAT CORTEX SLICES K.W. Kim,* Y. A. Eun, H. R. Lee and K. P. Cho Dept. of Pharmacol., Medical School and Institute for Medical Sciences Chonbuk National Univ. Chonju, 560-182, Rep. of Korea

It has been shown that cerebral ischemia alters brain monoamine metabolism, and brain monoamines are involved in regulating the development of ischemic brain damage. We have examined the ischemia-evoked release of [^{3}H]NE from the cortical and slices(250 μM thickness) of the rat loaded previously with the monoamine(15 nM for 15 min). Ischemia was induced by 5 min of deprivation of glucose and additional 5 min of deprivation of glucose and oxygen from Mg^{2+}-free Krebs-Henseleit buffer. During ischemic period, significant amount of total [^{3}H]NE was released into the incubation media. This ischemia-evoked release of [^{3}H]NE from cortex slices was attenuated by tetrodotoxin, 1.2 mM $MgSO_4$, dizocilpine, ketamine, DNQX, or carbetapentane. Ischemia-evoked [^{3}H]NE release was attenuated by nitric oxide synthase inhibitors (L-NAME or 7-nitroindazole), hemoglobin, and methylene blue, potentiated by 8-bromo-cGMP, zaprinast, and nitric oxide generator (SNAP). Nisoxetine inhibited the ischemia-induced release of [^{3}H]NE. Omission of Ca^{2+} from incubation media potentiated ischemia-evoked [^{3}H]NE release and the inhibitory effect of blockers for transporter. In conclusion, nitric oxide is involved in ischemia-evoked monoaminea release, and this release is achieved by Ca^{2+}-dependent exocytosis and reversal of transporters, and can be modulated by various neuronal mechanisms.
(Supported by 1995 Basic Medical Research Fund of Ministry of Education of Korea)

564

SYMPOSIUM. GENE TRANSFER: APPLICATIONS OF VIRAL VECTORS FOR THE STUDY AND TREATMENT OF CNS DISORDERS. L.S. Brady, NIMH & H.J. Federoff, Univ. of Rochester (Chairpersons); M.G. Kaplitt, Rockefeller Univ.; F.H. Gage, Salk Instit.; B.L. Davidson, Univ. of Iowa; X.O. Breakefield, Mass. Gen. Hosp.

The aim of this symposium is to describe recent advances in the use of viral vectors to transfer and express genes in target neurons of the CNS, with emphasis on their application to the study of brain function. M. Kaplitt will describe the use of adeno-associated virus (AAV) vectors to deliver gene products in experimental models of Parkinson's Disease, Alzheimer's Disease, and in neural protection. F. Gage will report on new retroviral and lentiviral vectors used to modify cells to express neurotransmitter and neurotrophic factors. He will describe the use of genetically-modified cells to study functional recovery in experimental models of CNS damage. B. Davidson will describe the use of replication-deficient adenovirus vectors to study disease in murine models of CNS dysfunction and the ability of the vectors to correct the underlying biochemical defects. X. Breakefield will describe the use of herpes simplex viral vectors to deliver genes for pro-drug activating enzymes in experimental brain tumors. She will present data showing that herpes vectors can be administered via multiple routes to target tumor cells disseminated throughout the brain. H. Federoff will summarize the advances and the limitations in the use of viral vectors to study CNS function.

565

SYMPOSIUM. BIRD SONG: TWENTY YEARS OF PROGRESS. E.A. Brenowitz, Univ. of Washington (Chairperson); D. Margoliash, Univ. of Chicago; B.A. Schlinger, UCLA; K. Nordeen, Univ. of Rochester; D.F. Clayton, Univ. of Illinois.

Over the past 20 years, the neural system that regulates song behavior in birds has emerged as an excellent model for study of fundamental issues including sexual differentiation of brain and behavior, sensitive periods in development, hormonal influences on brain function and development, the neural basis of learning and memory, plasticity in adult brains, and the organization of motor circuits. Birdsong is a learned, stereotyped behavior that is sexually dimorphic in many species. The learning, production, and perception of song are regulated by discrete neural circuits. These circuits show considerable morphological and physiological plasticity in adult birds.

This symposium will review progress in this field and identify important directions for future research. Brenowitz will provide a brief overview of the song system. Margoliash will discuss the implications of physiological data, from intracellular to chronic recordings, for understanding the functional organization of the song system. Schlinger will consider the profound influence of sex steroid hormones and their metabolites on development and function of the song system. Nordeen will describe anatomical and synaptic plasticity in the song system at the time of song learning. Clayton will present evidence that differential gene expression is important in determining the degree of plasticity found in the song control system.

568.1

EXPRESSION OF THE S182/PRESENILIN I PROTEIN IN NEURONAL CELL LINES. J. Shioi[1], H. Cai[1], N. Tezapsidis[1], H.-C. Li[2], S. Efthimiopoulos[1], N. K. Robakis*[1,3], Departments of Psychiatry[1] and Biochemistry[2] and Fishberg Research Center for Neurobiology[3], Mount Sinai Medical Center, New York, NY10029, USA.

Many individuals with familial Alzheimer's disease (FAD) have mutations in a chromosome 14 gene termed S182 or Presenilin I (PS-I), whose product has not been identified and its function remains unknown. We prepared affinity purified antibodies against synthetic peptides of the predicted amino acid sequence of PS-I. These antibodies recognized a glutathione S-transferase/PS-I bacterial fusion protein and were used to examine the cellular expression of PS-I. Western blot analysis of N2a neuroblastoma cell extracts detected a single antigen of approximately 50 kDa, in good agreement with the predicted molecular weight of the 467 amino acid protein. Immunoprecipitaion of the ^{35}S-labelled cell extracts also revealed a 50 kDa protein, whose detection was inhibited by the presence of the corresponding antigen peptide during precipitation. Immuno-cytochemistry of various cell lines demonstrated that only neural cell lines HS-SY5Y, N2a and NGF-induced PC12 cells were well stained with anti PS-1 antibodies, while non-neuronal cell lines, such as C6 rat glioma, fibroblast COS, 293 or CHO, were poorly stained. The staining was specific because it was inhibited by the presence of the competing peptide. In neuronal cells, both cell bodies and extended neurites were equally stained. High magnification showed a cytoskeletal structure-like staining pattern, and a double immunostaining with both anti PS-I and anti-cytoskeletal proteins is under way. These results are consistent with those of Elder et al (1996, J. Neurosci. Res., in press) showing that in tissue sections of mouse central nervous system, PS-I protein is found exclusively in neurons where it is located primarily within cell bodies and dendrites. These data suggest that PS-I is expressed mainly in neurons where it may perform a neuron-specific function. Supported by Grants AG08200 and AG05138.

568.2

SUBCELLULAR LOCALIZATION OF PRESENILIN-1 IN CULTURE AND IN THE ALZHEIMER BRAIN. Jorge Busciglio*, Alfredo Lorenzo, Henrike Hartmann, Matthias Staufenbiel+ and Bruce A. Yankner. Dept. Neurology, Harvard Medical School and the Children's Hospital, Boston, MA. +Sandoz Pharma Ltd., Basel, Switzerland.

Missense mutations in presenilin-1 (PS-1) have been shown to cause most cases of familial Alzheimer's disease (FAD). We examined the subcellular localization of PS-1 with antibodies to different regions of PS-1. Transiently transfected COS cells expressing full-length PS-1 showed strong perinuclear cytoplasmic staining which colocalized by double-labeling immunofluorescence with Golgi resident proteins βCOP and 58 kD protein and the ER resident protein BiP. PS-1 immunoreactivity was abolished by preabsorption of the antibodies with the corresponding antigenic peptides. Non-transfected COS cells exhibited a pattern of immunostaining restricted to the ER, suggesting an ER localization of PS-1 when the protein is not overexpressed. PS-1 immunolabeling was observed in neuronal cell bodies and neuritic processes of cultured rat hippocampal neurons. PS-1 immunoreactivity was also detected in oligodendrocytes, astrocytes and neuroblastoma cells. In the Alzheimer brain, PS-1 immunoreactivity was detected in pyramidal neurons of the hippocampal formation and neuronal cell bodies scattered throughout cortical areas, but not in granule cells of the dentate gyrus. PS-1 positive neurons were often seen adjacent and surrounding amyloid plaques, but PS-1 immunostaining was not observed in the plaque cores. Neurons stained with antibodies against phosphorylated tau were also frequently positive for PS-1. These results show that PS-1 is preferentially localized in neuronal populations that are particularly affected in Alzheimer's disease. The significance of these findings will be discussed.

568.3

EXPRESSION AND SUBCELLULAR LOCALIZATION OF PRESENILIN 1 AND 2. P. M. Mathews, B. Kao, J. L. Yang, X. Qin, J. L. Barnett, A. M. Cataldo, and R. A. Nixon*. Lab. for Molecular Neuroscience, McLean Hospital, Dept. of Psychiatry, Harvard Medical School, Belmont, MA 02178.

In order to study their expression and subcellular localization, we have generated a battery of antibodies against the recently identified chromosome 14 and chromosome 1 gene products (PS1 and PS2, respectively) linked to early-onset Alzheimer's disease. Multiple antibodies showed by Western blot analysis an ~50 kDa doublet in murine L-cells stably transfected with a human PS1 cDNA. Immunofluorescence labeling of these cells with a mAb specific for human PS1 localized the overexpressed protein to the ER. In addition to the transfected cells, an ER-like distribution was seen in various human cell lines expressing endogenous levels of PS1 and in pyramidal neurons from both human and monkey cortex. Antibodies against PS2 revealed a similar distribution in pyramidal neurons for this protein. Consistent with the initial report showing the broad distribution of PS1 mRNA, Western blot analysis of multiple human cell lines, of human brain, and of various tissues isolated from monkey has consistently detected PS1. The levels of PS1 immunoreactivity detected in frontal cortex from age-matched control and sporadic Alzheimer's disease cases were found to be similar both by immunocytochemistry and Western blot analysis. In both human and monkey brain, however, Western blot analysis gave evidence for substantial proteolytic processing of PS1 unrelated to the postmortem interval. (AG10916 and F2 NS09848)

568.4

CHARACTERIZATION OF THE PRESENILINS, A NEW CLUE TOWARDS UNDERSTANDING ALZHEIMER'S DISEASE. L. Lévesque, H. Chi, C. Bergeron*, W. S. Trimble, C. Haass, D. Selkoe, P. St George Hyslop and P.E. Fraser. Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario, Canada, M5S 3H2.

Presenilins (PS) I and II are part of a new class of proteins which are believed to play an important role in the development of Alzheimer's disease (AD). Missense mutations of these proteins are linked to families with early onset AD. Polyclonal antibodies were raised against different regions of the PS. Examination of neural (PC12) and non-neural cells (Cos and fibroblasts) by confocal microscopy revealed a PS distribution predominantly localized at the endoplasmic reticulum (ER), as well as some overlap with the golgi and nuclear envelop. Some unidentified vesicles were also labelled but no surface staining was detected. COS cells transfected with a c-myc-tagged PS construct showed a similar pattern of expression using either PS or c-myc antibodies. Electron microscopy of monkey cerebellum with immunogold-labelling appeared often in clusters and seemed to be associated with the ER membrane. Immunofluorescence of primary fibroblast from individuals with selected PS mutations showed no obvious different in PS distribution. Western blots and immunoprecipitation of native or expressed proteins with PS antibodies revealed a predominantly 40-45 kD band which is in agreement with its predicted size. Western blots of the different mutation-bearing fibroblast showed no difference in the level of PS expression. Therefore, mutations of PS does not seem to cause changes in its distribution pattern or its level of expression but may possibly cause functional changes. It is suggested that PS may play a role as protein processing or trafficking which may alter in some familial AD.

This work was supported by the Alzheimer Association of Canada and the Medical Research Council of Canada.

568.5

Analysis of Presenilin 1 in a Human Neuronal System: Localization in Cell Bodies and Dendrites, D.G. Cook*, J.C. Sung, T.E. Golde, K.M. Felsenstein, B.S. Wojczyk, R.E. Tanzi, J.Q. Trojanowski, V.M.-Y. Lee, and R.W. Doms. Dept. Pathology & Lab. Med., Univ. of Pennsylvania, Philadelphia, PA 19104.

Mutations have been found in two closely related genes located on chromosomes 14 and 1 that cause familial Alzheimer's disease. These genes, Presenilin 1 and 2 (PS1 and PS2), encode novel proteins for which little is known of their function or characteristics of expression.

To gain insight into PS1 biosynthesis we have expressed PS1 in non-neuronal BHK-21 cells and human neuronal NT2-N cells using the Semliki Forest virus (SFV) vector. PS1 was expressed both as a wild type protein, and with antigenic tags (the influenza HA tag was placed near the amino terminus and the Herpes Simplex Virus 1 gD tag was placed at the carboxyl terminus). For all three forms of PS1, immunoprecipitation showed two primary bands migrating at approximately 43 and 50kDa. Endoglycosidase analyses indicate that PS1 is not N or O-glycosylated. Immunofluorescence staining using antibodies against the N-terminus (anti HA), the C-terminus (anti-gD), and the 6th hydrophilic loop of untagged PS1 recognized PS1 only in permeabilized cells. This strongly suggests that PS1 is not expressed on the cell surface. In NT2-N cells, PS1 expression was restricted primarily to the cell bodies and dendrites, and co-localized with calnexin, a marker of the rough ER. At the light microscopic level, the A246E mutation did not alter the lacy, reticular expression pattern seen with PS1wt.

The use of SFV to express recombinant proteins in NT2-N cells provides an efficient system to investigate the function of wild type and mutant PS1 in human neuronal cells. Supported by NIH P01 AG 11542.

568.6

IDENTIFICATION AND NEURON SPECIFIC EXPRESSION OF THE S182 PRESENILIN I PROTEIN IN HUMAN AND RODENT BRAINS. G. A. Elder[1], N. Tezapsidis[1], J. Carter[2], J. Shioi[1]*, C. Bouras[4], H.-C. Li[3], J. M. Johnston[5], S. Efthimiopoulos[1], V. L. Friedrich Jr.[2] and N. K. Robakis[1]. Departments of Psychiatry[1], Molecular Biology[2], Biochemistry[3], The Mt. Sinai School of Medicine, New York, NY; Psychiatry[4], University of Geneva, Switzerland; Neurosurgery[5], Albert Einstein College of Medicine, Bronx N.Y.

Many individuals with familial Alzheimer's disease (FAD) have mutations in a gene termed S182 or presenilin I (PS-I). Presently, the PS-I gene product has been identified but its function remains unknown. Here we report that affinity purified antibodies against the predicted amino acid sequence of the PS-I gene product detected in homogenates of human, mouse and rat brains a single antigen of approximately 50 kDa. This antigen was also present in several neuronal cell lines. Brain tissue fractionation showed that all PS-I antigen was found in the membrane fraction. In stained tissue sections of mouse CNS, PS-I antigen was found only in neurons and could be located within cell bodies, axons and dendrites. Remarkably, the relative partition among these three compartments varied dramatically. A striking feature of PS-I expression was its intense concentration in some (but not all) dendrites, at levels substantially above those in the parent perikarya. In most of the cerebrum, PS-I staining in axons was very weak or undetectable. By contrast, many axons in portions of the brainstem and in the spinal cord showed marked PS-I immunoreactivity. Similarly, staining of sections from human temporal cortex showed that PS-I was present mainly in neuronal cell bodies and dendrites. These data show that in the CNS, PS-I is expressed mainly in neurons and suggests that this protein may perform a neuron specific function.

568.7

EXPRESSION OF PRESENILIN-1 IMMUNOREACTIVITY IN BRAIN AND TRANSFECTED CELLS. J.J. Lah, C.J. Heilman, H.D. Rees, H. Yi, K.E. Miller, S.E. Counts, D.B. Rye, M. Wakai, N.R. Nash, and A.I. Levey*. Dept. of Neurology, Emory University, Atlanta, GA. 30322

Mutations in two closely related genes *presenilin 1* (PS1) and *presenilin 2* (PS2) are responsible for the majority of cases of familial Alzheimer's disease (FAD). Here we examine the PS1 protein by western blot and immunocytochemistry utilizing an N-terminal specific monoclonal antibody. On immunoblots of brain tissue as well as PC12 and COS7 cells transfected with PS1 cDNA, this antibody recognizes a 53kD band, corresponding to the predicted size of the full-length protein. In addition, there is a more abundant polypeptide at 32kD, suggesting that native PS1 is processed into a smaller product. Samples from monkey frontal cortex, hippocampus, septum, thalamus, hypothalamus, striatum, substantia nigra, and cerebellum all revealed the predominance of the 32kD band with no apparent differences in regional abundance. Both bands are present in monkey peripheral tissues, with highest levels in testis and lung, intermediate levels in liver, spleen, salivary gland, and heart, and low or undetectable levels in skeletal muscle and pancreas. Immunocytochemical localization of PS-1 in brain sections shows labeling of neuronal cell bodies, with lower levels in glial cells and blood vessels. Large cortical pyramidal neurons and magnocellular basal forebrain neurons, vulnerable in Alzheimer's disease, express relatively high levels of PS1 immunoreactivity, although many other neuronal populations are also labeled. In transfected cells, immunofluorescent labeling reveals perinuclear and more widespread reticular distributions. Immuno-electron microscopy localizes the N-terminus of PS1 to the cytoplasmic face of discrete intracellular membrane compartments. Studies are in progress to clarify the subcellular localization and to assess the influence of mutations on the processing and localization of PS1.

568.8

Two types of monoclonal antibodies against Alzheimer's PS-1 molecule M. Okochi*, F. Kametani, M. Usami, N. Sahara, A. Yamamoto, T. Kondo, K. Tanaka, Y. Yahagi**, T. Shirasawa**, and H. Mori. Department of Molecular Biology, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156, Japan **Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173, Japan

We prepared several antibodies against 3 different domains in PS-1 to see *in vivo* function of PS-1; at the amino terminal, loop and carboxyl terminal regions of PS-1 as antigen peptides. Immunoprecipitation of PS-1 which were made *in vitro* using transcription/translational kits with PS-1 cDNA showed virtually a single strong band with Mr 43,000 when three antibodies were used as template. This was further confirmed in COS cells transfected with PS-1 cDNA. As we had a trouble to conclude PS-1 in brain tissues, we next prepared monoclonal antibodies to the same region of PS-1. Twelve of monoclonal antibodies were established against the same amino terminal antigen. We classified these antibodies into two groups by the following criterion. All 12 monoclonal antibodies precipitated PS-1-467 product. Four monoclonal antibodies precipitated PS-1-374, while 8 monoclonal antibodies hardly detected PS-1-374, though both presenilin molecules shared the same sequence in their amino terminal region. The lack of loop and carboxy terminal regions which are quite downstream from the epitope was found to affect the antigenicity in the amino terminal region. It suggested that both ends interact each other. Taken together with the established mutations in PS-1, the loop structure seemed to play an important role in the structure and functionn of PS-1.

568.9

ABNORMAL BEHAVIOR OF PS-1 IN GEL ELECTROPHORESIS. N. Sahara [1], A. Yamamoto[1], M. Usami[1], M. Okochi[1], T. Kondo[1], F. Kametani[1], K. Tanaka[1], Y. Yahagi[2], T. Shirasawa[2] and H. Mori*[1]. [1]Department of Molecular Biology, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagayaku, Tokyo 156, Japan. [2]Department of Molecular Pathology, Tokyo Metropolitan Institute of Gerontology, Itabashiku, Tokyo 173, Japan.

Presenilin 1 (PS-1) is the main causal gene for familial Alzheimer's disease. We have recently identified *in vitro* PS-1. Using this PS-1, we examined its biochemical property and found the abnormal behavior in gel electrophoresis. A single molecule with the apparent molecular size of 43,000 was identified as freshly synthesized PS-1-467 (the isoform consisting of 467 amino acids) and was found to disappear in one day after incubation at 37°C due to aggregation by its high content of hydrophobic domains. This aggregation changes underwent a formation of an intermediate aggregation with Mr 74,000/100,000 before the final formation of huge-sized aggregation retained at the gel top layer. Thus the 43,000-protein of PS-1-467 was found to be decreased with the reciprocal increase of 74,000/100,000-protein. Failure of identification of PS-1 in the brain may be partly explained by this abnormal property of PS-1.

568.10

PHOSPHORYLATION AND SUBCELLULAR LOCALIZATION OF THE ALZHEIMER'S DISEASE ASSOCIATED PRESENILIN PROTEINS, C. Haass[1*], A. Capell[1], J. Grünberg[1], B. Pesold[1], A. Schindzielorz[1], P. St George Hyslop[2], D. J. Selkoe[3] and J. Walter[1]. [1]Central Institute of Mental Health, Department of Molecular Biology, J5, 68159 Mannheim, GERMANY. [2]Center for Research into Neurodegenerative Diseases, University of Toronto, Toronto, Ontario, CANADA M5S 1A8. [3]Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA.

In about 10% of AD cases the disease is inherited as a fully penetrant autosomal trait. Of the genes involved in familial Alzheimer's disease (FAD), the Presenilin (PS) genes play a fundamental role in causing early onset AD. Here, we report the analysis of post translational modifications of the PS proteins. As shown by in vivo labeling and pulse chase experiments, PS proteins do not undergo sulfation or glycosylation. Interestingly, the PS-2 protein was found to be phosphorylated in vivo while little if any phosphorylation was observed for PS-1. The mutant form of PS-2 found in the Volga-German kindred is phosphorylated as well. Phosphorylation of PS-2 proteins was not only observed in transfected COS-7 cells but also in transfected CHO and kidney 293 cells. Phosphorylation of PS-2 takes place exclusively on serines residues. Although several potential recognition sites for protein kinase C (PKC) are present within the sequence of PS proteins, PS-2 is not phosphorylated by PKC, as analyzed by selective stimulation and inhibition of the kinase. By deletion analysis a major site of PS-2 phosphorylation was mapped to its N-terminal half. We also studied the effect of large C-terminal deletions on the subcellular localization of the PS proteins. Endogenous PS proteins as well as PS proteins overexpressed after transfection of a variety of cDNA clones were detected predominantly within the endoplasmic reticulum (ER) and early Golgi. Deleting the large hydrophilic loop and TM6 which contains a conserved aspartate did not alter the predominant localization of PS proteins within the ER.

568.11

DEVELOPMENTALLY REGULATED EXPRESSION OF ALZHEIMER-RELATED PRESENILIN GENES (PS-1 AND PS-2) MATCHES NOTCH IN MOUSE BRAIN. O. Berezovskaja, K. Page, MQ. Xia, V. Berezovskii*, W. Wasco, R. Tanzi and B. T. Hyman. Departments of Neurology and Neurobiology, Mass. General Hospital and Harvard Medical School, Boston, MA 02114.

Mutations in the PS1 and PS2 genes are related to early onset Alzheimer disease. The biological functions of the PSs in normal brain and in AD are unknown. One clue is the strong structural homology of PSs with the C.elegans gene product sel-12, which is believed to interact with lin12, a molecule important during neurogenesis. We studied the pattern of mRNA expression of PS1, PS2, and Notch (the mammalian homologue of lin12) throughout mouse embryogenesis and into adulthood using RT-PCR and in situ hybridization techniques. Strong expression of PSs was observed in embryos which decreased gradually as embryos developed. During early embryogenesis PS1 and PS2 mRNA were abundantly expressed in the majority of neuroepithelial cells. As embryogenesis continued, high levels of PSs were observed in the ventricular zone, containing precursor cells from which all cell types of the brain originate. Weaker signal was detected at postnatal day 1 and 7; even lower expression of both PSs was observed in an adult mouse brain, where there was very low level cortical expression with somewhat higher expression in the hippocampus and cerebellum. The time course and pattern of expression of PSs parallels that of Notch 1 and Notch 2. These observations suggest that PS could be related to Notch, analogous to the interactions between sel-12 and lin-12; we hypothesize that this role may involve the PSs in cell fate decisions such as neuronal differentiation, synaptogenesis, and/or apoptosis.

Supported by NIH grant 3P50 AG 05134.

568.12

The role of S182, a protein mutated in familial Alzheimer's disease, in membrane transport. **Thillai Koothan*, M. Maletic-Savatic and R. Malinow.** Jones Bldg, Cold Spring Harbor Lab., Cold Spring Harbor, NY 11724.

Missense mutations in S182 protein have been associated with some cases of familial Alzheimer's disease. The S182 gene product is predicted to contain multiple transmembrane domains and resembles an integral membrane protein. We have raised antipeptide antibodies against mouse S182 protein which reacts to both mouse and rat cultured hippocampal neurons. We see no immunostaining in astrocytes. The presence of perinuclear immunofluorescent staining and occasional dendritic punctuate staining indicates S182 protein might be present in the endoplasmic reticulum, Golgi apparatus and dentritic organelles. S182 protein might be involved in the transport of membrane-bound vesicles in the endosome-lysosome apparatus. Antisense oligonucleotides against rat S182 and the fluorescent membrane probe FM1-43 will be employed in cultured rat hippocampal neurons to investigate membrane-bound vesicle transport and exocytosis.

Supported by the Harold and Leila Y Mather's charitable foundation and an NIH award to R.M.

568.13

CHARACTERIZATION AND ANALYSIS OF PRESENILIN 2 IN MAMMALIAN CELLS: EFFECTS OF EXPRESSION ON CELL VIABILITY A.C. Crowley, D.E. Merriam, D.M. Kovacs, T.-W. Kim, and W. Wasco*, Genetics and Aging Unit, Department of Neurology, Massachusetts General Hospital-East, Harvard Medical School, Boston MA, 02129.

We have recently demonstrated that the expression patterns of PS1 and PS2 in the brain are extremely similar and that mRNA for both proteins is detected primarily in neuronal populations. In addition, we have found that both PS1 and PS2 localize intracellularly to the nuclear envelope, ER and Golgi compartments in transfected mammalian cells (*Nature Med.* 2, 224-229, 1996). These findings indicate that the pathogenic effect of FAD mutations may be initiated by alterations of processes associated with the localization of these proteins within neuronal intracellular membranes. We have continued to characterize the normal expression, processing and subcellular distribution patterns of PS2, and the effects of the FAD-associated mutations. Specifically, truncated and deleted FLAG-tagged expression vector constructs are being utilized to localize the PS2 ER/Golgi localization signals. Using H4 neuroglioma cell lines that have been stably transfected with constructs that allow for tetracycline-regulated induction of PS2, we have found that the induction of PS2 expression appears to compromise cell viability. We are currently exploring the possibility of PS2 induction of cell death by apoptotic mechanisms. Given that both PS1 and PS2 have been shown to undergo proteolytic processing into two major fragments (Kim et al, submitted), we are also testing for possible correlations between the proteolytic processing and degradation of PS2 and the occurrence of cell death.
Supported by grants from the NIA and NINDS.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CIRCUITRY AND PATTERN GENERATION IV

569.1

SILENT PERIOD INDUCED BY CERVICAL NERVE ROOT STIMULATION, P.C.Su, MD*, S.Pullman, MD Columbia University, The Neurological Institute, 710 W.168th street , NY, NY 10032

Objective:To determine onset, duration and latency of EMG silent period (SP) induced by cervical nerve root (CNR) stimulation (sti) compared with SP induced by wrist (W) sti. Background: SP has been used as an investigative tool in motor diseases such as Parkinsonism and dystonia.There is little information about SP induced by CNR. Methods:26 health subjects, age 20 to 65,10 men 16 women, were instructed to isometrically contract opponeus pollicis at 50 percent effort.Surface electrodes were used to record EMG activity.Magnetic sti was applied to C6-7 spinal process for root sti. Electric sti to W was employed in same subject and measurement was compared.Result:CNR resulted in a SP immediately following the M response which has latency of 14.1±5.1 mS. Total duration of SP following CNR sti is 97.6±11.4 mS and 112.2±14.3 mS with W sti. Post-sti vs pre-sti EMG amplitude ratio is 2.6 for CNR and 2.1 for W sti. Long spinal reflex (V2) seen in W sti is prominent in 2/3 subject following CNR sti and cannot be identified in 1/3 subject. V2 latency with W sti is 53.1±5.7 mS; 43.7±4.2 with elbow sti and 42.9±5.1 in CNR sti. In the 1/3 subjects where V2 is not discernable from the EMG activity which continued at pre-sti amplitude, there still is a higher EMG rebound at 96±15.3 mS.Conclusion:SP following the M response is most likely due to Renshaw Cell inhibition from antidromic motor impulse because of short latency. V2 may be a polysynaptic suprasegmental response since there are only a small differences in latency when sti was moved from W to elbow and to CNR. In 1/3 subjects, the SP appears to end at V2.This may be either due to decreased inhibition or relatively greater suprasegmental influences breaking through the SP folloing V2 response.

569.2

COMPARISON OF FUNCTIONAL ACTIVATION STUDIED BY WHOLE-BRAIN BOLD- AND CBF-BASED fMRI AND [^{15}O]WATER PET DURING SEQUENTIAL FINGER OPPOSITION. S-G. Kim, J.J. Sidtis, S.C. Strother, J.R. Anderson, K. Rehm, K. Ugurbil, D.A. Rottenberg*. Center for Magnetic Resonance Research, Departments of Radiology and Neurology, University of Minnesota, and VA Medical Center, Minneapolis, MN 55455 and 44517.

To compare the spatial patterns of functional activation obtained using fMRI and [^{15}O]water PET during the performance of a simple motor task, five normal volunteer subjects were studied during auditory-cued sequential left-hand finger-to-thumb opposition (1 Hz). All subjects had paired fMRI and [^{15}O]water PET scans, three within a two-week period. Functional MRI scans were acquired using a 4 Tesla MR system: each subject had three BOLD-based scans and one CBF-based FAIR scan. Thirty-four 5 mm coronal echo-planar images were acquired for whole-brain BOLD studies (TE = 25 ms, TR = 5 s); three contiguous 5 mm slices incorporating M1/S1 and SMA were acquired for CBF-based FAIR studies (TI = 1.4 s, TR = 2.8 s). BOLD and FAIR studies consisted of four control and three task epochs, each lasting one minute. [^{15}O]water PET studies consisted of 8-10 alternating baseline-activation scans (90 seconds/scan) separated by eight minutes. We found that contralateral M1/S1, SMA and ipsilateral cerebellum were typically activated in fMRI and PET images. For both PET and fMRI, the Talairach locations of activated foci were reproducible within and between modalities; however, the volume and intensity of individual foci were extremely variable. Inferior cerebellum and the deep gray nuclei were activated in some PET but in no BOLD fMRI studies The methodological and/or physiological basis of this variability must be understood in order to reliably interpret PET and fMRI functional activation studies. [Supported by NIH grants RR08079 and DA09246 and by grants from the Whitaker Foundation and the University of Minnesota.]

569.3

EFFECTS OF INTRATHECAL BACLOFEN ON ANALGESIA AND MOTOR PERFORMANCE IN THE RAT. P. Nance.* University of Manitoba, Winnipeg, Manitoba, Canada R3A 1M4.

Baclofen, a GABAb agonist, is well known as an analgesic and spasticity treatment. An often reported, but poorly documented, clinical side effect of baclofen is the induction of weakness. In the present study, tests of sensory and motor function were performed sequentially to examine the relationship, if any, of baclofen induced analgesia and motor impairment. In a group of four rats, three were cannulated for episodic injection of baclofen and one was not cannulated as a control. The treated animals received one injection per test-day of the following doses of baclofen: 0.5, 1.0, 1.5 µg. The baclofen was flushed through the cannulae with 10 µl of saline. Animals were tested three times prior to injection then every 20 minutes for 80 minutes after injection. The testing battery for sensory function included the following: tail flick, hot plate and paw pressure. Motor function was assessed by the inclined plane test and kinematic analysis of swimming. Data from the inclined plane test was not changed by baclofen treatment. However, motor performance on the swimming task was detectable at all doses. Only the hot plate test demonstrated significant analgesia at the highest baclofen dose tested. After baclofen injection, movements were slower and the alternating pattern of foot strokes was observed to change toward a non-alternating gallop style of swim stroke, $p<.001$. In conclusion, intrathecal baclofen induced changes in motor performance can be observed in sub-analgesic as well as analgesic dosages in the rat.
Supported by the Neuroscience Network of Canada.

569.4

COMPLEXITY IN INDIVIDUAL NEURONS DETERMINES WHICH PATTERNS ARE EXPRESSED IN A RING CIRCUIT MODEL OF GAIT GENERATION. D. A. Baxter*, C. C. Canavier, R. J. Butera, J. W. Clark, and J. H. Byrne. Dept. of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225 and Dept. of Electrical and Computer Engineering, Rice University, Houston, TX 77251.

In order to assess the relative contributions to pattern-generation of the intrinsic properties of individual neurons and of their connectivity, we examined a ring circuit composed of four complex physiologically-based oscillators. This circuit produced patterns that correspond to several quadrupedal gaits, including the walk, the bound, and the gallop. An analysis using the phase response curve (PRC) of an uncoupled oscillator accurately predicted all modes exhibited by this circuit and their phasic relationships. The key insights in the analysis were that in a gait pattern, all oscillators are entrained at the same frequency, hence the phase advance or delay caused by the action of each oscillator on its postsynaptic oscillator is the same, and the sum of the normalized phase differences around the ring is equal to an integer. As suggested by several previous studies, our analysis showed that the capacity to exhibit a large number of patterns is inherent in the ring circuit configuration. In addition, our analysis revealed that the shape of the PRC for the individual oscillators determines which of the theoretically possible modes can be generated using these oscillators as circuit elements. PRCs that have a complex shape enable a circuit to produce a wider variety of patterns, and since complex neurons tend to have complex PRCs, enriching the repertoire of patterns exhibited by a circuit may be the function of some intrinsic neuronal complexity. Our analysis showed that gait transitions in a ring circuit do not require rewiring the circuit or any changes in the strength of the connections. Instead, transitions can be achieved by using a control parameter, such as stimulus intensity, to sculpt the PRC so that it has the appropriate shape for the desired gait(s). A transition can then be achieved simply by changing the value of the control parameter. Our analysis illustrates the predictive value of PRCs in circuit analysis and can be extended to provide a design method for pattern-generating circuits.
Supported by a grant from the ONR.

569.5

FUNCTIONAL AND MOLECULAR DIVERSITY OF NEOCORTICAL INTERNEURONES. E. Audinat, B.Cauli, M.C. Angulo, B. Lambolez and J. Rossier*. CNRS URA 2054, ESPCI, 10 rue Vauquelin, 75005 Paris, France.

In the neocortex a large heterogeneity of GABAergic interneurones has been revealed by studying the expression of calcium-binding proteins and neuropeptides but this biochemical diversity bear no clear functional correlates. We have combined whole-cell patch-clamp recording with single-cell RT-PCR to study the expression of calbindin (CA), calretinin (CR), parvalbumin (PV), somatostatin, cholecystokinin, vasoactive intestinal peptide and neuropeptide Y in each recorded interneurone identified by infra-red video microscopy in slices of the rat motor and visual neocortex. The simultaneous amplification of seven different biochemical markers revealed that in 70% of the recorded cells at least one neuropeptide was expressed together with one or two calcium binding proteins. One third of the interneurones were characterized by nonadapting discharges of fast action potentials. All but 2 of the 31 recorded fast spiking cells expressed PV and none of them expressed CR. In half of these cells CA was co-expressed with PV. The remaining two third of the interneurones displayed a marked frequency adaptation of their action potential discharges. Most of these cells expressed either CA or CR which were rarely colocalized. This group of adapting interneurones could be further divided in different functional and biochemical subtypes.

Supported by the CNRS

569.6

COEXPRESSION OF CALRETININ AND VASOACTIVE INTESTINAL PEPTIDE (VIP) IN NEOCORTICAL BURSTING INTERNEURONES. B.Cauli, M.C. Angulo, E. Audinat, B. Lambolez* and J. Rossier. CNRS URA 2054, ESPCI, 10 rue Vauquelin, 75005 Paris, France.

Neocortical inhibitory interneurones are broadly divided in two main groups, interneurones displaying a marked adaptation of their action potential firing, and fast spiking non adapting cells. We have analysed the functional and molecular characteristics of single adapting non pyramidal cells by patch-clamp recordings and multiplex RT-PCR in acute slices of rat neocortex. The simultaneous detection of several biochemical markers (calbindin, calretinin, parvalbumin, neuropeptide Y, vasoactive intestinal peptide, somatostatin and cholecystokinin) and the firing properties revealed a large diversity in this population. However, this protocol has allowed us to characterised a well defined subpopulation of cells. Depolarization of these interneurones evoked an initial high frequency (>50 Hz) burst of action potentials followed by repetitive bursts emitted at low frequency (<20 Hz). RT-PCR indicated coexpression of calretinin and VIP in all these bursting cells. Their morphology was characterised by a bipolar vertically orientated dendritic arborization. Taken together, these results suggest that these bursting interneurones form a homogenous functional population.

Supported by the CNRS

569.7

COUNTING THE NUMBER OF *SHAL* 1 RNA MOLECULES IN IDENTIFIED PYLORIC NEURONS USING QUANTITATIVE SINGLE CELL RT-PCR. D.J. Baro*, R.M. Levini, C.L. Cole, and R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14850.

We study the motor pattern generated by the pyloric network in the stomatogastric ganglion of the spiny lobster. This network contains 14 neurons that fall into six cell types. Each cell type possesses a unique electrophysiological phenotype which defines the cell's role in the network. For example, when and how intensely a cell fires in the motor pattern is partly determined by the transient K^+ current (I_A), which is distinct in each cell type. We are interested in the molecular basis of I_A heterogeneity. Previously we cloned the open reading frame of the lobster *shal1* gene and found that it produced an I_A in *Xenopus* oocytes. Using a non-quantitative, single cell-reverse transcription-polymerase chain reaction (sc-RT-PCR) method we found that the *shal*1 gene was expressed in 94% of stomatogastric neurons, but that expression levels varied among cells. We have now developed a quantitative sc-RT-PCR method to determine if variable numbers of *shal*1 RNA molecules could account for somatic I_A heterogeneity. Using this method we found significant and reproducible differences in the numbers of *shal*1 RNA molecules in each cell type. For example, the PD cell contains 1,087±130 (n=6) *shal*1 RNA molecules, while the VD cell possesses 378±77 (n=5; p<.01). This difference reflects the size of the somatic I_A in each cell: the PD has the largest and the VD the smallest somatic I_A among all pyloric neurons. Presently, we hope to establish whether there is a positive correlation between the number of *shal*1 RNA molecules and the maximal conductance of I_A in the different cell types. Preliminary data indicates that unlike the other 5 cell types, the number of *shal*1 molecules in the AB cell varies significantly among individuals. This is similar to our previous finding in which we demonstrated that the level of *shab* gene expression was reproducible among individuals in all homogeneous cell types except the AB. Supported by NIH NS25915.

569.8

A MODEL FOR THE MCN1-ACTIVATED GASTRIC MILL RHYTHM IN THE CRAB, Y. Manor*[†], F. Nadim[†], E. Marder[‡] and M.P. Nusbaum*[‡]; [†]Volen Center, Brandeis Univ, Waltham, MA 02254 and [‡]Dept Neurosci, Univ Penn School of Medicine, Philadelphia, PA 19104.

The gastric mill rhythm (cycle period 7-15 s) in the stomatogastric ganglion of the crab *Cancer borealis* is elicited by direct activation of the projection neuron MCN1 (Coleman et al., Nature 378, 1995). MCN1 excites most gastric mill network neurons, including DG, Int1 and LG, the latter two of which make reciprocally inhibitory connections. Coleman et al. (1995) suggest that three ingredients are interacting to generate this slow frequency gastric mill oscillation: (1) presynaptic inhibition of MCN1 terminals by LG; (2) a voltage dependent electrical coupling between regions of the MCN1 arborization and the LG neuron; and (3) the different synaptic time scales of the MCN1 excitatory connections to Int1 and LG.

We present a model that reproduces the main experimental observations. MCN1, DG, Int1 and LG were modeled as Hodgkin-Huxley type neurons. MCN1 consisted of ten compartments, so that the electrically transmitting part of its axon and its transmitting-releasing terminals are spatially separated. Int1 is also inhibited by the pyloric network pacemaker (which produces the pyloric rhythm: period 0.5-2 s), so we added such an inhibition to the model. The model shows that the pyloric rhythm may have a prominent influence on the frequency and the duty cycle of the gastric rhythm. For the model gastric mill rhythm to persist in the presence of the pyloric rhythm influence, we found that it is essential for the inhibitory synapse between the model Int1 and LG to have a graded component, rather than to be merely spike-mediated.

Research supported by NS17813, MH46742, and the Alfred P. Sloan Foundation.

569.9

OBJECT-ORIENTED TECHNOLOGY ALLOWS UNIVERSAL ACCESS TO NEUROPHYSIOLOGY DATABASES. Daniel Gardner* and Steven M. Erde. Dept. of Physiology and Office of Academic Computing, Cornell University Medical College, New York, NY 10021.

The APLYSIA project is developing data types for queryable storage and entry of neurophysiological datasets, descriptions of identified neurons, and model parameter sets. Allied metadata include: investigator, date, and citation; neurons under study; scalable trace and time markers; query keywords; and experimental conditions. Anticipating parallel ongoing advances in database and multiplatform technology, we deferred implementation, concentrating initial development on specification of a functionally-defined compact yet comprehensive set of neurobiologically useful data types and subtypes. The maturation of object-oriented technologies now permits initiation of an Internet database including data type schema and methods that encompass complex time series and other multimedia abstract data types, data type expandability, universal access, and seamless multiplatform data exchange aiding integration of electrophysiology data from multiple laboratories. Data types are being encoded as C++ objects, made persistent using the Poet object-oriented database management system. Schemes under development for access, query, and entry of data will rely upon the evolving multiplatform object-oriented Java language system. Server-side storage of Java-deliverable methods avoids the need to develop, distribute, or update proprietary client tools. Familiar Web-based tools will combine with Java to shield users from most details of storage or implementation.

URL: http://ganglion.med.cornell.edu/Aplysia/Universal.html

Supported by NSF: BIR-9506171

569.10

NEURAL ANALOGUE OF OPERANT CONDITIONING OF FEEDING BEHAVIOR IN *APLYSIA*. R. Nargeot*, D.A. Baxter and J.H. Byrne. Dept. Neurobiol. and Anat., Univ. of Texas Med. Sch. at Houston, Houston, TX 77030.

Previous studies demonstrated that the consumatory feeding behavior in *Aplysia* (i.e., ingestion, rejection) can be operantly conditioned (Susswein *et al.*, 1986). In particular, successful ingestion of food functions as a reward and thus increases the frequency of ingestion behavior (the operant). Key elements of the central pattern generator (CPG) that mediates consumatory feeding behavior are located in the buccal ganglia. Moreover, the esophageal nerve (E n.) has been implicated as a reinforcing pathway. Thus, the isolated buccal ganglia may provide a simple neural preparation that manifests the essential features of this conditioning and that is amenable to analyses of the underlying cellular mechanisms. In this preparation, we monitored the neural correlates of consumatory feeding behavior with extracellular recordings from peripheral nerves, the I2 nerve, the nerve 2,1 and the radular nerve and intracellular recordings from identified cells of CPG for feeding. Tonic electrical stimulation of a nerve 2,3 was used to elicit ingestion-like and rejection-like motor programs, which are distinguished by the amount of overlap between the activities of closer and retractor motor neurons (Morton & Chiel, 1993). Three groups of preparations were examined: (1) a contingent reinforcement group in which the reinforcing stimuli of E n. were delivered each time an ingestion-like pattern was observed during a 10 min training period; (2) a yoke-control group in which the reinforcing stimuli were delivered but were not paired with the ongoing patterned activity; (3) a control group in which no reinforcing stimuli were delivered. Comparison of the motor programs elicited during a 10 min test period following the training period indicated that the contingent reinforcement group expressed a significantly higher frequency of ingestion-like patterns (i.e., the reinforced pattern) than either the yoke-control or control groups. No significant difference was observed between the yoke-control and control groups. Moreover, no differences in the frequency of other buccal motor patterns, which were not reinforced (e.g., rejection-like pattern), were observed among the three groups. Results from intracellular recordings are being examined to identify changes in synaptic connections and cellular properties that may have been induced by the contingent reinforcement paradigm. The results indicate that the isolated buccal ganglia preparation expresses an essential feature of operant conditioning (i.e., contingent reinforcement changed the frequency of a specific operant) and that this preparation will afford an opportunity to gain insights into the cellular mechanisms underlying operant conditioning. Supported by Fyssen Foundation and AFOSR.

570.1

LIMBIC EPILEPSY AND STRUCTURAL PLASTICITY IN MICE CARRYING A NULL MUTATION OF SYNAPSIN I. Dake Zheng*, Lian Li, Lih-Shen Chin, Paul Greengard and James O. McNamara. Department of Medicine, Duke University, Durham, NC27710. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

The cellular and molecular determinants underlying limbic epilepsy are incompletely understood. Synapsin I is a synaptic vesicle protein which has been postulated to modulate neurotransmitter release by controlling the availability of synaptic vesicles for exocytosis. Recent studies have shown that synapsin I-deficient mice exhibited an increased seizure propensity manifested by the striking enhancement of response to electric stimulation (Li et al, PNAS 92:9235, 1995). To further characterize the role of synapsin I in the modifications of neuronal function and structure in the hippocampus, we examined the behavioral and electrophysiological indices of kindling development and kindling-induced sprouting of hippocampal granule cell axons in wild type (+/+), heterozygous (+/-), and homozygous (-/-) mice carrying a null mutation of synapsin I gene. In contrast to wild type littermates, synapsin I-deficient mice exhibit a striking hyperexcitability. Thus the number of amygdala stimulations required to trigger three consecutive clonic motor seizures is significantly less in the -/- mice (6.0±1.0, n=5) compared to the +/+ mice (14.2±1.3, n=5; $p<0.005$). The afterdischarge duration is less in the -/- mice for the first nine stimulations, but there is no difference in the subsequent stimulations. Furthermore, the absence of synapsin I in the mutant mice does not impair the kindling-induced sprouting of granule cell axons measured by Timm staining. These findings underscore the powerful influence of synapsin I in controlling the excitability of the mammalian brain, yet kindling-induced structural plasticities are not affected by the null mutation. Supported by NS17771 from NINDS and NRSA NS10027.

570.2

RECIPROCAL CHANGES IN GENE EXPRESSION FOR *zif268* AND NMDA RECEPTOR SUBUNIT NR1 IN THE EPILEPTIC FOCUS AND "INHIBITORY SURROUND" INDUCED BY TETANUS TOXIN IN THE MOTOR CORTEX. F. Liang*, L.D. Le and E.G. Jones. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717

To reveal the mechanisms underlying epileptogenesis, we studied possible changes in gene expression in rats injected with tetanus toxin (TT, 2-35 ng) in the motor cortex. Immunocytochemistry for Zif268 protein and *in situ* hybridization histochemistry for NMDA (N-methyl-D-aspartate) receptor subunit 1 (NR1) and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate) receptor subunit 2 (GluR2) mRNAs were performed. Most of the rats that received 13-35 ng TT exhibited seizure signs and a cluster of increased Zif268 immunoreactivity at the injection site, surrounded by a cortical zone showing decreased Zif268 immunostaining. However, only rats that survived for more than one week after TT injection showed focal NR1 up- and GluR2 down-regulations at the TT injection site. This was surrounded by a zone showing decreased NR1 mRNA levels. Rats surviving for less than 8 days after TT injection showed no clear changes in NR1 mRNA levels, but some showed decreased GluR2 mRNA levels throughout most neocortical areas on the injected side. Cortical areas showing reciprocal changes in gene expression at the injection site and in the surround may correspond to the hyperactive epileptic focus and the hypoactive inhibitory surround, respectively. Based on the present results and our previous report of up-regulation of glutamic acid decarboxylase and down-regulation of Ca^{2+}/calmodulin-dependent protein kinase IIα gene expression in TT induced epilepsy (Liang and Jones, Soc. Neurosci. Abstr. 20:934, 1994), we propose: 1) that neurochemical segregation of the epileptic focus from other cortical areas may play an important role in the maintenance of seizure activity after TT has been cleared from the brain; 2) a model of epileptogenesis which emphasizes the disturbance of lateral inhibition among neighboring cortical columnar modules. Supported by NIH grant NS21377.

570.3

Seizure-like stimulation produces DNA fragmentation and Poly-ADP-ribose polymerase proteolysis in the immature dentate gyrus. K. Thompson, P. Popper, G. Cole and C. Wasterlain.* VHA Medical Center, Sepulveda CA 91343, Dept. of Neurology and Brain Research Institute, UCLA School of Medicine.

We have previously reported severe brain damage from perforant path stimulation in the immature rat both 1 day and 1 month following stimulation. To investigate the acute effects of status epilepticus in the immature brain we have combined histological techniques which show 1) moribund cells 2) DNA fragmentation and 3) proteolysis of the DNA repair enzyme Poly-ADP-ribose polymerase (PARP). Sixteen day old rats under metofane anesthesia were stereotaxically implanted with two bipolar electrodes, one into the perforant path and the second into the ipsilateral dentate gyrus. Awake animals were stimulated with 10 sec trains of a 20 Hz stimulus which was delivered once per minute for 16 hrs. Two hours after stimulation the animals were transcardially perfused with 4% paraformaldehyde. Tissue was processed for H&E staining, in situ end-labeling (ISEL), or immunohistochemical staining. For ISEL staining, fragmented DNA was labeled with biotinylated nucleotide dATP. For immunohistochemistry the tissue was exposed to antibodies directed against the proteolytic fragment of PARP. The biotinylated nucleotides and antibodies were visualized using the ABC peroxidase method. Our results show that neuronal injury is present immediately following perforant path stimulation. The combination of staining techniques shows that hilar damage is present solely in the stimulated hippocampus, with necrotic cells showing eosinophilia but no evidence of DNA fragmentation or PARP proteolysis. Interestingly, the inner layer of the dentate granule cells shows bilateral damage with eosinophilia, ISEL positivity, and immunoreactivity for the proteolytic fragment of PARP. These data suggest that the inner granule cell layer in the immature rat is extremely vulnerable to seizure-like discharge and that these granule cells experience rapid destruction of DNA and DNA repair enzymes. Supported by VHA Research Service and by research grant NS13515 from NINDS.

570.4

SEIZURES PROMOTE A RAPID TRANSCRIPTIONAL UPREGULATION OF PGHS-2 IN RAT HIPPOCAMPUS, WHICH IS INHIBITED BY THE PAF RECEPTOR ANTAGONIST BN-50730. Marcheselli V.L*., Cinar H.N., Stellingworth M.A., and Bazan N.G. LSU Neuroscience Center and LSU Eye Center, New Orleans, LA.

Seizures promote the accumulation of free fatty acids and diacylglycerols as well as the release of platelet-activating factor (PAF) in brain as a consequence of rapid activation of phospholipases A_2 and C. PAF, an inflammatory mediator, is a transcriptional activator of the immediate-early genes *zif*-268 and *c-fos* (Marcheselli and Bazan, *J. Nerosci. Res.* 37:54-61, 1994.). BN-50730, a specific antagonist for the intracellular PAF binding site, inhibits *zif*-268 mRNA accumulation in brain after a single electroconvulsive shock (ECS). The activation of phospholipase A_2 is a neural inflammatory response by which bioactive lipids become injury signals during repeated seizures (status epilepticus) thus promoting brain damage (Bazan, 1995. *Nature* 374:501-502.). Prostaglandin H synthase (cyclooxygenase, PGHS), which has a key function in the synthesis of PGE_2, $PGF_{2\alpha}$, PGD_2, prostacyclins and thromboxanes, presents two forms in brain tissues: the constitutive type PGHS-1 (COX-1) and the inducible type PGHS-2 (COX-2). Both kainic acid- and ECS-induced seizures promote a rapid increase in PGHS-2 mRNA in rat brain hippocampus which peaks about 3 hrs. However the kainic acid-induced seizures promoted an increase 20-fold larger and more sustained than ECS. Under the same conditions, *zif*-268 mRNA levels showed a smaller and very transient increase. *In vitro* transcription studies revealed the mRNA increases occurs through transcriptional gene activation, but not message stabilization. Western blot analysis showed an increase in PGHS-2 protein levels comparable to the mRNA increases. PGHS-1 protein levels did not change with seizures. Pretreatment with BN-50730 by icv injection 15 min before kainic acid administration or ECS largely inhibited PGHS-2 mRNA and protein accumulation, but did not promote changes in PGHS-1 levels. Distribution studies of PGHS-2 accumulation after seizures in rat brain indicated that hippocampus has the highest levels (a 35-fold increase), followed by brain cortex (8-fold increase), but no changes occurred in the cerebellum. This results are consistent with the involvement of PGHS-2 in synaptic plasticity, which, during epileptogenesis, may develop pathological forms of neuroplasticity. Therefore, intracellular PAF receptors may be the key to controlling the increase in PGHS-2 expression, which, in turn, can be blocked by BN-50730. This type of compound could be useful as a pharmacological tool to study and prevent epileptogenesis. NIH NS23002

570.5

LONG-TERM ALTERATIONS IN GROWTH FACTOR mRNA EXPRESSION FOLLOWING KAINATE INDUCED SEIZURE ACTIVITY. M. L. Garcia*, V. B. Garcia, E. E. Strehler, P. J. Isackson, and A. J. Windebank. Molecular Neuroscience Program, Mayo Clinic, Rochester, MN 55905 and Jacksonville, FL 32224 USA.

The role of growth factors in preventing cell death both *in vivo* and *in vitro* is well established. Changes in the mRNA of several growth factors are known to occur concurrently with neuronal insults *in vivo*. However, little is known about the long-term effects of neuronal insults on the expression pattern of growth factor mRNAs. As an assessment of potential effects on long-term growth factor mRNA expression, we have examined the effects of prolonged postictal times on the expression of NGF, BDNF and NT3 following KA-induced seizures. *In situ* hybridization was performed on tissue from adult male Sprague-Dawley rats sacrificed at various time points following intracranial ventricular injections of KA. Preliminary data indicate changes in all growth factors examined. NGF expression increases within the dentate gyrus and CA1 of the hippocampus ipsilateral to the injection site one week following KA injections. Contralaterally, NGF expression increased within the dentate gyrus, CA3 and CA1 relative to control. BDNF expression increased within the dentate gyrus both ipsi- and contralaterally to the injection site one week following KA injections. BDNF expression increased within CA3 contralateral to injection and within CA1 ipsilateral to the injection. NT3 expression was decreased within the dentate gyrus and CA2 one week following KA injections both ipsi- and contralateral to injection site. These observations provide evidence that alterations in growth factor mRNA expression occur even after prolonged postictal recovery suggesting a possible role for growth factors in recovery and continued maintenance of surviving neurons within limbic seizure foci (NIH, NS 14304).

570.6

GAP JUNCTION BLOCKADE BUT NOT POISONING OF GLIA BLOCKS SPREADING DEPRESSION IN RAT HIPPOCAMPAL SLICES. C. Largo[1], G.C. Tombaugh[2], P.G. Aitken[2], O. Herreras[1], and G.G. Somjen[2], [1]Depto de Investigacion, Hospital Ramon y Cajal, Madrid, Spain; [2]Dept. of Cell Biology, Duke Univ. Med. Ctr., Durham, NC 27710.

We investigated the role of gap junctions and glial function in the generation and propagation of spreading depression (SD). Waves of SD were triggered by microinjection of concentrated KCl (1.2M) into str. radiatum of CA1 in rat hippocampal slices from a micropipette. DC-coupled recordings of extracellular potential (Vo) were made at the injection site and at a second site approx. 1mm distant in str. radiatum or str. pyramidale. In some trials, antidromic population spikes were evoked by stimulating the alveus. Bath application of the gap junctional blocker heptanol (3mM) completely and reversibly prevented the propagation of the SD-related Vo shift without abolishing the ΔVo at the KCl injection site. Before blockade was complete, SD propagation velocity remained unchanged. Octanol (1mM) had a similar but less reliable effect on SD; fEPSPs and antidromic spikes were depressed by both alcohols. In isolated, patch-clamped CA1 neurons, heptanol (3mM) reversibly depressed voltage-dependent Na^+ currents. Fluoroacetate (5mM), a selective blocker of glial metabolism, virtually abolished synaptic transmission but did not inhibit SD propagation. Moreover, in the presence of fluoroacetate, the ability of heptanol to block SD was preserved. These results support the idea that patent gap junctions are required for SD propagation in mammalian brain tissue but that neither synaptic transmission nor intact glial function are essential for SD initiation or propagation.

Supported by NS18670 (NINDS) and 93/661 (Fondo de Investigaciones Sanitarias of Spain).

570.7

OPTICAL RECORDING OF THE SPATIAL AND TEMPORAL PATTERNS OF VOLTAGE TRANSIENTS IN RAT HIPPOCAMPAL SLICES

G. Golarai*, J. A. Connor ,The Lovelace Institutes, Albuquerque, NM 87108

To determine the functional consequences of synaptic reorganization in the hippocampal circuitry in epilepsy and after lesions, we developed a sensitive method with high spatial resolution to record evoked optical signals from voltage sensitive dyes in acute or cultured hippocampal slices. A CCD camera (array size =120X140), was used to capture fluorescent images of slices stained with a voltage sensitive dye (RH795 or di-2-ANEPPS) during a 4 ms exposure to excitation light. The ratio of two images, one taken before, and another at a fixed interval after the stimulation was obtained. Eight to 25 image ratios were averaged. A series of averaged ratio images collected at variable intervals following afferent stimulation, along with simultaneous field potential recordings revealed the spatial and temporal organization of evoked electrical activity. Activation of the perforant path (PP) evoked an optical signal in the stratum moleculare (str. ML) of the dentate gyrus (DG), that lasted for the duration of the electrically recorded field potential (~20 ms, n=17), consistent with depolarization of dendrites in this region. A concomitant depolariza-tion was also recorded optically in the str. Lacunosum ML of the CA1, consistent with parallel activation of the CA1 by the PP fibers (n=4). Optical signals indicating depolarization of the str. Granulosum as well as secondary synaptic potentials in the hilus of the DG and str. Lucidum, Pyramidal and Oriens of CA3 were recorded (n=3). These observations are consistent with previous reports on the organization of synaptic events in the normal hippocampus and suggest that our optical methods are sufficiently sensitive to detect alterations of synaptic events after reorganization of local connections in the hippocampal formation in lesion or epilepsy models.

570.8

CHANGES ON SYNAPTOSOMAL GLUTAMATE UPTAKE REGULATION WITH SUBCONVULSIVE DOSES OF PENTYLENETETRAZOLE.

O.I. Claudio, J.G.Ortiz*, J.Nieves, Dept. of Pharmacology, Univ. of Puerto Rico School of Medicine, P.O. Box 365067, San Juan, Puerto Rico 00936-5067

Previous studies in our laboratory revealed that high-affinity glutamate (GLU) uptake is altered in mice with audiogenic (genetic or induced) or pentylenetetrazole (ptz, 80 mg/kg) seizures. Arachidonic acid (ARA), nitric oxide (NO), protein kinase C and G-proteins have been implicated in the GLU uptake regulation. We administered subconvulsive doses of ptz (40 mg/kg, sc) to examine GLU uptake regulation in the absence of the effects that seizures could have. There was no apparent difference on GLU uptake regulation in synaptosomes from mice injected once (1Dx1Day) or three (3Dx1Day) doses a day. Similarly, there were no changes in the effects of pertussis or cholera toxin. However, synaptosomes from mice treated once for three days (1Dx3Days) displayed a biphasic effect at 5 μM GLU uptake, but not at 50 μM, when exposed to ARA, a protein kinase C activator. Nitroindazole, a nitric oxide synthase inhibitor, caused a decrease in 5 μM GLU uptake, but increased 50 μM GLU uptake. MCPG, a mGluR antagonist, caused an increase on 5μM GLU uptake, while a biphasic effect was observed on 50μM GLU uptake. These results indicate that GLU uptake regulation appears to be altered by repeated subconvulsive stimuli. (Supported by the NIH/MBRS Program GM S06 08224)

570.9

EPILEPTOGENESIS IN AMYGDALA KINDLING ENHANCES THE DEPRESSION OF EXCITATORY SYNAPTIC TRANSMISSION BY PRESYNAPTIC METABOTROPIC GLUTAMATE RECEPTORS.

V. Neugebauer*, N.B. Keele and P. Shinnick-Gallagher. DEPT. OF PHARMACOLOGY, THE UNIVERSITY OF TEXAS MEDICAL BRANCH, GALVESTON, TX 77555-1031.

Metabotropic glutamate receptors (mGluRs) play important roles in certain forms of neuroplasticity and neuropathological disorders. Modulation of excitatory synaptic transmission in the basolateral amygdala by presynaptic mGluRs was examined in brain slices from control rats and rats with amygdala kindled seizures.

Whole-cell recordings from basolateral amygdala neurons were performed in current-clamp and voltage-clamp. Monosynaptic EPSPs/EPSCs were evoked by stimulation in the lateral amygdala. Drugs were applied by superfusion.

The group II mGluR agonist L-CCG dose-dependently depressed monosynaptic EPSCs in normal and kindled neurons (EC_{50}: 36 nM and 1.2 nM, respectively). The group III mGluR agonist L-AP4 was less potent in normal and kindled neurons (EC_{50}: 297 nM and 10.8 nM, respectively). Thus in kindled neurons the affinity of each agonist for its respective mGluR is increased 30 fold. Both agonists also elevated thresholds for synaptically evoked bursting in kindled neurons. Neither L-CCG (up to 10 μM) nor L-AP4 (up to 50 μM) caused membrane currents and changes in current-voltage relationship. The novel mGluR antagonists MCCG (100 μM) and MAP4 (100 μM) selectively reversed the inhibition by L-CCG and L-AP4 to 61-73 % and 65-81 % of predrug responses, respectively. MCCG and MAP4 (up to 300 μM) themselves did not significantly affect synaptic transmission.

The enhanced potency in depressing synaptic activity in the kindling model of epilepsy and the lack of intrinsic activation suggest that the presynaptic group II and group III-like mGluRs might be useful targets for suppression of excessive synaptic activation in neurological disorders such as epilepsy.

Supported by NIH (NS 24643) and the Deutsche Forschungsgemeinschaft.

570.10

INTRACELLULAR CALCIUM CHELATOR (BAPTA) PREVENTS DECREMENT OF INHIBITORY POSTSYNAPTIC CURRENTS IN DENTATE GRANULE CELLS IN THE HUMAN AND RAT EPILEPTIC HIPPOCAMPUS.

Masako Isokawa*. Brain Research Institute, Sch. of Medicine, UCLA, Los Angeles, CA 90024-1761.

Intensive activation of the NMDA receptor is reported to temporarily reduce $GABA_A$ receptor-mediated inhibitory currents in the hippocampal dentate granule cells in human epilepsy and in the pilocarpine model of chronic epilepsy (Isokawa, *J. Neurophysiol.* 1996). The present study was designed to further characterize the mechanisms of this response decrement in the $GABA_A$ receptor-mediated synaptic transmission. The whole-cell patch clamp recording was conducted in the DGCs in hippocampal slices that were prepared from surgically resected temporal lobe specimens of epileptic patients and from pilocarpine-treated rats with chronic seizures. Inhibitory postsynaptic currents (IPSCs) were electrophysiologically isolated as outward currents at Vm= 0 mV near the reversal potential of glutamate EPSCs, and pharmacologically confirmed as $GABA_A$ IPSCs by 10 μM bicuculline methiodide. As previously shown, tetanic stimulation at -30 mV, at which NMDA receptors were activated, reduced IPSC amplitude at 0 mV in all epileptic DGCs. This reduction was APV (50 μM) sensitive. When 10 mM BAPTA was included in a recording pipette, the decrement of $GABA_A$ IPSCs following the tetanic activation of the NMDA current was prevented, indicating that an intracellular calcium increase was responsible for the reduction of the $GABA_A$ IPSCs. However, the effect of BAPTA was stronger than that of APV, suggesting that the source of this calcium increase may not be totally exclusive to the opening of the NMDA receptor. Supported by NINDS grants NS02808 and NS31180.

570.11

GAD-67 STAINED CELLS IN AREA CA1 OF HIPPOCAMPUS ARE PERMANENTLY REDUCED FOLLOWING PERINATAL HYPOXIA-INDUCED SEIZURES.

CB Geary*, R. Shanghvi, and FE Jensen. Neurology, Children's Hosp., Harvard Medical School, Boston, MA 02115.

We have previously demonstrated that perinatal hypoxia in rats (postnatal day (P) 10-12) results in acute seizures and permanently decreased seizure thresholds in adulthood. Depth electrode recordings during hypoxia in vivo reveal hyperexcitability in hippocampus and neocortex. Hippocampal slices removed from adult rats with previous perinatal hypoxia show increased excitability under convulsant conditions in vitro in area CA1. To determine whether this hyperexcitability is in part due to decreased inhibitory modulation of CA1 networks, we compared the density of GAD-67 stained cells in area CA1 in adult rats with previous hypoxia-induced seizures at P10. Rats were sacrificed at P70-75 and brains fixed and sectioned (40μm) for immunocytochemistry. A polyclonal antibody for the GABA synthetic enzyme GAD (GAD-67, Chemicon) was used for immunocytochemistry. The number of GAD stained cells was counted per $500\mu m^2$ areas in CA1 s. pyramidale and s. radiatum at stereotactically defined planes. Using the optical dissector method, the right and left hippocampi were evaluated for 10 previously hypoxic rats and 8 control rats. The mean number of GAD-positive cells was significantly lower in the previously hypoxic adults (23.2 ± 4.9, n=20) compared to litter mate controls (30.7 ± 5.9, $p<0.001$). Furthermore, the number of seizures observed during the hypoxic period at P10 negatively correlated with the number of GAD stained neurons ($r=0.5$, $p<0.002$). These results suggest that the chronic hyperexcitability induced by perinatal hypoxia is in part mediated by a loss of neurons synthesizing the inhibitory neurotransmitter GABA, and that the more severe seizure activity during the brief hypoxic episode may be related to the loss of these neurons, contributing to long term increases in seizure susceptibility. Supported by AHA, NS31718, NS32570 (FEJ)

570.12

ROLE OF $GABA_B$ RECEPTORS IN A GENETIC MODEL OF ABSENCE SEIZURES (GAERS).

R. Bernasconi*, P. Mathivet, C. Marescaux and H. Bittiger[1]. Unité INSERM U.398, F-67084 Strasbourg, [1] Ciba, CH-4002, Basel.

Marescaux et al. (J. Neural Transm. Suppl 35, 37-69, 1992) have shown that GAERS are an isomorphic model of absence seizures and that $GABA_B$ receptors play a central role in the control of absences, implying that the genetic susceptibility of GAERS might reside in an alteration of $GABA_B$ mediated mechanisms. To test this hypothesis we have assessed the kinetics of $GABA_B$ binding sites in membranes prepared from GAERS and from non-epileptic control rats in an agonist and in an antagonist binding assay. Using the agonist ^3H CGP 27492 as radioligand we observed a higher affinity of $GABA_B$ receptors in cortical membranes prepared from GAERS compared to controls, whereas the total number of $GABA_B$ receptors were similar in both strains. Saturation experiments with the antagonist radioligand ^3H CGP 54626 revealed two affinity states of $GABA_B$ receptors. The K_d and B_{max} of both binding sites were similar in cortical and thalamic membranes from GAERS and controls. We also compared the inhibition by the agonist R-(-)-baclofen of antagonist ^3H CGP 54626 specific binding to cortical membranes in absence and in presence of Gpp(NH)p. Hill numbers (nH) were less than unity suggesting two binding states of $GABA_B$ receptors. The IC_{50} values, the number of binding sites and nH were similar in both strains. Gpp(NH)p shifts were similar in GAERS and in controls suggesting that the interactions between $GABA_B$ receptors and G-proteins are similar in both strains. We examined the inhibition of ^3H CGP 27492 binding by Gpp(NH)p as an index of the allosteric interactions between the binding sites of $GABA_B$ receptor agonists and GTP. The displacement curves revealed two IC_{50} values implying two different interactions between $GABA_B$ receptors and GTP-binding sites. The binding parameters were similar in both strains, suggesting that the allosteric interactions betwen $GABA_B$ receptors and the GTP-binding sites are similar in GAERS and controls. Taken together, the results of this study suggest that the affinity of $GABA_B$ receptors in GAERS is increased compared to non-epileptic control rats whereas the interactions between G-proteins or GTP-binding sites and $GABA_B$ receptors are similar in both strains.

571.1

SLEEP AND WAKING AFTER 8-OH-DPAT GIVEN SYSTEMICALLY AS WELL AS PERFUSED INTO THE DORSAL RAPHE NUCLEUS IN RATS. B. Bjorvatn, K. Hole* and R. Ursin. Dept. of Physiology, University of Bergen, 5009 Bergen, Norway

The 5-HT_{1A} agonist 8-OH-DPAT is reported to increase waking and decrease slow wave sleep (SWS) at 0.375 mg/kg s.c., whereas 0.010 mg/kg s.c. as well as injection directly into the dorsal raphe nucleus (DRN) induced opposite effects. The interpretation has been that stimulation of 5-HT autoreceptors induces sleep, whereas stimulation of postsynaptic receptors induces waking. Both s.c. doses reduced REM sleep whereas DRN injection had no such effect. In cats, perfusion of 8-OH-DPAT (10 µM) in DRN through microdialysis probes increased REM sleep.

We investigated sleep and behavior in rats following identical doses s.c. as well as continuously perfusing 8-OH-DPAT into DRN through microdialysis probes. So far six rats have been stereotaxically implanted with electrodes for EEG/EMG recordings and a guide cannula aimed at the DRN. Each rat was given s.c. injections of saline, 8-OH-DPAT at 0.010 and 0.375 mg/kg in a balanced order blind design followed by 8-OH-DPAT (10 µM dissolved in aCSF) continuously perfused into the DRN. Six days separated the experiments. Microdialysis probes (CMA/12, 1 mm) were inserted and perfusion (1 µl/min) started one hour before EEG/EMG recordings. Histological examinations revealed that the probe was located in DRN in five rats.

8-OH-DPAT at 0.375 mg/kg increased waking initially, SWS-1 and REM sleep were reduced and the effect on SWS-2 was biphasic. Initially SWS-2 was clearly reduced, followed by an increase. This dose induced 5-HT behavioral syndrome with flat body posture, hindlimb abduction and increased locomotion. 8-OH-DPAT at 0.010 mg/kg did not change sleep or behavior. Continuous perfusion of 8-OH-DPAT (10 µM) in DRN reduced REM sleep, otherwise no significant changes were seen.

In conclusion, stimulation of 5-HT autoreceptors did not increase slow wave sleep, but instead seemed to reduce REM sleep.

Supported by the Norwegian Research Council.

571.2

SUPPRESSION OF MILK DRINKING IN RATS DUE TO ENHANCED SEROTONERGIC NEUROTRANSMISSION. S. K. Hemrick-Luecke*, D. C. Evans and R. W. Fuller. Lilly Research Labs., Eli Lilly and Co., Lilly Corporate Center., Indianapolis, IN 46285.

Serotonin (5HT) neurons are postulated to be involved in the regulation of food intake. The combination of fluoxetine, a selective 5HT reuptake inhibitor, and L-5HTP, a 5HT precursor, results in greater increases in extracellular 5HT concentrations than either fluoxetine or L-5HTP alone (Fuller et al., J. Pharm. Pharmacol. 48:68, 1996). In these experiments, fluoxetine (10 mg/kg, ip) or L-5HTP (20 mg/kg, ip) alone had no effect on milk drinking at 1 hr, while the combination decreased the ratio of sweetened, condensed milk drinking by 50%. The combination of 5HT reuptake inhibitors and $5HT_{1A}$ antagonists has also been reported to result in enhanced extracellular 5HT concentrations as compared to 5HT reuptake inhibitors alone (Fuller et al., 1996; Hjorth, J. Neurochem. 60: 776, 1993). Neither fluoxetine (10 mg/kg, ip) nor WAY100,635 (1 mg/kg, ip), a $5HT_{1A}$ antagonist, alone had an effect on milk drinking at 1 hr. The combination of fluoxetine and WAY100,635, however, decreased the ratio of milk drinking by 56%. Another $5HT_{1A}$ antagonist, S-(-)-206130, at 5 mg/kg, ip, also had no effect alone on milk drinking, while the combination of fluoxetine and S-(-)-206130 decreased milk drinking by 48% at 1 hr. The 5HT-releasing drug, d-fenfluramine, dose-dependently inhibited milk drinking at 1, 3 and 10 mg/kg ip (25%, 82% and 97% respectively) at 1 hr. D-Norfenfluramine, a metabolite of d-fenfluramine which is also a 5HT releaser, dose-dependently decreased milk drinking at 1, 3 and 10 mg/kg, ip (55%, 96% and 97% respectively) at 1 hr. These results suggest that decreases in milk consumption may be a functional response to enhanced serotonergic neurotransmission.

571.3

SEROTONERGIC CONTROL OF MELATONIN SECRETION: THE ROLE OF 5-HT_{1A} RECEPTORS. P.J. Nathan, G.D. Burrows, S.Y. Chai*, T.R. Norman. Dept. of Psychiatry, University of Melbourne, Austin and Repatriation Medical Centre, Melbourne, VIC 3084, Australia.

Melatonin (MT) is a hormone secreted from the pineal gland. The nocturnal rise in MT occurs by activation of β_1-adrenergic receptors in the pineal gland. α_1-adrenoceptors have also been shown to modulate MT secretion, by potentiating the effect of β_1-adrenoceptors. Although MT is synthesised from serotonin (5-HT), the role of serotonergic receptor subtypes in the control of melatonin production is not clear. We investigated the effect of some full (S-20499 & flesinoxan), partial (buspirone and ipsapirone) and indirectly acting 5-HT receptor agonists (d-fenfluramine & paroxetine), on day- and night-time MT in male rats. d-Fenfluramine and paroxetine (20mg/kg) were the only drugs that increased day-time MT ($p<0.01$). At night, d-fenfluramine and flesinoxan significantly increased melatonin at 20mg/kg ($p<0.01$). S(+)-20499 on the other hand significantly increased MT at both 10 mg/kg ($p<0.01$) and 20 mg/kg ($p<0.001$). The fact that only the indirectly acting agonists increased day-time melatonin suggests that acutely increasing 5-HT availability (in the pineal gland or an extra pineal source), increases MT. At night, all 5-HT agonists tended to produce a dose related increase in MT. d-Fenfluramine significantly increased MT at 20 mg/kg, further supporting the hypothesis that increasing the availability of 5-HT, stimulates both day- and night-time MT secretion. However both S(+)-20499 and flesinoxan significantly increased nocturnal MT, suggesting a role for 5-HT_{1A} receptors in the regulation of MT secretion. The fact that the latter drugs had no effect during the day would suggest that a functionally coupled β_1-adrenergic receptor may be required for the effect of 5-HT_{1A} receptor stimulation to be mediated. The mechanism by which 5-HT_{1A} receptors increase MT may be similar to that of α_1-adrenoceptors (i.e potentiation of the β_1-receptor response). (Funded by Servier)

571.4

Chronic Fluoxetine Reduces Serotonin Transporter mRNA and 5-HT_{1B} mRNA in a Sequential Manner in the Rat Dorsal Raphe Nucleus. J. F. Neumaier*, D. Root, and M. W. Hamblin. Harborview Medical Center, GRECC, SVAMC, Department of Psychiatry and Behavioral Sci., University of Washington, Seattle, WA 98104.

Depression has been associated with reduced serotonergic neurotransmission. SSRI antidepressants increase stimulated serotonin release and may do so by downregulating presynaptic terminal autoreceptors. We previously found increased 5-HT_{1B} mRNA in dorsal raphe nucleus (DRN) in learned helpless rats, and hypothesize that fluoxetine may protect against LH by reducing 5-HT_{1B} terminal autoreceptor synthesis. To test this hypothesis we measured the level of presynaptic 5-HT_{1B} mRNA in the DRN (a major source of serotonin projections to the forebrain) and other postsynaptic (nonserotonergic) regions that also express 5-HT_{1B} mRNA following subchronic fluoxetine treatment. This was compared to serotonin transporter (SERT) mRNA, which codes for fluoxetine's initial site of action.

Male rats were treated parenterally with fluoxetine or saline for 4-21 days prior to sacrifice and determination of 5-HT_{1B} and SERT mRNA by *in situ* hybridization. Fluoxetine produced a time-dependent and reversible reduction in DRN 5-HT_{1B} mRNA levels while other brain regions showed no significant changes. SERT mRNA was only temporarily reduced, and returned to sham values by 21 days. This time course study may help explain previous contradictory results regarding the effect of fluoxetine on SERT mRNA levels in DRN, and is relevant to hypotheses of initial and adaptive changes in gene expression following SSRI antidepressant treatment. These data support the hypothesis that increased 5-HT_{1B} autoreceptor activity may be important in animal models of depression and antidepressant action.

571.5

REGULATION OF SEROTONIN REUPTAKE TRANSPORTER (SERT) mRNA EXPRESSION BY ESTROGEN (E) AND PROGESTERONE (P) IN RHESUS MACAQUES. M. Pecins-Thompson, N.A. Brown, C.L.Bethea* Div. of Repro. Sci., Oregon Regional Primate Center, Beaverton, OR 97006

Ovarian steroids regulate prolactin secretion and modulate mood. Both prolactin and mood are regulated by serotonin neurons which we have found contain nuclear receptors for steroid hormones. This study examined the effect of E±P on the expression of mRNA for SERT in monkeys using *in situ* hybridization. A recombinant subclone of the 5′ cytoplasmic domain of the human SERT was generated with a 253 bp insert. The subclone was generated by RT- PCR using the human SERT cDNA (gift from R.D. Blakely) and primers based on published sequence. Monkeys were ovariectomized (OVX)-control, E treated (28 days) and E+P treated (14 days E + 14 days E+P). Perfusion fixed midbrain sections containing the dorsal raphe (10µ) were hybridized at 50°C with ^{35}S antisense cRNA. After a final wash in 0.1X SSC at 60°, sections were apposed to β-max film for 3 days. Adjacent sections were immunostained for serotonin to confirm the location of the dorsal raphe. Densitometric analysis of autoradiographs with graylevel thresholding was performed at 5 levels of the dorsal raphe. The number of pixels exceeding background in defined areas was obtained (*pixel number*) and normalized by the total number of pixels counted (*pixel fraction*). Average pixel number was 22,280 ± 3517, 15,227 ± 1714, 14,827 ± 2042 for ovx, E and E+P treated groups, respectively. There was a 32% and 33% decrease in SERT mRNA signal represented by pixel number in E and E+P treated macaques compared to controls (ANOVA, $p< 0.05$). These data indicate that E with or without P reduces SERT mRNA expression which could result in an overall increase in serotonin neurotransmission. Supported by HD17269, HD18185, DK9098, RR00163.

571.6

REGULATION OF PERIPHERAL 5-HT_{2A} RECEPTORS IN MAJOR DEPRESSION: AN *IN VITRO* MODEL. S. J. Garlow*. Dept. of Psychiatry and Behav. Sci., Emory Univ. Sch. of Med., Atlanta, GA 30322.

One of the most consistent peripheral phenotypes of Major Depression is an increase in the B_{max} of the 5-HT_{2A} receptor on platelets. The other consistent platelet phenotype of depression is the decrease in B_{max} for the serotonin transporter (5-HTT). The factors that regulate the numbers of 5-HT_{2A} receptors on platelets is not known. The hypothesis tested in this project is that the numbers of 5-HT_{2A} receptors on platelets is determined by the rate of transcription of the 5-HT_{2A} receptor gene in megakaryocytes. Megakaryocytes are the nucleated cells from which platelets are derived. A series of soluble factors are being tested for their ability to affect the rate of transcription of the 5-HT_{2A} receptor gene in megakaryocytes. A second hypothesis tested in this project is that the same factor that causes the increase in the B_{max} of the 5-HT_{2A} receptor also causes the decrease in the B_{max} of the 5-HTT. These experiments are a prelude to studying the regulation of transcription of the 5-HT_{2A} receptor and 5-HTT in depressed patients.

SUPPORT: Grant; Dept. of Psychiatry, Emory University School of Medicine

571.7

5-HT_{2C} RECEPTOR ACTIVATION MODULATES MELATONIN PRODUCTION IN RAT PINEAL GLAND L. Steardo*, P. Monteleone, M.R. Carratù, D. Ancona, V. Cuomo. Institute of Pharmacology, School of Medicine, University of Bari, Italy.

5-HT receptors have been detected on the rat pinealocyte membranes; however, at present the precise role played by serotonin in the *in vivo* regulation of rat pineal gland activity remains still not identified. The present investigation has been performed to evaluate the effect of the 5-HT_{2C} agonist, mCPP (meta-chlorophenylpiperazine), on the synthesis of melatonin in rat pineal gland. In comparison to saline, mCPP (0.5 and 2.5 mg/kg, i.p.) increases the nocturnal rise of both pineal N-acetyltransferase (NAT) activity and melatonin-content ($p<0.001$), whereas it has been not able to influence the biosynthetic activity of the gland during the day. Furthermore, mCPP has potentiated the increase of the NAT activity and melatonin production provoked by β_1 stimulation (isoproterenol 1 mg/kg, i.p.) induced during the day ($p<0.001$). The findings suggest that the 5-HT_{2C} receptor stimulation is a part of the physiological mechanism involved in the modulation of rat pineal activity.

571.8

SPECIES- AND TISSUE-SPECIFIC DIFFERENCES IN TRYPTOPHAN HYDROXYLASE K.E. Vrana*, J.W. Haycock†, C.A. Stockmeier‡, and S.C. Kumer. Dept. of Physiology and Pharmacology, Bowman Gray Sch. of Med., Medical Center Blvd., Winston-Salem, NC 27157; †Dept. of Biochem. And Mol. Biol., LSU Medical Center, New Orleans, LA 70119; ‡Dept. Of Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

Tryptophan hydroxylase subserves disparate functions in the brain and in the pineal gland, where it mediates serotonin and melatonin biosynthesis, respectively. Whereas some differences in the biochemical characteristics of raphe and pineal TPH have been reported, both enzymes appear to be encoded by the same mRNA sequence. In the present studies, we report immunochemical differences in TPH from rat and human dorsal raphe and pineal gland using blot immunolabeling with four different antibodies that recognize TPH. TPH immunoreactivity from the four sources was present as a 50-55 kDa band, although the apparent MW of human pineal TPH was several kDa lower than that of the other three. Each of the antibodies produced a different pattern of reactivity with TPH from the four sources: WH3, a mouse monoclonal, was raphe TPH-preferring; a rabbit polyclonal was rat pineal TPH-preferring; and PH8, another mouse monoclonal, was relatively pan TPH-specific. In addition, although TPH activity (with Trp as substrate) appeared to be substantially higher in homogenates of rat pineal gland as compared with rat dorsal raphe, dilution/mixing experiments indicated that endogenous inhibitors in the dorsal raphe homogenates were, in large part, responsible for this difference. These immunochemical results provide further evidence for differential posttranslational processing of TPH in dorsal raphe and pineal gland. [Supported by GM38931 (K.E.V.), MH00967 and NS25134 (J.W.H.), MH45488 (C.A.S.), and T32DA07246 (S.C.K.)]

571.9

DOPAMINE D1 AND SEROTONIN 5HT-2A/2C RECEPTOR SYNERGISTIC STIMULATION OF *c-fos* AND *zif 268* mRNA IN THE STRIATUM. RF Paletzki * and C R Gerfen. Lab of Neurophysiology, NIMH, Bethesda, MD 20892.

The output of the basal ganglia is determined by the relative activity of the "direct" and "indirect" pathways from the striatum to the substantia nigra. Striatal neurotransmitter systems modulate these pathways in a variety of ways, in some cases through the distribution of receptor subtypes with different signal transduction effects on functionally distinct subpopulations of striatal neurons. For example, dopamine D1 and D2 receptor subtypes, which are segregated to direct and indirect output pathway neurons, oppositely affect these pathways due to the respective facilitation and inhibition of adenylate cyclase. The multiplicity of serotonin receptor subtypes provide other patterns of modulation of striatal output pathways. The 5HT-2A/C receptor subtype is expressed by select subsets of both direct and indirect striatal output neurons. This serotonin receptor subtype was studied with quantitative in situ hybridization histochemical localization of the induction of the mRNAs encoding the immediate early genes c-fos and zif268 following treatment with 5HT-2A/2C agonists and antagonists alone and in combination with the dopamine D1 receptor agonist SKF38393 in rats with unilateral striatal dopamine depletion. The 5HT-2A/2C agonist m-CPP (1 mg/kg) did not have an affect on basal immediate early gene mRNA levels in striatum. D1 receptor mediated induction of mRNAs encoding both c-fos and zif268 in direct projection neurons was evident in the dopamine depleted striatum of animals treated with SKF38393 (2 mg/kg) alone. The level of this induction was further increased with combined SKF38393 and m-CPP treatment. These results demonstrate an interaction between D1 dopamine and serotonin 5HT-2A/2C receptors (supported by the NIH).

571.10

CELL-SPECIFIC GLUCOCORTICOID REGULATION OF TRYPTOPHAN HYDROXYLASE MRNA LEVELS IN THE BRAIN. Michael S. Clark* and Andrew F. Russo. Department of Physiology and Biophysics. University of Iowa, Iowa City, IA 52242.

A potential target of glucocorticoid modulation of mood and sleep patterns is the enzyme tryptophan hydroxylase (TPH); which is required for the synthesis of serotonin in the raphe nuclei, and melatonin the pineal gland. We have used a sensitive competitive RT-PCR assay to examine the effect of glucocorticoid treatment on TPH mRNA levels in the rat brain. Chronic treatment increases TPH mRNA levels by 16-fold in the pineal, while decreasing them 6-fold in the raphe nuclei. Using a model serotonergic cell line, we show that glucocorticoids are capable of directly repressing TPH mRNA levels. Hence, glucocorticoids can potentially alter serotonin and melatonin biosynthetic capacity by cell-specific modulation of the TPH gene.

This work supportted by NIH Grant HD25969 (to A.R) and an individual NIMH NRSA (MH10752) to M.S.C.

POTASSIUM CHANNELS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION

572.1

A *HERG*-LIKE K^+ CURRENT IN PROLIFERATING MYOBLASTS BEFORE FUSION

E. Wanke*, A. Arcangeli^, M. Olivotto^, L. Faravelli and R. Bonecchi. Dept. of Gen. Physiology and Biochemistry, Univ. of Milan, Via Celoria 26, I-20133 Milano and ^Inst. of Gen. Pathology, Univ. of Florence, Vle. Morgagni 50, I-50134 Firenze, ITALY.

A novel inactivating inward-rectifying current (I_{IR}), described in murine and human neuroblastoma cells (Arcangeli et al., 1993 *J. Cell Biol.* **122**:1131; Arcangeli et al., 1995 *J.Physiol.*, 489:455-471, 1995), turned out to be biophysically and pharmacologically similar to the heart current I_{Kr} and to the currents expressed in oocytes after injection of *HERG* cDNA. Here we show that a *HERG*-like current is expressed also in myoblasts derived from young mouse muscles. Hindlimb muscle was dissected from 1 to 2-week-old animals. Satellite cells in culture were obtained after incubation in 80% DMEM, 20% FCS, 10 μM/ml insuline, 2 mM glutamine, 10 ng/ml EGF and 50 ng/ml bFGF. The percentage of cells displying the I_{IR}, the anomalous rectifier current (I_{K1}) or both is 25/40/35% respectively, during proliferation of myoblast. The I_{IR} current tend to disappear being substituted by I_{K1} after differentiation and fusion to form myotubes. Very rarely a small fraction of I_{IR} was found in myotubes. The various currents were pharmacologically isolated with Cs^+ (5mM) which is a blocker of all inward rectifier currents and E-4031 which is considered selective for *HERG*-like currents. These findings suggest that, at certain stages of cell differentiation, a novel type of inward rectifying channel is expressed, whose biophysical properties could coincide to those encoded in oocytes by the *HERG* gene. In conclusion our findings should help to understand not only the physiological role of the novel channel but also its linkage to the proliferating phase of normal and cancer cells.

Supported by CNR, Comitato Promotore Telethon and MURST to E.W.

572.2

ACTIVATION AND INHIBITION OF THE G PROTEIN-ACTIVATED K^+ CHANNEL, GIRK1, BY DIFFERENT TYPES OF METABOTROPIC GLUTAMATE RECEPTORS. Dahlia Sharon, Dmitry Vorobiov and Nathan Dascal*. Dept. Physiol. Pharmacol., Sackler Sch. Med., Tel Aviv University, Ramat Aviv 69978, Israel.

Metabotropic glutamate receptors (mGluRs) control intracellular signaling cascades through activation of G proteins. The inward-rectifying K^+ channel, GIRK, is activated by the βγ subunits of G_i proteins and is widely expressed in the brain. We investigated whether an interaction between mGluRs and GIRK is possible, using *Xenopus* oocytes expressing mGluRs and a cardiac/brain subunit of GIRK, GIRK1. Adenylyl cyclase-inhibiting mGluRs (types 2, 3, 4, 6 and 7) activated the GIRK1 channel. This is consistent with the activation of GIRK1 by G_i coupled receptors. In contrast, phospholipase C-activating mGluR1 and mGluR5, presumably coupled to G_q, inhibited the channel's activity. This inhibition was suppressed by a protein kinase inhibitor, staurosporin, and thus appeared to be mediated by a protein kinase, possibly protein kinase C. These modulations could be involved in the mGluRs' effects on neuronal excitability in the brain. Inhibition of GIRK1 by phospholipase C-activating mGluRs bears upon the problem of specificity of G protein - GIRK1 interaction, explaining why receptors coupled to G_q are inefficient in activating GIRK1.

572.3

K^+ - GATED K^+,Na^+-PERMEABLE INWARD RECTIFIER IN THE HIPPOCAMPAL PYRAMIDAL NEURONS O. Krishtal*, V.Pinchenko, V. Filippov, S. Zhenochin & T. Volkova. Bogomoletz Institute of Physiology, Dept. of Cellular Membranology, Bogomoletz Str. 4, 252024 Kiev, Ukraine.

The concentration of K^+ in the interstitial fluid is subjected to transient changes in the course of normal functioning of densely packed central neurons. We have studied the reaction of acutely isolated pyramidal neurons to the step-like elevation of the external K^+. In many cases the shift in external K^+ from O- 2 mM to 2-20 mM was followed by the slow development of the current which had inward direction when measured at negative holding voltages from -120 to -50 mV. This current was clearly additive to the "normal" quick leakage component. The amplitude of the slow current (but not its kinetics) was determined by the K^+ concentration. External Na^+ strongly influenced the kinetics and to a smaller extent the amplitude of this current: with the increase in the external Na^+ from 0 to 150 mM the current was becoming larger and substantially slower. Thus, the time constant of activation of this current was around 80 ms when measured at external $[K^+]$ = 20 mM and $[Na^+]$ = 20 mM and 120 ms at 20 mM and 150 mM. respectively (isotonic K phosphate inside). The development of this current was due to the increase in K^+ and, to a smaller extent, in Na^+ permeability of the membrane. We suggest that the K^+ - activated inward rectifier may serve as a sensor of external K^+ concentration subjected to transient changes in the course of functioning of the central neurons.

572.4

Functional effects of the *weaver* mutation on GIRK2 function in *Xenopus* oocytes and in cerebellar granule cells. P.A. Slesinger*, N. Patil#, Y.N. Jan and L.Y. Jan. HHMI and Dept. of Physiology, Univ. of California, San Francisco, San Francisco, CA 94143. #Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305.

A mutation (G156S) in the putative pore region of a G protein-gated inwardly rectifying K^+ channel, GIRK2, has been identified in the *weaver* mouse (*Nature Genetics* **11**, 126, 1995); an ataxic and hyperactive mutant mouse in which neurons in the cerebellum and substantia nigra are developmentally impaired and subsequently die. We our interested in determining the mechanism by which mutant GIRK2 *wv* channels lead to the *weaver* phenotype. We found previously that the expression of GIRK2 *wv* channels in oocytes forms G protein-activated inwardly rectifying channels that lose their selectivity for K^+ and Na^+, whereas the co-expression of GIRK1 with GIRK2 *wv* appears to form heteromeric channels with reduced channel activity (*Neuron,* **16**:321, 1996). These results suggest that the effect of the *weaver* mutation on neuronal function would depend on the relative expression levels of other GIRK subunits. To test the hypothesis that GIRK1 and GIRK2 *wv* heteromeric channels are non-functional, we have examined the expression of dimers in oocytes. The GIRK1-GIRK2 dimer but not the GIRK1-GIRK2 *wv* dimer shows large receptor-activated inwardly rectifying K^+ currents. To determine whether the *weaver* mutation leads to a gain of function (Na^+ permeability) or loss of function (non-functional heteromeric channels) in cerebellar granule cells, we have begun to record G protein-activated inwardly rectifying K^+ currents from wild-type and *weaver* granule cells. In wild-type granule cells grown *in vitro*, intracellular perfusion with 200 μM GTPγS activates an inwardly rectifying K^+ current which can be blocked by 500 μM external Ba^{2+}. Stimulation of $GABA_B$ receptors by 100 μM (±)-baclofen also activates an inwardly rectifying K^+ current. We are now examining the GIRK currents in *weaver* granule cells and using immunohistochemistry to determine the subcellular distribution of GIRK2 and GIRK1 channel proteins in cultured granule cells. (supported by HHMI & NIH)

572.5

ATP-SENSITIVE K^+ CHANNELS ARE MODULATED BY CYTOPLASMIC pH AND LACTATE IN RAT CEREBRAL CORTICAL NEURONS. Z.-Q.TONG*, P.SU and F ZOU. Dept.of Physilo.,First Military Medical Univ., Guangzhou 510515.P.R.Chian

Recent studies have proved that increase of K-ATP channels activity could protect hypoxic-ischemic brain damage. Nevertheless, the absence of intracellular ATP concentration in hypoxic-ischemic damage, in vivo,is not enough to active K-ATP channels, which suggested some metabolic substances, such as H^+ and lactate, maybe modulate K-ATP channels. The effects of pH and lactate on K-ATP channels activity have been studied on inside-out membrane patches of neurons acutely dissociated from rat.The pipette filled with 140mM KCl solution and bath solution was identical (with 1 mM EGTA, pH 6.0,7.3 or 8.0). The results were as follows: (1) Open probability (Po) and mean open times of K-ATP channels were 0.275 ± 0.068, 0.056 ± 0.01, 0.011 ± 0.003 and 12.7 ± 6.4 ms, 4.3 ± 2.2 ms,3.2 ± 1.8 ms observed in pH 6.0,7.3 and 8.0, respectively .holding potential at 60 mV(n=10,p<0.05 compared each other).The cytoplasmic ATP levels had an IC_{50} for inhibiting Po of K-ATP channels approximately (mM): 2.5 ± 1.4 , 0.124 ± 0. 023 and 0.085 ± 0. 019 in various pH (n=6,p<0.05). (2) Po and mean open time of K-ATP channels were dependent on the cytoplasmic lactate levels, which were 0.060 ± 0.012, 0.153 ± 0.043,0.265 ± 0.073,0.299 ± 0.098 and 15.6 ± 3.4 ms,20.5 ± 5. 8 ms,56.7 ± 11. 2 ms,95.7 ± 20. 8 ms, in 5,10,20 and 30 mM lactate ,respectively. An IC_{50} of ATP were 1.2 ± 05 mM in 20 mM lactate . (3)Single channel currents often became multichannel activities in pH 6.0 or 20 mM lactate levels .The results demonstrated that K-ATP channels activity related to cytoplasmic pH and lactate ,easily evoked in acid condition with lower sensitivity to ATP concentration, which maybe involved mechanism of protection of hypoxic-ischemic brain damage.

572.6

CLONING AND CHARACTERIZATION OF A MODULATORY ELEMENT FOR POTASSIUM CHANNELS. Fuxiong Lu, Dengfeng Xia, Zhengxing Qu, David H Solomon* Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, NY 10032.

Calcium-activated potassium (K_{Ca}) channels play an important role in a wide variety of physiological processes. Previous studies indicated that K_{Ca} channels are modulated by the cAMP dependent protein kinase (A. Carl et al. Am. J. Physiol. **261**, C387, 1991), protein kinase C and protein phosphatase (P. Reinhart & B. Levitan. J. Neurosci. **15,** 4572, 1995). Although regulation of potassium channel activity by N-terminal modulation has been extensively studied at the molecular level, the effect of C-terminal modulation on its activity is poorly understood. In an effort to search for intracellular proteins that can bind to the COOH-terminus of K_{Ca} channels and modulate their activity, we used the yeast two-hybrid system to screen proteins that associate with the COOH-terminus of this channel. PCR products encoding the cytoplasmic COOH-tails of two *Aplysia* K_{Ca} channels were subcloned into a yeast expression vector and fused with the GAL4 DNA binding domain. An *Aplysia* cDNA library was constructed into a vector containing the sequence encoding the GAL4 activation domain. The target constructs were cotransfected with the cDNA library into yeast cells. One clone obtained by this procedure, clone L6, contained a 1.2 kb cDNA. A 2.6 kb full-length cDNA of clone L6 was obtained and sequenced. Southern blot analysis suggests this is a unique gene. A search of protein sequence databases with BLAST revealed similarity of the NH_2 terminal 481-amino acids of *Aplysia* L6 to regions found in a phosphotyrosine independent ligand p62B for the Lck SH2 domain, a neuronal acetylcholine receptor, and dystrophin. It is postulated that this new protein may modulate K_{Ca} channel activity by associating with the C-terminus of the channel. We are functionally studying, by co-injection of L6 mRNA into *Xenopus* oocytes, whether this protein can modulate channel activity. Biochemical experiments are examining co-localization of L6 protein with K_{Ca} channel to determine if this new protein organizes localization of K_{Ca} channels.

572.7

BAPTA POTENTIATES CA^{2+} DEPENDENT AHPs WITHOUT ALTERING ASSOCIATED CA^{2+} SIGNALS IN CA1 HIPPOCAMPAL NEURONS IN RAT BRAIN SLICES. B.S. Jahromi*[1], L. Zhang[1], P.S. Pennefather[1,3], M. Tymianski[1], M.P. Charlton[1,2] and P.L. Carlen[1,2]. *Playfair Neuroscience Unit[1], Toronto Hospital Research Institute and Depts. of Physiology[2] and Pharmacy[3], University of Toronto, Toronto, Ontario, Canada, M5S 1A8.*

We have shown previously that intracellular application of the fast calcium chelator BAPTA potentiates the Ca^{2+}-dependent slow afterhyperpolarization (sAHP) and underlying K^+ current (I_{sAHP}) in rat CA1 cells. Here we examined whether this potentiation of AHP by BAPTA is associated with altered intracellular Ca^{2+} dynamics. Using whole-cell patch-clamp techniques, depolarizing pulses were used to generate AHP's in hippocampal CA1 neurons of acute rat brain slices. The resulting sAHP or I_{sAHP} was recorded in tandem with evoked Ca^{2+} transients by inclusion of fluo3 or calcium green (2-5μM) in the patch pipette solution. The observed cell body Ca^{2+} fluorescence signals took a time course similar to that of the I_{sAHP}, in accordance with data published elsewhere (Knöpfel et al., 1992). Addition of 1 mM BAPTA + 0.1 mM $CaCl_2$ to the intracellular dialysis solution caused potentiation and an alteration of the I_{sAHP} time course. However, simultaneously measured Ca^{2+} signals were not significantly different from those recorded in the absence of BAPTA. Possible interpretations involving the buffered diffusion of Ca^{2+}, localization of AHP channels, and limitations of the Ca^{2+} imaging method will be presented.

This work was supported by the Medical Research Council of Canada. L.Z. is a Scholar of the Canadian Heart and Stroke Foundation.

572.8

GATING OF BK CHANNELS CAN BE INDEPENDENT OF CALCIUM OR VOLTAGE. Brad S. Rothberg* and Karl L. Magleby. Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33101.

Large conductance calcium-activated K^+ (BK) channels are activated by membrane depolarization and micromolar levels of intracellular Ca^{2+}. In order to study the interaction between Ca^{2+} and voltage in the channel gating mechanism, currents were recorded from single BK channels using the patch clamp technique from inside-out patches of membrane excised from cultured rat myotubes. The intracellular surface of the patch was exposed to bathing solutions containing free Ca^{2+} at concentrations ranging from 0.3 nM to 1000 μM. At each $[Ca^{2+}]$, currents were recorded at several different membrane potentials (V_m's) ranging from -80 to +120 mV. Analysis was limited to data during the normal mode, which includes 96% of the open and shut intervals.

Very low levels of activity were observed at $[Ca^{2+}] < 30$ nM (open probability (P_o) < 5×10^{-5} at +30 mV). This activity appeared to be Ca^{2+}-independent, since increasing the free $[Ca^{2+}]$ 100-fold (from 0.3 to 30 nM) did not significantly increase P_o at a given V_m, although the P_o was increased at these $[Ca^{2+}]$ by depolarization. Therefore it appears that the gating mechanism contains V_m-dependent transitions which are Ca^{2+}-independent for $[Ca^{2+}] < 30$ nM. Changing V_m in the negative direction decreased P_o, and this decrease leveled off at a minimum value which depended on the $[Ca^{2+}]$. Therefore some gating transitions may be V_m -independent.

A fit to the data projected a $V_{0.5}$ (V_m resulting in half-maximal activation) of about +165 mV as $[Ca^{2+}] \rightarrow 0$ and about -75 mV as $[Ca^{2+}] \rightarrow \infty$. The effective gating charge (estimated from single Boltzmann fits) appeared to be Ca^{2+}-independent over the examined range of $[Ca^{2+}]$'s. Supported by grants from the NIH and Muscular Dystrophy Association to K.L.M.

572.9

h-CURRENT MODULATION BY NOREPINEPHRINE, SEROTONIN, DOPAMINE, HISTAMINE AND CYCLIC-AMP ANALOGUES IN RAT HIPPOCAMPAL NEURONS J.F. Storm*, T. Winther & P. Pedarzani.# *Inst. of Neurophysiol. University of Oslo, Norway, and #Dept. Molec. Biol. Neur. Signals, Max-Planck Institute for Experimental Medicine, Göttingen, Germany.*
Norepinephrine, acting via cyclicAMP (cAMP), enhances the hyperpolarization-activated cation current I_h (=I_Q) in hippocampal neurons, in a protein kinase A(PKA)-independent manner (Pedarzani & Storm, *PNAS* 92: 11716-20, 1995), thus causing depolarization. We now report that three other transmitters acting via cAMP, serotonin (5-HT), dopamine (DA) and histamine (HA) have a similar effect on I_h. Furthermore, the cAMP-analogue Rp-cAMPS occludes the I_h modulation by these monoamines, suggesting a direct nucleotide action on the channel. ---- I_h was activated by hyperpolarizing voltage steps during whole-cell recording from CA1 pyramidal cells (n = 55) in slices from young rats. 5-HT (10-30 μM), DA (10-30 μM), HA (10-30 μM) or the β-adrenergic agonist isoproterenol (Iso, 1-5 μM) enhanced I_h, and caused an inward shift in the holding current at negative voltages where I_h was activated. (5-HT was applied in the presence of a blocker of $5HT_{1A}$ receptors). In cells loaded with intracellular Rp-cAMPS (500 μM in the pipette), I_h was large and Iso, 5-HT, DA or HA caused no further increase in I_h (n = 4-5 for each), nor the corresponding inward shift in the holding current at negative potentials (n = 4 for Iso). These data suggest that Rp-cAMPS can enhance I_h in a manner similar to cAMP, thus occluding the effect of the monoamines. A similar action of Rp-cAMPS (known as a PKA inhibitor), and cAMP, both enhancing I_h, suggests a direct cyclic nucleotide effect on the h-channels, as previously shown in heart SA node (DiFrancesco & Tortora, *Nature* 351:145-47, 1991). These results further suggest a convergent modulation of I_h by several transmitters involved in brain state control and arousal [Supported by NFR].

572.10

CALMODULIN (CaM) AND CaMK MODULATE COEXISTING Kv1.3 AND INTERMEDIATE-CONDUCTANCE K_{Ca} CHANNELS IN A SIMILAR MANNER. M.C. Chang and L.C. Schlichter* Dept. of Physiology, Univ. of Toronto, and Playfair Neurosci. Unit, Toronto Hospital, Toronto, ON M5T 2S8 Canada.

Voltage-activated (Kv1.3), and intermediate-conductance (IK) K_{Ca} channels are present in lymphocytes and some CNS neurons. In the immune system, Kv1.3 and K_{Ca} are involved in T lymphocyte activation, proliferation and volume regulation, partly by regulating Ca^{2+} signalling. In some CNS neurons, similar IK channels may produce after-hyperpolarizations, and Kv1.3 may contribute to repolarization of specific neurons. Several enzymes that are important for T-cell activation lie downstream of the Ca^{2+} entry: calmodulin (CaM), calcineurin (a CaM-dependent phosphatase that is highly expressed in lymphocytes and CNS neurons), and Ca^{2+}/CaM dependent-protein kinases, of which T cells express CaMKII, and CaMK-Gr (IV). We investigated a possible regulatory loop whereby Ca^{2+} entry, which requires K^+ channel activity, feeds back to up-regulate the function of those K^+ channels. Whole-cell Kv1.3 and IK K_{Ca} currents were measured in mitogen-stimulated human T lymphoblasts. Three membrane-permeant CaM antagonists (trifluoperazine, W-7, calmidazolium) dose-dependently reduced both currents, and, as expected for competitive binding, excess internal CaM significantly relieved this inhibition. To test for involvement of CaM kinases, intact T lymphoblasts were treated with KN-62 (originally developed as a CAMKII inhibitor), before establishing whole-cell recordings: both K^+ currents were significantly inhibited. Thus, CaM and CaMK appear to regulate both IK K_{Ca} and Kv1.3 channels, and would be expected to promote channel activity after Ca^{2+} rises during T-cell activation. A broader implication is that a transient Ca^{2+} elevation, which can persistently activate CaMK, may produce long-term regulation of ion channels. In this, there may even be parallels between mechanisms underlying neuronal memory (e.g. LTP, LTD) and immune cell memory. *Supported by MRC, OGS, Canada.*

572.11

EFFECT OF ANOXIA AND METABOLIC INHIBITION ON MEMBRANE POTENTIAL AND K^+ CHANNELS IN PYRAMIDAL CELLS IN RAT BRAIN SLICE. L. Hyllienmark and T. Brismar*. Dept Clinical Neurophysiology, University Hospital, S-58185 Linköping, Sweden.

Metabolic inhibition or anoxia causes hyperpolarization due to an increase in membrane K^+ conductance. The aim of the present study was to analyse this mechanism in pyramidal cells and to elucidate the type of ion channel involved. Brain slices (300 μm) were obtained from 10 to 19 days old rats and individual cells were visualized with infra-red differential contrast microscopy. Whole-cell recordings were performed on pyramidal cells from the CA1 field in the hippocampus with patch-clamp technique (1-3 MOhm pipettes). Slices were perfused with Ringer-glucose solution and in most experiments 300 nM tetrodotoxin. Anoxia was induced by switching the aerating gas from 95%O_2-5%CO_2 to 95%N_2-5%CO_2. The resting potential (E) was -56.0 ±0.22 mV (n=171, mean, SEM). Anoxia caused hyperpolarization which appeared with a latency of about 4 min and reached a maximum of -3.4 ±0.42 mV (n=16) after 8 minutes. Addition of the metabolic inhibitor dinitrophenol for two minutes changed E by -3.9 ±0.76 mV (n=59). The hyperpolarization was accompanied by an incresed K^+ conductance at resting potential which could be blocked by tolbutamide but not by glibenclamide or addition of intracellular ATP. The effect of metabolic inhibition could partly be inhibited by cadmium suggesting that an increase in intracellular Ca^{2+} may be involved. In addition, metabolic inhibition and anoxia caused a decline in the A-current.

In conclusion, the short-term effects of anoxia or metabolic inhibition in pyramidal cells in brain slices are similar and resembles those previously described in cultured cells. Although the hyperpolarization and incresed K^+ conductance at resting potential were mediated by a tolbutamide-sensitive channel it was not of a classical ATP-sensitive type.

Supported by the Swedish Medical Research Council (project no. 14x-4255).

INVERTEBRATE LEARNING AND BEHAVIOR V

573.1

LONG-TERM MEMORY OF OPERANT CONDITIONED BEHAVIOUR IN *LYMNAEA*. K. Lukowiak*, G. Spencer, T. Inoue, R. Cotter and N. Syed. Neuroscience and Respiratory Research Groups. University of Calgary, Calgary, Alberta, T2N 4N1 and Arabian Gulf University/CMMS, Manama, Bahrain.

Lymnaea are bimodal breathers being able to respire either through their skin or when at the surface via a rudimentary lung (aerial respiration). Aerial respiratory behaviour increases significantly when the animal is in an hypoxic environment. This aerial respiratory behaviour can be operantly conditioned by the contingent presentation of a tactile stimulus to the respiratory orifice, the pneumostome, each time the snail attempts to breathe in the hypoxic pond water. A training period of 0.5 h per day, twice daily for 2.5 days results in learning which persists for up to 1 week. A second group of animals (N=15) received a similar initial 2.5 day training regime but were then retrained at one week intervals for 3 more weeks. This group thus received 8 training sessions over a 4 week period (*spaced training group*). Learning in this group was found to persist for up to one month. In a third group of animals (N=15) the same number of training sessions (8) were given, but over only a 4 day period, (*massed training group*). In this *massed training group* learning did not persist for one month. *Yoked Control* animals (N=15 to both spaced and massed regimes) neither exhibited significant changes in their aerial respiratory behaviour during their "training period", nor did they exhibit any retention of learned behaviour when tested at a one week interval.

Supported by MRC (Canada)

573.2

BEHAVIORS OF THE GILL IN FREELY--MOVING APLYSIA CALIFORNICA. J.L. Leonard*, Mark O. Hatfield Marine Science Center, Oregon State Univ., Newport, OR 97365 and Marine Biological Laboratory, Woods Hole, MA 02543.

The neural control of movement in the *Aplysia* gill has been an important model system for the study of behavioral plasticity. Many studies have used reduced preparations and/or restrained intact animals to examine the physiological mechanisms of behavioral phenomena identified in freely-moving animals. The behavior of the siphon has often been used to infer gill behavior in intact *Aplysia*. In *in vitro* preparations siphon movement is not necessarily a good predictor of gill movement (Mpitsos and Lukowiak 1985; Schaefer and Brownell 1986). To better understand the behavior of the gill under "normal" conditions, the right parapodium was surgically removed from *Aplysia* weighing from 19-300 g and both spontaneous and evoked behaviors of the gill recorded on videotape. The most surprising result was the variety of patterns of spontaneous gill and mantle organ contractions. In intact but restrained *Aplysia*, gill movements such as the SGM or LUM seen associated with INT II activity in *in vitro* preparations, are reliably associated with the "stereotyped" Pumping behavior (Pinsker 1982-3; Leonard and Lukowiak 1986; Leonard et al. 1989). This was not the case in hemiparapodectomized (HPX) *Aplysia*. In HPX animals spontaneous LUMs occurred in several contexts; a) with contractions of the siphon, mantle and parapodia, the classic Pumping; b) preceeding contraction of other mantle organs, the "gill-first" pumping; and c) alone, without contraction of other mantle organs. Similarly, contractions of the siphon were often observed that were not followed or accompanied by gill movements, and on occasion, coordinated contractions of siphon, mantle and parapodium were not accompanied by detectable gill contraction. The types of gill movements seen in HPX animals were consistent with those described previously (Leonard et al. 1989), with the addition of a new Act, Quiver. Supported by an NIH grant (NS-08437) to L.B. Cohen.

573.3

CONTRIBUTION OF MONOSYNAPTIC EPSPS FROM LE SIPHON SENSORY NEURONS TO MEDIATION AND HABITUATION OF THE GILL- AND SIPHON WITHDRAWAL REFLEX IN *APLYSIA*. R.D. Hawkins* and L. Frost. Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

Monosynaptic connections from LE siphon sensory neurons to motor neurons are thought to contribute to the gill- and siphon-withdrawal reflex in *Aplysia* and its modification during simple forms of learning. However, other sensory neurons and interneurons are also thought to contribute (Hawkins & Frost, 1995). To estimate the relative contribution of monosynaptic EPSPs from LE neurons, we compared them to the complex PSP produced in LFS siphon motor neurons by tactile stimulation of the siphon. In rested preparations, the monosynaptic EPSP was 34.5 ± 5.1% of the amplitude and 6.6 ± 1.8% of the area of the complex PSP (n = 15). Siphon stimulation produced 2.6 ± 0.7 spikes in an LE cell when it was within the cell's receptive field ("on-field"). Following 5 siphon stimulations at 5 min intervals, which produces behavioral habituation in this preparation (Hawkins et al., 1990), the area of the complex PSP was significantly reduced (81 ± 9%, n = 12, $p < .05$). There was no change in the number of spikes in on-field LE neurons, but the area of monosynaptic PSPs from those neurons was significantly reduced (43 ± 10%, n = 7, $p < .01$). By contrast, the area of monosynaptic PSPs from off-field LE neurons was not changed (n = 5). In preliminary experiments (n = 5) we have obtained quantitatively similar results when recording from the motor neuron LDG1, which mediates most of the gill-withdrawal reflex in this preparation (Hawkins et al., 1992). These results suggest that monosynaptic EPSPs from LE siphon sensory neurons make a substantial contribution to the gill- and siphon-withdrawal reflex and its modification during habituation. Supported by NIMH.

573.4

5-HT ENHANCES SPONTANEOUS ACTIVITY OF ISOLATED NERVES AND INCREASES EXCITABILITY OF *APLYSIA* SENSORY NEURON AXONS. J.D. Gunstream*, G.A.Castro, and E.T. Walters. Dept. Integrative Biology, Univ. Texas - Houston Med. School, TX 77030.

Strong, noxious stimulation of *Aplysia* is likely to damage peripheral nerves and trigger the release of 5-HT at various sites (see Walters & Ambron, *Trends Neurosci.* 18:137, 1995). To begin to test whether 5-HT directly modulates peripheral axons following nerve injury, we examined the effects of 5-HT on extracellularly recorded activity from a segment of peripheral nerve p9 severed from both the tail and CNS. In every case, isolated segments showed some spontaneous activity that persisted for the duration of the experiment (up to 2 d). Application of 10 μM 5-HT increased spontaneous activity in 8 of 8 isolated nerves tested. Clear increases in activity were seen for doses of 1-250 μM (n=2 preparations). Enhanced activity was also seen after 5-HT application to surgically desheathed segments (n=2). 5-HT did not cause obvious enhancement of activity in central (pleuroabdominal) connectives. Selective application of 5-HT to nerve p9 while attached to the CNS never activated pleural sensory neurons (SNs). However, local application of 5-HT to nerve p9 increased the activity evoked by a 1-s nerve shock in 3 of 3 cells tested (from 11.4 to 17.6 spikes), under conditions (2 μM external Ca^{2+}) expected to reduce Ca^{2+}-dependent release of endogenous modulators. 2-hr nerve application of 10 μM 5-HT does not appear sufficient to induce long-term hyperexcitability of pleural SN somata (n=5) or to enhance hyperexcitability induced by nerve crush (n=4). Peripheral 5-HT may enhance SN injury discharge and activate axons of neurons that have widespread modulatory effects (see Liao & Walters, this volume).

Supported by NIH grants MH38726 to E.T.W. and DK37260 to G.A.C.

573.5

INDIRECT AND DIRECT EFFECTS OF 5-HT ON SENSORIMOTOR SYNAPSES IN *APLYSIA*. X. Liao* and E.T. Walters. Dept. Integrative Biology, Univ. Texas - Houston Medical School, TX 77030.

We are testing whether 5-HT application to *Aplysia* ganglia activates modulatory neurons that facilitate synapses between pleural sensory neurons (SNs) and pedal motor neurons. Six of 8 connections measured in 5 μM 5-HT in artificial seawater (ASW) showed facilitation, to a mean for the entire group of 169% of their pre-5-HT values. Surprisingly, only 1 of 9 connections measured in 5-HT medium containing elevated divalent cation levels ("hi-di" solution - 2.2 X Mg^{2+} and 1.25 X Ca^{2+}) was facilitated (to 106%), while the group mean was depressed to 88% of baseline. Long-term treatment was conducted 4 hr after dissection with 2 hr application of 5 μM 5-HT. Connections were tested before and 1 d after treatment. Treating with 5-HT in ASW caused significant long-term facilitation (LTF)(from 7.7 to 11.3 mV, n=12 ganglia). Untreated controls showed no change (from 7.5 to 7.5 mV, n=10). Treatment with 5-HT in hi-di caused no significant change (from 9.4 to 8.2 mV, n=10). Similarly, treatment with 5-HT mixed with 60 μM TTX, resulted in mean EPSP depression in 4 of 6 ganglia, while TTX alone had no obvious long-term effect (n=4). Initial results (n=2) showing that hi-di solution does not block 5-HT facilitation in 3 d old dissociated cell cultures, suggests that blocking effects of hi-di are not due to direct actions on 5-HT pathways in SNs. Interestingly, hi-di fails to block LTF in ganglia treated with 5-HT 3-5 d after CNS dissection (22 of 24 connections facilitated, from a mean of 6.9 to 11.5 mV). Our results show that 5-HT applied to ganglia produces complex facilitatory effects, and suggest that axon injury may cause a delayed increase in the responsiveness of SNs to 5-HT.

Supported by NIH grant MH38726 to E.T.W.

573.6

LONG-TERM HYPEREXCITABILITY OF *APLYSIA* SENSORY NEURONS FOLLOWING cAMP INJECTION: INVOLVEMENT OF Ca^{2+} AND OTHER SIGNALS. M.R. Lewin and E.T. Walters*. Dept. Integrative Biology, Univ. of Texas - Houston Medical School, TX 77030.

One d after injection of cAMP into *Aplysia* sensory neurons (SNs) K^+ currents in the cell soma are depressed (Sholz & Byrne, *Science* 240:1664, 1988) and apparent synaptic growth occurs (Nazif et al., *Brain Res* 539:324,1991). Because of likely overlap of injury and memory signals (Walters, *Biol. Bull.* 180:241,1991) and because impalement/injection procedures cause potentially significant cellular damage, we are testing the possible involvement of injury-related signals in triggering long-term hyperexcitability during pressure injection of cAMP and fast green (FG) dye into pleural SN somata. One d after cAMP injection, 28.9 spikes were evoked in SN somata by a 1-s depolarizing test pulse at 2.5X threshold (n=6 ganglia and 24 cells). This significantly exceeded the number of spikes evoked 1 d after injecting 1) cAMP and the Ca^{2+}-chelator BAPTA (16.5 spikes, n=7 ganglia and 25 cells), 2) cAMP and BAPTA while extracellular Ca^{2+} was reduced by 5000X (13.9 spikes, n=9 ganglia and 37 cells), or 3) FG alone (15.5 spikes, n=8 ganglia and 57 cells) ($p<.01$ in each case). The attenuation of hyperexcitability by BAPTA injection and low extracellular Ca^{2+} suggests that concomitant Ca^{2+} transients contribute to long-term effects of cAMP signals. FG-injected cells were more excitable than uninjected cells in the same ganglia (15.5 vs 6.2 spikes, $p<.002$), suggesting that this dye or other aspects of injection can also induce persistent responses. The relatively low excitability seen after FG injection in low extracellular Ca^{2+} (6.8 spikes, 4 ganglia and 37 cells) suggests that other (injury-related) signals may also be Ca^{2+} dependent.

Supported by NIH grant MH38726 to E.T.W.

573.7

Rp-cAMPS BLOCKS THE AXOTOMY-INDUCED INCREASE IN EXCITABILITY AND GROWTH OF ISOLATED *APLYSIA* SENSORY NEURONS IN CELL CULTURE. S. S. Bedi* and D. L. Glanzman. Department of Physiological Science, UCLA, Los Angeles, CA 90024.

Axotomy of *Aplysia* sensory neurons in dissociated cell culture induces long-term changes in these cells which resemble those induced by repeated applications of the modulatory neurotransmitter serotonin (5-HT). Among these changes are hyperexcitability (Salim and Glanzman, *Soc. Neurosci. Abstr.* **21**:1267, 1995) and increased neurite outgrowth. Serotonin's long-term effects on sensory neurons are mediated by activation of the cAMP second messenger pathway. To determine whether the long-term effects of axotomy are also mediated by cAMP-dependent intracellular signals, we axotomized cultured sensory neurons in the presence or absence of Rp-cAMPS, a specific inhibitor of protein kinase A (PKA).

The excitability of isolated sensory neurons in culture was tested and the number of branch points on their neurites quantified. Then some of the sensory neurons were axotomized. Twenty-four hr later the excitability of the neurons was retested and their neurites re-inspected. Axotomized neurons left in normal cell culture medium exhibited significantly greater excitability and neurite outgrowth, as indicated by an increase in the number of branch points on their neurites, than did unaxotomized (control) sensory neurons. By contrast, axotomized sensory neurons placed into cell culture medium containing Rp-cAMPS (500 μM) did not exhibit an increase either in excitability or outgrowth when examined 24 hr after axotomy. Interestingly, Rp-cAMPS also blocked the more modest increase in excitability and outgrowth observed in control cells left in normal medium for 24 hr. Our results indicate that injury stimulates cAMP-dependent gene transcription in *Aplysia* sensory neurons and provide further support for the idea (Walters and Ambron, 1995) that the cellular pathways activated by injury and those activated by 5-HT in sensory neurons overlap. Supported by NSF and the Alzheimer's Disease Program, State of California.

573.8

ROLE OF PROTEIN KINASES IN UBIQUITIN-MEDIATED TURNOVER OF C/EBP DURING LONG-TERM FACILITATION IN *APLYSIA* SENSORY NEURONS. **N. Yamamoto*, A.N. Hegde, D.G. Chain and J.H. Schwartz. Center for Neurobiology & Behavior, Columbia University, New York, NY 10032.**

Rapid induction of mRNA encoding the transcription factor ApC/EBP is necessary for long-term facilitation (LTF) of the sensory-to-motor synapses that mediate defensive reflexes (Alberini et al., *Cell* 76:1099, 1994). We found that DNA-binding activity of ApC/EBP protein is maximal at 3 h after beginning sensitization treatment *in vivo*, and then falls to basal levels by 6 h, indicating that the transcription factor acts during a critical time period. To examine the molecular mechanism of the turnover of ApC/EBP, we incubated recombinant ApC/EBP with *Aplysia* nervous tissue extracts. The protein was rapidly degraded. The proteolysis depended on the presence of ATP and proteasomes, and gave rise to multiple ubiquitin ladders as intermediate products. The degradation occurred only in nervous tissue, suggesting some tissue-specific factor is required. This ATP-ubiquitin-proteasome-mediated degradation was enhanced by phosphatase inhibitors, suggesting that the factor is a protein kinase. From studies with various kinase specific inhibitors, we found that both MAP kinase (MAPK) and protein kinase C (PKC) are necessary for the degradation of ApC/EBP. In addition, we have evidence that activation of ApC/EBP (DNA binding) requires direct phosphorylation by MAPK, which is activated by PKC. These results suggest that MAPK and PKC regulate both activation and turnover of ApC/EBP. (Supported by NIH grants MH48850 and MH00921 [JHS])

573.9

INHIBITORS OF PROTEASOMES BLOCK LONG-TERM FACILITATION IN *APLYSIA*. D.G. Chain*, S. Schacher, A.N. Hegde, A. Casadio, A.L. Goldberg and J.H. Schwartz. Center for Neurobiology & Behavior, Columbia University, NY, NY 10032 and Harvard Medical School, Boston, MA 02115.

The cAMP-dependent protein kinase (PKA) becomes persistently activated during long-term facilitation (LTF) in *Aplysia* sensory neurons (SNs). Persistence of PKA results from proteolysis of its regulatory (R) subunits that are degraded in tissue extracts by the ubiquitin system (Hegde et al., *PNAS* 90:7436, 1993). Down-regulation of R is a nervous system-specific process dependent on factors that modulate proteasomes (Hegde et al., *Soc. Neurosci. Abs.* 20:1072, 1994; Chain et al., *J. Neurosci.* 15:7592, 1995). We have characterized the proteasomes biochemically and used inhibitors to establish whether they have a critical role in LTF. We tested the action of a transition-state peptide aldehyde inhibitor, N-Carbobenzyl-leu-leu-leunorvalinaldehyde (MG115) by monitoring the hydrolysis of fluorogenic peptide substrates using purified proteasomes. We found that MG115 blocks chymotryptic activity by > 90%. We tested MG132 (N-Carbobenzyl-leu-leu-leu-leucinaldehyde), a more potent, closely-related structural analog, in cell-culture. SNs and motor neurons reestablish connections after 4 days and undergo LTF after repeated applications of 5-HT (Montarolo et al., *Science* 234:1249, 1986). The amplitude of the EPSP was recorded for each culture before and 24 h after 5 applications of control solution at 20 min intervals followed by 22 h treatment with MG132 alone, 5 applications of 5-HT at 20 min intervals followed by 22 h treatment with the inhibitor alone, or 5 applications of 5-HT alone. A one-factor Anova indicated a significant effect of the treatment (DF=2,17; F+13.725; p<0.001). Multicomparison tests (Dunnett's) indicated that 5-HT significantly increased the amplitude of the EPSP compared to the MG132 treatment (T=3.88; p,0.01). Treatment with MG132 after 5-HT evoked a change not significantly different from treatment with MG132 alone (t=0.55). These results suggest that degradation by proteasomes has an important function in the maintenance of LTF. (Supported by NIH grants NS29255 and MH00921 [JHS])

573.10

MUTATION IN MAP KINASE PHOSPHORYLATION SITES BLOCKS LEARNING-RELATED INTERNALIZATION OF apCAM IN *APLYSIA* SENSORY NEURONS. C.H. Bailey*, B.-K. Kaang, M. Chen, and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032, & Inst. Mol. Biol. & Genetics, Seoul Natl. Univ., Korea.

The synaptic growth that accompanies 5-HT-induced long-term facilitation of the sensory-to-motor connection in dissociated cell culture is associated with a down-regulation of cell adhesion molecules (apCAMs) on the surface membrane of the sensory neuron (Mayford et al., 1992). Down-regulation is achieved by activation of the endosomal pathway leading to internalization and apparent degradation of apCAM (Bailey et al., 1992). Of the two apCAM isoforms (membrane bound and GPI-linked), only the transmembrane form is internalized. Thus, a 1 hr exposure to 5-HT leads to a 68% decrease in the density of gold-labeled complexes bound to the transmembrane form at the surface membrane and a 24-fold increase in its internalization. The selective internalization of the transmembrane form highlights the potential regulatory significance of its intracellular domain, which contains a PEST sequence (thought to target degradation) and two consensus sites for MAP kinase phosphorylation. To explore further the functional role of the intracellular domain, we have overexpressed specific epitope-tagged deletion and mutant transmembrane constructs and find both 5-HT-induced down-regulation at the surface membrane and internalization can be blocked by removing either the entire cytoplasmic tail; or the region containing PEST or by simply substituting alanine for threonine in the two MAP kinase consensus sites. These data suggest phosphorylation by MAP kinase may be important in the learning-related internalization of apCAMs.

Supported by NIH grants MH37134 and GM32099 to C.H.B., and the Howard Hughes Medical Institute to E.R.K.

SUBCORTICAL VISUAL PATHWAYS III

574.1

FUNCTIONAL ORGANIZATION OF THE AMPHIBIAN OPTIC TECTUM
G. Roth*, U. Dicke and M. Wallstein. Brain Research Institute, University of Bremen, PO Box 330440, 28334 Bremen, Germany.

Tracer injections and intracellular labeling with Biocytin (Sigma) reveal 3 types of tectal projection neurons in salamanders. The 1st type arborizes predominantly in the *superficial* layer of retinal afferents containing information about contrast. The axon descends to the *contralateral* medulla; no ascending projections exist. The 2nd type arborizes in the *intermediate* layer of retinal afferents containing information about motion. The axon descends *ipsilaterally* and *laterally* inside the medulla; ascending axons project bi- or ipsilaterally to pretectum and thalamus. The flat dendritic tree of the 3rd type arborizes in the *deep* layer of retinal afferents containing information about ambient illumination and in the efferent fiber layers. Ascending projections are the same as in the 2nd type; the descending axon runs in the *medial ipsilateral* medulla. Tracer studies in various species of frogs reveal essentially the same types of projection neurons. In salamanders and frogs, interneurons have small dendritic trees arborizing in one or more of the retinal layers. Intracellular recording and labeling reveals extensive electrical coupling among different types of neurons. Projection neurons show excitatory or excitatory-inhibitory responses at short latencies (7.6 ms on average) at electrical stimulation of the optic nerve, while interneurons respond at longer latencies (33.4 ms) mostly with inhibition or excitation-inhibition.

Experiments using polyclonal primary antibodies against GABA and glycine (Sigma, Chemicon) and a monoclonal primary antibody against glutamate (Sigma) suggests that most projection neurons are exclusively glutamatergic, while a small number appears to contain GABA and/or glycine in addition. Many tectal interneurons, in addition to glutamate, contain GABA or GABA/glycine.

The 3 types of projection neurons plus subtypes of interneurons constitute 3 tectal subsystems concerning shape, motion and ambient illumination. The result of interaction among these subsystems plus integration of ascending and descending systems from other brain parts is sent via anatomically separate pathways to different premotor and motor regions in the medulla and rostral spinal cord.

Supported by DFG Ro 481/11-3 and SFB 517

574.2

TEMPORAL RELATIONS BETWEEN RESPONSES IN VISUAL CORTICAL AREAS AND SUPERIOR COLLICULUS OF THE CAT.
A.K. Engel* and M. Brecht. Max-Planck-Institut für Hirnforschung, 60528 Frankfurt, Germany

The functions of cortex and superior colliculus (SC) are intimately related in mammals. Corticotectal projections originate from almost the entire neocortex and collicular feedback is provided via thalamic structures. Here, we have aimed at testing the hypothesis that temporal codes may be important for the corticotectal interplay. To this end, we applied correlation analysis to visually evoked multiunit responses that were simultaneously recorded from superficial SC layers and from visual cortical areas 17, 18, PMLS and PLLS in anesthetized cats. We frequently observed temporal correlations between responses in these cortical areas and the SC. Many of the correlograms showed broad peaks (width >20 ms) but occasionally also narrow peaks were seen. In addition, we frequently observed oscillatory side peaks indicating rhythmic firing at frequencies between 5-80 Hz. Corticotectal interactions were similar for all cortical areas studied. These interactions occurred mainly for cells with overlapping receptive fields and tended to be weaker than those observed within the cortex. In addition, corticotectal interactions did not occur at 0-time lag but systematic phase shifts were observed, the cortical units leading 0-25 ms over collicular responses. Furthermore, our data indicate that the incidence of corticotectal synchrony is correlated with the occurrence of synchrony between the cortical areas. Our data demonstrate for the first time that the corticotectal pathway affects spike timing in the SC. Thus, SC neurons might be able to "read" relations within cortical populations defined by synchronized firing. Supported by the MPG, by the Heisenberg Program of the DFG and by the Minna-James-Heineman Foundation.

574.3

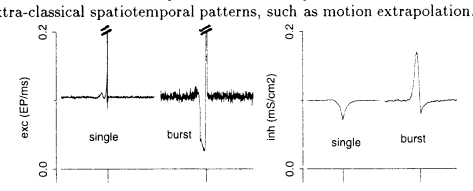
TEMPORAL CODING IN THE CAT SUPERIOR COLLICULUS.
M. Brecht*, W. Singer and A.K. Engel. Max-Planck-Institut für Hirnforschung, 60528 Frankfurt, Germany

Evidence is accumulating that synchronous firing of neurons plays an important role for visual information processing. Recent work on the visual cortex has suggested that correlated activity may serve for the integration of activity into coherent representational states and for the selection of distributed responses for further joint processing. Since population coding is also assumed to be important for sensorimotor transformations carried out in the superior colliculus (SC), we have now studied temporal relationships between spatially separate neurons in the tectum. Using arrays of multiple electrodes, we have recorded from superficial layers of the SC in anesthetized and paralyzed cats. Responses to visual stimulation were subjected to correlation analysis. Our results demonstrate that neurons in the SC can readily synchonize their activity. Synchronized activity was observed for neurons with overlapping as well as nonoverlapping receptive fields and occurred over distances of several millimeters. Correlograms showed both narrow as well as broad peaks (width >20 ms) that were centered around 0 ms phase shift. In many cases, the interactions were accompanied by oscillatory components of varying frequency (5-80 Hz). In a number of cases, we have obtained preliminary evidence that the interactions depend on the configuration of visual stimuli. Taken together, the data are compatible with the notion that synchrony may be important for processing in the SC and may allow the selection of stimulus-specific motor responses from spatially nondiscrete activity patterns. Supported by the MPG, by the Heisenberg Program of the Deutsche Forschungsgemeinschaft and by the Minna-James-Heineman Foundation.

574.4

MODELING THALAMUS AS A PREDICTIVE COMPARATOR
W. Softky* Mathematical Research Branch, NIDDK, NIH

This work proposes that the combined influences of cortical/reticular (nRT) feedback to a thalamic "relay" cell may provide it with a very specific contextual signal—an inhibitory prediction of the cell's imminent sensory input--and that under normal sensory conditions the cell acts as a comparator, conveying to cortex approximately the difference between the prediction and the actual sensory input. Such predictive inhibition would implement several useful features: nonlinear reduced-redundancy coding in space and time; compensation for feedback's inevitable delays; and refining the brain's represenation by emphasizing its mistakes. This preliminary test evaluated whether a relay cell could transmit both signs of prediction error by means of its two known spiking patterns, single (tonic) spikes and post-inhibitory-rebound bursts. Reverse-correlations (±300 *ms*, below) of random excitatory and inhibitory input to a simulated thalamic cell showed, over a wide range of firing rates, that on average tonic spikes signalled excess excitation and reduced inhibition in the preceding 30-100 *msec*, while bursts signalled approximately the opposite. These simulations predict that the receptive fields reconstructed from bursts vs. from spikes should be different and—in the postulated case of predictive inhibition—senstive to extra-classical spatiotemporal patterns, such as motion extrapolation.



574.5

MECHANISMS UNDERLYING THE GENERATION OF THE SLOW AFTERHYPERPOLARIZATION (AHP) IN PERIGENICULATE (PGN) NEURONS AND ITS ROLE IN SYNCHRONIZED OSCILLATIONS. U. Kim*, T. Bal, and D.A. McCormick. Sec. Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510

During the generation of spindle waves in vitro perigeniculate neurons progressively hyperpolarize and this appears to contribute to the "waning" of these synchronized oscillations. Intracellular recordings in PGN neurons revealed that this afterhyperpolarization was associated with a reduced responsiveness to barrages of EPSPs arising from burst firing in LGNd thalamocortical cells, as well as a decreased ability of a burst of activity in thalamocortical cells to generate a burst of action potentials in PGN neurons. This afterhyperpolarization appears to be generated through intrinsic mechanisms since the generation of a single burst of action potentials (500-600 Hz) induced by an intracellular injection of a depolarizing current pulse (50-100 msec) is followed by a slow AHP of about 1 sec in duration. The induction of repetitive burst discharges at the frequency of 5-10 Hz augments the amplitude and duration of AHP up to 5-10 sec.

The AHP was found to have multiple components, including a voltage-dependent rectification of the membrane (including activation and de-activation of the persistent sodium current), a Ca^{2+}-activated K^+ current, and possibly a Na^+-dependent K^+ current. Intracellular injection of depolarizing and hyperpolarizing ramps resulted in the generation of apparent inward rectification at depolarized membrane potentials. Block of the persistent Na^+ current with TTX reduced this rectification, but did not abolish it. The slow AHP following repetitive depolarizing current pulses was associated with an increase in membrane conductance, its equilibrium potential was shifted to more positive levels with increases in $[K^+]_o$ and was abolished following the abolition of action potentials with TTX. These results suggest that is may be mediated in part by a Na^+ and/or Ca^{2+}-dependent K^+ current. Supported by NIH and the Klingenstein Fund.

574.6

HISTAMINE INHIBITS PERIGENICULATE NEURONS THROUGH ACTIVATION OF A Cl^- CONDUCTANCE. K. Lee* and D.A. McCormick. Section of Neurobiology, Yale Univ. Sch. Med. New Haven, CT 06510.

The nucleus reticularis thalami (nRt) and its visual counterpart, the perigeniculate nucleus (PGN), is innervated by histaminergic fibers presumably arising from neurons in the tuberomammillary nucleus of the hypothalamus. Local application of histamine to the PGN neurons in the ferret geniculate slice maintained in vitro resulted in a decrease in input resistance and a slow membrane depolarization or hyperpolarization towards about -73 mV, depending on resting membrane potential. These responses were not abolished by blockade of synaptic transmission with local application of tetrodotoxin, indicating that they are direct postsynaptic actions. Voltage clamp analysis revealed that the membrane potential change induced by histamine or the $GABA_A$ agonist muscimol was associated with a current that reversed around -73 mV when recorded with either 2 M potassium acetate or 3 M cesium acetate-filled electrodes. When cells were loaded with Cl^- by recording with 3 M potassium chloride-filled electrodes, the reversal potential of histamine and GABA responses both shifted to a similar extent to more depolarized membrane potentials. These results strongly suggest that activation of histaminergic receptors in the GABAergic PGN/nRt neurons results in an increase in a chloride, but not a potassium, conductance. Interestingly, application of histamine to the PGN could halt the generation of spindle waves, indicating that increased activity in the tuberomammillary histaminergic system may play a functional role in dampening thalamic oscillations through increases in Cl^- conductance in PGN cells. Supported by NIH and the Klingenstein Fund.

574.7

LATERAL INHIBITION IN THE PERIGENICULATE NUCLEUS OF THE FERRET. M. V. Sánchez-Vives* and D. A. McCormick. Sec. Neurobiology, Yale Univ. School of Medicine, New Haven, CT 06510.

The GABAergic neurons of the perigeniculate nucleus (PGN) form reciprocal connections with thalamocortical cells of the LGN and with other PGN neurons. These reciprocal interconnections allow for the generation of both normal (e.g. spindle waves) and abnormal (e.g. spike-and-wave seizures) patterns of activity, probably depending upon lateral inhibition within the PGN. Here we demonstrate that activation of PGN neurons with the local application of glutamate typically results in IPSPs that exhibit reversal potentials of around -78 mV and are blocked by the $GABA_A$ receptor antagonists bicuculline and picrotoxin. In addition, PGN neurons also possess $GABA_B$ receptors and lateral inhibition can generate slow IPSPs that may be mediated by these receptors.

Local application of bicuculline results in a marked prolongation of rebound bursts of action potentials and the rebound LT Ca^{2+} spike in PGN neurons in normal and TTX containing solution, respectively. These results suggest that the activation $GABA_A$ receptors strongly regulates the duration of burst discharges in PGN neurons. However, the burst prolongation induced by picrotoxin is always much weaker, even when it is effective in blocking IPSPs and the response to muscimol. These observations suggest that this effect of bicuculline is mediated by an unusual $GABA_A$ receptor in PGN cells or due to an uncharacterized effect of bicuculline on a membrane conductance out of the GABA receptor complex.

Supported by NIH and the Klingenstein Fund.

574.8

MUSCARINIC RECEPTOR-MEDIATED RESPONSES IN THALAMIC LOCAL INTERNEURONS. J. J. Zhu*, D.J. Uhlrich, and W.W. Lytton. Neuroscience Training Program and Depts. of Anatomy and Neurology, University of Wisconsin Medical School and the Wm. S. Middleton VA Hospital, Madison, WI 53706

Thalamic cells, including local interneurons, have both bursting and tonic firing modes. Many neurotransmitters switch firing modes in thalamocortical and thalamic reticular cells, reflecting behavioral state transitions. We wanted to determine whether these neurotransmitters have similar effects on thalamic local interneurons.

We studied muscarinic receptor-mediated effects on identified local interneurons (n=53) in the adult rat lateral geniculate nucleus slice. As expected, a brief application of a muscarinic agonist, methacholine (Mch), induced a hyperpolarization due to an increase a K^+ conductance. However, with the whole-cell recording configuration, this was often followed by a slow depolarization. Both muscarinic responses, which were accompanied by a decrease in input resistance, were insensitive to TTX and blocked by gallamine, suggesting the direct involvement of the M_2 receptor. The depolarization was selectively suppressed when high concentration EGTA was included in the patch electrode solution, suggesting a Ca^{2+}-mediated event, and voltage clamp analysis revealed that the depolarization was due to an increase in two Ca^{2+}-dependent conductances, I_h and I_{can}. Interneurons, when hyperpolarized or at rest, assume the burst mode of firing. Mch application switched them into the tonic firing mode. Computer simulation demonstrated that this switch from bursting to tonic mode could be explained by a combination of the decreased input resistance and the pre-activation of I_{can}, preempting the initial burst response. Our study provides a possible cellular mechanism for different firing modes observed in thalamic local interneurons in different behavioral states. Supported by NEI, NINDS, and the VA

574.9

A RETINOGENICULATE PATHWAY EXPRESSES THE α SUBUNIT OF CAM II KINASE IN THE PRIMATE. D.J. Calkins* and S.H.C. Hendry, Krieger Mind/Brain Institute, Johns Hopkins University, Baltimore, MD 21218.

Neurons in the intercalated regions ventral to each magnocellular (M) and parvocellular (P) layer of the primate lateral geniculate nucleus (LGN) are differentiated from M and P neurons by immunoreactivity for the α subunit of type II calmodulin-dependent kinase (Cam II kinase). These intercalated neurons provide multiple direct pathways to the primary visual cortex and, like the M and P pathways, are likely to transmit visual information from the retina. However, whether the intercalated neurons are postsynaptic to one or more types of retinal ganglion cell, and whether these also are differentiated by Cam II kinase immunoreactivity, is unknown. Immunostaining for the α subunit of Cam II kinase in wholemounts and vertical sections of *Macaca mulatta* retina revealed labeled amacrine cells, with a corresponding band of dense immunoreactivity in the inner plexiform layer (IPL) just below the inner nuclear layer, and an intensely labeled population of ganglion cells, with a corresponding band of dense immunoreactivity along the border between the IPL and ganglion cell layer (GCL). A weaker band was also present in the middle IPL that could indicate additional populations of labeled amacrine or ganglion cells. The intensely labeled ganglion cells had broad dendritic trees stratifying narrowly over the GCL. These cells, some with dendritic trees nearly 1 mm in diameter, branched sparsely and resembled γ ganglion cells, but whether they comprise only a single type is undetermined. Ganglion cell axons were also intensely labeled, as were axon terminals in LGN confirmed by electron microscopy to be presynaptic to α Cam II kinase labeled dendrites of intercalated neurons. These results are consistent with one or more retinogeniculocortical pathways immunoreactive for Cam II kinase that originate in large-field ganglion cells distinct from the "parasol" and "midget" ganglion cells underlying the M and P pathways. Supported by NIH EY06432.

574.10

FUNCTIONAL ACTIVATION OF LATERAL GENICULATE NUCLEUS AND PRIMARY VISUAL CORTEX DURING VISUAL IMAGERY IN HUMAN BRAIN. T. Kato, W. Chen, X.-H. Zhu, D. Tank#, S. Ogawa# and K. Ugurbil*, CMRR, University of Minnesota, School of Medicine, Minneapolis, MN 55455, #Lucent Technologies, Bell Labs, Murray Hill, NJ 07974, USA.

Whether visual imagery process is subserved by the similar neural substrate as visual perception is one of the major controversies in visual neuroscience. Our goal in this study was first to demonstrate that the LGN activation in the human brain can be robustly detected during visual perception using fMRI with blipped EPI at high (4 Tesla) magnetic fields, subsequently to examine if V1 and the LGN activation are both present during visual mental imagery tasks and finally to study the relationship between the visual imagery patterns and the V1 activation. The flashing light created by a goggle was used for visual perception task. Five visual imagery tasks were designed for this study. Each task involved different view field and spatial resolution of imagined picture and memory. The activation in V1, LGN and pulvinar was detected during visual perception task using fMRI. The results showed a coupled relationship between the LGN and V1 activation. Most visual imagery experiments also showed the activation in V1, LGN, and pulvinar. Inferior pulvinar was more involved in visual perception and lateral and medial pulvinars were more involved in visual imagery. Our results demonstrated that activated location in V1 areas during visual imagery tasks depend on the view size, fine details (or spatial resolution) of imagined pictures and the ability to recall and recreate imagined pictures. It was also demonstrated for the first time that the LGN was activated during visual imagery processing in human brain together with V1 and pulvinar activation. These data suggest that both the LGN and V1 involves in visual perception and visual imagery processing and that other thalamic nuclei such as the pulvinar also contributes to the processing of mental visual imagery.

574.11

LUMINANCE CHANGES ACTIVATE THE LATERAL GENICULATE NUCLEUS IN THE HUMAN BRAIN. B. Gulyás*, J. Larsson and P. E. Roland, Division of Human Brain Research, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

With the purpose of exploring the brain structures activated by changes in luminance levels under Ganzfeld viewing conditions, we measured regional cerebral blood flow (rCBF) changes in ten male volunteers with positron emission tomography (PET) and 15O-butanol, as the tracer, while the subjects performed a visual detection task. During the task the luminance level of the isochromatic Ganzfeld visual scene gradually changed in either direction (brighter or dimmer).The subjects had to press a response key when they detected the change. In the reference condition, no luminance changes were present in the isochromatic visual scene, however, the subjects had to press the response key at the same rhythm as in the experimental condition. Significant changes in rCBF levels were determined with 3-D cluster analysis (Roland et al. Human Brain Mapping 1(1993): 3-19).

During luminance detection, there was activation in the lateral geniculate nucleus (LGN) (HBA [Roland et al., Human Brain Mapping 1(1994):173-184] coordinates: x = 24, y = -25, z = -2 mm), as well as in extrastriate visual cortical regions (lateral occipital gyri, lingual and fusiform gyri), and the temporal and prefrontal cortex.

The present findings are in line with earlier observations on the neuronal role of LGN cells in primates in luminance detection, and demonstrate, for the first time, that activation in the human LGN can be shown with a functional brain imaging technique, in combination with a high performance computerised brain atlas system.

Acknowledgement: Swedish Medical Research Council (B96-14X-11586-01A)

574.12

LOCALIZATION OF GLUTAMATE IN THE RELAY NEURONS OF CAT LATERAL GENICULATE NUCLEUS Qilin Cao, Zhonghao Liu, Dahua Lu, Xuegang Luo* Department of Anatomy and Neurobiolgy,Hunan Medical University, Changsha 410078,P.R. China

The geniculocortical pathway in cat has been studied thoroughly morphologically and physiologically, and is composed of at least three channels(X,Y,W). The transmitters used by the pathway and the variation of the trasmitters used by the different channels however, remained to be elucidated. In present study, the immunogold silver staining(IGSS) technique combined to the retrograde tracing methods, was used in 6 adult cat lateral geniculate nucleus(LGN) to analysize the distribution of Glutamate-containing neurons and to determine the locolization of Glutamate in the relay cells of cat dLGN. The glutamate-immunoreactive(Glu-ir) neurons distributed in all three laminaes (A, A1 and C) of cat dLGN, and can be divided into small, medium and large cells. Laminae C contained only small Glu-ir neurons, while laminae A and A1 had all the three types of Glu-ir neurons. Following the HRP injection into the primary visual area(area 17), the retrograde labeling neurons distributed in laminae A and A1. But not laminae C, consisting with the previous studies. The results of double-labeling study showed most HRP labeling neurons were also Glu-immunoreactive, and the double-staining neurons were composed of a large proportion of medium cells and a smaller proportion of large size cells. The proportion of large double-staining neurons increases from medial to lateral parts of the laminae A and A1. Our result indicates most relay cells of cat dLGN are Glu-immunoreactive, and suggests the Glu are one of the major transmitters in the geniculocortical pathway in cat. Support by CNSF39270269

574.13

IMAGING CALCIUM TRANSIENTS IN RELAY NEURONS IN THALAMIC SLICES REVEALS BOTH LOW- AND HIGH-THRESHOLD CALCIUM CHANNELS IN THE SOMA AND PROXIMAL DENDRITES. Q. Zhou*, D.W. Godwin, D.O'Malley and P.R.Adams, Dept. Neurobio. SUNY Stony Brook, NY 11794.

Thalamic relay neurons respond to sensory inputs in one of two modes, burst or tonic, which partially depend on the underlying Ca^{2+} currents. Because synaptic inputs to relay neurons may segregate upon relay cell dendrites, the location of voltage-gated Ca^{2+} channels may influence the effectiveness of synaptic inputs. Ca^{2+} transients in thalamic relay neurons in brain slices of postnatal (10 to 21 day old) rats were examined using confocal imaging and the fluorescent indicator calcium green dextran. Electrical activity was controlled by whole cell recording. In response to a ramp depolarizing current injection, we observed Ca^{2+} transients in relay cells in two membrane potential ranges: one at relatively hyperpolarized values corresponding to the burst firing mode where both low- and high-threshold Ca^{2+} channels are active, and the other at more depolarized values corresponding to the tonic mode where Ca^{2+} influx through high-threshold Ca^{2+} channels dominates. These Ca^{2+} transients have been observed in the cell body and throughout the part of the dendritic field examined so far (up to 100 µm from the soma, including branches up to the fourth order). At different low firing frequencies (up to 5 Hz for Na^+ action potentials, 3 Hz for LTS), individual Ca^{2+} transients of constant sizes corresponding to single Na^+ action potentials or LTS can be seen throughout the dendritic field. At higher firing rates, e.g., in the range of spindle-wave activity, the signals became larger and merged. The location of the Ca^{2+} currents underlying these Ca^{2+} transients was examined using voltage protocols to selectively activate either low- or high-threshold Ca^{2+} channels. We measured fluorescence changes along the soma and proximal dendrites using rapid line scans and observed larger Ca^{2+} signals in the proximal dendrites. These Ca^{2+} signals rose simultaneously at both sites indicating the presence of both types of channel throughout this portion of the cell. Fluorescence-voltage curves were obtained at different points along the dendrites for high-threshold Ca^{2+} channels, and preliminary results indicate that they are similar to those observed in the soma. This suggests adequate voltage control of dendrites up to 90 µm away from the cell body, in agreement with the proposed electrotonic compactness of relay neurons. Because primary afferents synapse predominantly on the proximal dendrites of the relay neurons, these Ca^{2+} channels are likely to be activated by physiological stimuli. (D.W.G. supported by EY03038)

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XII

575.1

THE ROLE OF THE BASAL GANGLIA IN MEMORY: EVIDENCE FROM PATIENTS WITH PARKINSON'S DISEASE AND HUNTINGTON'S DISEASE. W.J. Lombardi*, K. Marder, E.D. Louis, D. Thorne, L.J. Côté, and Y. Stern. Department of Neurology, Columbia University, New York, NY 10032.

Nondemented patients with Parkinson's disease (PD) and Huntington's disease (HD) have a qualitatively similar memory disorder characterized by a recall deficit accompanied by normal recognition memory. This recall deficit is presumed to reflect the contributions of frontal-subcortical circuits to memory performance. If the structures of the basal ganglia play a unique role within these circuits, it should be possible to demonstrate some differences in memory performance among patients with different types of basal ganglia dysfunction. We provide evidence for such a dissociation between PD and HD patients using the proactive interference paradigm. Subjects studied a target list of word pairs (e.g., VIOLIN-MUSIC), some of which had been preceded by an interfering pair (e.g., VIOLIN-STRING) on a previous list (the interference condition) and some of which had not (the control condition). HD patients and normal subjects recalled fewer interference than control word pairs. PD patients, however, recalled an approximately equal number of pairs from the interference and control conditions. The proactive interference effect appears to occur in subjects with normal basal ganglia function as well as in subjects with an imbalance of basal ganglia pathways favoring those which decrease basal ganglia output (HD). Proactive interference does not appear to occur in subjects with an imbalance of basal ganglia pathways favoring those which increase basal ganglia output (PD). These data suggest that the basal ganglia play an important role in the memory processes which give rise to interference phenomena.

This research was supported by NINDS NRSA F32NS09755 and the Huntington's Disease Society of America.

575.2

CALLOSAL AND CORTICAL CONTRIBUTION TO PROCEDURAL LEARNING. E. de Guise[1], A. Quattrini[2], I. Papo[2], M del Pesce[2], N. Foschi[2] and M. Lassonde[1]*.1-Groupe de Recherche en Neuropsychologie Expérimentale, Univ. de Montréal, Qué., Canada. 2- Centro dell'Epilessia, Ospedale Torrette, Ancona, Italia.

The present study thus examined whether callosotomized (CS) and acallosal subjects (AS) could learn a visuomotor skill that involved a motor control from either both or only one hemisphere. Eleven adult patients, five CS and six AS, were examined in this study. Five of these patients had frontal epileptic foci and three had temporal foci. Furthermore, one of the AS had a bilateral prefrontal atrophy consecutive to surgical removal of an orbito-frontal kyst. The performance of these experimental subjects was compared to that of eleven control subjects, matched for age and I.Q., on a modified version of a serial reaction time task (Nissen and Bullemer, 1987). On each trial, a light appeared at one of four locations. Subjects were instructed to press the one key, out of a set of four keys, that was direcly below the light. For the bimanual (interhemispheric) condition, subjects rested the middle and index fingers of both hands on the four keys. In the unimanual (intrahemispheric) condition, subjects rested the index, middle, third and little fingers of one hand on the four keys. In each condition, the subjects received 16 blocks of 100 trials. Each block comprised 10 repetitions of a 10-trial sequence. None of the experimental subjects learned the task in the bimanual, interhemispheric, condition. Patients with frontal epileptic foci or orbito-frontal damage (n=6) also failed to learn the task in the unimanual condition when they were using the hand controlateral to the damaged hemisphere. All other subjects, including the CS and AS with temporal foci, learned the visuomotor skill as well as their controls in the unimanual condition. These findings indicate that the corpus callosum and the frontal cortical areas are important for procedural learning of a visuomotor skill and question the widespread conception of a predominantly subcortical involvement in procedural learning.

575.3

DISSOCIATING THE ROLES OF THE BASAL GANGLIA AND FRONTAL LOBES IN HABIT LEARNING B.J. Knowlton* L.R. Squire[1] and J. Mangels[2] Dept of Psychology, UCLA, Los Angeles CA 90095, [1]Depts of Psychiatry and Neurosciences, UCSD and VAMC San Diego 92161 and [2]Dept of Psychology, UC Berkeley, CA, 94720.

Patients with Parkinson's disease (PD) and amnesic patients exhibit a double dissociation between performance on a nondeclarative probabilistic classification task and performance on a declarative memory test for the training episode. These results demonstrate the existence of independent systems for declarative memory and gradually formed associations akin to habit learning. The former depends on medial temporal lobe structures while the latter appears to depend on the basal ganglia. However, frontal lobe dysfunction present in PD may have impaired habit learning in these patients. To test this possibility, 10 patients with frontal lobe damage were tested on the probabilistic classification task. They performed as well as control subjects. There was also a significant interaction between the performance of the frontal patients and PD patients across 150 trials, with frontal patients learning more rapidly. Finally, performance was uncorrelated with severity of frontal signs in frontal patients, yet was correlated with PD symptom severity in PD patients. These results suggest that habit learning depends on the basal ganglia and that cognitive deficits in PD and frontal lobe dysfunction are dissociable. Supported by NIMH and Dept. of Veterans Affairs.

575.4

RECOGNITION JUDGMENTS: NO EVIDENCE FOR A CONTRIBUTION FROM IMPLICIT MEMORY. P.J.Reber* and L.R.Squire. Departments of Psychiatry and Neurosciences, UCSD and VA Medical Center, San Diego, 92161.

Nondeclarative (implicit) memory for previously seen stimuli does not depend on the brain structures that support declarative memory. However, questions have been raised about whether the two kinds of memory operate fully independently, and specifically, whether implicit memory can support recognition judgments. Dorfman et al. (1996) reported that patients receiving ECT improved their recognition performance when they were encouraged to relax their criteria for endorsing test items as familiar. It was suggested that implicit memory helped patients make recognition judgments, because they could use information about the familiarity of the test items. We adopted this paradigm for a study of amnesic patients. Subjects studied a list of 72 words and, after a 5 min. delay, took two yes/no recognition tests in sequence. The instructions encouraged either a 'Low' or 'High' criterion for test item endorsement. The Low-criterion recognition test encouraged subjects to say 'yes' if the word was "at all familiar," and the High-criterion test instructed subjects to say 'yes' only when they were relatively certain the word had been presented. In accordance with the instructions, the amnesic patients endorsed more items in the Low-criterion test. However, their recognition performance was identical in the two conditions by percent correct or d' measures. Three control groups tested at different study-test delays (5 min., 1-2 d. or 1 w.) exhibited poorer performance at longer delays but, like the amnesic patients, performed the same on both tests. The findings of Dorfman et al. may be specific to patients tested one hour after ECT. Improved performance in the Low-criterion test may reflect a partial lifting by instructions of a meta-memory deficit (or confusion) present after ECT but not present in stable amnesia. Our results provide no evidence that the intact implicit memory of amnesic patients can contribute to recognition memory performance, consistent with previous reports (Haist, Shimamura & Squire, 1992) These results suggest that implicit memory is encapsulated and separate from from declarative memory. Support: Dept. of Veterans Affairs, NIMH grant MH24600 and NIA grant 5T32AG00216-04.

575.5

INTACT PRIMING FOR NOVEL LETTER STRINGS IN AMNESIA. S.Hamann* and L.R. Squire. VA Med Ctr. and Dept. of Psychiatry and Neurosciences. UCSD, La Jolla, CA 92093. Recent studies have challenged the idea that priming for ostensibly novel stimuli such as pseudowords (REAB) reflects the creation of new representations. Priming for such stimuli could instead reflect the activation of familiar memory representations that are orthographically similar (READ), and/or the activation of familiar subparts of stimuli (component letters). We tested this possibility in 3 experiments that assessed perceptual identification priming and recognition for novel and familiar letter strings in amnesic patients and control subjects. Priming for words (READ), pseudowords (REAB), and orthographically illegal nonwords (KXZL) was fully intact in the amnesic patients following a single exposure, whereas recognition was impaired for the same items. Thus, priming can occur for stimuli that are unlikely to have preexisting representations and that are dissimilar to real words. Words and pseudowords exhibited twice as much priming as illegal nonwords, suggesting that activation may contribute to priming for words and wordlike stimuli. Two additional results suggested that the priming effect for illegal nonwords must result from the formation of new perceptual associations among the component letters of each nonword rather than the activation of individual letter representations. First, robust priming was demonstrated when conditions allowed the formation and use of new perceptual associations but prevented any contribution to priming from activation of component letters (by including the same letters in the primed and baseline lists: study: KXZL; primed: KXZL, baseline: LZXK). Second, no priming occurred when only activation of individual letters could contribute to the priming effect (study: KXZL; primed: LZXK, baseline: DJRQ). In summary, the results demonstrate that priming following a single exposure can depend on the creation of new perceptual representations for novel letter strings. This process does not depend on the brain structures important for declarative memory that are damaged in amnesia. Support: Dept. of Veterans Affairs and NIMH Grant MH24600.

575.6

BEHAVIORAL AND FUNCTIONAL NEUROIMAGING EVIDENCE FOR PRESERVED CONCEPTUAL IMPLICIT MEMORY IN GLOBAL AMNESIA. J.D.E. Gabrieli*, E.V. Sullivan, J.E. Desmond, G.T. Stebbins, C.J. Vaidya, M.M. Keane, A.D. Wagner, M.M. Zarella, G.H. Glover, and A. Pfefferbaum. Depts. of Psychology, Psychiatry, and Radiology, Stanford University, Stanford, CA 94305, Dept. of Neurological Sciences, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612, Memory Disorders Research Center, Boston VA, Boston, MA 02130, and Dept. of Psychology, Wellesley College, MA 02181.

Studies with amnesic patients and normal subjects have provided evidence for functional and anatomic dissociations between the memory systems that mediate performance on explicit memory tasks (recall, recognition) and implicit memory tasks (repetition priming). Functional neuroimaging studies have revealed multiple neocortical memory systems that appear to mediate distinct forms of repetition priming. In our prior functional magnetic resonance imaging (fMRI) studies, subjects judged whether words referred to abstract or concrete concepts. Judgments for repeated relative to initial presentations of words yielded shorter response latencies and reduced activity in left inferior prefrontal cortex (LIPC). The present study tested the hypothesis that the reduced LIPC activity reflects priming of implicit conceptual retrieval processes by examining whether amnesic patients show normal behavioral priming and reduced LIPC activation when performing abstract/concrete judgments on repeated words. In the behavioral study, 14 patients with global amnesia (7 with alcoholic Korsakoff's syndrome, 7 with other etiologies) and 14 control subjects (7 alcoholic, 7 normal) made abstract/concrete judgments on two lists of words, followed by either an implicit memory test (abstract/concrete judgments for repeated and novel words) or an explicit recognition memory test. The amnesic patients showed a normal gain in speed of response to repeated versus novel words despite impaired explicit recognition for repeated words. Three other amnesic patients (two with alcoholic Korsakoff's syndrome and one with closed head injury) were scanned with fMRI while making abstract/concrete judgments on alternating sets of novel and repeated words. Each patient showed a distinct reduction in LIPC activity during conceptual judgments for repeated relative to initial word presentations. The preservation of repetition priming and reduced LIPC activity in amnesia suggests that the reduction in LIPC activity for repeated conceptual judgments reflects implicit retrieval of memory gained during initial conceptual judgments. Supported by grants from the NIA, NIAAA, NIMH, and NINDS.

575.7

DISSOCIABLE CONCEPTUAL PRIMING PROCESSES: EVIDENCE FROM ALZHEIMER'S DISEASE C. J. Vaidya*, J. D. E. Gabrieli, K. L. Lange, and D. A. Fleischman. Dept. of Psychology, Stanford University, Stanford, CA 94305; Dept. of Neurological Sciences, Rush-Presbyterian St. Luke's Medical Center, Chicago, IL 60612.

We report a neuroanatomical dissociation between two forms of conceptual implicit memory. Past studies of patients with Alzheimer's disease (AD) have shown impaired conceptual priming using tests that require generation of responses based upon knowledge of semantic-category relations (category association: e.g., FRUIT-APPLE, name the first FRUIT that comes to mind), and conceptual associations between words (word association: e.g., SALT-PEPPER, SALT - ? say the first word that comes to mind). Studies with normal participants have shown functional dissociations between these two forms of conceptual priming, using manipulations such as divided attention (reduces category- but not word-association priming) and semantic processing. Semantic processing enhances priming for strongly related category-exemplar pairs, but not for strongly associated words. Thus, distinct conceptual processes may underlie priming of strongly related stimuli, in category association and word association tests. We hypothesized that AD patients will show intact priming on word association using strong word-associates, and impaired priming on category association using strong category-exemplar pairs. Indeed, AD patients showed normal word-association priming, but impaired category-association priming. Our findings differ from past studies of word-association priming in AD because those studies included stimuli that were moderately associated and did not distinguish between category-exemplar and other kinds of word associates. Thus, conceptual priming is impaired in AD only to the extent that response-generation depends on knowledge of semantic-category relations. Conceptual priming may be intact in AD to the extent that response-generation depends on unitized representations, such as strongly associated words.
Supported by grants from NIA (AG 11121).

575.8

DISSOCIATION BETWEEN TWO FORMS OF CONCEPTUAL IMPLICIT MEMORY IN ALZHEIMER'S DISEASE: ROLE OF ATTENTION. N. Askari*, C.J. Vaidya, B. Jacobson, S.L. Thompson-Schill, K.L. Lange, D.A. Fleischman, J.D.E. Gabrieli. Dept. of Psychology, Stanford, University, Stanford, CA 94305 and Dept. of Neurological Sciences, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612.

Patients with Alzheimer's Disease (AD) typically show preserved priming on some perceptual implicit memory tasks (e.g., perceptual identification and picture naming) but reduced priming on conceptual implicit memory tasks (e.g., category exemplar generation). In a previous report, however, we found a dissociation between forms of conceptual priming in AD: impaired priming on a test that requires the 'generation' of a response (e.g., category generation: "name 8 types of VEGETABLES") but intact priming on a test that requires the 'identification' of an item (e.g., category verification: "Is this a type of vegetable?, LETTUCE). In the present report, this dissociation was replicated with a larger sample of AD patients (AD, N=36; NC, N=36). We hypothesized that AD patients may be impaired on conceptual priming tests that are more demanding of attentional capacity (e.g., category generation), but intact on tests that are less demanding of attentional capacity (e.g., category verification). To test this hypothesis, college students performed these two tasks under divided or focused attention conditions. Dividing attention reduced priming on category generation but not on category verification, thereby paralleling the dissociation found in AD patients. Thus, it appears that the conceptual priming deficits seen in AD patients may be attributable, at least in part, to reduced attentional capacity in AD.
Supported by NIA.

575.9

NEUROPHYSIOLOGICAL (ERP) CORRELATES OF ENCODING AND RETRIEVAL FROM VERBAL EPISODIC MEMORY. J. A. Mangels*, T. W. Picton, and F. I. M. Craik. Rotman Research Institute, Baycrest Centre for Geriatric Care, North York, Ontario, Canada M6A 2E1.

The frontal lobes play an important role in strategic, attentionally controlled processes related to the encoding and retrieval of episodic memories. Recent neuroimaging studies (PET) indicate this role is functionally lateralized. Episodic encoding differentially activates left prefrontal cortex, whereas episodic retrieval differentially activates right prefrontal cortex.

To assess the time course of neural activity during encoding and retrieval, we recorded event-related potentials (ERPs) from 46 electrodes as subjects studied and recognized words. During encoding, subjects studied words under full attention or while performing a secondary task (divided attention). The secondary task required subjects to make motor responses to tones presented in either a predictable pattern ("easy") or a random sequence ("difficult"). During retrieval, subjects classified items depending on whether recognition was accompanied by conscious recollection of the study-episode ("remember") or whether recognition was based only on the familiarity of the stimulus ("know"). In all encoding and retrieval conditions, the N180 observed over occipito-temporal sites was larger over the left hemisphere and may reflect processes associated with the automatic identification of verbal stimuli. From 400 to 2400 msec, a sustained positive wave emerged maximally over prefrontal sites. This frontal positivity was lateralized to the left hemisphere at encoding and right hemisphere at retrieval and was modulated by attention at encoding and by successful recognition at retrieval. Specifically, frontal positivity was evident in the full attention and "easy" divided-attention encoding conditions, but was not apparent in the "difficult" divided-attention condition. Frontal positivity also was associated with correctly recognized words, but not with new words. "Remember" responses were further differentiated by a centro-parietal positive wave (P650) that was larger than that observed for "know" or "new" responses.

This research was supported by the Rotman Research Institute and Medical Research Council of Canada.

575.10

DISSOCIATION OF MEDIAL TEMPORAL LOBE STRUCTURES INVOLVED IN ENCODING AND RETRIEVAL: AN FMRI STUDY. J. B. Brewer*, J. E. Desmond, G. H. Glover, and J. D. E. Gabrieli. Neuroscience Program, Depts. of Psychology and Radiology, Stanford University, Stanford, CA 94305.

The medial temporal region is essential for the formation of declarative memories in humans and other primates. Animal studies have provided evidence that different components of the medial temporal region make distinct contributions to declarative memory performance. The present study used functional magnetic resonance imaging (fMRI) to examine whether different components of the medial temporal region make distinct contributions to human memory. Subjects performed retrieval and encoding memory tasks. In the retrieval task, subjects discriminated between visually presented words that corresponded to studied ("OLD") and unstudied ("NEW") line drawings. Functional data were acquired at 1.5 T using a gradient echo spiral sequence sensitive to T2* (TR/slice=90; total TR=720; TE=40; flip angle=65°). Eight alternating blocks of 20 words were used. Half consisted of 18 OLD and 2 NEW items where subjects responded to OLD items, and half consisted of 18 NEW and 2 OLD items where subjects responded to NEW items. Functional data analysis using the cross-correlation method described by Friston et al. [Human Brain Mapping, 1994] revealed in 5 subjects increased activation in the subiculum region of the hippocampus when subjects were responding to OLD relative to NEW items. In the encoding task, subjects saw 50 color photographs, 25 indoor and 25 outdoor, arranged in12 alternating blocks. Blocks of 8 novel pictures were alternated with blocks of 2 pseudorandomly repeating familiar pictures. Subjects were instructed to respond to indoor photographs. In 5 subjects, there was greater activation in the parahippocampal gyrus during viewing of novel versus repeated pictures. These results demonstrate the potential of fMRI to resolve activity in the closely spaced structures of the hippocampal region and to shed light upon their distinct contributions to memory formation.

This study supported by NIH GM07365 and by NIA.

575.11

LEFT MEDIAL TEMPORAL ACTIVATION DURING VERBAL EPISODIC MEMORY CONSOLIDATION: A FUNCTIONAL MRI STUDY. J. R. Binder*, P. S. Bellgowan, J. A. Frost, T. A. Hammeke, J. A. Springer, S. M. Rao, and R. W. Cox. Dept. of Neurology, Medical College of Wisconsin, Milwaukee, WI 53226.

Functional imaging studies of declarative memory have only rarely demonstrated activation of the medial temporal lobe. We hypothesize that this is because these memory systems are "on" under most circumstances, making it difficult to elicit activity contrasts. This idea was tested by contrasting activation during an episodic verbal encoding task with either a "rest" state or a perceptual "distractor" task that lacked distinct, verbally encodable episodes. Whole-brain, echoplanar functional magnetic resonance imaging was performed at 1.5T in 30 normal subjects. In the **semantic decision** task, subjects heard spoken nouns and pressed a button if a word met specified semantic criteria [Binder et al., Arch. Neurol. 1995]. This was compared to either a **"rest"** state or to a **tone decision** task in which subjects heard tone sequences and responded to specified target sequences. Individual maps of the difference between conditions in each voxel, represented as a *t* statistic, were resampled in stereotaxic space and spatially averaged across subjects. Averaged *t* values with a voxel-wise probability of $p<.00001$ were considered significant.

Activation occurred in the left inferomedial temporal lobe during the semantic decision task in comparison to the tone decision task. This region included the middle portion of the fusiform gyrus, adjacent collateral sulcus, anterior and middle portions of the parahippocampus, and anterior hippocampus up to but not including the amygdala. No significant activation occurred in the right inferomedial temporal lobe. There was also activation in the left retrosplenial cortex. No significant activation was observed in these areas when the semantic decision task was compared to "rest."

These results suggest that the left medial temporal memory system is relatively active during semantic-level processing of verbally encodable episodes, and that this state description also applies to the condition called "rest." This left medial temporal area is less active during perceptual-level processing of non-verbally encoded material. (Supported by NIH NS33576.)

575.12

MEDIAL TEMPORAL PATHOLOGY AND GLOBAL ANTEROGRADE AMNESIA DURING CHILDHOOD. F. Vargha-Khadem[1], A. Connelly[2], K.E. Watkins[1], W. Van Paesschen[2], M.Mishkin[3] and D.G. Gadian[2]. [1]Neurosciences and [2]Radiology and Physics Units, Institute of Child Health, London WC1N 1EH, UK and [3]NIMH, NIH, Bethesda MD 20892 USA. (SPON: European Brain and Behaviour Society)

Combined magnetic resonance (MR) and neuropsychological findings are presented for three adolescents with profound amnesia due to medial temporal damage sustained in one case at age 9, in a second at age 4, and in the third at birth. With the advent of quantitative MR techniques, such cases provide an unusual opportunity to determine not only which specific medial temporal-lobe structures are critical for memory functions but also when they become critical. MR imaging and spectroscopy were carried out on a 1.5T clinical imaging system. The protocol included acquisition of 3-D data sets for volumetric analysis of the hippocampi, T2 maps through the hippocampi, and ^{1}H spectra of 2x2x2 cm cubes centred on the medial regions of the temporal lobes. Neuropsychological evaluations showed normal immediate memory but impaired long-term memory for both verbal and nonverbal material in all three cases. The amnesic syndrome was as pronounced in the perinatal case as in the others. All three cases had bilaterally elevated hippocampal T2 values and bilaterally small hippocampal volumes. In contrast, magnetic resonance spectra were normal or displayed only mild unilateral abnormalities, suggesting that bilaterally symmetrical pathology was largely confined to the hippocampal formation. The results not only confirm the importance of this structure for long-term memory but also suggest that, even when reorganizational capacity of the brain is at its peak, no other structure can compensate for damage to this one.

SUPPORTED BY THE WELLCOME TRUST

CORTEX: HUMAN STUDIES III

576.1

A PET STUDY OF SIMPLE FINGER MOVEMENTS INSTRUCTED BY AUDITORY, SOMATOSENSORY, VISUAL AND INTERNAL CUES. RA Weeks*, M-J Catalan, M Honda and M Hallett. Human Motor Control Section, NINDS, NIH, Bethesda, Maryland, USA.

Externally guided movements have been shown to activate premotor areas. We sought to determine whether the pattern of cerebral activation is dependent upon the modality of sensory instruction during choice reaction finger movements with visual, auditory and somatosensory instructions and comparisons with internally generated movements.

We measured rCBF in 16 healthy volunteers after bolus intravenous injection of $H_2{}^{15}0$. 4 movement and 4 control scans were performed in each subject. Data was analysed using subtraction techniques and statistical parametric mapping. Both within condition and between condition comparisons were made at Omnibus significance levels of $p<0.001$.

Within condition analysis revealed that all movements activated the contralateral sensorimotor cortex, premotor, supplementary motor and ipsilateral cerebellar areas. Between condition analysis showed there were no statistical differences in the cortical motor areas activated by the three externally instructed conditions. Internally generated movements were associated with increased activation in the contralateral anterior SMA and bilateral parietal areas when compared to the external tasks. The somatosensory task showed increased activation in the ipsilateral thalamus when compared to all other movements and may be related to attentional mechanisms.

Internally generated movements showed increased contralateral anterior SMA activity, while externally instructed finger movements regardless of modality of instruction are associated with similar patterns of cortical motor activation.

Supported by NIH

576.2

GAZE DIRECTION MODIFIES HUMAN MOTOR CORTICAL HAND REPRESENTATIONS. J. N. Sanes*, J. P. Donoghue, B. Siewert, R. R. Edelman, S. Warach, Dept. Neuroscience, Brown University, Providence, RI 02912, Depts. Neurology and Radiology, Beth Israel Hospital, Harvard Medical School, Boston, MA 02115.

Direction of gaze influences neuronal discharge in primate parietal and frontal cortical areas involved in controlling arm movements suggesting that neuronal registration of gaze with limb motor output occurs broadly across cerebral cortex. The current work investigated whether gaze angle affected activation patterns in human cortex during performance of sequential finger movements. With the BOLD EPI method (Siemens 1.5 T MR system), we obtained functional MR images (TR = 2 s) while subjects alternated between 30 s periods of repetitive finger movements using the right dominant hand and no finger movement. Subjects lay supine in the MR system with their right arm positioned next to the right side. Gaze was actively maintained leftward, centrally or rightward. Regions activated by finger movements were determined using cross-correlation statistics. Gaze angle modified activation patterns in contralateral primary motor cortex, lateral and medial premotor cortex, and parietal areas. In each cortical region, the least activation occurred when subjects directed gaze leftward, with significant increases in the number of activated voxels when subjects looked centrally or rightward. Activation greater than that obtained during leftward gaze was nearly equivalent during central and rightward gaze for some subjects, while it was greater for others during central or rightward gaze. These data demonstrate that gaze can immediately shift brain activation patterns occurring in multiple motor cortical areas during performance of hand movement and suggest that primary and non-primary motor cortical areas are influenced by gaze signals. The results provide further support for dynamic interactions between gaze signals and neural mechanisms encoding somatic motor mappings. Support: NIH AG10634, NS01634, NS25074; McDonnell-Pew Program in Cognitive Neuroscience; and Harcourt General Charitable Trust.

576.3

FUNCTIONAL NEUROANATOMY ASSOCIATED WITH PERFORMANCE OF HETEROGENEOUS FINGER MOVEMENT SEQUENCES. S. M. Rao*, K. Y. Haaland, D. L. Harrington, A. C. Rosen, J. A. Bobholz, S. J. Woodley, T. A. Hammeke, S. A. Fuller, J. M. Cunningham, J. R. Binder and R. W. Cox. Department of Neurology, Medical College of Wisconsin, Milwaukee, WI 53226.

Cognitive-motor theory contends that voluntary, sequential movements are governed by motor programs thought to be hierarchical in nature. The duration of motor programming prior to movement is affected by many characteristics of the action, including sequence complexity. In this whole-brain echoplanar 1.5T FMRI experiment, we systematically manipulated sequence complexity to better understand the functional neuroanatomy underlying planning and controlling of sequential events. Ten right-handed volunteers performed right finger keypresses in response to 5-digit sequences (1=index, 2=middle, 3=ring) presented visually. One condition involved repetitive sequences (11111, 22222, or 33333) and the remaining seven conditions used heterogeneous sequences varying systematically in the number of transitions (2-4) and postures (2-3). Functional images were generated using a cross-correlation technique, blurred (4 mm FWHM), co-registered with high resolution anatomic images, and converted to stereotaxic space. Paired t-tests (t [9]=3.7, $p < 0.005$) were used to subtract the functional images derived from the repetitive sequence condition from those generated from each of the 7 heterogeneous sequence conditions on a pixel-by-pixel basis.

Mean reaction time to the first keypress and the total activated brain area (heterogeneous minus repetitive conditions) increased significantly as a function of sequence complexity. This increased activity was observed primarily in the posterior parietal, extrastriate, and lateral premotor areas bilaterally, and in the left lateral cerebellum (contralateral to the side of movement). We conclude that these brain regions are critical in the translation of an external visuospatial stimulus into heterogeneous movement sequences. Our findings are in contrast to previous functional imaging studies demonstrating increased activation within the supplementary motor area (SMA) during the performance of memorized finger sequences. These results suggest that the SMA is activated only during the performance of internally generated sequences held in working memory. (Supported by NIH P01-MH51358.)

576.4

THE ROLE OF THE RIGHT PREMOTOR CORTEX AND SMA IN THE COORDINATION OF BIMANUAL MOVEMENTS. N. Sadato*, Y. Yonekura, A. Waki, H. Yamada and Y. Ishii. Biomedical Imaging Research Center, Fukui Medical School, Fukui, Japan

Previous electrophysiological study showed that SMA is required for the synchrony of bimanual movements and for the bimanual coordination of parallel movements (Pascual-Leone, et al. Neurology 44:A329, 1994). To obtain better understanding of cortical representation of the bimanual coordination, we measured regional cerebral blood flow with O-15 water and PET. We studied 12 right handed normal volunteers. A complete session consisted of 3 rest scans, 6 scans with acoustically paced (1 Hz), bimanual sequential movements , mirror or parallel version of the sequence. To determine if the cortical representation of bimanual coordination is sequence dependent, other 9 volunteers were recruited. Each subject performed acoustically paced abduction-adduction movement of the index finger: right only, left only, mirror and parallel version were performed twice. The right dorsal premotor area extending to the right anterior SMA showed significantly larger activation during parallel sequential movement compared to the mirror sequential movement ($p < 0.05$ with correction for multiple comparisons). With lesser degree, the same areas were activated by parallel abduction-adduction movement significantly greater than by the mirror version. The right dorsal premotor area and SMA are related to the bimanual coordination of parallel movements.

Study supported by grant-in-aid for scientific research, from Japan Ministry of Education, Science and Culture.

576.5

MULTI-MODALITY CEREBRAL ACTIVATION IN MOTOR FUNCTION BY PET AND MEG. J.R.Pedersen[1], P.Johannsen[2], K.Særmark[1], and A.Gjedde*[2]. Biomagnetic Group, Physics Dept, The Technical University of Denmark, Lyngby,[1], PET Centre, Aarhus University Hospitals, Aarhus, Denmark.[2]

We report the use of positron emission tomography (PET) to fix the location of magnetoencephalography (MEG) locations of cerebral activity. MEG is the direct measurement of magnetic fields created by electrical activity of populations of neurons. It has excellent temporal resolution but uncertain spatial resolution. To map cerebral activation in voluntary movement of the right index finger, we applied PET to evaluate the location and MEG to evaluate the temporal sequence. MEG signals were recorded in 35 channel positions, using a 7-channel, second order gradiometer SQUID-system. Data was collected from 1s before to 2s after the movement. The "inverse problem" was solved using the MUSIC-algorithm. The PET data were co-registered to the individual MRI and the Talairach co-ordinate system. Voluntary movements followed rest in three scans each. A t-statistic [2] was applied to the subtracted PET images (FWHM = 18mm). For $t>4.30$, the change in cerebral blood flow was significant at $p<0.05$. PET showed significant activation in SMA, pre-motor and motor cortex. The MEG sources were placed within 1 cm of the PET positions. A non-significant PET peak in the left middle frontal gyrus, ($x=-31, y=46, z=21$; $t=3.16$) coincided with MEG and was active 800-200 ms before the movement. The study points to a center in the left middle frontal gyrus, involved in the early planning of voluntary finger movements (MRC of Denmark).

576.6

EQUIVALENT ELECTRICAL DIPOLE MODELING OF HUMAN MOVEMENT-RELATED CORTICAL POTENTIALS FOR FINGER, FOOT AND MOUTH MOVEMENTS. Ina M. Tarkka*[1], Garrett W. Milliken[2] and Dobrivoje S. Stokić[2]. [1]Department of Neurosurgery, University of Texas-Houston Medical School, and [2]Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030.

Self-paced voluntary movements are reflected in scalp-recorded movement-related cortical potentials (MRCP). In an attempt to delineate which of the MRCP components reflect cortical somatotopic organization, noninvasive equivalent electrical dipole modeling of MRCP data derived from movements of the right index finger, right foot, and mouth was performed. Generator locations for MRCPs were identified in each of the 8 healthy volunteers. EEG was recorded with 30 Ag-AgCl electrodes placed on the scalp (bandwidth DC-50 Hz). MRCPs were created by averaging according to the onset of the rectified surface EMG of the active muscle (400 ms pre- and 300 ms post-onset). Individual wave forms consisted of 60-80 sweeps. Models for both grand average and individual data were developed for each movement type using Brain Electrical Source Analysis software with a 4-shell spherical head model and individual head radii. The spatio-temporal models covered the activation profile of different brain regions throughout the 700 ms window. MRCP of the index finger movement was sufficiently explained by 8-dipole model, unilateral foot movement by 5-dipole model and pucker of the mouth by 7-dipole model. Residual variances of all the models were below 6%. Superimposition of dipole coordinates on a stereotaxic atlas identified active regions in the cerebellar cortex, thalamus and supplementary motor area. These general regions were common for the three movement types but the primary sensory and primary motor cortical areas, active for about 300 ms, showed distinct somatotopic organization. This work was supported in part by the Vivian L. Smith Foundation for Restorative Neurology, Houston, and in part by NCMRR (NIH) grant #T32 HD 07465 (GWM).

576.7

FRONTAL LOBE INVOLVEMENT IN THE SPONTANEOUS EMERGENCE OF ANTICIPATORY VISUOMOTOR BEHAVIOR, S.L. Bressler*, G.V. Wallenstein, J.A.S. Kelso. Program in Complex Systems & Brain Sciences, Florida Atlantic University, Boca Raton, FL 33431.

The dynamics of a visuomotor coordination task show that simple reactive behavior spontaneously shifts to synchronized behavior as the rate of stimulus presentation is increased, even though the inter-stimulus interval remains sufficient to allow reactive behavior (Engstrom, Kelso & Holroyd, in press). Behavior is attracted to a synchronized state as the perception of a rhythmic pattern in the stimulus train allows anticipation of each succeeding stimulus.

Ten subjects performed sixty runs of the task, flexing the right index finger in coordination with an LED stimulus. In each run, ten stimuli (called a plateau) were presented at rates stepped from 0.167 to 2.0 Hz. Behavior was strictly reactive at the lowest rates (mean RT=298±194 ms; minimum=78 ms), but at 1.25 Hz and above became quasi-synchronized, showing a mixture of anticipatory and reactive flexions.

To monitor the topography of brain electrical activity as synchronized behavior emerged, EEGs were recorded at 61 scalp sites. Fourier analysis revealed maximal spectral power at the frequency corresponding to the rate of stimulus presentation and movement in each plateau. Maps of the topographic distribution of spectral power at this frequency were plotted for each plateau. Separate maps were made for reactive and anticipatory types of behavior for plateaus with both types.

During reactive behavior, the maxima of significant power spectral density occurred at left central and antero-central sites (contralateral to motor output). During anticipatory behavior, additional maxima occurred at frontal sites, the power being significantly greater than during reactive behavior in the same plateaus. Selective frontal involvement in anticipatory behavior supports a role for frontal cortex in the generation of movement based on expected events, particularly when they induce an internalized temporal pattern. Supported by NIMH Grants MH42900 & MH19116.

576.8

COHERENT BILATERAL ACTIVITY OF THE HUMAN PREMOTOR AND SENSORIMOTOR CORTEX DURING INTERNALLY- AND EXTERNALLY-CUED FINGER MOVEMENTS C. Gerloff*, J. Hadley, J.M. DeLeo, J. Classen, C. Toro and M. Hallett Human Motor Control Section, Medical Neurology Branch, NINDS, National Institutes of Health, Bethesda, MD 20892, U.S.A.

EEG coherence analysis can be used to assess functional cooperativity of different cortical areas. We investigated EEG coherence patterns during metronome- and self-paced finger movements in 8 normal subjects.

EEG was recorded during 4 task conditions from 28 scalp electrodes. The 4 conditions were continuous metronome-paced finger extensions at 2 Hz (right hand), self-paced movements at the same rate (right hand), listening to the metronome without moving, and neither metronome sound nor movement. For coherence analysis, the EEG was segmented into epochs of 2048 ms, and the normalized cross-power-spectra were computed for each condition, all electrode pairs, and different frequency bands (alpha:8-12.9, beta1:13-20.9, beta2:21-30.9, gamma:31-50 Hz). Task-related coherence was obtained by subtraction of task and respective rest conditions. Multiple t-tests and Bonferroni correction were used for significance testing.

For all frequency bands, task-related increases of coherent EEG activity were maximal in electrode pairs overlying the left and right lateral and the mesial centroparietal cortex. For these regions, coherence was generally higher during internally- than during externally-cued movements. Maximum differences were found in the beta bands in electrode pairs C3-C4, C3-CP4, C3-CPz, C3-Pz, Fz-TP7, FCz-TP7, FCz-CPz, FCz-Pz, FCz-C4, FCz-CP4, Fz-P4, Fz-C4, C4-FC4 ($p<0.05$).

These results indicate (1) a highly bilateral, synchronized cortical processing for unilateral finger movements, and (2) a higher demand for functional cooperativity between lateral and mesial premotor and sensorimotor areas with internal pacing.

576.9

POSTERIOR CORTICAL AREAS MEDIATE BIMANUAL COORDINATION OF VISUALLY INSTRUCTED MOVEMENTS: PRE- AND POST-OPERATIVE STUDY OF A SPLIT-BRAIN SUBJECT. J.C. Eliassen*†, K. Baynes† and M.S. Gazzaniga§
†Center for Neuroscience, University of California, Davis
§Department of Psychology, Dartmouth College, Hanover NH

Callosotomy patients provide a unique population for the study of two-handed coordination. The corpus callosum allows for the smooth coordination of two handed movements. In its absence subjects coordinate the two arms poorly via subcortical pathways or by using visual guidance. We followed a patient through two stage callosotomy testing her on two handed line-drawing tasks. Before surgery she exhibited spatial coupling, the tendency of one arm to adopt the other's movement characteristics when executing dissimilar trajectories. Spatial coupling persisted following anterior callosotomy but significantly diminished after posterior section.

Additionally, before surgery RT's and movement times (MT's) for bimanual movements were greater when the subject made two different movements than when making identical movements. The RT and MT differences persisted after anterior callosotomy but vanished following posterior callosotomy.

This suggests that the posterior callosum mediates the integration of spatial information for bimanual movements during both the planning and execution stages of visually instructed bimanual arm movements.

Supported by NIH/NIDCD grant R29 DC00811 to K.B. and NIH/NINDS grant PO1 NS 17778-14 and the McDonnell-Pew Foundation to M.S.G.

576.10

USE-DEPENDENT MODULATION OF MOVEMENT REPRESENTATION IN THE HUMAN MOTOR CORTEX. J Classen*, J Liepert, M Hallett, and LG Cohen. Human Cortical Physiology Unit, Human Motor Control Section, NINDS, NIH, Bethesda, U.S.A.)

In animals, movement representations in the primary motor cortex can be reshaped by various manipulations. In the present study we investigated whether movement representations in the human motor cortex can be modulated by repetition of simple motor actions. In nine right-handed healthy volunteers (Ss) focal transcranial magnetic stimulation (TMS) over the motor cortex induced isolated, consistent, and reproducible movements of the thumb at a stimulation intensity of 5 % above relaxed motor threshold. The coil and head position were held stable by an aluminium frame. Thumb movements were recorded with miniature accelerometers fixed to the proximal phalanx, and quantified using the first-peak acceleration (FPA) in the axes of the principal movement plane. A baseline movement vector was calculated by averaging FPAs obtained over 10 min of TMS at 0.1 Hz. Training consisted of brisk repetitive thumb movements (1 Hz for 30 min) in a direction opposite to that induced by TMS. Posttraining, TMS (0.1 Hz for 40 min) induced movements with a direction opposite to pretraining movements and similar to training movements. FPA vectors were calculated and their angular deviations from the baseline FPA-vector determined. FPA vectors changed in the direction of training ($P<0.001$) and returned to near baseline value after about 20 min.

This study provides direct physiological evidence of plasticity of movement representation in the human motor cortex.

This study was supported by DFG grant Cl 95/2-1

576.11

DIRECTIONAL MODULATION OF MOTOR CORTEX PLASTICITY INDUCED BY SYNCHRONICITY OF MOTOR OUTPUTS IN HUMANS. Leonardo G. Cohen*, Christian Gerloff, Lala Faiz, Norimichi Uenishi, Joseph Classen, Joachim Liepert and Mark Hallett. Human Cortical Physiology Unit, Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892.

Synchronous movements (SM) of hand and shoulder can trigger motor plasticity (Soc. Neurosci. Abstr. 21:517). The purpose of this study was to determine if plasticity of a motor representation is directionally modulated by the interaction with other body parts involved in a synchronous task. We studied plastic changes associated with (a) SM of hand and shoulder, (b) SM of hand and mouth, (c) unskilled and (d) skilled asynchronous movements of hand and shoulder, and (e) of hand alone.

Five groups of 8-11 subjects each participated in this study. Abductor pollicis brevis (APB) was mapped twice a day using transcranial magnetic stimulation before and after training in one of the 5 tasks. In each mapping session, we determined motor thresholds, maps, and centers of gravity (COG).

Training resulted in improved performance in tasks a, b, and d. Motor thresholds did not change in any condition. Motor maps were larger in tasks involving skill acquisition (a, b, d), but not in those involving unskilled movements (c, e). APB COG moved laterally with hand-mouth training (b) and medially with hand-shoulder training (a) but remained unchanged in other conditions. We conclude that training can induce directionally selective plasticity in the adult human motor cortex and that synchronous engagement of different body parts is a powerful trigger for this type of plasticity.

576.12

REORGANIZATION OF DOMINANT HAND MOTOR FUNCTION ASSOCIATED WITH ARTERIOVENOUS MALFORMATION-A FUNCTIONAL MRI STUDY J. R. Meyer *, D.R. Gitelman, J Schweitzer, C. Callahan, T. Parrish, H. Batjer, E.J. Russell, M-M Mesulam, Northwestern University Medical School, and Northwestern Memorial Hospital, Chicago Illinois

Brain lesions early in development lead to functional reorganization as an expression of plasticity, but the evidence for this in the human brain has so far been mostly indirect. We present an fMRI study consistent with reorganization of motor function in a 20 y. o. right-handed male with a left hemispheric precentral AVM that appears to involve the motor area for the hand.

fMRI of motor function was performed using a 1.5T Siemens Vision MR scanner with 25mT/m gradients. Multi-slice, single shot, blipped-EPI was performed centered on the motor cortex,parallel to the ac-pc line while the subject performed a finger tapping task with each hand. Analysis of fMRI data was performed using SPM-95 software for correction of motion and statistical analysis, and AVS software was used for image overlay, image segmentation, scalp editing and volume rendering. Time series analysis with rest vs. active tapping was performed. Functional data was overlayed on high resolution volume rendered images.

With movement of the left hand, localized activation was seen in the contralateral sensory-motor cortex. Movement of the right hand demonstrated localized activation in the ipsilateral sensory-motor cortex, as well activation medially in area 6 bilaterally. Right hand movement did not lead to significant activation in the contralateral motor cortex (i.e., the region of the AVM) but some activation was seen in the sensory cortex lateral to the AVM.

These observations are consistent with a reorganization of dominant (right) hand function within the left and right hemispheres. The potential advantage of fMRI in studying reorganization is that it gives a much more comprehensive picture of the full anatomical re-mapping of the target function. Correlation with intra-operative corticography is still necessary in this patient to exclude the possibility of movement-induced activation of left motor cortex that may have been obscured by fast blood flow within an AVM.

576.13

MOTOR CORTEX MAY DRIVE THE MOTONEURON POOL DURING SUSTAINED CONTRACTION S. Salenius*, K. Portin, R. Salmelin, M. Kajola, R. Hari. Brain Research Unit, Low Temperature Laboratory, Helsinki University of Technology, FIN-02150 Espoo, Finland.

We recorded cortical rhythms with a Neuromag-122™ whole-scalp neuromagnetometer, during weak sustained contractions of the right and left first interosseus muscles from 8 healthy right-handed subjects.

We found coherence between cortical and motor unit rhythmicity in all subjects at frequencies ranging from 14 to 33 Hz. Sources coherent with EMG were identified in contralateral motor cortices. Averaging with respect to EMG bursts also revealed clear responses in the motor cortex. The strongest MEG responses preceded the peak of the EMG burst by 10 and 30 ms. Cooling the skin over the interosseus muscle to about 15 °C prior to contraction increased the time-lag between MEG and EMG by 7–10 ms.

These results implicate that motor unit activity is modulated by cortical activity at frequencies of 14–33 Hz and that the motor cortex may drive the spinal motoneuron pool during sustained contractions.

Supported by the Academy of Finland and the Gyllenberg Foundation.

577.1

INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-5 EXPRESSION IS INCREASED WITH SCHWANN CELL DIFFERENTIATION. H-L. Cheng* and E.L. Feldman. Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, MI 48109.

The IGF family of ligands, receptors and binding proteins are essential for normal nervous system development and instrumental in nervous system regeneration. We have reported that Schwann cells (SCs) express insulin-like growth factor binding protein-5 (IGFBP-5). IGFBP-5 is enhanced during differentiation in multiple cell types and may play a role in the maintenance of a differentiated phenotype. In the presence of cAMP analogues, we have observed morphological differentiation of SCs and expression of myelin proteins. In the current study, we examined the levels of IGFBP-5 produced by SCs upon differentiation with cAMP. SCs were incubated in serum free media in the presence or absence of 1 mM 8-bromo cAMP and/or 10 nM IGF-I. Conditioned media were collected, concentrated, and proteins were separated by SDS-PAGE followed by IGFBP-5 immunoblotting. As in our previous experiments, we observed that secreted IGFBP-5 was degraded in the absence but protected in the presence of IGF-I. 1 mM 8-bromo cAMP significantly enhanced the expression of IGFBP-5 in the presence of 10 nM IGF-I. Forskolin and dibutyryl cAMP had similar effects to 8-bromo cAMP and enhanced IGFBP-5 expression in SC conditioned media in the presence of IGF-I.

To determine if the effects of cAMP on IGFBP-5 protein expression was secondary to enhanced IGFBP-5 gene expression, Northern analysis was performed using RNA isolated from SCs cultured under the same conditions as the immunoblot experiments. IGF-I had no effect on IGFBP-5 gene expression, however, addition of cAMP increased IGFBP-5 gene expression 5 fold. In summary, we believe terminal SC differentiation is augmented by the ability of SCs to secrete IGFBP-5, which, in turn, may bind SC basement membrane and increase local IGF-I bioavailability.

Supported by R29 NS32843 and grants from the American Diabetes Association and the Juvenile Diabetes Foundation International.

577.2

TAU PROTEIN HAS A KEY ROLE IN EXTENSION FORMATION OF CULTURED OLIGODENDROCYTES. P. LoPresti* and G. Dawson Dept. Pediatrics, University of Chicago, Chicago, IL 60637.

The Microtubule-Associated Protein (MAP) tau was found in cultured oligodendrocytes (OLGs) from adult brain (LoPresti et al, Proc Natl Acad Sci USA 92,10369-10373; 1995). In these cells, tau and its mRNA were present in OLG soma, processes and tips. In addition, Western blot analysis detected as many as 8 tau isoforms. In this study we address the question of the role of tau in neonatal oligodendrocytes. By Western blot analysis, we have examined the electrophoretic pattern of tau both in CG4 cells and in neonatal rat OLGs. In both CG4 cell line and primary OLG cultures, we detected a similar electrophoretic pattern of tau. We found that 3 tau isoforms were present in both undifferentiated CG4 cells and freshly isolated OLGs, however tau protein levels increase both in differentiated CG4cells and in one-week old cultured neonatal OLGs. By using tau antisense oligonucleotides (50uM) in neonatal OLGs, we founded almost complete inhibition of extension formation. As a control, sense-treated or untreated OLGs show the same extent in the length of processes. Western blot analysis of tau antisense treated OLG cultures showed absence of any detectable levels of tau protein.

The above findings suggest that a) tau protein is upregulated during active process formation during myelination and that b) suppression of tau protein will prevent extension in OLGs. (This work was supported by Children's Research Foundation Grant to P.L. and HD-06426 to G.D.).

577.3

ANTIOXIDANTS SUPPORT MYELINATION OF DRG AXONS IN FULLY DEFINED MEDIA *IN VITRO*. A. J. Windebank* and J. L. Podratz. Molecular Neuroscience Program, Mayo Clinic, Rochester, MN 55905 USA.

Dorsal root ganglion neurons myelinate when cultured in medium containing serum and ascorbic acid. Ascorbic acid plays a key role in the development of basement membrane and myelination does not occur without it. B27 is a defined serum substitute that contains antioxidants such as catalase and α-tolcopherol (vitamin E) but does not contain ascorbic acid. Our studies looked at effects of B27, catalase and vitamin E on myelination. Catalase and vitamin E were tested individually in both N2 serum-free and serum-containing media. DRG neurons were cultured for 4 weeks in collagen-coated ACLAR plastic dishes and myelin was stained with Sudan black. Myelination was observed in cultures containing B27 without the addition of ascorbic acid. Vitamin E induced myelination in medium-containing serum but not in N2 serum-free medium. Catalase did not induce myelination with or without serum. These results indicate that vitamin E plays a major role in the ability of B27 to induce myelination. This is the first demonstration of myelination in fully defined media *in vitro* and confirms the critical importance of antioxidants in myelination (NIH, NS 14304).

577.4

THE EXPRESSION, REGULATION, AND FUNCTIONS OF HIPPOCAMPAL SPECIFIC METALLOTHIONEIN ISOFORM. M. Baxi, M. Hiramatsu, I. Ahmad, H. El Refaey, G. Helal, F.M. Hamada, R. Hao, R.F. Pfeiffer, P. Rojas, D.R. Cerutis, and M. Ebadi. Depts. of Pharmacology, Cell Biology and Anatomy, and Neurology Univ. of Neb. Coll. of Med., Omaha, NE 68198-6260, Inst. for Life Support Techn., Yamagata, Japan; and Dept. of Neurology, Univ. of Tennessee, Memphis, TN 38163

Metallothionein (MT), first described in the brain in our laboratory (M. Itoh, M. Ebadi, and S. Swanson, J. Neurochem. 41:823-829, 1983), is now known to consist of four similar but distinct isoforms, designated as MT I - MT IV. MTs I and II are present in glial and ependymal cells, and MT III which is found in high concentration in the hippocampus is found mostly, but not solely, in neurons. The zinc containing neurons in the brain are a subclass of the glutamatergic neurons, which are found predominantly in the telencephalon, and are viewed as an endogenous modulator of ligand- and voltage-gated ion channels. In this communication, we report that the hippocampal MT and its MT mRNA are synthesized on a continuous basis and hence may play a role in regulating the transport, accumulation, and compartmentation of zinc in the hippocampus. In addition, since MT is able to regulate the intracellular redox potential, we have taken a group of experiments to learn the role of MT in oxidative stress. By using electron spin resonance spectrometry (ESR) we have shown that 6-hydroxydopamine induces hydroxyl radicals and superoxide anions and dramatically enhanced MT mRNA in several brain areas, but especially in the hippocampal subfields. The results of these studies are interpreted to suggest that MT plays a major role in the defense against oxidative reactions, especially in the areas of brain possessing high concentrations of zinc. (Supported in part by a grant from USPHS-NS34566).

577.5

CULTURED MICROGLIA EXHIBIT IMPORTANT ASPECTS OF THE "RESTING" MICROGLIAL PHENOTYPE IN SERUM–FREE, ENDOTOXIN-FREE MEDIA. C.J.M. Kane*, I.R. Niesman, W. Chen, Z.Q. Yang and S.A. Gilmore. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Cultured CNS microglia are useful for modeling the interaction of microglia with neurons. However, the established methods of microglial culture are limited to proliferating, ameboid cells expressing MHC class II, complement receptor 3, and ED1 antigen. This cellular phenotype resembles activated microglia at sites of pathology. Understanding the induction of microglial activation in CNS disease has been thwarted by the absence of a routine method for culture of "resting" microglia.

A method was developed to culture microglial cells expressing important aspects of the resting microglial phenotype associated with ramified microglia. Primary cultures of mixed glia were prepared from the cerebrum, cerebellum, or spinal cord of one-day old Charles River CD rats. The loosely attached microglia were separated from subconfluent cultures by orbital shaking. The microglial cells were plated on uncoated glass chamber slides in DMEM + 10% FBS. After 24 hrs, the culture medium was replaced with serum-free, endotoxin-free medium. The majority of *Griffonia simplicifolia* lectin-labeled microglial cells extended ramified processes and the rate of microglial mitosis was markedly decreased within 24 hrs. Immunocytochemistry revealed decreased expression of MHC class II, complement receptor 3, and ED1 antigen. Thus, microglia can be cultured routinely under conditions that eliminate activation signals and promote expression of a cellular phenotype similar to that of resting, ramified microglia *in vivo*. This *in vitro* model of "resting" microglia permits cellular and molecular analysis of the mechanisms of microglial activation and analysis of constitutive interactions between resting microglia and neurons. (NIH AG12411).

577.6

MICROGLIAL CELLS IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES REGAIN A RESTING STATUS FOLLOWING INITIAL ACTIVATION. N.P.Hailer*, J.D.Järhult, R.Nitsch, Humboldt University Clinic (Charité), Institute of Anatomy, D-10098 Berlin, Federal Republic of Germany.

Numerous studies indicate that neurons in organotypic hippocampal slice cultures (OHSC) preserve morphological and physiological features of the *in vivo*-situation, however, there is little knowledge about the properties of microglial cells under these *in vitro*-conditions. We therefore addressed the question whether microglial cells in OHSC are initially activated following explantation but return to a resting state during *in vitro*-cultivation. Thus, we analyzed a) microglial cell morphology, b) microglial cell distribution, and c) expression of integrin adhesion molecules as putative markers of microglial activation at 0, 3, 6 and 9 days after explantation. Hippocampal slices fixed immediately following explantation showed only resting microglial cells, mainly located in the paraventricular regions. After 3 days *in vitro* (div) OHSC surfaces were covered by activated microglia, whereas intermediate layers contained fewer microglial cells. After 3 div microglial cells in intermediate layers of OHSC showed activated morphology with ovaloid cytoplasm and no or merely few cytoplasmic processes, after 6 div, however, an increasing degree of ramification could be observed. After 9 div microglia in intermediate layers had almost regained the morphological appearance of resting cells with filigrane cytoplasmic processes extended in all directions. The integrin adhesion molecules LFA-1 and VLA-4 were expressed on most microglial cells with activated morphology, as verified by co-localization with double immunofluorescence labeling for LFA-1 or VLA-4 and *Griffonia simplicifolia* isolectin B_4 (GFS-B_4). However, LFA-1 or VLA-4 were never found on ramified cells, and co-localization for LFA-1 or VLA-4 with ramified GFS-B_4⁺-microglia failed. We conclude that a) originally resting microglial cells become activated in an early phase *in vitro* but regain a resting status after at least 6 div; b) integrin adhesion molecules LFA-1 and VLA-4 are potential markers of microglial activation, as they were only found on activated but not resting microglial cells. This enables further investigations on immunological and electrophysiological features of resting and activated microglial cells under *in vitro*-conditions. Supported by the DFG: SFB 507/C1

577.7

MICROGLIAL CELLS AND ASTROCYTES INCORPORATE DEGENERATING PERFORANT PATH AXONS IN THE RAT DENTATE MOLECULAR LAYER FOLLOWING ENTORHINAL LESION Bechmann, I. and Nitsch, R.* Humboldt University Clinic (Charité), Institute of Anatomy, D-10098 Berlin, Germany

Entorhinal lesion results in anterograde degeneration of axons normally terminating in the outer portions of the dentate molecular layer. This is accompanied by an activation of microglial cells and astrocytes which play a key role in various neurodegenerative disorders. In order to further analyze the involvement of glial cells in mechanisms of hippocampal reorganisation following lesion, we studied the process of phagocytosis of degenerating axon terminals by activated microglial cells and astrocytes. Crystals of a rhodamine- and biotin-coupled dextran-amine (Mini-Ruby; allowing for light double- and confocal-fluorescence as well as electron microscopical analysis) were stereotaxically placed in the entorhinal cortex of adult rats. This resulted in staining of the entire perforant path including terminal branches in the middle and outer dentate molecular layer and which was stable at least until 20 days following application. One day after, animals received an electrolytic entorhinal cortex lesion. Microglial cells were detected by means of lectin staining (GSI-B4) or an antibody against a microglial cell surface protein (MUC-102) while astrocytes were immunostained for GFAP, all visualized using FITC-fluorescence. Following lesion, typical axons and their boutons were no longer visible but the rhodamine was arranged in characteristic granules. Double fluorescence for both rhodamine and FITC unraveled microglial cells which exhibited typical characteristics of activation, i.e., a round shape and the lack of ramification by one day and astrocytes at least by four days post lesion (dpl) to be associated with these granules. High power double fluorescence and confocal microscopy 2 dpl detected rhodamine granules signifying degenerating entorhinal axons to be contained in microglial cells which were labeled by FITC. Double fluorescence and electron microscopy revealed labeled and degenerating axons to be contained in astrocytes by 4 dpl. These data demonstrate that both microglial cells and astrocytes incorporate degenerating perforant path axons following entorhinal lesion. Thus, both cell types are essential components for the removal of degenerating material. Our approach allows further studies on a correlation of glial cell phagocytosis and cell surface protein expression in an experimental model of anterograde degeneration on a single cell level. Supported by the DFG: SFB 507/C1

577.8

TISSUE PLASMINOGEN ACTIVATOR MEDIATES MICROGLIAL ACTIVATION VIA A PROTEASE-INDEPENDENT MECHANISM. A. D. Rogove*, S. Strickland and S. E. Tsirka . Department of Pharmacology State University of New York at Stony Brook, Stony Brook, NY 11794

The serine protease tissue plasminogen activator (tPA) has been shown to mediate the activation of microglial cells. The participation of tPA in microglial activation was found to be independent of the conversion of plasminogen to plasmin. To further elucidate the mechanism by which tPA mediates microglial activation, microglial cells in primary mixed cortical cultures prepared from mice deficient in tPA ($tPA^{-/-}$)were tested for their ability to activate in response to the addition of lipopolysaccharide (LPS) to the culture media. The activation microglia in cultures from $tPA^{-/-}$ mice was incomplete (only 19% of the microglia were partially activated, compared to complete activation seen in 84% of microglia in cultures from wild-type mice). Furthermore, the addition of either rtPA or rtPA irreversibly inactivated with the protease inhibitor PMSF (rtPA/PMSF) to $tPA^{-/-}$ cultures prior to LPS addition restored the ability of microglia in these mixed cortical cultures to respond to LPS to nearly wild-type levels (70% and 71% of the microglia were completely activated in $tPA^{-/-}$ cultures supplemented with rtPA and rtPA/PMSF, respectively). These results demonstrate that tPA affects microglial activation in a protease-independent manner and defines the first physiological role of tPA which is independent of its proteolytic activity.

Funding sources: AFAR/Glenn Foundation Scholarship and MSTP Fellowship to A.D.R., NIH grant to S.S. and IHFSPO to S.E.T.

577.9

HUMAN FETAL ASTROCYTES EXPRESS FIBRONECTIN NON-SPECIFICALLY IN VITRO AND SOLELY AT THE BLOOD-BRAIN BARRIER IN VIVO. D.M. Kerkovich*, K.M. Weidenheim, W.D. Lyman. Path. Dept., Albert Einstein Coll. Med., Bronx, NY 10461.

Until recently, fibronectin was believed to be absent in the central nervous system (CNS) with the exception of basement membranes (BM) surrounding blood vessels and in the pia mater. BM fibronectin is a produced by endothelial cells and, in the pia, by fibroblasts. In vitro, however, enriched cultures of rat and mouse astrocytes can express fibronectin. In this study, we examined whether human fetal astrocytes synthesize and secrete fibronectin. We have addressed this question using Western blot analysis and single- and double-label immunocytochemistry of enriched astrocyte cultures from 16 to 24 week human fetal CNS tissue. Fibronectin expression was also examined in vivo by immunohistochemical staining of frozen tissue sections. Furthermore, we utilized a novel co-culture model of the human blood-brain barrier (BBB) to examine fibronectin expression. Results showed that greater than 50% of GFAP+ astrocytes in culture expressed fibronectin whereas, in vivo, fibronectin positivity was only detected in profiles consistent with astrocyte foot processes abutting the abluminal surface of microvessels. This localization of staining was also found in the BBB model.

(Supported in part by USPHS grants MH 47667 and MH 46815)

577.10

ELECTROPHYSIOLOGICAL HETEROGENEITY OF CORTICAL ASTROCYTES. G.M McKhann II*, R. D'Ambrosio and D. Janigro. Dept. of Neurosurgery, Univ. of Washington, Seattle, WA 98104

Astrocytes have traditionally been thought of as a relatively homogeneous population of cells characterized by a negative resting membrane potential (RMP) and low input resistance due to high potassium permeability. However, in situ recordings from hippocampal and corpus callosum slice astrocytes have revealed a wide range of RMP. Additionally, astrocytes from different areas of the central nervous system have been shown to exhibit regional variations in ion channel expression and intercellular gap junction coupling. We hypothesized that astrocytes within a single brain region constitute a heterogeneous population of cells. Utilizing whole cell and gramicicdin-perforated patch clamp recordings from cultured rat neocortical astrocytes, we found that these cells exhibit a bimodal distribution of RMP (range -20 to -84). RMP did not depend on the recording configuration (whole cell vs. perforated), cell proliferation, cell differentiation, or time in culture. Cells could be divided into "hyperpolarized" cells (HC, RMP<-60 mV) and "depolarized" cells (DC, RMP>-60 mV). HCs were characterized by a higher membrane conductance (43.9±0.14 nS) and a nearly linear I-V profile. DCs had a lower membrane conductance (25.3±0.14 nS) and displayed inward rectification. Pair recordings from adjacent cells suggested that DCs and HCs are often electrically coupled. The ion current profile as well as the pharmacological properties of a given cell were often dynamic, depending on the degree of intercellular coupling with other astrocytes. The heterogeneity of astrocyte ion current mechanisms may allow for specialized regulation of the extracellular environment in different areas of the central nervous system. Supported by NIH NHLB 51624 and NS 07144.

577.11

SYNAPTIC ACTIVITY-DEPENDENT COMMUNICATION BETWEEN NEURONS AND ASTROCYTES . G. Carmignoto*, L. Pasti and T. Pozzan. Dept. of Biomedical Sciences, University of Padova, 35127 Padova, Italy.

Glutamate and neuronal stimulation can trigger $[Ca^{2+}]_i$ oscillations and waves in cultured astrocytes and, when repetitively applied, long-term changes in their $[Ca^{2+}]_i$ oscillation frequency. Oscillating astrocytes can, in turn, signal back to neurons by increasing their $[Ca^{2+}]_i$. The occurrence of similar phenomena in the brain would suggest the involvement of astrocytes as active cells in the astrocyte-neuron networtk. We, therefore, used acute brain slice preparations and confocal fluorescence microscopy to monitor the spatio-temporal features of $[Ca^{2+}]_i$ changes in hippocampal and visual cortical astrocytes after loading with Indo-1. We found that, similarly to cultured astrocytes, the frequency of oscillations in astrocytes in situ increased upon repetitive activations of mGluRs by *t*-ACPD (5-30μM) (0.75±0.04 and 1.38±0.01 peaks/min at the 1st and 3rd stimulation, respectively; mean change 102±18 %; $p > 0.001$; n= 52). Electrical stimulation of presynaptic afferents at 0.2 Hz (100-200 msec train at 20-50 Hz; 100 pA) induced $[Ca^{2+}]_i$ oscillations in astrocytes sorrounding stimulated neurons. Repetitive $[Ca^{2+}]_i$ increases could occur at discrete loci along astrocyte processes. An identical stimulus applied several minutes after the first induced in 10 of 21 cells a significant increase in oscillation frequency indicating that neuronal stimulation can induce long-term changes in the astrocyte response. Glutamate released at the synapse can, therefore, trigger $[Ca^{2+}]_i$ elevations in both postsynaptic neurons and astrocytes. These observations suggest the existence of a synaptic-like form of communication between synaptic terminals and astrocyte processes and prompt the evaluation of a new aspect in the study of brain's plasticity.

Grants from CNR "Biotechnology" and Thelethon Italy.

578.1

CALCITONIN GENE RELATED PEPTIDE-IMMUNOREACTIVITY (CGRP-IR) EXPRESSION IN HIPPOCAMPAL CA1 NEURONS FOLLOWING ISCHEMIA MAY SUPPRESS INFLAMMATORY IMMUNE RESPONSES TO DYING PYRAMIDAL CELLS. K. Bulloch[1*], T.A. Milner[2], L.P. Reagan[1], N.G. Weiland,[1] G. Buzsaki[3], M. Hsu[3] and B.S. McEwen[1],. Lab Neuroendocrinol, Rockefeller U.); Dept Neurol& Neurosci, Cornell U. Med Col, New York, NY 10021 (2) Center Mol Behav Neurosci., Rutgers U., Newark, NJ 07102.

We have shown that CGRP derived from hilar mossy cells is increased in the inner molecular layer of the dentate gyrus after adrenalectomy and colchicine destruction of granule neurons (Bulloch et al. NeuroReport V.7 in press). Moreover, GCRP contains cellular immune responses in specific organs such as the thymus (Bulloch et al Ann NY Acad Sci.V.741,p.126,1994). Given the immunoregulatory role of this peptide, we have continued to characterize the distribution of CGRP in another model of insult, ischemia. Following the rat four vessel occlusion model of ischemia, CGRP-immunoreactivity (ir) was found in a distinct band of cells distributed diffusely within the CA1 pyramidal cell layer and in varicose fibers within the CA2 pyramidal cell layer. Control rats showed no CGRP-ir within these regions. Sections double-labeled for CGRP and nitric oxide synthase, or calbindin, or parvalbumin, showed that CGRP co-localized with calbindin in some neurons. Electron microscopic analysis of the ischemic CA1 region showed that CGRP-ir is primarily found in terminals containing small clear vesicles which form symmetric synapses (inhibitory type) with the perikarya and large dendrites, some identified as originating from pyramidal cells. Together, these data indicate that in addition to impaired pyramidal cells, CGRP-ir was primarily localized to axon terminals of basket cells in response to ischemia. Moreover, in conjunction with our previous studies, these findings also support a discrete regional immunoregulatory role for CGRP following CNS trauma. (Supported by MH 41256, The Arthur Vining Davis Foundation (BSM,KB); MH42834 and HL18974(TAM)

578.2

INTERLEUKIN-12 GENE EXPRESSION IS INDUCED IN THE CNS AND IN CULTURED ASTROCYTES AND MICROGLIA BY LPS. J.L. Campbell*, C-S Chiang, N.C. Yu, F.E. Bloom and A.K. Stalder. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, 92037

Interleukin-12 (IL-12) plays a central role in the regulation of cell-mediated immunity and is implicated in antiviral and antitumor host responses in the CNS as well as in the pathogenesis of the autoimmune demyelinating disorders EAE and MS. Here we examined whether the IL-12 p35 and p40 genes that encode the subunits of the biologically active IL-12 heterodimer can be induced in the brain following peripheral LPS administration. Mice were injected ip with either a single or two staggered sub-lethal doses of LPS and IL-12 p35 or p40 mRNA levels analyzed in the brain by RNase protection assay (RPA). Expression of IL-12 p 35 mRNA was constitutive, the levels of which did not alter following either LPS treatment protocol. In contrast, IL-12 p40 mRNA was not detectable in brain from control mice or mice given a single injection of LPS . However, significant induction of IL-12 p40 RNA was seen in mice given two LPS injections. Analysis of IL-12 p40 RNA expression by in situ hybridization revealed positive cells with the morphological appearance of microglia and astrocytes, scattered throughout the brain parenchyma. To confirm the possibility that microglia and astrocytes express the IL-12 p40 gene, purified cultures of these cells were prepared, treated with LPS and IL-12 p40 RNA levels assessed. No detectable IL-12 p40 mRNA was found in control cultures. However, following exposure to LPS, significant induction of IL-12 p40 mRNA occurred in both astrocytes and microglia. Taken together the findings indicate IL-12 gene expression can be activated in the brain, with microglia and astrocytes likely providing a significant local source for production of this cytokine. Localized production of IL-12 may have a significant impact on the development of cell-mediated immunity within the CNS. Supported by USPHS grants MH 50426 and MH 47680.

578.3

LYMPHOCYTIC MENINGOENCEPHALOMYELITIS INDUCED BY TRANSGENIC EXPRESSION OF TNF-α IN THE CNS. A. K. Stalder*, A. Pagenstecher, I. L. Campbell, The Scripps Research Institute, Dept. of Neuropharmacology, La Jolla, CA 92037.

TNFα is a proinflammatory cytokine that may play a role in many inflammatory CNS disorders e.g. bacterial meningitis, HIV encephalopathy and MS. To study the mechanisms by which TNFα may contribute to the clinicopathological alterations in these disorders, we used a transgenic (tg) approach to target expression of TNFα to astrocytes using a GFAP/TNFα fusion gene. Two GFAP-TNFα lines were established in which TNFα mRNA expression was demonstrated predominantly in the brain stem, cerebellum and spinal cord (GT-8 line), or throughout the brain and the spinal cord (GT-2 line). A profound phenotype developed in both lines which differed for each. The GT-8 line showed a progressive development of ataxia with muscle spasms and hind-limb dysfunction, while the GT-2 had sudden onset of total paralysis affecting the lower body. In the brain and spinal cord from tg mice, significant pathological changes were present that overlapped with areas of TNFα expression. In the GT-2 line, extensive meningoencephalomyelitis with predominantly lymphocytic infiltrates, was seen in the spinal cord and cerebellum. The GT-8 line showed focal perivascular and meningial infiltrates in the neocortex, cerebellum and pons. In both lines, tissue damage was evident in both gray and white matter regions of the spinal cord and brain and overlapped with the mononuclear infiltrates. Furthermore, areas of hemorrhage were observed within the infiltrates suggestive of vascular injury. Pronounced astrocytosis was found around the infiltrates. Apoptosis is a well described effect of TNFα, we analyzed for apoptosis in the CNS. Apoptotic nuclei were rarely if at all present in the CNS tissue from wild type mice. In contrast, in the CNS tissue of GFAP-TNFα mice, numerous apoptotic nuclei were observed surrounding, and to a lesser extent within the mononuclear infiltrates. These findings indicate that overexpression of TNFα in the CNS promotes the recruitment and infiltration of mononuclear cells, in which the resultant lymphocytic meningoencephalomyelitis, together with increased apoptosis, leads to extensive neural tissue destruction, and likely underlies the observed phenotype of these GFAP-TNFα tg mice. These tg mice should be a valuable tool to further define the mechanics of TNFα actions in the CNS. Supported by USPHS grant MH 50426.

578.4

LEUKEMIA INHIBITORY FACTOR CAN ACT AS AN ANTI-INFLAMMATORY CYTOKINE. L. R. Banner*[†], C. J. Woolf[‡] Steve Poole[‡] and P.H. Patterson[†]. [†]Biology Division 216-76, Caltech, Pasadena, CA 91125 and [‡]Dept. of Anatomy, University College London, London WC1E 6BT.

The neuropoietic cytokine leukemia inhibitory factor (LIF) plays a role in the response to injury in both the peripheral and central nervous systems. To test whether LIF regulates neurogenic inflammation, we have studied its role in adjuvant-mediated inflammation. Intraplantar injection of complete Freund's adjuvant (CFA) produces an inflammatory response characterized by local swelling, sensory hypersensitivity and a rapid up-regulation of IL-1β and NGF, followed by substance P and CGRP in sensory neurons. We find that LIF mRNA levels in the footpad increase following either CFA or IL-1 injections. Intraplantar injection of LIF results in a decrease in IL-1 protein, suggesting that LIF may down-regulate the inflammatory response. Further evidence comes from results obtained with LIF knockout mice. Intraplantar CFA injection in these mice yields a significant increase in inflammatory edema over that seen in wild type mice. Moreover, peak IL-1 levels in plantar skin of wild type mice are lower and return to baseline faster than in mutant mice. These results provide evidence for an anti-inflammatory role for LIF during peripheral inflammation.

This work is supported by an NIH grant to PHP, an NRSA to LRB and CJW is supported by the MRC.

578.5

VAGOTOMY DELAYS, BUT DOES NOT BLOCK LPS-INDUCED FEVER. Sheba M. J. MohanKumar, P. S. MohanKumar and S. K. Quadri*

Neuroendocrine Research Laboratory, Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506.

Lipopolysaccharide (LPS) has been reported to induce a number of central effects including sleep, fever, anorexia, analgesia etc. The mechanisms by which LPS produces its central effects are unclear. This study was done to investigate the possibility that LPS-induced fever is mediated through the vagus nerve. Sub-diaphragmatic vagotomy was performed in adult male Sprague-Dawley rats, followed 2 h later by an intraperitoneal injection of saline or LPS in saline (10μg/kg BW). Rectal temperature (°C) was monitored at half-hour intervals for 5 h. Saline treatment produced no significant changes in rectal temperature either in sham-operated or in vagotomized rats. LPS treatment increased ($p<0.05$) rectal temperature in sham-operated rats from 37.1±0.2 to 37.9±0.2 within 1 h and kept it elevated for 4 h. A similar LPS treatment did not produce a significant increase in rectal temperature in vagotomized rats (from 37.1±0.3 to 38±0.3) until 2 h after treatment, after which the temperature remained elevated for 3 h. The delay in fever induction in vagotomized rats indicates that the immediate stimulus for LPS-induced fever is transmitted to the central nervous system through the vagus, whereas another mediator, possibly interleukin-1, is involved in the delayed increase in temperature such as that observed in vagotomized animals.

578.6

INTERLEUKIN-1 RECEPTOR ANTAGONIST ATTENUATES INFLUENZA-VIRUS-INDUCED EFFECTS ON INGESTIVE BEHAVIOR. A. H. Swiergiel*, and A. J. Dunn.
Dept. Pharmacology, Louisiana State Univ. Med. Ctr., Shreveport, LA 71130

As infection with influenza virus develops, animals display fever, decreased exploratory and feeding behavior, and loss of body weight. These responses may be mediated by cytokines, specifically by interleukin-1 (IL-1). We assessed the effects of a naturally occurring IL-1-receptor antagonist (IL-1ra) on food and milk intake in mice injected with IL-1, lipopolysaccharide (LPS), or inoculation with influenza virus (PR8 H1N1).

IL-1ra significantly attenuated the reductions of the ingestion of sweetened condensed milk in a 30-minute period following intraperitoneal (ip) injection of IL-1α or IL-1β (100 ng), but only attenuated that caused by LPS (1-5 μg ip).

In the influenza virus infection model, mice were inoculated intranasally with a lethal or sublethal doses of the virus or vehicle and were either given repeated injections of IL-1ra, or IL-1ra or vehicle was delivered by subcutaneous osmotic minipumps. The IL-1ra treatment did not reverse the effects of the virus infection on body weight, 23-hour food pellet intake, or milk intake. However, in several experiments, IL-ra significantly attenuated the reductions in body weight, food intake and milk ingestion. In most experiments, there was also a significant decrease in mortality caused by lethal doses of the virus. It is concluded that the IL-1ra treatment may attenuate some aspects of sickness behavior, suggesting that IL-1 may mediate some of the effects of infection with influenza virus on ingestive behavior.

Supported by NIMH (MH46261), NINDS (NS25370) and Amgen

578.7

THE INTERLEUKIN-1-RECEPTOR ANTAGONIST FAILS TO ALTER THE NEUROCHEMICAL AND HPA RESPONSES TO LPS

Adrian J. Dunn and Rhonda F. Brown*
Dept. Pharmacology, LSU Med. Ctr., Shreveport, LA 71130

Endotoxin (LPS) administration stimulates the secretion of interleukin-1 (IL-1). LPS and IL-1 both activate the hypothalamo-pituitary-adrenocortical (HPA) axis and increase cerebral catecholamine and indolamine metabolism. The responses to LPS and IL-1 are remarkably similar with peak responses in plasma ACTH and corticosterone and in the norepinephrine catabolite, MHPG, around 2 h, and elevations of brain tryptophan and 5-hydroxyindoleacetic acid (5-HIAA) somewhat later. The results are not compatible with the simple hypothesis that IL-1 mediates these responses to LPS, because peak IL-1 production after LPS does not occur until around 2 hours, so that the responses to LPS ought to be delayed more than is actually observed.

The HPA and neurochemical responses to LPS were examined using doses of the IL-1-receptor antagonist (IL-1ra) more than adequate to block responses to administered mouse IL-1β. We failed to observe statistically significant attenuation of the neurochemical or HPA responses to LPS 1-6 h following injection of low doses of LPS (1-5 μg IP), although slight attenuations were observed at 4 and 6 h in one experiment. Icv doses of IL-1ra up to 200 μg failed to attenuate the responses to IP LPS at 1-2h, but occasionally produced a small attenuation at 4h.

We conclude that although IL-1 probably contributes to the HPA and neurochemical responses to LPS, it is not the only factor, and probably not the major one. Moreover, LPS does not activate the HPA axis or neurochemical changes solely by inducing IL-1 synthesis within the brain.

Supported by NIMH (MH46261) and NINDS (NS25370)

578.8

ANTIPYRETIC ROLE OF ENDOGENOUS BRAIN MELANOCORTINS DURING FEVER.

J.B. Tatro*[1], M.L. Entwistle[1], J.D. Alvaro[2], R.S. Duman[2], S. Sharma[3], V.J. Hruby[3], and O.-H. Huang[1]. [1]Endocrine Div., New Eng. Med. Ctr./Tufts Univ. Sch. Med., Boston, MA 02111; [2]Lab. Mol. Psych., Yale Univ. Sch. Med., New Haven, CT, 06508; [3]Dept. Chemistry, Univ. Arizona, Tucson, AZ 85721.

Bacterial infection causes fever, an adaptive but potentially self-destructive response, in the host. Exogenous melanocortin (α-MSH-related) peptides are antipyretic, and forebrain structures important in thermoregulation are innervated by MSH-containing neurons and contain melanocortin receptors (MCR). To test the hypothesis that endogenous central melanocortins exert antipyretic effects during fever, we tested the effect of intracerebroventricular (icv) injection of a novel synthetic MCR antagonist (MCR-Ant) in endotoxin-challenged rats. In vitro, MCR-Ant inhibited α-MSH-induced adenylate cyclase activation in heterologous cells expressing either rat MC3 or rat MC4 (the major MCR subtypes expressed in brain; IC_{50} = 1 nM for each), but showed no intrinsic agonism. Rats received *E. coli* lipopolysaccharide (LPS, 25 ug/kg) or saline ip, followed 30 min later by 4 μL (168 pmol) MCR-Ant or saline, icv. Body temperatures (T_b) were monitored for 8 hr. LPS fevers were 77% greater in MCR-Ant-injected rats (11.4±1.1, N=11; vs. 6.4±0.9 °C.hr, N=12; area under curve for Tb rise)($p<.01$). Effect of MCR-Ant was negligible in control rats. The results suggest a protective antipyretic role of central MSH neurons during infection.

Support: NIH MH44694, DA08227, DK17420.

578.9

LIPOPOLYSACCHARIDE, BUT NOT TURPENTINE INDUCES EXACERBATED FEVERS IN TNF DOUBLE RECEPTOR KNOCKOUT MICE.

L. R. Leon, W. Kozak, J. Peschon, and M. J. Kluger*. The Lovelace Institutes, Albuquerque, NM 87108 and Immunex Research Corporation, Seattle, WA 98101.

Tumor necrosis factor-α (TNF-α) is capable of causing fever, lethargy, body weight loss and anorexia in several species. Under several experimental conditions, TNF-α has endogenous cryogenic or antipyretic properties; that is, it reduces fever.

We used TNF double receptor (TNFR) knockout mice to examine the effect of peripheral injections of lipopolysaccharide (LPS) or turpentine on fever, lethargy (motor activity), body weight and food intake. Body temperature and motor activity of mice was measured using biotelemetry. TNFR knockout mice injected ip with a high dose (2.5 mg/kg) of LPS developed exacerbated fevers compared to wildtype mice. This enhancement was seen during the early (3-15h), but not late phase (16-24h) of fever to this dose of LPS (ANOVA, Scheffe, $p < 0.05$). Motor activity, body weight and food intake were similarly depressed in both groups of mice to the high dose of LPS. A low dose of LPS (50 μg/kg, ip) or turpentine (100 μl/mouse, sc) induced similar fevers, lethargy, loss of body weight and anorexia in TNFR knockout and wildtype mice.

These results support the hypotheses that (1) endogenous TNF-α is a key inhibitor of the febrile response to a high dose of ip injected LPS, but not a low dose of ip LPS or sc turpentine, and (2) endogenous TNF-α is not a key mediator of the lethargy, loss of body weight or anorexia induced to ip injected LPS or sc turpentine in mice.

Research supported by NIH AI27556.

578.10

NEURAL-IMMUNE ABNORMALITIES IN DEPRESSED PATIENTS AND HEALTHY CONTROLS,

G.R. Heninger*, L.H. Price, R. T. Malison, and G. H. Pelton, Dept. of Psychiatry, Yale University, New Haven, CT 06510. Immune abnormalities have been demonstrated in patients with depression, but their role in pathogenesis is unknown. METHODS: In order to evaluate possible abnormalities in immune mediated neuroendocrine function in patients with depression, we have administered, in a placebo-controlled manner, a 5 min. i.v. infusion of the potent immune stimulant, lippopolysaccharide (LPS), 2 ng/kg to 6 depressed patients (DP) and 6 healthy controls (HC). Before and after the LPS or placebo infusion, repeated measures were made of subjective symptoms, body temp., blood leukocytes, IL-1 beta, IL-6, TNF alpha, C-reactive protein, alpha 1-acid glycoprotein, haptoglobin, ACTH, cortisol, and growth hormone (GH). RESULTS: LPS produced reliable and robust increases in all the measures listed above. In comparison to the HC, the DP had a 60% reduction of subjective reports of chills and body temp, but total leukocytes were doubled at early time points. There was a trend for the IL-6 and TNF-alpha responses to be lower in DP, but the response of C-reactive protein and haptoglobin tended to be higher. The ACTH, cortisol, and GH response to LPS of DP were reduced to less than 20%, 50%, and 25% of the response of HC respectively. CONCLUSION: Depressed patients have a blunted symptomatic, body temp., and neuroendocrine response to LPS in the face of a normal or increased immune response. The data is consistent with the hypothesis that a chronic hyper immune state in depression leads to downregulation of the central nervous system response to immune stimulation. (Supported by the Stanley Foundation).

OCULOMOTOR SYSTEM: SACCADES

579.1

ACTIVITY OF PREFRONTAL NEURONS DURING MEMORY-GUIDED EYE MOVEMENT AND FIXATION.

R.G. Erickson*, LNP, NIMH, Poolesville, MD 20837.

Cortical neuronal responses during delayed response tasks have previously been proposed to reflect either working memory or motor planning. To determine how primates execute memory-guided as opposed to visually-guided eye movements I hypothesized that parts of the prefrontal cortex serve as a selective relay allowing stored rather than current sensory information to be sent to motor areas. As a test, extracellular recordings were obtained from prefrontal cortex along the principal sulcus of rhesus monkeys trained to perform memory-guided eye movements followed by memory-guided ocular search in total darkness. Three types of randomly interleaved trials were used to require eye movements and fixation guided by either spatial memory in complete darkness, memory for one of multiple visible targets, or in response to movement of a single visible target. In a sample of 102 cells from one monkey, two predominant activity patterns were observed. First, when significant modulation of delay-period activity occurred on spatial memory trials (18 cells), the activity did not depend upon presence or absence of a visible cue at the intended saccadic target zone. In addition, the delay-period activity invariably ended with saccade execution and response modulation did not persist during a subsequent period of memory-guided search (18 of 18 cells). Second, a larger group of cells (42) had significant modulation of activity during memory-guided fixation without significant modulation of delay-period activity, but no cells exhibited modulation which persisted through both phases of the task.

These data suggest that both remembered and current sensory information is available to prefrontal 'delay' and 'memory-fixation' neurons and is selectively accessed as part of the process of selecting a particular response. The results do not support the hypothesis that prefrontal cells actually store spatial memories.

579.2

DISSOCIATION OF TARGET SELECTION FROM SACCADE PLANNING IN MACAQUE FRONTAL EYE FIELD.

N.P. Bichot*, K.G. Thompson & J.D. Schall. Department of Psychology, Vanderbilt University, Nashville, TN 37240

To determine if target discrimination in macaque frontal eye field (FEF) is contingent on saccade planning, the activity of FEF neurons was recorded in two monkeys during blocked GO and NOGO visual search trials. In the GO search task, the monkey was rewarded for directing gaze to the salient oddball target among distractors distinguished by color. In the NOGO search task, a cue to withhold saccades was presented 500 ms before the appearance of the search display, and the monkey was rewarded for maintaining fixation on a central fixation point for up to 1000 ms.

To account for possible saccade planning even during successful NOGO trials, we analyzed the eye movements made by the monkeys within a 500 ms time window after they were rewarded for maintaining fixation. The monkeys made saccades to the oddball after reward in less than 15% of NOGO trials during recordings from 20 out of 22 cells. The lack of saccade planning was reflected by an attenuation of visual responses in NOGO trials compared to GO trials, similar to what had been observed by Bushnell and Goldberg (*J. Neurophysiol.* 46:773-787) during visual detection. The evolution of activity signalling target location was characterized with an analysis based on Signal Detection Theory. In the GO task neural activity evolved to signal target location at a consistent time after stimulus presentation and did not predict when the eyes would move (Thompson et al., *Soc. Neuro. Abs.* 21:1270). In the NOGO task, for the majority of neurons, activity evolved to signal the location of the oddball stimulus to a comparable degree and over a similar timecourse. Thus, the selection of a salient visual stimulus by FEF neurons is not contingent on saccade production but rather may reflect the outcome of an "automatic" bottom-up visual selection process. (Supported by F32-EY06495, McDonnell-Pew and R01-EY08890)

579.3

NEURAL BASIS OF SACCADE REACTION TIME: FRONTAL EYE FIELD
D.P. Hanes* & J.D. Schall. Department of Psychology, Vanderbilt University, Nashville, TN 37240

Understanding the neural basis of the duration and variability of reaction times (RT) is a central problem of cognitive neuroscience. Many conceptual models have been proposed to account for the distribution of RTs. One class of models utilizes a decision process in which a response is initiated when a growing signal reaches a threshold. By stochastically varying either the rate of the growth (variable rate model) or the threshold that triggers the movement (variable threshold model) RT distributions are generated. Physiological approaches that test such models are essential for understanding the processes that regulate movement initiation. We have been investigating these decision processes using a countermanding task that manipulates a subject's ability to inhibit a saccade during different stages of its preparation. Cells with movement-related activity were recorded in the frontal eye fields of two rhesus monkeys performing the countermanding task. In 23 of 25 cells, the discharge level 10-20 ms before the saccade did not vary with saccade latency (SL). Also, the rate of growth of the activity significantly declined with increasing SL in 22 of 25 cells. Both of these results are predicted by the variable rate, but not the variable threshold model. If the variable rate model is correct, the level of neural activity on successfully inhibited trials should not reach the critical threshold level. In 19 of 25 cells the neural activity when monkeys withheld saccades was significantly less than the threshold level. We implemented a simple mathematical model to further test the variable rate model. The parameters of the model were derived directly from corresponding measures of activity of individual neurons. In 15 of 22 cells the mean of the model SL distribution was not different from the actual SL distribution. In 9 of 22 cells the both the mean and the shape of the distributions were not different. (Supported by R01-MH55806 & F31-MH11178)

579.4

FUNCTIONAL INTERACTIONS BETWEEN THE TWO FRONTAL EYE FIELDS IN MONKEY.
J. Schlag, P. Dassonville, M. Schlag-Rey and M. S. Letinsky*. Brain Research Institute, Depts. of Neurobiology and Physiology, UCLA, Los Angeles, CA 90095.

Movement cells in the FEF discharge before and during purposeful saccades of particular direction and amplitude. At the same time, cells that encode a different saccade vector are silent. What prevents different populations of saccade-related cells from issuing competing commands? That is: why do oculomotor centers always seem to agree on the next orientation of gaze? To study the mechanism of this remarkable cooperation, we have recorded from one FEF in trained monkeys while saccades were evoked by electrically stimulating the other FEF. The preferred saccade vectors of cells at both the recording and stimulation sites were first determined. When the vectors were different, the cells were either unaffected (51%) or inhibited (49%) by the contralateral stimulation. Reciprocally, in all 14 cases in which the cells in both FEFs had similar vector preferences, the stimulation produced an excitation in the contralateral cells. These results are comparable to those found earlier in studying the effects of FEF stimulation on burst cells in the superior colliculus (Schlag-Rey et al., J. Neurophysiol., 1992, 67: 1003). We suggest that the winner-take-all phenomenon in oculomotor structures is implemented by connections such that cells that command similar movements mutually excite each other while silencing cells that would produce conflicting movements. (Supported by USPHS grants EY05879 and EY02305).

579.5

ACTIVITY DURING INTERRUPTED SACCADES OF ROSTRAL SUPERIOR COLLICULUS NEURONS PROJECTING TO THE OMNIPAUSE REGION.
N. J. Gandhi* and E. L. Keller. Graduate Group in Bioengineering, UCSF, San Francisco and The Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115.

Neurons found in the deeper layers of the rostral third of the superior colliculus (SC) are active during fixation and inhibited during large saccades. An anatomic study has reported that the rostral SC projects to omnipause neurons (OPNs), and previous physiological studies have shown that stimulation of the rostral SC during large saccades produced interrupted saccades similar to those produced by OPN stimulation. These findings suggest that the rostral SC provides an input signal to the OPNs that may be critical in controlling and ending saccades. To clarify the role of the rostral SC projections to the OPNs in saccade control, we stimulated the region of the OPNs to test for antidromic activation of SC neurons and recorded neural activity in the rostral SC during saccades interrupted by OPN stimulation. We specifically wished to determine if rostral SC neurons were reactivated during the period of interruption.

The probability of antidromic activation from the OPN region was greatest for the most rostral SC neurons (0.7) and decreased linearly to 0.2 for the caudal SC. A resumption of discharge in rostral neurons was rarely observed during the interrupted period. Reactivation of these neurons occurred prior to or after the end of the resumed saccade. For truncated movements, however, resumed activity was observed at the time of truncation. Therefore, neurons in the deeper layers of the rostral SC partially reflect the eye perturbations induced by OPN stimulation and may contribute to the control of OPNs and saccade termination, but their discharge profiles are not nearly as tightly coupled to saccadic termination as the latter group of cells. The fact that rostral SC neurons do not resume discharging during saccadic interruption may help to explain why interrupted saccades are normally orthometric,when control theories with a resetable neural integrator would predict hypometric errors.

Research supported by NIH grant EY06860.

579.6

A NEW MODEL OF THE SACCADIC SYSTEM: I. SEPARATE MODULES FOR SPECIFYING DIRECTION AND DURATION
L.M. Optican*, C. Quaia, P. Lefèvre, LSR, National Eye Institute, NIH, Bethesda, MD 20892.

Saccades rapidly change the orientation of the eye in the head, despite the viscous and elastic forces of the extraocular muscles and orbital tissues. This requires a pulse-step of innervation. Existing models focus on generating the pulse of innervation, and obtain the step of innervation by integrating that pulse.

Robinson (1975) proposed a closed-loop feedback controller that generated the pulse simply as a function of the dynamic motor error (difference between desired and current eye orientations). Models based on this feedback controller obtain burst neuron activity as a nonlinear function of either horizontal or vertical motor error. Experimental results, however, reveal that the activities of brain stem burst neurons have directional tuning curves with a wide range of preferred on-directions. Thus, the input to the burst neurons can not come from a decomposition of the motor error into horizontal and vertical components, but must come from a vectorial error. However, a vectorial error feedback loop would always generate horizontal and vertical components of the same duration, whereas saccades can have components of different durations. Without a vectorial motor error feedback loop, the duration of the pulse must be determined elsewhere. Only lesions of the cerebellum result in hypermetric saccades, which are, essentially, saccades that do not stop when they should. We infer from this that the cerebellum plays a crucial role in ending the saccade.

These three abstracts present a new approach to modeling the saccadic system that uses a distributed representation of neurons whose activity more closely matches the experimentally observed behavior of neuronal populations in the brain stem, superior colliculus, and cerebellum. A model based on this distributed approach generates the saccadic pulse through the interaction of a collicular mechanism providing a directional drive, and a cerebellar mechanism controlling duration.

Supported by The National Eye Institute and the FNRS and SSTC (Belgium).

579.7

A NEW MODEL OF THE SACCADIC SYSTEM: II. DIRECTIONAL DRIVE BY THE SUPERIOR COLLICULUS.
P. Lefèvre*, C. Quaia, L.M. Optican, LSR, National Eye Institute, NIH, Bethesda, MD 20892.

The intermediate layers of the superior colliculus (SC) are believed to be responsible for controlling saccades. Three types of neurons contribute to this control: burst neurons that burst during saccades; fixation neurons that pause during saccades; and buildup neurons that begin firing before, and can burst during saccades. The locus of activity in the SC is retinotopically mapped, and the locus of the active burst cells seems to encode the amplitude and direction of the movement. The activity of the buildup neurons is initially localized to the same place, but during the saccade it appears to spread in a rostral direction.

These findings have led to models of the SC that propose that the locus of active burst neurons determines the desired ocular displacement, and the spatial distribution of activity among the buildup neurons forms the displacement integrator of the feedback controller. However, experimental data from Aizawa and Wurtz (1994) show that muscimol lesions in the intermediate layers of the SC lead to curved saccade trajectories that attempt to "go around" the site of the lesion. This would not be possible if the displacement integrator were in the SC, as damaging the integrator would eliminate the brain's knowledge of where the eye is currently. This would lead to an incorrect estimate of the motor error, and an inappropriate saccade.

In the new model proposed here, the burst neurons determine the desired ocular displacement, and the buildup neurons provide a directional drive to the brain stem. The saccade starts when the interaction between the burst and fixation neurons turns off the brain stem omnipause neurons. A novel feature of the new model is that the integrator is in the cerebellum, not the SC. Thus, the end of the saccade is controlled by the cerebellum. If the cerebellum is lesioned, saccades eventually stop because the collicular fixation neurons reactivate.

Supported by The National Eye Institute and the FNRS and SSTC (Belgium).

579.8

A NEW MODEL OF THE SACCADIC SYSTEM: III. FEEDBACK CONTROL OF DURATION BY A SPATIAL INTEGRATOR IN THE CEREBELLUM.
C. Quaia*, P. Lefèvre, L.M. Optican, LSR, National Eye Institute, NIH, Bethesda, MD 20892.

Lesions of the cerebellum result in permanent dysmetria of saccades, in most cases a hypermetria. The hypermetric saccades, although larger, are slower than normal. This leads us to the inference that the cerebellum contains the saccadic integrator and contributes to the dynamics of the saccade. We infer from recording studies of the vermis and fastigial nuclei that the saccadic integrator may be represented by a wave of activity that moves through the fastigial nuclei during a saccade. The output of the fastigial nuclei is responsible for accelerating the eye at the beginning of the saccade, and stopping the eye at the end of the saccade by inactivating the drive to the motor neurons. Thus, a saccade can cease even though an input to the burst neuron remains, and the pontine omnipause neurons have not been reactivated.

One consequence of the fastigial output is that the activity of individual motor neurons is shut down abruptly, in a process we call *dynamical derecruitment*. This derecruitment allows the total drive of the oculomotor neurons to decrease gradually, even though experimental evidence shows that the burst on individual motor neurons shuts off abruptly.

One advantage of a dual controller for the saccadic system is that it provides redundancy. If either the cerebellum or the superior colliculus is lesioned, saccades are still possible. However, they will show deficits associated with the loss of the damaged part of the system. In the case of cerebellectomy, saccades will be too large. This hypermetria results from the longer duration of the saccade, which only stops when the fixation neurons in the SC come back on, and turn on the pontine omnipause neurons. In contrast, lesions of the SC lead to errors in the initial direction of a saccade, and lower saccade velocity. The final eye position, however, would be correct, because of the cerebellar output.

Supported by The National Eye Institute and the FNRS and SSTC (Belgium).

579.9

DEMONSTRATION OF SACCADE-RELATED SINGLE-UNIT ACTIVITY IN THE DORSOLATERAL PONTINE NUCLEUS (DLPN) OF THE RHESUS MONKEY. P. Thier*[1], P. W. Dicke[1], S. Barash[2], U. Ilg[1]. [1]Sektion Visuelle Sensomotorik, Neurologische Universitätsklinik, 72076 Tübingen, Germany and [2]The Weizmann Institute, 76100 Rehovot, Israel.

The DLPN is connected to saccade-related regions, but saccade-related neuronal activity has not been reported in the DLPN. The DLPN receives strong input from cortical area LIP and the FEF, and it projects to vermal lobules VI,VII. The involvement of all these areas in saccades is well established. Nevertheless, only pursuit-related activity, not saccade-related activity, has been reported in the DLPN. The question, whether saccade-related activity exists in the DLPN is particularly interesting in view of current thinking about cortical contributions to goal-directed eye movements, which favors the dichotomy of a cortico-pontine pathway for smooth-pursuit eye movements and a cortico-tectal pathway for saccades.

We have started to record single-units from the DLPN of a rhesus monkey trained to execute saccades to remembered locations („memory saccade task"). So far, we have recorded from 50 single-units, we would provisionally assign to the DLPN, based on their locations relative to conspicuous electrophysiological landmarks such as the oculomotor nuclei and additional information derived from MRI scans. Out of these, 8 exhibited clearcut directionally selective saccade-related responses, characterized by strong pre- or perisaccadic bursts of discharge. In several cases, these bursts were preceded by a tonic increase of activity in the „memory period", in which the monkey was required to memorize the spatial location of the peripheral target in the absence of visual information. These preliminary electrophysiological observations strengthen the hypothesis of a cortico-pontine pathway for saccades, which involves the DLPN, and bypasses the classical cortico-tectal pathway for saccades.

Supported by the German-Israeli-Foundation

579.10

SACCADIC GAIN ADAPTATION IS JUST THAT. J. Wallman*, A. F. Fuchs† and M. Pong†. Biology Dept., City College and Graduate School, CUNY, NY, NY 10031 and †Dept.of Physiol. & Biophysics, Univ. of Washington, Seattle, WA 98195

The brain maintains the accuracy of saccadic eye movements by adjusting their amplitudes relative to the distance of the target (the saccadic gain) based on the errors of recent saccades. Thus, in the classic paradigm, if one arranges for saccades to land beyond the target (by surreptitiously moving the target during the saccades), the saccade gain decreases over a few hundred saccades. We suppose that this saccadic gain adaptation occurs at the motoric level and uses a visual error signal. However, the error signal might be motoric (the brain noting whether the corrective saccades are in the same or opposite direction as the preceding primary saccade). Also, the gain change might simply reflect a remapping of visual space in the CNS. We tested these possibilities by two experiments in humans and rhesus monkeys.

To test whether the error signal is motoric, we used a paradigm suggested by Deubel. During the primary saccade to an initial target step, we stepped the target backward only briefly, so that fewer than 10% of corrective saccades were backward (~200 msec). The saccadic gain decreased over a few hundred saccades, even though most of the corrective saccades were forward by the end of the adaptation. Therefore, corrective saccades do not guide the direction of saccadic gain adaptation.

To test whether the gain adaptation involves visual remapping, we decreased the gain of 10° horizontal saccades by adaptation according to the classic paradigm described above, and then tested them to targets in retinal positions unused during the adaptation procedure, by having targets make a double step—the first vertical and the second horizontal—before the first saccade is made. The adapted gain transfers to 10° horizontal saccades made to these new locations.

We conclude that saccadic gain adaptation involves an error signal that is visual, not motor, but the adaptation is motor, not visual.

Supported by NIH EY00745, RR00166 and the National Science Foundation

579.11

EFFECT OF DORSAL CEREBELLAR VERMAL LESIONS ON SACCADES AND PURSUIT IN MONKEYS, M. Takagi, D.S. Zee*, R. Tamargo. Neurology Dept., Johns Hopkins Hospital, Baltimore, MD, 21287.

We investigated the effect of aspiration of the dorsal cerebellar vermis (predominantly lobules 6 and 7) on saccades and pursuit in three rhesus monkeys. We analyzed immediate effects, on saccade accuracy and dynamics (velocity and acceleration) to target steps, and on pursuit initial acceleration and steady-state gain to step-ramp stimuli. We also measured saccade and pursuit adaptive capabilities using double step paradigms (position steps for saccades and velocity steps for pursuit).

Saccades: In most cases saccades became hypometric postop, (82% on average). Only one monkey showed a transient hypermetria, for leftward saccades. In some cases saccade dynamics were also altered, especially changes in acceleration and deceleration, but these did not necessarily correlate with changes in accuracy. Saccade gain adaptation (to a decreasing double-step stimulus, preop value about 10-15% decrease after 200 trials) was abolished in two monkeys, and impaired (50% of preop value) in a third.

Pursuit: Pursuit initial acceleration (avg accel in first 100ms of smooth tracking) was decreased in all monkeys (76% of preop). Steady-state tracking gains were also slightly decreased (92% of preop). Pursuit adaptation (to a double-step stimulus, target velocity doubled or halved at the onset of smooth tracking) was also impaired in all monkeys (78% of preop value for increasing paradigm and 52% for decreasing paradigm).

These results implicate the dorsal cerebellar vermis directly in both immediate (online) and in adaptive control of saccades and pursuit. They also suggest that cerebellar cortex alone has an important role in saccade and pursuit adaptation.

Supported by NIH EY01849.

LONG-TERM POTENTIATION: PHYSIOLOGY III

580.1

THREE-DIMENSIONAL ORGANISATION OF SYNAPTIC VESICLE ENSEMBLES IN RAT HIPPOCAMPUS IN RELATION TO SYNAPTIC EFFICACY. D.A.Rusakov*, M.G.Stewart, H.A.Davies, G.Richter-Levin and T.V.P.Bliss. (SPON: Brain Research Association). Dept. of Biology, The Open University, Milton Keynes MK7 6AA, UK; Dept. of Psychology, Univ. of Haifa, 31905 Israel; National Institute for Medical Research, London NW7 1AA, UK.

The spatial organisation of synaptic vesicles (SVs) in pre-synaptic boutons is believed to play a functional role in synaptic transmission. Visible groupings of SVs in excitatory synapses from area CA1 of rat hippocampus were examined in an electron microscopic study. In each animal, 50-90 single section micrographs of synapses were sampled randomly. Euclidean co-ordinates of each SV and of related active synaptic zone (AZ) edges were transported to a data base. The data were used in order: (i) to establish the probabilities of SV-AZ distance classes, including stereological estimation via analytical unfolding; (ii) to estimate frequencies of the positioning of SVs with respect to AZs within synaptic boutons. In several synapses, the organisation of SV ensembles in space was reconstructed and visualised via a serial section study.

Analysis of SV co-ordinates has revealed two distinct sub-population of synapses, one with relatively high, and one with low, accumulation of SVs in the proximity of AZs. A phenomenon showing the lower occurrence of SVs in the centre, compared to the edges, of AZs has also been discovered.

The results provide insights into the possible molecular machinery of synaptic transmission where SV-AZ distance reflects the probability of recruitment of a SV for mediator release. The second stage of the study will compare SV ensembles contralaterally within the paradigm of long-term potentiation induced in one hemisphere of the rat hippocampus.

Supported by *BBSRC Grant S02085*

580.2

SYNERGISTIC ACTION OF GABA-A AND NMDA RECEPTORS IN THE NEONATAL RAT HIPPOCAMPUS.

Y. Ben-Ari *, R. Khazipov, I. Khalilov, & X. Leinekugel
INSERM Unite 29, 123, Bd de Port-Royal, Paris, 75674, FRANCE

In the neonatal hippocampus, GABA acting via GABA-A receptors has a depolarizing action because of elevated intracellular Cl-. We have used now non dyalizing patch-clamp recording technique and confocal microscopy to study the interactions between GABA and glutamate mediated currents in neonatal hippocampus. Our observations can be summarized as followings: 1) Electrical stimulation of stratum radiatum evoked 3.9±1.2 spikes in cells-attached recordings from pyramidal cells (n=13). The AMPA-R antagonist CNQX (10μM) did not modify significantly the response, whereas D-APV (50μM) reduced it to 1.3±0.2 spikes, that were blocked by further application of bicuculline (10μM). This suggests that GABA-A and NMDA receptors mediate most of the excitatory synaptic transmission in neonatal slices, whereas AMPA-Rs are quiscent; 2) GABA-A agonist isoguvacine attenuated the voltage-dependent Mg-block of single NMDA channels recorded in cell-attached configuration; 3) Isoguvacine also augmented the calcium influx through NMDA channels as visualized with confocal microscopy in neurons loaded by Fluo-3AM. We conclude that in neonatal hippocampus, GABA is the principal "fast" acting excitatory transmitter. GABA activates NMDA receptor by removing Mg-block, i.e. it plays the role conferred to AMPA-R in adult. This synergistic action underlies a novel form of LTD that prevails at this early stage (see McLean et al.) and probably other roles subserved by GABA and glutamate receptors in the developing brain.

Supported by INSERM and Ministère de la Recherche et de l'Espace (MRE).

580.3

A NOVEL LONG TERM DEPRESSION IN THE NEONATAL RAT HIPPOCAMPUS REQUIRING SYNERGISTIC ACTIVATION OF $GABA_A$ AND NMDA RECEPTORS

H.A. McLean, O. Caillard, Y. Ben-Ari, A. Majllart* and J-L Gaiarsa
INSERM Unité 29. 123 Bd de Port Royal. Paris 75674, France.

During the first 4 days of postnatal life (P0-P4), GABA provides the main excitatory drive in the CA3 region of the rat hippocampus. At the same time, GABA provides sufficient membrane depolarization for NMDA-R activation (see abstract: Ben-Ari et al). We have discovered that this $GABA_A$ / NMDA synergy underlies a novel form of tetanic LTD expressed by $GABA_A$-R (LTD_{GABA-A}). Tetanic stimuli (TS; 100 Hz, 1 second) delivered to the stratum radiatum in the presence of CNQX (10 µM) induced a sustained (> 60 min) depression of the peak amplitude and the initial slope of monosynaptic $GABA_A$ postsynaptic potentials (PSPs) to 57% and 49% of control values respectively. Tetanic LTD_{GABA-A} requires: (1) membrane depolarization during TS mediated by $GABA_A$-R as LTD_{GABA-A} was prevented by either voltage clamping cells at -60 mV during TS (n=5) or by bath application of bicuculline (10 µM) during TS (n=6); (2) activation of NMDA-R as LTD_{GABA-A} was prevented by bath application of D-APV (50 µM) during TS (n=5); (3) an increase in intracellular calcium levels as LTD_{GABA-A} was prevented by loading cells with BAPTA (50 mM) (n=5). Furthermore, bath application of NMDA (30µM) at potentials more positive than -40 mV induced sustained (>45 min) decreases in both the amplitude and initial slope of $GABA_A$ PSPs to 63% and 56% of control values respectively. LTD_{GABA-A} is a novel form of synaptic plasticity demonstrating that synergistic actions of GABA and NMDA receptors have important functional consequences in the developing hippocampus. Supported by INSERM.

580.4

DEFICIENCY IN INDUCTION BUT NORMAL EXPRESSION OF LTP IN HIPPOCAMPAL SLICES FROM YOUNG RATS.

D. Liao* & R. Malinow, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY11724 and *Dept. of Physiology & Biophysics, U. of Iowa, Iowa City, IA52242.

We have investigated the developmental changes of LTP in the CA1 region of hippocampal slices from rats of postnatal day 4-14. Consistent with previous results, we found that tetanus-induced LTP in field recordings is diminished in slices from younger animals ($n=30$, $p<0.01$). Tetanus-induced LTP in whole-cell current-clamp recordings is also diminished in younger animals ($n=20$, $p<0.02$). However, robust LTP can be induced in young animals if sufficient postsynaptic depolarization is directly provided by the recording electrode with a pairing protocol (average age = 7.3 days; $n=12$, $p<0.01$). In whole-cell current-clamp recordings, we found a positive correlation between the number of action potential spikes during tetanus and age of animal ($n=25$, $p<0.05$), and a positive correlation between the amount of potentiation and the number of spikes ($n=19$, $p<0.04$). Several measures of synaptic function also suggest that synaptic density is lower in younger animals. These results indicate that the smaller LTP in field and whole-cell current-clamp recordings are due to insufficient postsynaptic depolarization during LTP induction rather than a defect in expression mechanisms.

Supported by an NIH grant to RM.

580.5

ADENOSINE A2 RECEPTORS UPREGULATE THE POSTSYNAPTIC AMPA COMPONENT IN HIPPOCAMPAL LTP.

D.J. Mogul* & K. Kessey. Depts. of Biomed Engineering & Neurobiol. Northwestern University, Evanston, IL 60208 USA

Increases in neuronal firing elevate levels of extracellular adenosine in the brain. We examined the role of A_2 receptors in both normal synaptic transmission and tetanus-induced long-term potentiation (LTP) in rat hippocampal slices. Extracellular postsynaptic field potentials (EPSP) recorded in stratum radiatum of CA1 were measured in response to presynaptic stimulation of the Schaffer collaterals. Addition of the A_2 agonist DPMA increased the EPSP evoked by low frequency test pulses (0.033Hz). DPMA had no effect on paired-pulse facilitation suggesting that the effects were not presynaptic. Consistent with this, DPMA did not affect the NMDA component of the EPSP but selectively increased the AMPA component. The A_2 antagonist DMPX blocked or significantly reduced the induction of tetanus-induced LTP (100Hz; 1s) but had no effect on LTP maintenance when the A_2 antagonist was perfused after LTP induction in control solution. Furthermore, LTP induction normally blocked with the NMDA antagonist AP5 could be induced by concurrent A_2 activation. However, this induction was occluded by a train of saturating tetani applied prior to AP5 and DPMA indicating the two potentiations were acting via a convergent pathway. These results suggest that A_2 receptors may play an important role in the modulation of hippocampal transmission and LTP by regulating postsynaptic AMPA receptor-channels. Supported by the NIH (NINDS NS31764) and the Whitaker Foundation.

580.6

Metabotropic glutamate receptor antagonists inhibit both NMDA receptor-dependent and -independent LTP in the dentate gyrus *in vivo*.

D. Manahan- Vaughan and K.G. Reymann*. Federal Institute for Neurobiology, Brenneckestr. 6, D-39118 Magdeburg, Germany.

This study examined the role of metabotropic glutamate (mGluR) receptors in NMDA receptor-dependent and -independent long-term potentiation (LTP). The group 1 mGluR antagonist (S)-4-carboxyphenylglycine (4CPG), and the group 1 and 2 antagonist (RS) -α -methyl -4 -carboxyphenyl -glycine (MCPG) were used. D (-)-2- amino-5-phosphonopentanoic acid (AP5) was used to confirm the NMDA receptor contribution in the LTP recorded.

Male wistar rats underwent implantation of stimulating and recording electrodes into the granule cell layer of the dentate gyrus (DG). Following 5 days recovery, the field excitatory post-synaptic potential slope function (fEPSP) and population spike amplitude (PS) were measured from freely moving animals. Drug concentrations used were 4 -30 mM 4CPG, 20 mM AP5 and 200 mM MCPG in an injection volume of 5 µl. Drugs were applied 30 min before tetanus via a cannula implanted the lateral cerebral ventricle.

200 Hz tetanisation produced an LTP of fEPSP and PS which was inhibited by pre-tetanus application of AP5. MCPG completely inhibited LTP by 2h post-tetanus. A similar effect, which was dose-dependent, was obtained with 4CPG. Neither drug had an effect on baseline when compared with vehicle injected controls. LTP induced by 400Hz tetanisation was not inhibited by AP5. However MCPG and 4CPG inhibited the development of this LTP by 2h. These results support a role for mGluRs in both NMDA receptor-dependent and -independent LTP, and implicate group 1 mGluRs in these phenomena.

580.7

12-HYDROPEROXYEICOSATETRAENOIC ACID INDUCED THE LONG-TERM POTENTIATION IN RAT HIPPOCAMPAL CA1.

M. Nishiyama*[1], N. Hori[2], T. Watanabe[1], T. Hori[1], K. Suzuki[3], E. Maru[3] and T. Shimizu[4] [1]Div. of Neurosurg., Inst. of Neurol. Scis., Tottori Univ. Sch. of Med., Yonago 683, [2]Dept. of Pharmacol., Kyushu Univ., Fac. of Dent., Fukuoka 812, [3]Dept. of Physiol., Nippon Med. Sch., Tokyo 113, [4]Dept. of Biochem., Fac. of Med., Univ. of Tokyo, Tokyo 113, Japan.

We previously demonstrated that at least two distinct receptors for 12-hydroperoxyeicosatetraenoic acid (12-HPETE) existed in the canine hippocampus and that 12-lipoxygenase translocated in the synaptic area, according to the enhancement of synaptic potential by seizure in vivo. In this report, we confirmed these results by using brain slices in vitro. Intracelluar response (EPSP), population spikes and population EPSP (pEPSP) induced by Schaffer collateral stimulation were recorded from the pyramidal cell, cell layer and the dendric trees in CA1 area, respectively. Spike potentiation, induced by tetanic stimulation is remarkably augmented by the pretreatment of 100 nM 12-HPETE(267.8± 44.0%, n=8) and 500 nM arachidonic acid (197.0 ±18.5%, n=8), as compared with control ones (144.0 ± 10.1%, n=16). 12-HPETE (100 nM) itself induced LTP-like potentiation (pEPSP: 196.0±31.6%, n=10; EPSP: 162.3 ±7.4%, n=4), which were not blocked by 50 µM APV. Furthermore, 12-HPETE did not affect the impedance of postsynaptic membrane, or enhance the postsynaptic responses to iontophoretically applied quisqualate and NMDA. Theta burst stimulations also induce the LTP (pEPSP: 150.9 ±9.5%, n=8; EPSP: 148.8 ± 22.3%, n=4) without the enhancement of postsynaptic glutamate responses. Thus, 12-HPETE is suggested to be one of retrograde messengers, which predominantly acts in the presynaptic site, playing a critical role in the synaptic plasticity.

580.8

DISSOCIATION OF LTP OF AMPA AND NMDA RECEPTOR-MEDIATED SIGNALS: EVIDENCE FOR GLUTAMATE SPILL-OVER AND PRESYNAPTIC CONTRIBUTION TO EXPRESSION OF LTP.

Dimitri M. Kullmann* & Fredrik Asztély. Dept. Clinical Neurology, Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom.

Is LTP of NMDA receptor-mediated signals always accompanied by LTP of AMPA signals? To prevent induction of conventional LTP we first dialysed 25 CA1 pyramidal cells from guinea pig hippocampal slices for 45 minutes with whole-cell pipettes containing 5 mM BAPTA. Pure NMDA receptor-mediated EPSCs were elicited by holding the cells at +40 mV in 10 µM DNQX, and stimulating two pathways at 0.1 Hz. Tetanic stimulation (2 x 50 pulses, 100 Hz) while holding the cells at - 80 mV caused a small potentiation when compared to the control pathway at 25 minutes (11 ± 5 %, mean ± S.E.M., p = 0.033, paired t test). In 17 other slices we simultaneously recorded AMPA receptor-mediated signals with whole-cell and extracellular field electrodes. We elicited LTP of the field EPSPs with two 100 pulse 100 Hz tetani (54 ± 5 %, $p < 10^{-6}$). By clamping individual pyramidal cells at -80 mV during the tetani, LTP of the AMPA EPSCs was prevented (1 ± 6 %, p = 0.870). In contrast, when the NMDA component was subsequently compared to the control pathway, it was again significantly potentiated (14 ± 6 %, p = 0.036). Tetanic LTP of NMDA EPSCs can thus be elicited under conditions designed to prevent LTP of AMPA signals. We propose that NMDA receptors sense glutamate spill-over from terminals which are presynaptic to neighboring cells. LTP at these synapses potentiates presynaptic glutamate release, causing an increase in the NMDA signal, but not in the AMPA signal because of the lower affinity of AMPA receptors. This explains (i) differential potentiation of AMPA and NMDA signals; (ii) increased quantal content with LTP; (iii) greater variability of AMPA than NMDA components of EPSCs; and (iv) the observation that pure NMDA EPSCs can be elicited with minimal stimulation. (Supported by the MRC and the Wellcome Trust)

580.9

TETANIC LTP OF NMDA RECEPTOR-MEDIATED EPSCS IN CA1 IS ASSOCIATED WITH AN INCREASED RATE OF DEPRESSION BY MK-801. Gül Erdemli* & Dimitri M. Kullmann. Dept. Clinical Neurology, Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom

Manabe & Nicoll (*Science* 265:1888, 1992) looked for presynaptic expression of LTP in CA1 by measuring the rate at which the use-dependent blocker MK-801 depressed NMDA receptor-mediated EPSCs. Prior induction of LTP by pairing low-frequency stimulation with postsynaptic depolarization had no detectable effect on the rate at which the EPSCs decayed with repeated stimulation, implying no change in the opening probability of NMDA receptors. Pairing-induced LTP is however associated with very little potentiation of the NMDA component. We have therefore repeated these experiments with tetanic LTP, since this has been reported to be associated with an increase in the NMDA component. We elicited EPSCs in CA1 pyramidal cells of guinea pig hippocampal slices (holding potential -80 mV). Tetanic LTP was elicited in one pathway (2 x 100 pulses at 100 Hz) while holding the cells at 0 mV. Non-NMDA receptors were subsequently blocked by DNQX (10 μM), and the cells held at +40 mV. When compared to the control pathway, the NMDA component was also potentiated, although less than the non-NMDA component (21 ± 9 % and 63 ± 11 %, respectively, means ± S.E.M.; n = 25). MK-801 (40 μM) caused a faster decay of successive NMDA receptor-mediated EPSCs in the tetanized pathway than in the control pathway (difference in single exponential time constants: 20 ± 8 %, $p < 0.02$). Our results support an increase in glutamate release probability. To explain the apparent disagreement with Manabe & Nicoll (1992), we propose that much of the NMDA signal arises from spill-over of glutamate from terminals which are presynaptic to neighboring cells. Tetani, but not pairing, induce LTP at these synapses, increasing the spill-over, and explaining the larger potentiation of the NMDA receptor-mediated signals and faster decay with MK-801. (Supported by the MRC)

580.10

2-DEOXYGLUCOSE (2DG)-INDUCED LTP IN HIPPOCAMPAL CA1 NEURONS REQUIRES Ca^{2+} RELEASED FROM INTERNAL STORES. **S. Tekkök* and K. Krnjević Anaesthesia Research and Physiology Depts., McGill Univ., Montréal, PQ H3G 1Y6 Canada.**

In hippocampal slices from Sprague-Dawley rats, a temporary block of glycolysis by 2DG is followed by a sustained increase in EPSPs (2DG LTP) (Tekkök and Krnjević, J. Neurophysiol. 74:2763, 1995). To investigate a possible role of Ca^{2+} in 2DG LTP, the effects of 2DG on EPSPs were tested in slices exposed to Ca^{2+}-free media (at 33° C). When 8 slices were perfused for 25 min with Ca^{2+}-free ASCF (in 6 cases with 100-200 μM EGTA added), and then for 13 min with Ca^{2+}-free ACSF containing 10 mM 2-DG instead of glucose, the return to standard ASCF was followed by the usual 2DG LTP -- the slope of the afferent volley-EPSP (V-E) relation increased by 88 ± 10.5 % (SEM). But after Ca^{2+}-free treatment for 77 min, similar 13 min applications of 2DG caused only a very small LTP (V-E slope increased by 17 ± 7.3 %, n=6, P<0.05). To control for a possible loss of synaptic plasticity after prolonged Ca^{2+}-free perfusion, 5 slices were perfused with Ca^{2+}-free medium for 90 min, and then with standard ACSF for 60 min before the 2DG application: there was a 2-DG LTP by 34 %, indicating substantial conservation of synaptic plasticity. In similar experiments on NMDAR-mediated EPSPs, 25 min of Ca^{2+}-free perfusion did not prevent a large 2DG LTP (V-E slope increased by 96 ± 9.9%, n=2). But after longer Ca^{2+}-free treatment, NMDAR-mediated EPSPs failed to recover from 2-DG effect (n=6).

These results suggest that Ca^{2+} is required for the induction of 2-DG LTP; but the necessary Ca^{2+} probably comes from an internal stores which is not readily depleted by Ca^{2+}-free perfusion.

(Supported by Canadian MRC and Hacettepe University, Ankara)

580.11

2-DEOXY-D-GLUCOSE (2DG) CAUSES A RAPID BUT REVERSIBLE INCREASE IN CYTOPLASMIC $[Ca^{2+}]$ IN HIPPOCAMPAL NEURONS. **K. Krnjević*, I. Medina and Y. Ben-Ari. INSERM Unité 29, 123 bd Port Royal, 75014 Paris, France.**

In view of the very predictable sustained potentiation of EPSPs produced by 2DG in CA1 neurons (Tekkök and Krnjević, *J. Neurophysiol.*, 74: 2763, 1995) - and evidence that like other types of LTP, 2DG-induced LTP is Ca-dependent (Tekkök and Krnjević, this meeting) - we were interested in possible changes in $[Ca^{2+}]$ produced by temporary applications of 2DG.

As in the previous experiments, 2DG (10 mM) was substituted for glucose in the ACSF. Changes in intracellular calcium concentration $[Ca^{2+}]_i$ were estimated by measuring Fluo-3 fluorescence using confocal scanning microscopy. Fluo-3 was loaded in two ways: 1) by local external application of 1 μM Fluo-3 AM in CA1 pyramidal layer; 2) by loading of 10 μM Fluo-3 using a whole-cell patch micropipette.

In all experiments, whether on cultured hippocampal neurons (at room temperature) or in slices (kept near 30°C), from Wistar rats at 5, 15 or 30 days, 2DG applications lasting 5-12 min induced without fail a marked increase in fluorescence of CA3 and CA1 pyramidal neurons. The change was always detectable within 1-2 min. It usually reached a plateau near the end of the 2DG applications and persisted for at least 10 min subsequently. In 4 CA1 cells (in slices at P30), further washing with glucose-containing ACSF for a total of 30 min led to full return of fluorescence to the initial baseline.

Major suppression of inward currents by TTX, Cd^{2+} and CNQX did not prevent the rise in $[Ca^{2+}]_i$. In preliminary tests, this effect of 2DG appeared to be reduced by dantrolene but not by thapsigargin (both 10 μM). We conclude that 2DG consistently raises $[Ca]_i$ of hippocampal neurons and that this may be the intracellular signal that initiates the 2DG-induced LTP.

580.12

LTP DEVELOPMENT IS RESTRICTED TO TETANIZED SIDE IN HIPPOCAMPUS. Janea Mack and Sanika Chirwa*. Physiology, Meharry Medical College, 1005 DB Todd Blvd, Nashville, TN 37208

The objective of the present study was to characterize the use-dependent changes in synaptic efficacy occurring across ipsilateral and contralateral CA1 synapses following paired-pulse (interpulse intervals, 30 msec at 0.01 Hz, for 5-10 pairs) or brief tetanic stimulation (100 Hz, 1 sec; 3 trials given at 5 sec intervals) applied to ipsilateral CA3 afferents. For this purpose, 5 male guinea pigs (gp) weighing 150-200 g, anesthetized with urethane (1500 mg/kg IP) had small access holes made in the skull to allow for stereotaxic placement of a stimulating electrode in ipsilateral CA3 region. Similarly, an electrode was positioned in the ipsilateral and in the contralateral CA1 dendritic regions to record field EPSPs. Subsequently, the CA3 afferents were activated (0.02 Hz) to elicit CA1 dendritic field EPSPs (0.5-1.0 mV). It was found that both paired-pulse facilitation (4 of 5 gp) and LTP (3 of 5 gp) were readily induced in the ipsilateral CA1 dendrites. In contrast, LTP development did not occur in the contralateral CA1. In addition, the contralateral CA1 exhibited no change (4 of 5 gp) or some depression (1 of 5 gp) to paired pulse stimulation. These results suggest that development of synaptic LTP across ipsilateral CA1 synapses does not co-occur with LTP across the contralateral CA1 synapses. This attribute may be important in determining bi-directionality during spatial navigation, as LTP in the hippocampus appears to be involved in this process. Supported by AID Grant HNE-5053-G-00-5093-00, NIH RCMI Grant G12-RR03032 and NIH NIGMS Fellowship 5-F31-GM17065-02.

ISCHEMIA: MECHANISMS

581.1

NITRIC OXIDE AND PEPTIDE GROWTH FACTORS DIRECTLY MODULATE PROTEIN KINASE C ACTIVITY. M. TenBroeke, I. Kue, R. P. Lisak*, and K. Maiese. Dept. of Neurology, Center for Molecular Medicine, Wayne State University School of Medicine, Detroit, MI, 48201

Both the protective ability of peptide growth factors and the neuronal degeneration following exposure to nitric oxide (NO) are dependent upon the pharmacological down-regulation of the signal transduction pathways of protein kinase C (PKC) (Maiese, et al. *J Neurosci Res* 36: 77-87, 1993; Maiese, K. and Boccone, L. *J Cereb Blood Flow Metab* 15: 440-449, 1995). In primary hippocampal neurons, we characterized the ability of NO and the trophic factors, basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF), to directly regulate the cellular activity of PKC. PKC activity was measured by a commercial P^{32} enzyme assay and results were expressed as a percent change from baseline. Initially, neuronal cultures were exposed to the NO generators sodium nitroprusside (300μM) and SIN-1 (300 μM) for 5 minutes and PKC activity was assessed at 2, 6, and 24 hours post NO exposure. PKC activity rapidly increased over baseline within 2 hours (31±8%) and gradually returned to baseline at 6 hours (19±8%) and 24 hours (4±5%) following NO administration (n=13, p<0.001). Treatment with the phorbol ester phorbol 12-myristate 13-acetate (PMA) at a neuroprotective concentration of 1μM down-regulated PKC activity by 88±5% in normoxic cultures and actively reversed the NO induced increase in PKC activity by 79±6% (n=6, p<0.001). When administered alone, the trophic factors bFGF and EGF increased PKC activity over baseline by 26±5% and 54±11%, respectively (n=8, p<0.001). In contrast, bFGF and EGF individually reduced PKC activity in the presence of NO by approximately 65% (n=8, p<0.001). We demonstrate that the pharmacological inhibition of PKC activity directly antagonizes NO induced elevations and that the peptide growth factors actively down-regulate PKC activity during NO toxicity. These results suggest that NO toxicity and neuroprotection by bFGF and EGF are directly linked, in part, to the active modulation of the signal transduction pathways of PKC.

Supported by the Alzheimer's Association, AHA (National), J&J Focused Giving Award, NIH, and the United Cerebral Palsy Foundation.

581.2

PEPTIDE GROWTH FACTORS ACTIVELY REVERSE NITRIC OXIDE INDUCED PROGRAMMED CELL DEATH. K. Maiese*, I. Kue, and M. TenBroeke. Dept. of Neurology, Center for Molecular Medicine, Wayne State University School of Medicine, Detroit, MI, 48201

Peptide growth factors, and the subsequent signal transduction pathways that they modulate, are neuroprotective during nitric oxide (NO) induced neurodegeneration (Maiese, K. and Boccone, L. *J Cereb Blood Flow Metab* 15: 440-449, 1995). Yet, the molecular mechanisms of neuronal protection are not well defined. In primary hippocampal neurons, we characterized the role of programmed cell death (PCD) during administration of NO and the peptide growth factors basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF)wear condensation and DNA fragmentation was considered consistent with PCD and was documented by hematoxylin and eosin stain, terminal-deoxynucleotide transferase-mediated dUTP-digoxigenin nick end-labeling, and transmission electron microscopy. NO toxicity was achieved with the administration of the NO generators sodium nitroprusside (300 μM) and SIN-1 (300 μM). Induction of PCD following NO exposure alone was both robust and rapid with neurons positive for PCD increasing from 20±4% (0 minutes) to 50±5% (60 minutes) and to 75±6% (24 hours). Consistent with PCD, NO induced neuronal degeneration was reduced from 79±6% to 28±5% by the inhibition of either transcriptional or translational protein synthesis confirmed by an S^{35} methionine assay. Basic FGF (10 ng/ml) or EGF (10 ng/ml) administered 24 hours prior to NO exposure increased neuronal survival from 30±5% (NO only) to 68±4% (bFGF) and to 70±4% (EGF) (n=10, p<0.001). In addition, the peptide growth factors actively decreased PCD expression over a 24 hour period from 80±7% (NO only) to 30±4% (bFGF) and to 32±4% (EGF) (n=10, p<0.001). Assessment of protein synthesis during trophic factor administration revealed a reduction in NO induced protein synthesis by both bFGF and EGF of approximately 20%. Thus, NO induced neurodegeneration is a dynamic process that is directly reversible at the molecular level by peptide growth factors.

Supported by the Alzheimer's Association, AHA (National),. J&J Focused Giving Award, NIH, and the United Cerebral Palsy Foundation.

581.3

NEURONAL NITRIC OXIDE SYNTHASE (NOS) INHIBITION ATTENUATES ISCHEMIC EXCITATORY NEUROTRANSMITTER RELEASE.

R Kahn, M Panah, S Kiffel, J Weinberger*, Departments of Anesthesiology and Neurology, The Mount Sinai Medical Center, New York, NY 10029.

Nitric oxide (NO) is an important modulator of excitatory neurotransmitter release (ENR) during cerebral ischemia/reperfusion (ISC/R). In this study, we examined the role of specific neuronal NOS inhibition (nNOSI) on ENR and GABA release during global cerebral ISC/R using the novel nNOSI 7-nitroindazole (7-NI).

Microdialysis probes were stereotaxically inserted into the striatum of 24 Sprague-Dawley rats. Dialysates were analyzed by HPLC for neurotransmitter release. Rats received 0, 5, 10 or 20 mg/kg 7-NI IP. Global forebrain ischemia was induced for 15 minutes (ISC). ENR continued to be measured in 15 minute epochs for one hour during reperfusion (R1-R4).

Large increases in glutamate (GLU), glycine (GLY), GABA, and aspartate (ASP) occurred during ISC, which returned to basal levels during reperfusion. 7-NI administration resulted in a dose-dependent attenuation of GLU release during ISC/R. Significant decreases in GLY release as well as a more rapid return to basal levels were observed with nNOSI compared to placebo. Although trends towards decreases in ASP and GABA release with nNOSI were observed, these differences were not statistically significant.

Previous studies using non-specific constitutive NOSI did not demonstrate differences in ENR during ischemia and did demonstrate increases in ENR during reperfusion.[1] This nNOSI mediated attenuation of GLU and GLY release as well as the avoidance of sustained GLY release during reperfusion may help elucidate the role of NO in ischemic damage in the striatum.

1. Stroke 1995;26:298-304.

581.4

EFFECTS OF CEREBRAL ISCHEMIA ON PROSTAGLANDIN (PG)-INDUCED VASODILATION IN PIGLETS.

D. Busija*[1], F. Bari,[1,2] R. Errico,[1] and T. Louis.[3] [1] Bowman Gray Sch. of Med., Winston-Salem, NC 27157, [2]Albert Szent-Györgyi Med. Univ., Szeged, Hungary H-6720, and [3]East Carolina Univ. Sch. of Med., Greenville, NC 27858

Prostaglandins are important regulators of the cerebral circulation, but effects of ischemia on PG-induced dilation are unclear. We examined the effects of iloprost, a stable prostacyclin analog, and PGE_2 on cerebral arterioles prior to ischemia, and 1, 2, and 4 hours following 10 minutes of total, global ischemia. Baseline arteriolar diameters in piglets were ~100 μm. Ischemia was induced by increasing intracranial pressure. Topical application of iloprost dilated arterioles by 10±0.3% at 0.1 μg/ml and by 21±3% at 1 μg/ml, and ischemia reduced dilation at 1 hour to 3±1% and 8±1% for 0.1 and 1 μg/ml, respectively (n=6). However, arteriolar dilation returned towards normal over 2-4 hours. In contrast, PGE_2-induced dilation was unaffected by ischemia (n=6). In control animals, we found that glibenclamide, an inhibitor of ATP-sensitive K^+ channels (K_{ATP}), blocked arteriolar dilation to iloprost (n=6) but not PGE_2 (n=4). Finally, administration of indomethacin (5 mg/kg) prior to ischemia preserved dilation to iloprost (n=5). We conclude that transient changes in vascular reactivity to iloprost are caused by impaired K_{ATP} function following ischemia, and that reduced responsiveness to iloprost can be prevented by prior treatment with indomethacin. Supported by HL-30260, HL-46558, and HL-50587.

581.5

INDOMETHACIN ATTENUATES INCREASES IN iHSP 70 FOLLOWING GLOBAL CEREBRAL ISCHEMIA IN PIGLETS.

T. C. Beasley* [1], F. Bari[1,3], C. Thore[1], T. Louis[2], N. Thrikawala[1], and D. W. Busija[1], [1]Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157, [2]East Carolina Medical School, Greenville, NC 27858, and [3]Albert Szent-Györgyi Medical University, Szeged, Hungary.

Changes in protein metabolism following ischemia may determine the course of brain damage and/or recovery. In anesthetized, newborn pigs, we investigated indomethacin modulation of ischemia related changes in inducible heat shock protein 70 (iHSP 70) levels in brain. Total global ischemia was produced for 5-10' by raising intracranial pressure. Cessation of blood flow was verified visually and by microsphere technique. iHSP 70 levels were evaluated by western blotting and localized by immunohistochemistry. Silver staining demonstrated moderate neuronal necrosis following 5' ischemia compared to time-control tissue while 10' ischemia resulted in extensive neuronal necrosis. Western blotting revealed a significant ($p<0.05$) elevation in iHSP 70 levels after 6 hours of reperfusion over time control samples in hippocampus (195±69%, n=8), cerebellum (136±45%, n=7), and cerebral cortex (111±39%, n=6). iHSP 70 immunoreactivity (IR) was observed primarily in neurons. Ischemia also increased glial fibrillary acidic protein (GFAP) IR of astrocytes. Administration of 5 mg/kg indomethacin (i.v.), prior to ischemia attenuated the ischemia-induced increase in iHSP 70 observed in the hippocampus and cerebral cortex (89±30% and 7±30%, respectively n=5, $p<0.05$ compared to ischemia). Similarly, indomethacin administration reduced the extent of GFAP(+) IR. Indomethacin pretreatment had no apparent effect on the iHSP 70 increase in the cerebellum (186±65%, n=5, $p<0.05$). We conclude that indomethacin pretreatment attenuates ischemia-induced increases in iHSP 70 and GFAP in hippocampus and cerebral cortex. Supported by NIH grants HL30260, HL46558, and HL50587.

581.6

Modulation of heat shock factor proteins by glutamate receptor antagonists in focal cerebral ischemia.

Stephen L. Minger*, James Geddes, Mary Holtz, Susan Craddock and L. Creed Pettigrew. Department of Neurology and Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY.

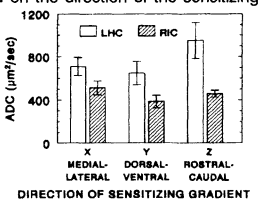
Heat shock proteins, molecular chaperones that function as markers of neuronal stress, are induced following experimental brain injury or neurodegenerative disease. We examined whether glutamate receptor antagonists could modulate neuronal injury responses that occur in the wake of permanent focal cerebral ischemia. Male spontaneously hypertensive rats pretreated with either 30 mg/kg NBQX (i.p., 30 minutes prior),10 mg/kg CGS 19755 (i.v., 5 minutes prior) or saline were subjected to tandem common carotid and middle cerebral arterial (CCA-MCA) occlusion. After 24 hours, only CGS 19755 pretreatment significantly reduced infarct volume, whereas both NBQX or CGS 19755 ameliorated cytoskeletal proteolysis and calpain activation (Minger et al., 1996. *Stroke*. 26, 191). In ischemic controls, expression of heat shock proteins (hsp) -72 and -32, well-characterized markers of neuronal stress, were detected only after 24 hours of CCA-MCA occlusion. Hsp72 immunoreactivity was observed in discrete neuronal populations in healthy tissue outside the infarct, with hsp32 immunoreactivity restricted to glia surrounding the infarction border. Pretreatment with either glutamate receptor antagonist, however, significantly altered the distribution and temporal expression of both heat shock proteins after focal ischemia. Large numbers of hsp72 immunoreactive neurons were now detected within the infarct after 24 hours, whereas cellular hsp32 expression was induced after 6 hours and a shift from exclusively glial to mixed neuronal and glial hsp32 expression was observed after 24 hours in the infarcted cortex. These studies are among the first to report that glutamate receptor antagonism modifies both the distribution and cellular pattern of heat shock protein expression following ischemia. Supported by the American Health Assistance Foundation/National Heart Foundation (SLM) and R01 NS33773-01A1 (LCP).

581.7

ANISOTROPY IN APPARENT DIFFUSION COEFFICIENT (ADC) IN THE EARLY HOURS FOLLOWING FOCAL CEREBRAL ISCHEMIA.

A.D. Perez-Trepichio, A.W. Majors, Y. Wang, A.J. Furlan, T.C. Ng, S.C. Jones*. Cerebrovascular Research Laboratory, Cleveland Clinic Foundation, Cleveland OH 44195.

Diffusion-weighted imaging (DWI) has enormous potential in the early detection of brain ischemic insult. We studied the contribution of the direction of the diffusion-sensitizing gradient pulses in the ADC in rat focal cerebral ischemia. Seven Sprague-Dawley rats were anesthetized (isoflurane, N_2O, O_2) and underwent permanent direct occlusion of the right middle cerebral artery and both common carotid arteries. MR-DWI were obtained with a GE 4.7 T magnet (TR=1500 ms/TE=35 ms; Slice thickness 2 mm; 2 slices, b=0, 261, 586 and 1042 sec/mm^2). ADC images with an acquisition window of 101 ± 33 min (mean ± sd) from stroke onset were generated. Mean ADC values (± sd) in the x (medial-lateral), y (dorsal-ventral), z (rostral-caudal) axis in the core of the hypointense area on the right ischemic cortex (RIC) and the left homologous area (LHC) are shown in the Figure (RIC vs LHC, $p<0.01$, n=7, RM-ANOVA). Expectedly, each ADC is lower on the ischemic side. The percent decreases (RIC/LHC) in $ADC_{x,y,z}$ are 72, 61, and 50%, respectively, (x vs z, $p<0.025$, Newman-Keuls) with the largest change being in the z direction. Changes in ADC during ischemia depend on the direction of the sensitizing gradient. It is possible that the z axis is particularly sensitive to the cortical columnar neuroarchitectural organization of the cerebral cortex. These results suggest that anisotropic changes of water diffusion occur in ischemia and that the utilization of separate axes for the estimation of ADC can better demonstrate the damaged area following focal cerebral ischemia in gray matter. (Support: NIH NS30839)



581.8

SERIAL DIFFUSION (DWI) AND PERFUSION MR IMAGING (PI) IN ACUTE STROKE PATIENTS DEMONSTRATES REMARKABLE VARIABILITY IN THE EVOLUTION OF TISSUE INJURY IN THE ISCHEMIC ZONE.

LH Schwamm, AG Sorensen, W Copen, G Gonzalez, W Koroshetz*, Neurology and Radiology, Massachusetts General Hospital, Harvard Medical School, Boston MA 02114.

PI traces the entry of an intravenous bolus of contrast to non-invasively assess cerebral blood volume as well as kinetic characteristics related to blood flow. DWI identifies regions of lowered water diffusion occurring when ATP levels fall. PI/DWI images localize decreased perfusion and early ischemic tissue changes in acute stroke patients (Radiology 1996). The evolution of ischemia and ischemic tissue injury has been determined using these techniques in animal stroke models.

We performed serial PI/DWI and conventional MR imaging in 8 patients with presumed embolic strokes. Each patient received up to 5 studies over the first 3 days after stroke onset. The initial scan was always performed within 3-12 hours of symptom onset and a final scan at 7 days. The studies in these patients highlight the remarkable variability and relative slowness in the evolution of clinical stroke and ischemic tissue injury. Within regions of abnormal blood flow (abnormal PI), it was common to find early ischemic tissue changes (abnormal DWI) continue to develop 12-36 hours after stroke onset (6/8 patients). In general, regions that became abnormal on DWI progressed to completed infarction, whereas regions abnormal on PI demonstrated significant variability in the progression to infarction. Improved perfusion occurred over a wide range of times during the study in some patients (5 hours-4 days), and not at all in others.

These data demonstrate that ischemic stroke can be a prolonged and dynamic process in humans. Brain regions that eventually infarct may remain normal on DWI (preserved ATP levels) for hours after stroke onset, before progressing to infarction. The MRI-defined "time window" of reversible brain injury (i.e., abnormal PI without abnormal DWI) is much longer in humans than has been published in studies of animal stroke models.

Supported by a grant from the Pfizer Corporation

581.9

SERIAL DIFFUSION MAPPING OF SPREADING DEPRESSION LIKE DEPOLARIZATIONS IN NORMO- AND HYPERGLYCEMIC RATS

T. Els, J. Röther, C. Beaulieu, A. de Crespigny, D. Kunis*, M. Moseley. Lucas MRS Imaging Center, Dept. of Radiology, Stanford University Medical School, Stanford, California, USA

We investigated the hypothesis, that hyperglycemia influences the initiation and propagation of spreading depression like periinfarct ischemic depolarizations (SD) in focal cerebral ischemia of rats. Rats (n=6) were rendered hyperglycemic by a single i.p. injection of streptozotocin (60mg/kg BW) 36 hours before induction of focal cerebral ischemia using the intraluminal suture model. Results of control rats (n=7) were previously published (Röther et al., *JCBFM* 1996;16:214-220) and re-evaluated according to a standardized protocol. The initiation and propagation of remotely induced focal ischemia and periinfarct SD was monitored using ultrafast diffusion weighted MR imaging. Maps of the apparent diffusion coefficient (ADC) were calculated. SD related transient ADC decreases were followed with a temporal resolution of 12-18 sec. Hyperglycemic rats revealed a significant prolongation of the latency time between induction of ischemia and start of the ADC decrease as compared to controls (3.7±1.2 min. vs. 1.5±1.3 min.; p=0.04). The time to the maximal ADC decrease was significantly increased in hyperglycemic rats (5.1±1.8 min. vs. 2.8±0.7 min.; p=0.01). Transient ADC decreases occurred in the periinfarct tissue of both experimental groups that represent extra- / intracellular water shifts during SD. Hyperglycemic rats showed significantly faster recovery of transient ADC declines in the area adjacent to the ischemic core (4.72±1.29 min. vs. 7.3±2.66 min. in controls, p=0.026). Tissue perfusion as assessed by dynamic contrast enhanced susceptibility MRI did not reveal significant differences between the two groups. Conclusions: 1) Hyperglycemia delays the anoxic depolarization after focal ischemia and 2) supports a faster repolarization in severely mal-perfused areas indicating the increased demand of energy in the ischemic core and periinfarct tissue

581.10

INCREASED PLASMA LIPID PEROXIDATION IN PATIENTS WITH STROKE.

M.C. Polidori*, W. Koroshetz, B. Frei, G. Rordorf and M.F. Beal. Neurology Service, Massachusetts General Hospital, Boston, MA 02114 and Boston University, Boston, MA

Cerebral ischemia followed by reperfusion, may stimulate lipid peroxidation. We measured cholesterol ester hydroperoxides (CE-OOH) in 10 patients with lacunar stroke (6M, 4F, 76±9.7 yo) and 19 patients with large cortical infarcts (16M, 3F, 64.4±16.8 yo) diagnosed on the basis of the TOAST subtype classification system. Four plasma samples were obtained from each patient every other day. Lipid peroxidative damage was measured with a sensitive and selective HPLC assay with chemiluminescence detection. The neurological deficit was monitored in all patients with daily administration of NIH Stroke Scale and Glascow Coma Scale. We found higher levels of CE-OOH (nmol/l) in the plasma of the cortical stroke patients with respect to lacunar stroke patients at all timepoints. There was a strong positive correlation between the levels of lipoperoxides and the NIH Stroke Scale scores ($r2=0.7$, $p<0.002$) and a negative correlation between CE-OOH concentrations and Glascow Coma Scale scores ($r2=-0.7$, $p<0.003$), both at the 7th day from the stroke onset. Levels of plasma CE-OOH are an excellent biological marker of clinical outcome in stroke. Supported by NS108288.

581.11

DIMENSIONAL COMPLEXITY OF THE EEG IN A CASE OF SUBCORTICAL STROKE

M. Molnár*[a], Gy. Gács[b], J. E. Skinner[c], G. Ujvári[a] and G. Karmos[a]

[a]Institute for Psychology, Hungarian Academy of Sciences, Budapest, H-1394 Hungary

[b]Peterfy Hospital, Budapest, Hungary

[c]Tott's Gap Medical Research Laboratories, Bangor, USA

The application of recently developed methods of chaos theory makes it possible to use deterministic measures to the analysis of time series such as the EEG. By these means inferences can be drawn as to the complexity of the generator producing the analyzed signal. Consequently, the functional integrity of the cortical areas where the analyzed EEG was recorded from can be assessed. The case of a 62 year old female patient is described who suffered embolism from the heart in the area of the right internal capsule and lentiform nucleus - as shown by CT scan - causing left sided faciobrachial hemiparesis. The EEG was recorded by 22 scalp electrodes in "eyes open" and "eyes closed" conditions. A low dimensional area was seen in the right parietal area the size of which changed dynamically corresponding to the different recording conditions. Conventional analysis of the EEG (power spectra) revealed no apparent slow activity in the same region. It is suggested that the observed low dimensional EEG activity was probably caused by the ischemic damage of the thalamocortical projection system on the same side. The non-linear methods of analysis may in cases be more sensitive to detect pathology than the conventionally used procedures.

581.12

Antimyoclonic effects of riluzole and benzamide in posthypoxic myoclonus.

A.G. Kanthasamy, T.Q. Vu, A. Tran and D.D. Truong.* Parkinson & Move. Disord. Lab., Dept. of Neurology, Univ. of California, Irvine, CA 92717.

Since neuronal overexcitation has been linked to neurological consequences in posthypoxic conditions, our previous study using nitric oxide synthase inhibitors (L-NAME and 7-nitroindazole) demonstrated that nitric oxide plays a neuromodulatory role in posthypoxic myoclonus. In the present study, to further confirm that a nitric oxide cascade is involved in posthypoxic myoclonus, compounds that intervene in upstream and downstream events of nitric oxide synthase activation, namely riluzole (glutamate release inhibitor) and benzamide (inhibitor of poly-ADP-ribose-synthetase), were tested in a cardiac arrest-induced posthypoxic animal model of myoclonus. The test compounds were administered to cardiac arrested rats and audiogenic myoclonus was quantitated for 3 hr. Riluzole (4-12 mg/kg i.p.) dose-dependently attenuated myoclonus and higher doses produced a rapid and long lasting response persisting for more than 3 hr. Preliminary histological examination of riluzole-treated animals showed decreased neuronal damage in the cortex, basal ganglia and hippocampus as compared to untreated cardiac arrested rats. Also, benzamide at 75 and 150 mg/kg, i.p. significantly ($p<0.01$) attenuated audiogenic myoclonus. Taken together with data showing antimyoclonic effects of several serotonergic agents, the results suggest that nitric oxide-mediated neuromodulation of serotonergic system may be involved in the pathophysiology of posthypoxic myoclonus. Further mechanistic studies underway to explore the interaction between nitrergic and serotonergic systems.

CELL DIFFERENTIATION AND MIGRATION IX

582.1

MECHANISMS OF ANTERIOR PITUITARY CELL TRANSDIFFERENTATION.

S.A. DeRiemer*, Division of Biomedical Sciences, Meharry Medical College, Nashville, TN 37208.

Somatotrophs from the adult rat anterior pituitary gland transdifferentiate into mammotrophs via a dual hormone secreting mammosomatotroph intermediate. This process, which may occur *in vivo* during the normal pregnancy/lactation cycle of females, can be induced *in vitro* by addition of serum or a partially purified serum factor to FACS purified somatotrophs. Two sets of experiments have been carried out to extend these findings: 1) Characterization of other possible inducers which has identified a soluble factor released by rat pituitary and human epidermal fibroblasts and 2) Analysis of the mechanism of the phenotypic respecification which has identified changes is the pattern of tyrosine phosphorylated proteins. Methods: Mixed populations of pituitary cells were prepared by percoll density gradient centrifugation of cells dissociated from adult male or female Sprague-Dawley rats. Cells were cultured in serum free defined medium with or without the addition of hormones/growth factors or fibroblast condictioned medium for up to 2 weeks. The extent of transdifferentiation was assayed by immunocytochemistry or reverse hemolytic plaque assay with PRL and GH antisera (NIDDK). Western blots were probed with anti-phosphotyrosine antibodies to monitor phosphorylation Results: Mixed cultures had previously been shown to have a lower response to serum than FACS purified cells. Panning to remove fibroblasts prior to plating increased the response to serum. Fibroblast conditioned medium could substitute for serum addition and human epidermal fibroblast conditioned medium was as effective as medium conditioned by pituitary fibroblasts. Incubation with 10% horse serum or fibroblast conditioned medium for 15 min. -24 hrs induced increased tyrosine phosphorylation of at least two proteins of Mr. 75 kd and 56 kd. Supported by RCMI (5-G12-RR03032); MBRS (2-SO6-GM-08037); and MRCE (HRD-92-55157.

582.2

A MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISATION OF PITUITARY ADENYLATE CYCLASE ACTIVATING PEPTIDE (PACAP) INDUCED NEURONAL DIFFERENTIATION OF PC12 CELLS

A. P. Barrie, G. Ballantyne, J. N. Brown and J. M. Allen*
Division of Biochemistry and Molecular Biology, Glasgow University, Glasgow G12 8QQ, U.K.

When exposed to Nerve Growth Factor (NGF) PC12 cells differentiate into a sympathetic neuronal phenotype and have been used to map in detail the NGF induced MAP kinase cascade.

Receptors for PACAP have been found on these cells, and we find that a low concentration of PACAP (5nM) induces the cells to differentiate into a neuronal phenotype distinct from that of NGF.

In morphological studies, TRITC-phalloidin staining of actin filaments, shows that whereas NGF treated cell neurites have many branches and filopodia the PACAP treatment results in longer, less branched neurites with fewer filopodia.

Western blotting shows that PACAP unlike NGF does not induce expression of the growth cone protein, GAP-43.

The effect of PACAP on MAP kinase activation was measured and compared to the response of NGF. PACAP (5nM) causes a prompt increase in MAP kinase activity which remains elevated for at least 1 hr after stimulation. In contrast to NGF, the PACAP response is independent of ras, and comparable stimulations are seen in ras negative and wild type PC12 cells. The effects of various kinase inhibitors on the PACAP stimulation will be shown.

Supported by grants from the Wolfson and Wellcome Trusts.

582.3

CONNEXIN43 TRANSFECTION DELAYS NEURONAL DIFFERENTIATION OF PC12 CELLS. A.F. Andrade-Rozental*, R. Rozental, M. Urban, M. El-Sabban and D.C. Spray. Dept. Neurosci., A. Einstein Coll. Med., Bronx, NY 10461, Inst. Biophys. "Carlos Chagas Filho", Fed. Univ. Rio de Janeiro, Brazil.

We have shown that there is a reciprocal relationship between electrotonic coupling *via* gap junctions and expression of neuronal phenotype during differentiation (*Dev. Biol.* **167**: 350, 1995). However, whether this temporal relation implies functional connection remained to be determined. To investigate this relationship, we have used PC12 cells, a rat pheochromocytoma cell line which can differentiate into neurons. Specifically, we evaluated whether constitutive expression of Cx43, a Cx-type expressed in immature neuroblasts (see Rozental *et al.*), prevents the differentiation of PC12 cells treated with NGF (50 ng/ml). We have used molecular biological immunocytochemical and electrophysiological techniques. Clonal sublines were established from the parental population. Among the clones selected, we have concentrated on two termed "D" and "J". The "D" clone expressed low incidence of dye coupling (LY; 20%) and g_j (200 -2000 pS). By contrast "J" clone was virtually uncoupled and fully differentiated; this clone when transfected with Cx43 ("J"-Cx43; 60% coupled by LY) did not undergo pronounced differentiation. We show an inverse relationship between differentiation and strength of coupling such as "J" clones > "D" clones > > > > stably transfected "J"-Cx43. These results support the hypothesis that intercellular coupling modulates neuronal differentiation.

582.4

EXPRESSION OF UBIQUITIN GENES IN PC12H CELLS INDUCED BY NGF TREATMENT.

R. Ohtani-Kaneko*, K. Takada(1), M. Asahara(2), K. Kakuta(3), M. Hara, M. Iigo, K. Ohkawa(1), H. Yokosawa(4), K. Hirata. Dept. of Anatomy, St. Mariannna Univ. Sch. of Med., Kawasaki, Japan 216. (1) Dept. of Biochem.[1], Jikei Univ. Sch. of Med., Tokyo, Japan. (2) Dept. of Pediatrics, St. Marianna Univ. Sch. of Med., Kawasaki, Japan. (3) SRL Inc., Tokyo, Japan. (4) Dept. of Biochem. Faculty of Pharmacological Science, Hokkaido Univ. Sapporo, Japan

Ubiquitin is a highly conserved 76 amino acid protein, functioning in a variety of cellular activities. Recently, ubiquitin has been suggested to be involved in neuronal differentiation. Takada et al. ('94) showed that levels of high molecular weight (HMW) ubiquitin-protein conjugates were increased in PC12h cells after NGF treatment and that inhibition and promotion of neurite outgrowth resulted in blockade and induction, respectively, of HMW ubiquitin-conjugates. Furthermore, we found that the level of monoubiquitin was decreased and that of multi-ubiquitin chains was increased in water-soluble nuclear extract of NGF-treated PC12h cells. All these results suggested the possibility that ubiquitination, making multi-ubiquitin chains on target proteins to form HMW ubiquitin-protein conjugates, was induced by NGF.

In the present study, we investigated effects of NGF on gene expression of ubiquitin. Ubiquitin genes have been cloned in various species which are divided into three types depending on the number of repeated ubiquitin. We compared NGF-induced changes in expression of these three ubiquitin genes by Northern hybridization, using an porcine ubiquitin coding region probe.

582.5

IDENTIFICATION AND CLONING OF GENES REGULATED BY NERVE GROWTH FACTOR M. Grønborg, M. Lauritzen* and S. Gammeltoft, Dep. of Biochemical Chemistry, Glostrup Hospital, DK-2600 Glostrup

Nerve Growth Factor (NGF) induces growth arrest and neurite outgrowth in PC12 rat pheochromocytoma cells, whereas other growth factors such as Epidermal Growth Factor (EGF) and Insulin-like Growth Factors (IGFs) support survival and stimulate growth. NGF-induced genes includes the immediate early genes (IEGs), which encode transcription factors like *c-fos*, NGF-IA, NGF-IB and *c-myc*. The products of the IEGs are thought to be involved in regulating expression of genes, which are associated with the neuronal phenotype such as various neurofilaments, peripherin, GAP43 and transin. However, IEGs known to be regulated by NGF presently are also induced by EGF and IGFs indicating, that although they are essential, they are not sufficient for neuronal differentiation. In order to identify new early genes involved in neuronal differentiation, we have analyzed gene expression in PC12 cells before and after 2 hr of NGF stimulation by differential PCR-display. A total of 10 bands potentially representing NGF-regulated mRNAs have been observed. These have been cloned and sequenced and represents both known genes encoding thrombospondin, the LDL receptor and cytokeratin in addition to sequences with unknown functions. Characterization of expression pattern of the unknown sequences and their functional significance during neuronal differentiation are in progress.

This work were funded by Glostrup Hospital and The Danish Research Council.

582.6

EFFECTS OF BONE MARROW STROMAL CELLS ON NEURONAL DIFFERENTIATION OF NEUROBLASTOMA CELLS. L. H. So, K. O. Lai, N. Y. Ip and M. F. Leung*. Dept. of Biology, Hong Kong Univ. of Science & Technology, Hong Kong.

Bone marrow stromal cells have been shown to play an important role in the proliferation and differentiation of hemopoietic cells. The effect of bone marrow stromal cells on the growth regulation of other cell types is not clear. In human, it has been shown that malignant neuroblastoma cells can metastasize into bone marrow. The neuroblastoma cells isolated from the affected bone marrow can attach to the stromal cell layer to grow and extend neurite outgrowth. To study the effects of bone marrow stromal cells on the growth and differentiation of neuroblastoma, we have isolated bone marrow stromal cells from cultured bone marrow of leukemia patients. Phase-bright cell bodies and neurite extensions typical of neuronal cells were observed in PC-12 and IMR-32 cells after treatment with either conditioned medium or cellular protein extracts of the bone marrow stromal cells. Co-culture of bone marrow stromal cells and neuroblastoma NG108-15 cells also resulted in neuronal phenotype. A stromal cell line developed by mutageniting the primary stromal cells with *N*-methyl-*N'*-nitrosoguanidine resulted in a more pronounced effect in inducing neuronal differentiation. Our findings suggest that in addition to their roles in hemopoietic cells development, bone marrow stromal cells may also affect neuronal differentiation.

582.7

PROTHROMBIN SYNTHESIS IN HUMAN NEURONS IS REGULATED BY RETINOIC ACID. V.B. Mahajan, D.D. Cunningham*. Dept. of Microbiology and Molecular Genetics, University of California, Irvine CA 92717.

In neuronal culture systems thrombin influences growth cone collapse, neurite retraction, viability and differentiation while thrombin inhibitors reduce synapse elimination and cell migration. Although prothrombin mRNA has been detected in the CNS, it remains uncertain whether an endogenous, regulated source of thrombin protein is available in the brain to activate thrombin receptors. In this study, prothrombin synthesis was demonstrated in cultured human retinoblasts (Ad12Her10) and in a human neuroblastoma (SK-N-SH) cell line. Anti-prothrombin antibody was incubated with conditioned medium from cells labeled labeled with [^{35}S]-methionine and [^{35}S]-cysteine. Following SDS-PAGE of immunoprecipitates, a 72 kD band was detected by autoradiography. This band corresponded to the molecular weight of prothrombin and an identical band isolated from human liver cells (HepG2). Furthermore, the 72 kD band was specifically eliminated when excess, unlabeled prothrombin was added to conditioned medium. To exclude the possibility that the band was a complex formed by serum-derived thrombin with labeled thrombin inhibitors, hirudin was added to the conditioned medium; hirudin is a potent inhibitor of thrombin and prevents the formation of thrombin complexes. Even with hirudin treatment, the 72 kD band remained intact. To investigate the regulation of prothrombin synthesis and secretion, factors important during neurodevelopment were screened. Interestingly, application of retinoic acid caused a rapid time and dose dependent decrease in prothrombin synthesis and secretion. Recognized as a potent regulatory and differentiating factor during development, retinoic acid mediates effects opposite to those of thrombin such as neurite extension. Thus, brain-synthesized prothrombin may serve as a developmentally regulated source of thrombin in the nervous system. (Supported by NIH Grant AG 00583)

582.8

Neural Crest Derived Frontonasal Mesenchymal Cells May Provide Retinoid Signals To Regulate Olfactory Pathway Morphogenesis. Raymond M. Anchan* and Anthony-Samuel LaMantia. Department of Neurobiology, Duke University Medical Center, Durham, N.C. 27710

Our recent observations suggest that the olfactory epithelium and bulbs do not develop in PAX-6 mutant (small eye-*sey/sey*) mice due to a failure in retinoic acid (RA) signaling from the frontonasal mesenchyme. This embryonic mesenchyme is a mosaic of cells including a GAP-43 immunoreactive subpopulation that is absent in *sey/sey* mice. We asked whether these GAP-43 immunoreactive cells have characteristics that imply they are neural crest derived and produce RA. If so, their absence in *sey/sey* mice may reflect migratory anomalies of the cranial neural crest reported previously in *sey/sey* rats (Matsuo et al., (1993) Nat Genet: 299). Frontonasal mesenchymal cells in the mouse express neural crest associated molecules including the low-affinity nerve growth factor receptor, cellular RA binding proteins, and NCAM. Some of these antigens are coexpressed by GAP-43 immunoreactive cells. Using an RA responsive indicator cell assay, we correlated RA signaling with populations of GAP-43 immunoreactive cells. Next we compared RA responses elicited by explants of various mesenchymal regions from different age embryos. At early stages RA signals generated by mesenchyme from either the branchial arch or optic stalk are at least 2X greater than that from frontonasal mesenchyme. At later stages RA signaling from the olfactory epithelium mesenchyme increases. Thus changes in RA signaling coincide with cranial crest migration. It is therefore possible that a distinct population of neural crest derived RA-producing cells migrates to the frontonasal mesenchyme and subsequently provides local RA signals essential for olfactory pathway formation. Supported by NRSA 1 F32 DC00244-01 to R.M.A. and NIH HD29178 to A-S.L.

582.9

THE UBIQUITOUS EXPRESSION OF A PERLECAN- RELATED PROTEOGLYCAN WHICH ACTIVATES FIBROBLAST GROWTH FACTORS IN NEURAL DEVELOPMENT. S. J. Joseph , M. D. Ford, V. Nurcombe* and U. Greferath. Dept. of Anatomy and Cell Biology, Univ. of Melbourne, Parkville, Vic. Australia , 3052.

A heparan sulfate proteoglycan, called BRM (brain regulated molecule),was isolated through its ability to potentiate neural cell responses to either FGF-1 or FGF-2 (1). Amino acid sequence of the core protein of BRM is homologous to the large proteoglycan, Perlecan . However, BRM has a significantly smaller core protein of 45 kD as compared to the 400 kD Perlecan core. Analysis of an embryonic mouse neuroepithelial cell line indicated that a 96 bp cDNA probe homologous to both Perlecan and BRM detected Perlecan at 12.6 kb, as well as novel transcripts at 6.5 and 3.5 kilobases. The latter species appears by virtue of its size and abundance to be the BRM transcript. In addition polyclonal antibody specific to BRM detects a single species at 290 kD , whereas a polyclonal anti-Perlecan antibodies cross-reacts with BRM as well as detecting native Perlecan and three other variant isoforms. These results suggest that Perlecan variants exist on an RNA and protein level, and that BRM is a homologous variant of Perlecan which is differentially glycosylated in a manner that confers specific function. Immunohistochemical studies reveal a differential staining pattern for BRM as compared to Perlecan; BRM is highly expressed in neuroepithelial cells through-out embryonic neural development. The BRM antibody also detects a single species in various tissues of adult mouse, and has recently been shown to have a broad distribution. This ubiquitous proteoglycan is found in the extracellular matrix of several tissues and is expressed by various epithelial cells . This proteoglycan, which plays a critical role in neural development appears to have a function in many different tissues.

1. Nurcombe *et al*. (1993). *Science*, **260**, 103-106.

NH&MRC grant, Australia

582.10

EFFECTS OF ACETYLCHOLINE AND ITS ANALOGUES ON MEDIAL SEPTAL NEURONS IN PRIMARY CULTURE, R.L. Kenigsberg* and Y. Hong, Centre de Recherche Pediatrique, Hôpital Ste-Justine, Montréal, Québec, Canada H3T 1C5

Although forebrain cholinergic cell loss has been documented to be associated with learning and memory deficits in aging and Alzheimer's disease, cholinergic replacement therapy does not appear to sufficiently prevent intellectual decline. Furthermore, the roles of acetylcholine (ACh) in the basal forebrain are actually not fully understood. We thus recently examined the effects of ACh and its analogues on the survival and differentiation of cholinergic and GABAergic neurons derived from an important basal forebrain cholinergic nucleus, the medial septal nucleus from fetal rat brain (embryonic day 17) maintained in culture. After the cells were cultured in serum-free chemically-defined media in the presence of muscarinic or nicotinic receptor agonists or antagonists for 7 days, the cells were harvested and choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) activities measured by radiometric assays. We found ACh to markedly elevate both ChAT and GAD activities without altering general cell survival. These effects were mimicked by bethanechol (muscarinic agonist) as well as nicotine. Surprisingly, pirenzepine (M_1 antagonist) also increased ChAT activity, although it did not affect GAD. When glia were eliminated from these cultures by treating with the antimitotic 5-fluorodeoxyuridine (10^{-5}), the cholinergic response was significantly attenuated while the induction of GAD was unaltered. Furthermore, exclusion of glia from these cultures did not change the effects of pirenzepine on either neuronal cell type. These results suggest that activation of cholinergic receptors (both M and N) up-regulates medial septal neuronal maturation and glia are implicated in these effects on cholinergic but not GABAergic neurons. The pirenzepine-induced increase in ChAT implicates a differential distribution of M_1 receptors on some groups of cells, such as the GABAergic neurons for example. (Supported by MRC of Canada)

582.11

THE ROLE OF CALCIUM CHANNELS IN CHROMAFFIN CELL DIFFERENTIATION BY LOW FREQUENCY MAGNETIC FIELD STIMULATION (LFM).

Consuelo Morgado-Valle[1], Leticia Verdugo-Díaz[2] and René Drucker-Colín[1-2]*

[1] Instituto de Fisiología Celular, and [2]Departamento de Fisiología, Facultad de Medicina, UNAM, México.

Adrenal chromaffin cells have an endocrine phenotype and secrete adrenaline. When these cells are cultured with nerve growth factor (NGF) , they differentiate into a neural-like phenotype with a conspicuos neurite-outgrowth and preferentially secrete noradrenaline.The exact mechanism of differentiation is not well understood, since multiple events are involved. One such mechanism may be related to calcium movements across the plasma membrane, through voltage-dependent calcium channels. In this work we have studied the role of calcium channels in the differentiation process, while comparing NGF and LFM field stimulation as the differentiation stimuli. Chromaffin cells were cultured in the presence or absence of NGF or under LFM stimulation of 7 Gauss during 7 days (4hrs/day). We tested L and N type calcium channel blockers (Nifedipine 0.1 and 0.3 uM, ω-conotoxin 0.5 and 1 uM) and L- type channel agonist Bay K 8644 (5 uM) , to determine the participation of calcium ions in the mechanism of neurite outgrowth. In another set of experiments we tested K^+ induced noradrenaline release in nifedipine cultured chromaffin cells, with or without LFM field stimulation.. Our results indicate that there is a significant inhibition of neurite outgrowth when cells are cultured in presence of chronic nifedipine only in cells stimulated with LFM field ($p<0.001$). ω-conotoxin a potent N-type calcium channel blocker did not affect neurite outgrowth of cells neither in the presence of NGF or LFM High K^+ induced noradrenaline release, is not affected when cells are cultured in the presence of chronic nifedipine with NGF, LFM field or both. These results suggest that L-type Ca^{2+} channels are involved in chromaffin cell differentiation induced by LFM stimulation .

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING VI

583.1

THE EFFECT OF VARIOUS SUBSTRATES ON THE ORIENTATION OF CHICK DRG NEURONS IN APPLIED ELECTRIC FIELDS M. E. McGinnis*, B. A. Green, E. L. Tuck, O. Jarrett, and B. Livingston. Biology Department, Spelman College, Atlanta, GA 30314-4399.

The orientation of neurons in an electric field is a well described phenomenon. Neurites usually extend toward the cathode of an electric field, resulting in asymmetric distribution of neurites. The purpose of this study is to compare the behavior of neurons grown on various substrates when placed in electric fields. Adhesion and motility of growth cones are highly dependent on both specific and nonspecific characteristics of the substrate molecules. Redistribution of the cellular receptors for these molecules in electric fields may be one of the mechanisms for the galvanic effect.

DRG neurons from 8.5 - 9 day old chick embryos were isolated and seeded onto glass coverslips coated with various substrates such as glass, collagen type IV, laminin, fibronectin, and matrigel. These coverslips were then placed in chambers for applying uniform DC electric fields. The orientation, length, and point of origin of neurites were determined from high resolution video images. Quantitative measures of orientation were determined for a range of field strengths (1.0 mV/mm – 100 mV/mm). The resulting dose response curves were compared for the different substrates.

Supported by NIH grant # S06GM08241-11 to M. E. M.

583.2

CLONING OF HUMAN NEOGENIN AN IG SUPERFAMILY PROTEIN RELATED TO DCC

J. Vielmetter*, X. Cheng, K Yamakawa, R. Lane, F. Miskevich, J. Korenberg, and W. J. Dreyer. Division of Biology, California Institute of Technology, Pasadena, CA 91125

Neogenin is a cell surface protein belonging to the immunoglobulin (Ig) superfamily and was first characterized in the chicken. Neogenin's structure deduced from its amino acid sequence is predicted to be composed of four Ig domains followed by six fibronectin type III (FnIII) domains, a transmembrane domain and an alternatively spliced intracellular domain. Neogenin shares this domain structure with the human tumor suppressor molecule Deleted in Colorectal Carcinoma (DCC). Neogenin also shares a significant sequence similarity with DCC, and both molecules therefore define a subgroup of the Ig superfamily proteins that is structurally distinct from other Ig molecules such as for example N-CAM, Ng-CAM, Bravo/Nr-CAM, axonin, and contactin. Expression studies in the chicken embryo using a monoclonal antibody against chicken neogenin have revealed that neogenin expression correlates with the onset of neuronal differentiation. We have now cloned the human homolog of neogenin. Human and chicken neogenin share over 90% similarity and are both alternatively spliced in identical regions in the intracellular domain. Human neogenin is expressed in the human embryonic and adult brain and maps to the chromosomal location 15q22.2-22.3 as determined by fluorescence insitu hybridization (FISH). Neogenin maps within a gene locus associated with the Bardet Biedel syndrom, a genetic defect causing severe mental retardation, obesity and several other defects. The cloning and sequencing of human neogenin not only proves that neogenin and DCC are paralogs and not species homologs but also allows to determine genetic linkage of neogenin to known or yet unknown genetic defects in humans. Supported by NEI

583.3

NEUROTRIMIN, A MEMBER OF A SUBFAMILY OF NEURAL CELL ADHESION MOLECULES, PROMOTES HOMOPHILIC ADHESION AND NEURONAL OUTGROWTH. O. D. Gil*, G. Zanazzi, A. Struyk, and J. Salzer, Department of Cell Biology, New York University Medical Center, New York, NY 10016.

Several cell adhesion molecules of the immunoglobulin superfamily (IgCAMs) have been implicated in the development of specific neuronal projections. Neurotrimin (Ntm) is a member of a differentially expressed subfamily of IgCAMs that also includes the opioid binding cell adhesion molecule (OBCAM) and the limbic system associated membrane protein (LAMP). These proteins have three Ig-like domains and are attached to the plasma membrane via a glycosyl-phosphatidylinositol (GPI) anchor. To characterize the function of Ntm, we generated a soluble recombinant chimera in which Ntm is fused to a human Fc domain (Ntm-Fc). We also expressed a myc-tagged Ntm in CHO cells and observed that Ntm accumulates at sites of cell contact. In addition, Ntm-Fc binds to Ntm expressing CHO cells but not to control cells transfected with the vector alone. Ntm-Fc also binds extensively to E-15 dorsal root ganglia (DRGs) primary neurons which express high levels of Ntm but not to E-21 superior cervical ganglia (SCGs) primary neurons which do not. The binding of Ntm-Fc to DRGs and to transfected cells was eliminated by pretreating the cells with PIPLC which specifically cleaves GPI anchored proteins. In neurite outgrowth assays, Ntm-Fc absorbed onto a nitrocellulose coated coverslip promotes the outgrowth of DRGs but not of SCGs. Taken together, these results indicate that Ntm mediates homophilic interactions that may be important in the formation of specific neuronal circuits by promoting adhesion and neurite outgrowth. Supported by NIH grant RO1NS33165 and NS26001.

583.4

IDENTIFICATION OF A PEPTIDE SEQUENCE INVOLVED IN HOMOPHILIC BINDING IN THE NEURAL CELL ADHESION MOLECULE L1. X. Zhao* and C.-H. Siu. Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5G 1L6, Canada.

L1 plays an important role in neural development and has been implicated in X-linked hydrocephalus and related neurological diseases. The extracellular region of L1 contains six immunoglobulin (Ig)-like domains, followed by five fibronectin type III-like repeats. We have previously demonstrated that the second Ig-like domain (Ig2) of L1 contains both homophilic binding and neuritogenic activities (Zhao and Siu, 1995, *J. Biol. Chem.* 270:29413-29421). Two Hydrocephalus/MASA syndrome-related mutations (R184Q and H210Q) have been mapped to the Ig2 domain. The R184Q mutation led to a complete loss of both homophilic binding and neuritogenic activities, while the H210Q mutation resulted only in a partial loss, suggesting that Arg184 might play an important role in L1 homophilic binding. Oligopeptides corresponding to sequences flanking Arg184 were synthesized, and their effects on L1 homophilic binding and neuritogenic activities were examined. The peptide PL1-2A corresponding to the sequence between His-178 and Gly-191 inhibited both Ig2 fusion protein and L1-mediated cell-cell adhesion, while several control peptides and peptide analogs had no effect. When neural retinal cells were cultured on a monolayer of L1 transfected cells, peptide PL1-2A was a potent inhibitor of L1-induced neurite outgrowth. Taken together, these results indicate that the homophilic binding site of L1 resides within the sequence HIKQDERVTMGQNG of Ig2.
(Supported by the Medical Research Council of Canada.)

583.5

RECOMBINANT FRAGMENTS OF Ng-CAM WITH DIFFERENT EFFECTS ON CNS AND PNS NEURITE GROWTH. S. Chikramane, D.R. Friedlander and M. Grumet*. Depts. of Pharmacology and Neurology, NYU Medical Center, 550 First Avenue, New York, NY 10016.

Ng-CAM is a cell adhesion molecule expressed on neurons and Schwann cells and is one of the most potent promoters of neurite growth in culture. To assess its potential use as a promoter of axonal regeneration in vivo, specific domains of Ng-CAM were analyzed for their stimulation of neurite outgrowth in vitro. Recombinant baculovirus proteins representing the 6 Ig domains (Ng16), the first 3 Ig domains (Ng13) and the 80 kD subunit that normally occurs in vivo (Ng80) were produced and isolated without denaturation. Dissociated DRG neurons extended processes on substrates coated with each recombinant protein. Neurite extension was highest for Ng16 and at levels comparable with purified Ng-CAM. Second was Ng13, and Ng80 was third. These observations suggest that the 135 kD is more potent than the 80 kD subunit in stimulating neurite outgrowth of DRG cells.

Dissociated neurons from CNS tissues including spinal cord, retina and optic tectum extended neurites on substrates coated with Ng16 at levels comparable to those elicited by purified Ng-CAM. In contrast, minimal neurite extension was observed on Ng80, and intermediate levels on Ng13. When explants or reaggregates were placed on these substrates, numerous very long processes extended on Ng16. In sharp contrast, only few, short neurites extended on Ng13 or Ng80, although a meshwork of neurites was present at the explant boundary. Given that dissociated cells extend neurites on Ng13, the lack of neuritic extension from explants on Ng13 may be due to a choice by neuronal growth cones to remain in the cellular environment rather than extending on Ng13. The superior activity of Ng16 suggests that multiple domains in Ng-CAM are required for optimal stimulation of neurite outgrowth and that Ng16 may have application as a promoter of axonal regeneration. *Supported by NSF IBN-9310731 and Acorda Therapeutics, Inc.*

583.6

A NOVEL CADHERIN-RELATED MOLECULE IS REGULATED BY SYNAPTIC ACTIVITY AND IN THE DEVELOPING CENTRAL NERVOUS SYSTEM. K.I. Andreasson*, C.A.Barnes and P. F. Worley. Depts of Neurology and Neuroscience, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205 and Depts. of Neurology, Psychology and Div. of Neural Systems, Univ. of Arizona, Tuscon, AZ 84724.

We have utilized differential cDNA cloning to identify genes rapidly regulated by activity in the rat hippocampus. One of the cDNAs isolated has an open reading frame that encodes a predicted protein which shows some homology to the family of protocadherins. The mRNA for IEG8 is regulated by a variety of activity-dependent stimuli. Two hours following a maximal electroconvulsive seizure, expression is induced by Northern analysis and *in situ* hybridization in the dentate gyrus and amygdala, peaks at four hours and is back to baseline at 12 hours. In chronically implanted animals, IEG8 mRNA was strongly induced in hippocampal granule cells ipsilateral to high frequency LTP stimulus two hours after stimulation. This induction was eliminated with pretreatment of the animals with MK-801. During fetal and post natal development, IEG59 is expressed in discrete patterns in developing cortex and spinal cord. These findings suggest a role of cadherin-related molecules in synaptic and developmental plasticity. Supported by MH53608, AG9219, and HD00992.

583.7

TERMINAL FIELD FORMATION BY THE DORSAL P NEURON IN THE LEECH: THE ROLE OF INTERACTIONS BETWEEN SIBLING PROCESSES.

H. Wang* and E. R. Macagno, Dept. Biol. Sci., Columbia University, New York, NY 10027

Pressure-sensitive (P) neurons in the leech CNS form terminal fields in the body wall of the animal early in embryogenesis. A characteristic of these terminal fields is that individual branches form arbors that do not overlap with one another, a property that has been called self-avoidance. For dorsal P neurons, the major field comprises six subfields that are established by six first-order branches and the higher order branches they produce. Time-lapse studies indicate that sibling processes from neighboring subfields have frequent but transient overlaps that last several minutes, the overlapping processes then retracting. Analysis of 3D images obtained from serial optical sections using a deconvolution algorithm suggest that at least half of such overlaps do not include direct contact of the respective processes. However, mechanically detaching parts of the terminal field induces the invasion of the processes from neighboring subfields within 24 hours. We are currently using a laser microbeam to investigate this further. These results imply that there are very strong growth-inhibiting interactions between sibling processes, but that they do not necessarily involve direct contact. Supported by NIH Grant NS-34545.

583.8

SELECTIVE AND STEREOTYPED PATHFINDING BY A DEVELOPMENTALLY MALLEABLE MOTOR NEURON IN THE MEDICINAL LEECH. J. Jellies*, T.M. Harik and B.M. Campbell. Department of Biological Sciences, Western Michigan University, Kalamazoo, MI 49008.

The neurons innervating the heart tubes in *Hirudo medicinalis* (the heart excitors, HE's) exhibit transient overgrowth of peripheral projections followed by loss of supernumerary terminals after innervation of the heart. The emerging heart muscle exerts a local influence over the peripheral terminals as well as a retrograde influence on recruitment of the HE's into the central motor circuit driving rhythmic activity (Jellies & Kopp, *Invert. Neurosci.* 1995. 1:145). As initial steps to investigate the heart-derived signals and their influence over HE development we have; 1) used intracellular labeling and immunohistology to examine the earliest choices of HE growth cones *in situ*, 2) confirmed the specificity of heart innervation by retrograde DiI tracing , and 3) begun to examine HE outgrowth using muscle-neuron co-culture. In the heart system, pathfinding and target selection seem to be distinct events and the initial overgrowth of HE axons is not random. HE growth cones all exit an existing pathway at stereotyped locations and pioneer longitudinally-directed arbors, including those that will be subsequently lost. Just after emergence of the heart, the HE makes very close appositions with it as evidenced by retrogradely labeled HEs following DiI injections into the embryonic heart. In adults, focal DiI injections into heart muscle in 14 segments labeled the HEs (and no other neurons) in 8 ganglia and no neurons in 6. Thus, while ultimate target choice is highly specific, the specificity of initial outgrowth does not play a direct role in target recognition or choice. Innervation of the heart arises not from target-dependent pruning of random projections but rather by virtue of a heart-derived influence that induces a distinct change in phenotype. We have begun to examine this by culturing denervated segments of heart muscle with CNS ganglia. To date, both heart muscle and regenerating axons adhere well to a polylysine substrate on glass, survive in L-15 medium, and exhibit profuse outgrowth after 3-5 days. We are currently examining this outgrowth for evidence of reinnervation and specificity. Supported by NSF 9609701 and a Sloan Foundation Fellowship (JJ).

583.9

DIFFERENTIATION OF NEURONAL PROCESSES DETERMINES THEIR RESPONSE TO INHIBITORY ENVIRONMENTAL CUES. A. Shibata*, M.V. Wright, and S.B. Kater,+ Department of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523; +Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, Utah, 84132.

Mammalian CNS neurons develop into polarized cells with distinct axonal and dendritic processes. Hippocampal neurons initiate undifferentiated minor processes, one of which becomes the axon (1.5 days in vitro (DIV)) and the rest mature into dendrites (after 4 DIV) (Dotti et al, 1988). Axons and dendrites are unique in many ways including their morphology, cytoskeletal organization, and mRNA content. Studies on other cell types have shown that inhibitory cues found on oligodendrocytes inhibit axonal outgrowth. The response of dendrites and axons from the same neuron to oligodendrocytes is not known. Based on the intrinsic differences between axonal and dendritic processes, we tested the idea that dendrites and axons of rat hippocampal neurons behave differently when encountering oligodendrocytes.

We have developed a high resolution culture system in which primary cultured oligodendrocytes (and control 3T3 fibroblasts) are presented to the growth cones of minor processes, dendrites, or axons of rat hippocampal neurons. Our studies show that axonal growth cones of neurons cultured for 1 or 6 DIV collapse upon encountering oligodendrocytes 88.5% (n= 26) and 93.3% (n=15), within an average of 12.2 min and 9.1 min, respectively. Similarly, the majority (65.4%, n=26) of minor processes collapse within ~11.3 min. after contacting oligodendrocytes. In contrast, only 17.4% (n=23) of MAP2-positive dendrites of hippocampal neurons cultured for 6 DIV collapse when encountering oligodendrocytes. These results suggest that as minor processes differentiate into axons and dendrites, dendrites appear to become unresponsive to oligodendrocytes whereas axons remain sensitive to this inhibitory environmental cue. Taken together the data reveal several previously unrecognized points including that collapse of growth cones following contact with oligodendrocytes is by no means an obligatory reaction.

Supported by NIMH 5F31 MH108-60-02 and NS 24683.

583.10

MYELIN-ASSOCIATED GLYCOPROTEIN (MAG) INDUCES COLLAPSE OF HIPPOCAMPAL AXONAL GROWTH CONES. S.B. Kater , P. E. Braun*, S. David~, L. McKerracher~ and A. Shibata# Dept. of Neurobiology and Anat., Univ. of Utah Sch. of Med., Salt Lake City, UT; +Dept. of Biochm,. McGill Univ., Montreal CA; ~Cent. for Res.in Neuroscience, Montreal Gen. Hosp.Res. Inst., Montreal, CA; #Dept. Anat. and Neurobiology, Colorado State Univ., Ft. Collins, CO.

Both during development and in the mature mammalian CNS the outgrowth of neuronal processes is constrained by inhibitory environmental signals. Molecules on oligodendrocytes appear in large part responsible for the inability of adult axons to regrow following injury. Two molecules associated with oligodendrocytes are reported to contribute to inhibitory activity: NI-35/ NI-250 and MAG. The native and recombinant MAG have been shown to inhibit neurite initiation *in vitro* (McKerracher et al, 1994, Mukhopadhyay et al. 1994) although its ability to inhibit elongating growth cones is unknown. Previously, inhibitory activity of particular substrates has been determined by their ability to elicit growth cone collapse *in vitro*. In a preceding abstract we employed a growth cone collapse assay to shown that axonal growth cones are inhibited by oligodendrocytes which expresses MAG and GAL-C in culture. Here, we utilized the same growth cone collapse assay to evaluate the inhibitory activity of MAG to cultured CNS neurons. Axonal growth cones of rat hippocampal pyramidal neurons were presented the with MAG coated polystyrene beads. Our results reveal that MAG coated beads (N=15) cause 10X more collapse (73%) of axonal growth cones than do uncoated beads (N=14). During collapse the growth cone becomes phase dark, losing an average of 80% of its lamellipodial surface area within approximately 25+/- 0.5min after initial contact. Unlike NI-35, this collapse response is not mediated through signaling by intracellular Ca2+. Axonal growth cones loaded with Fura-2AM showed no significant changes in intracellular Ca2+ levels preceding, during, or following the time of collapse (N=6). These results show that MAG, as a substrate bound molecule, inhibits axonal elongation of CNS neurons tin a manner similar to oligodendrocyte -induced collapse.

Supported by NIMH 5F31MH1086002, NS 24683, and Spinal Cord Res. Foundation.

583.11

DOES GRASSHOPPER SEMAPHORIN-1 INDUCE COLLAPSE OF NEURONAL GROWTH CONES *IN VIVO*? J. Wong, W. Yu, and T. P. O'Connor*. Dept. of Anatomy and Zoology, Vancouver B.C., Canada.

Members of the semaphorin/collapsin family of transmembrane and secreted proteins have been proposed to act as growth cone guidance signals in vertebrates and invertebrates. A number of studies have indicated that these molecules inhibit neurite outgrowth and stimulate growth cone collapse *in vitro*. Whether these molecules have similar functions *in vivo* is uncertain. We have used the developing grasshopper limb bud (Shistocerca gregaria) to study the effects of grasshopper semaphorin 1 (G-Sema 1) *in vivo*. In the embryonic limb-bud, neurons of the subgenual organ (SGO) contact and fasciculate with a pathway pioneered by the Ti 1 neurons. Elimination of the Ti 1 pathway by heat shock (Klose and Bentley 1989, Science 245:897) causes arrest of the proximal extension of the SGO growth cones. Since semaphorins have been implicated to have growth inhibitory properties, we examined the possibility that the arrest/collapse of the SGO axon is mediated by a band of epithelial cells expressing G-Sema 1. Using fluorescence double-labeling, we demonstrate that SGO axons extend into the band of G-Sema 1 expression by 42% of embryonic development. At this stage, the SGO neurons are typically within filopodial reach of the Ti 1 neurons. In the absence of the Ti 1 pathway, the proximal extension of the SGO growth cones is arrested within the band of G-Sema 1 expression. In addition, the growth cones do not display a collapsed phenotype, and typically extend to the proximal border of the G-Sema 1 expressing band. These results suggest that inhibition of growth cone extension *in vivo* may be due to signals other than growth cone collapse.

Funded by the Medical Research Council of Canada.

583.12

LESION-INDUCED SPROUTING OF CORTICOSPINAL FIBERS IS STRONGLY INCREASED IN MYELIN-FREE RAT SPINAL CORD. Michaela Thallmair, Patrik Vanek, Martin E. Schwab and Josef P.Kapfhammer*§. Brain Research Institute, University of Zürich, August-Forel-Str. 1, 8029 Zürich, Switzerland,§ Department of Anatomy, University of Freiburg, Germany.

Myelin and its associated neurite growth inhibitors play a crucial role in preventing fibers from regeneration in adult rats *(Schnell et al., Nature 367:170, 1994)*. An inverse regional distribution of myelin and GAP-43, a putative marker for axonal growth and plasticity, has been described in normal rats *(Kapfhammer and Schwab, JCN 340:194, 1994)*; Regions in the mature CNS which are only lightly myelinated retain a high expression of GAP-43 and in parallel high capacity for sprouting and regrowth. After suppression of myelination in the lumbar spinal cord by neonatal X-irradiation GAP-43 expression was strongly enhanced *(Kapfhammer and Schwab, Eur.J.Neurosci.6:403,1994)*. This increased GAP-43 expression correlated with enhanced sprouting of primary afferents after dorsal root lesions *(Schwegler et al., J.Neurosci.15:2756, 1995)*. These findings suggest that myelin and its inhibitors restrict sprouting and structural plasticity in the adult CNS.

To further investigate the physiological role of myelin-associated neurite growth inhibitors unilateral pyramidotomies were performed in X-irradiated and normal 3-4wk old rats. Sprouting was investigated using WGA-HRP or BDA. We found that collateral sprouting of unlesioned fibers was increased in the myelin-free parts of the spinal cord as compared to controls: Fibers crossed the midline and terminated in the denervated area. We obtained similar results in pilot-experiments in which specific antibodies (IN-1) against the neurite growth inhibitors NI-35/250 were applied. This supports our hypothesis that myelin and its associated neurite growth inhibitors contribute to the restriction of collateral sprouting and plasticity in the adult CNS.

Supported by the Swiss National Science Foundation.

583.13

CORTICO-EFFERENT PLASTICITY IS REGULATED BY MYELIN ASSOCIATED NEURITE GROWTH INHIBITORS IN THE ADULT RAT. G.L. Tillotson*, M.K. Schulz, L. Schnell and M.E. Schwab. Dept. of Neurology, Hines VA Hospital/Loyola University, Maywood, IL, 60153, USA; Dept. of Anatomy and Cell Biology, Odense University, Denmark and Brain Research Institute, University of Zürich, Zürich, Switzerland.

Following unilateral cortical lesions in neonatal rats, the spared unablated hemisphere is known to demonstrate remarkable neuroanatomic plasticity in cortico-efferent connectivity. This same type of structural plasticity is not seen after similar lesions in adult rats. One possibility for the lack of such a plastic response in the adult CNS may be the presence of myelin associated growth inhibitory proteins NI 35/250. These proteins have been found to play a crucial role in preventing fibers from regenerating in adult rat spinal cord lesions (Schnell et al., Nature 367:170, 1994). The aim of this study was to determine if blocking these inhibitory proteins by the application of the specific antibody IN-1 would enhance cortico-efferent plasticity in adult rats.

6-8 week old Lewis rats underwent unilateral aspiration lesion of the sensorimotor cortex. Animals were then treated with either IN-1 antibody or a control antibody. After a survival period of 12 weeks, the spared sensorimotor cortex was stereotaxically injected with the anterograde tracer biotinylated dextrane amine (BDA), and cortico-efferent fibers were analyzed. We found an increase in BDA positive fibers crossing the midline at the level of the corpus callosum in animals treated with IN-1 antibody, as compared to controls. These results indicate that myelin associated neurite inhibitory proteins play an important role in regulation of cortico-efferent plasticity in the adult. Fiber distributions at subcortical levels including thalamic and pontine nuclei are currently under investigation.

Supported by VA Merit Award and the Swiss National Science Foundation.

583.14

Two new chondroitin sulfate proteoglycans (CSPGs) in the developing chick nervous system

W. Halfter, Dept. of Neurobiology; University of Pittsburgh

Two CSPGs were detected in the developing chick brain. Immunostaining shows that the 4C10 antigen is present in CNS but not PNS. The antigen is particularly abundant in white matter of the spinal cord, axonal tracts in the brain, the optic fiber layer and the plexiform layers of the retina. The antigen is present in embryonic but not in posthatched chick CNS. In western blots, the 4C10 antigen appears as a broad smear with a MW of 300kD. After chondroitinase treatment, the immunoreactiv sinal decreases to two sharp bands with molecular weights of 90 and 45kD. Under reducing conditions, a single band at 45kD is detected, suggesting that the core protein of the CSPG exists as a disulfide-linked dimer with subunits of 45kD. The second CSPG (1D10 antigen) has a very restricted distribution. It is abundant in the optic nerve from the optic disc to the onset of the diencephalon, on the surface of macrophages and in the amnion. The expression of the antigen is restricted to only embryonic tissues. The molecular weight of the antigen is 300kD with a core protein of 70kD.

583.15

THE DSD-1 EPITOPE REPRESENTS A CHONDROITIN SULFATE MODIFICATION EXPRESSED ON DSD-1-PROTEOGLYCAN AND PROMOTES NEURITE OUTGROWTH OF HIPPOCAMPAL NEURONS

A. Clement, S. Nadanaka[1], K. Masayama[1], C. Ueoka[1], K. Sugahara[1], C. Mandl, and A. Faissner*, Dept. for Neurobiology, University of Heidelberg, D-69120 Heidelberg, Germany; [1] Dept. of Biochemistry, Kobe Pharmaceutical University, J-658 Kobe, Japan

The chondroitin sulfate (CS) proteoglycan (PG) DSD-1-PG has been identified with the monoclonal antibody (mAb) 473HD. The results of digestion experiments with glycosaminoglycan (Gag) lyases suggest that the epitope of the mAb 473HD is composed of a dermatan (DS)/CS C hybrid (designated DSD-1 for dermatan sulfate dependent). DSD-1-PG is expressd by immature glial cell in a developmentally regulated fashion and Western blot analysis of mouse brain extracts documented maximal expression during early postnatal stages. When coated as substrate, DSD-1-PG promotes neurite outgrowth of embryonic day 18 (E18) hippocampal neurons and the DSD-1 epitope is required for this effect, which suggests functional relevance of a particular Gag in neuronal process outgrowth. Therefore, the structural characterization of DSD-1 was attempted. Pharmacological agents which block sulfation abolished the epitope in the DSD-1-PG expressing oligodendroglial Oli-neu precursor cell line, as shown by immunoprecipitation and immunocytochemistry. This conclusion is consistent with competition ELISAs using several CS of similar structure, but distinct sulfate composition. In order to advance the purification of the DSD-1 structure, various CS carbohydrates were enriched on a 473HD column by affinity chromatography. The retained eluates proved functionally active, while the flow through was not efficient in a neurite outgrowth test. These results indicate that the DSD-1 Gag by itself might be a neurite outgrowth promoting factor. (Supported by DFG (Fa 159/5-1,2,3) and H.-L.-Schilling Stiftung)

583.16

ACTIVITY-DEPENDENT REGULATION OF NEURON-SPECIFIC GROWTH-ASSOCIATED GENES: ROLE OF PROTEOGLYCANS. W. Wang and K.E. Dow*, Dept. of Pediatrics, Queen's University, Kingston, Ontario Canada K7L 2V7.

Excitatory amino acid (EAA) neurotransmitters have been shown to play important roles as molecular signals influencing the structure of neurons during development. We have previously provided evidence that EAA agonists induce the synthesis and release of proteoglycans (PGs) with neurite-promoting activity from fetal hippocampal neurons (Sugiura and Dow, Dev. Biol. 1994; 164:102). In the present studies, we analyzed the regulation by EAA receptor activation of the expression of the neuron-specific growth-associated genes Tα1 α-tubulin (Tα1), microtubule-associated protein-2 (MAP-2), growth-associated protein-43 (GAP-43) and two developmentally regulated PGs, cerebroglycan and neurocan, and correlated this expression with morphological indicators of growth.

Primary cultures prepared from E19 rat hippocampal neurons were exposed to glutamate (100 μM) for 5 minutes and mRNA levels were quantitated by competitive reverse transcription polymerase chain reaction (RT-PCR). Neurite growth was also quantitated by morphological assessment at different time points following exposure. Exposure to glutamate resulted in 2.0-, 1.5- and 2.2-fold increases in Tα1, MAP-2 and GAP-43 mRNA levels respectively, which peaked at between 8 and 12 hours following exposure ($p < 0.05$ vs control) and increases in neurite growth measured at 48 hours following exposure ($p < 0.05$ vs control). Heparin inhibited the glutamate-induced induction of Tα1, MAP-2 and GAP-43 mRNA expression and neurite growth when added to culture medium at 25 μg/ml following glutamate exposure while chondroitin sulphate had no effect. Exposure to glutamate produced a 3.6-fold increase in mRNA levels of the integral membrane heparan sulphate PG (HSPG) cerebroglycan ($p < 0.05$ vs control) while mRNA levels of neurocan, a developmentally regulated CSPG, were not significantly altered. These studies suggest that neuronal HSPGs regulated by activation of EAA receptors may mediate autocrine growth responses.

583.17

DYNAMIC MICROTUBULES ARE REQUIRED FOR GROWTH CONE TURNING TO AVOID SUBSTRATUM-BOUND CHONDROITIN SULFATE PROTEOGLYCAN. J. F. Challacombe*, D. M. Snow and P. C. Letourneau, The University of Minnesota, Dept. of Cell Biology and Neuroanatomy, Minneapolis, MN 55455.

We previously examined the organization of actin filaments (AFs) and stable and dynamic microtubules (MTs) in growth cones turning to avoid substratum-bound chondroitin sulfate proteoglycan (CSPG) (Challacombe, et al., 1995, Soc. Neurosci. Abs. **21**:13). Using cytochalasin B, we showed that intact AF bundles are necessary for the MT reorientation that is crucial for growth cone turning.

In the present study, E9-11 chick sensory neurons were exposed to low concentrations of the MT-specific drugs taxol and vinblastine (VB) to reduce MT dynamics. Growth cones migrating on fibronectin were monitored by phase contrast video microscopy as approached and contacted CSPG borders. In contrast to controls, taxol- and VB-treated growth cones did not turn to avoid CSPG. Instead, they either stopped, retracted or sidestepped laterally along the border. When the drugs were washed out, normal turning behavior resumed. Following fixation of recorded growth cones, dynamic and stable MTs were immunofluorescently labeled and imaged with a laser scanning confocal microscope. In untreated growth cones and in those exposed to MT-specific drugs that were subsequently washed out, dynamic MT ends extended farther toward the leading margin than stable MTs. In growth cones exposed to low concentrations of taxol and VB, the labeling of dynamic and stable MTs were nearly coincident, and dynamic MT ends did not extend as far distally. Based on the effects of taxol and VB on growth cone behavior, and on the organization of dynamic and stable MTs, we propose that dynamic extension of MT ends toward the leading edge is necessary for growth cone turning to avoid CSPG. Supported by NIH grants NS09971 (JFC), EY10545 (DMS) and HD19950 (PCL).

583.18

OKADAIC ACID AFFECTS SUBSTRATE DEPENDENT NEURITE OUTGROWTH J.Viti* and C. Lagenaur. Dept. of Neurobiology, Univ. of Pittsburgh Pittsburgh PA 15261.

Phosphorylation events within the neuron are thought to be important mediators of neurite outgrowth. We have shown that okadaic acid, a serine/threonine phosphatase inhibitor, can significantly alter neurite outgrowth and morphology. When okadaic acid is added to mouse cerebellar granule cells cultured on purified N-CAM, growth cones disappear and the cells produce multiple processes along the cell body. The effect is substrate-dependent. Using time-lapse cinematography, okadaic acid was shown to cause complete neurite retraction within 20 min. Over the course of several hours, new neurite-like structures(without growth cones) formed around the cell body. Vesicular transport was seen along these processes. Double labeling experiments showed that these processes induced by okadaic acid have a core of tubulin in their center surrounded by a thin sheet of actin This is the same pattern seen in normal neurites. Also, taxol, a drug which stabilizes tubulin and does not allow it to depolymerize, causes these neurons to produce multiple processes around their cell bodies These experiments suggest that the structures induced by okadaic acid are neurites and that okadaic acid is somehow acting on the polymerization state of tubulin. KN-62, an inhibitor of calcium/calmodulin protein kinase II, was shown to attenuate the effect of okadaic acid, suggesting that this class of kinases might be might play a role in substrate-dependent neurite formation. Supported by NIH grant EY 05308

583.19

TENASCIN/CYTOTACTIN (TN-C) EXPRESSION IN THE RAT DENTATE GYRUS AFTER UNILATERAL ENTORHINAL CORTEX LESION.

T. Deller[1]*, C. A. Haas[1], T. Naumann[1], A. Joester[2], A. Faissner[2], and M. Frotscher[1]. Anatomical Institute, Univ. of Freiburg, F.R.G. (1), Department of Neurobiology, Univ. of Heidelberg, F.R.G. (2).

Unilateral entorhinal cortex lesion (ECL) induces a massive sprouting response in the denervated outer molecular layer of the rat dentate gyrus. Recent *in vitro* data suggest, that the glial derived extracellular matrix protein TN-C could be involved in these axonal growth processes. The expression of TN-C was analyzed in the dentate gyrus of adult rats 2, 6, 10, 14d, 4wks, and 6 months after ECL using immunocytochemistry and *in situ* hybridization. TN-C immunoreactivity increased throughout the denervated outer molecular layer by day 2, reached a maximum around day 10, and was back to control levels by 4 weeks. Using electron microscopy, single intensely TN-C-immunoreactive cells could be identified as reactive astrocytes that phagocytose degenerated terminals. *In situ* hybridization for TN-C mRNA revealed cellular profiles in the denervated zone, most likely astrocytes. These data suggest that glial derived TN-C is involved in axon growth *in vivo* and indicate that neuron-glia interactions are essential for the sprouting response after ECL (supported by the DFG).

583.20

DIFFERENTIAL RESPONSE OF NEURONS TO TENASCIN SPLICE VARIANTS, S. Meiners, S. Y. Ho, and H. M. Geller*. Dept. of Pharmacology, Robert Wood Johnson Medical School-UMDNJ, Piscataway, NJ 08854.

Tenascin-C (TN) is a 6-armed multidomain extracellular matrix (ECM) protein which has been implicated in the regulation of neurite outgrowth both during development and after injury. Each arm of TN is a polypeptide of 220-320 kDa, depending on species and alternative RNA splicing. The largest (HxB.L) and smallest splice (HxB.S) variants differ in that the former contains several fibronectin type III (FN-III) domains which are missing in the latter. We compared neurite outgrowth on baby hamster kidney (BHK) cells transfected with HxB.L or HxB.S (the generous gift of Dr. Harold P. Erickson, Duke University of Medicine). Outgrowth was significantly higher on cells transfected with HxB.L and significantly lower on cells transfected with HxB.S in comparison to outgrowth on control, untransfected cells.

To determine whether the splice variants exerted their effects in a substrate-bound or a soluble form, HxB.L and HxB.S were isolated from the conditioned media of transfected cells, added to cultures of untransfected BHK cells for 24 hr, and then washed away. Under these conditions, both HxB.L and HxB.S bound to the surface of BHK cells and promoted neurite outgrowth, although bound HxB.L was a somewhat better growth substrate than bound HxB.S. In contrast, soluble HxB.S inhibited neurite outgrowth in a dose-dependent manner, whereas soluble HxB.L failed to inhibit outgrowth. These results indicate that the effect of TN on neurite outgrowth is a function of splice variants, as well as the form or conformation of those splice variants.

This work was supported by NIH Grant NS 25168 to H. G. and by NJ Governor's Council Advanced Research Fellowship P2MF6C to S. M.

584.1

Isolation of a *Drosophila* Eph receptor protein tyrosine kinase expressed in the nervous system. A.L. Scully[1], M. McKeown[2], R. Dyck*[1], J.B. Thomas[1], 1) Molecular Neurobiology Laboratory, 2) Molecular Biology & Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92186.

We are examining the role of receptor protein tyrosine kinases (RPTKs) in axon pathfinding during development. In order to identify *Drosophila* RPTKs which may be involved in neuronal pathfinding, we carried out a PCR-based screen using degenerate primers to two conserved regions within the kinase domain of RPTKs (Lai and Lemke, Neuron *6*, 691-704). One of the kinase domains identified in the screen shows similarity to the Eph subfamily member Cek7 and its homologues in rat and mouse, Rek7 and Bsk. Sequence analysis of a full-length cDNA confirms that it shares significant sequence homology to Cek7/Rek7/Bsk within both the kinase and extracellular domains. We have thus tentatively named this *Drosophila* RPTK Dek. Rek7 has been shown to be expressed on subsets of developing axons within the brain and eye (Winslow et al., Neuron *14*, 973-81). *In situ* hybridization of *dek* cDNA probes to embryos reveals a pattern of expression restricted to the CNS during the period of axon elongation. We are currently screening for mutations in *dek* and expressing dominant negative forms of the protein in order to elucidate its possible role in axon guidance. Supp. by NIH grants and a Pew Scholars Award from Pew Memorial Trusts to John B. Thomas.

584.2

TYROSINE PHOSPHORYLATION OF NILE/L1 BY THE EPH KINASE CEK5
A.H. Zisch, L.D. Chong, W.B. Stallcup, J.A. Holash, H. Voshol#, M. Schachner# and E.B. Pasquale*. The Burnham Institute, La Jolla CA 92037, USA and #ETH, CH-8093 Zurich, Switzerland.

NILE/L1 is a transmembrane cell adhesion molecule of the immunoglobulin superfamily that has been implicated in neurite outgrowth and neuronal migration. Binding of ligands to NILE/L1 results in the activation of intracellular signaling pathways, in some cases through the association of NILE/L1 with kinases. For example, NILE/L1 interacts in cis with FGF receptor 1 and complexes with cytoplasmic serine/threonine kinases. Recently it was demonstrated that crosslinking of Ng-CAM, the putative chick homologue of NILE/L1, on the surface of chick primary neurons leads to tyrosine phosphorylation of the cytoplasmic domain. Here we address whether NILE/L1 is a target of receptor tyrosine kinases of the Eph family, since the Eph kinases are highly expressed during development of the nervous system. In vitro kinase assays showed that the recombinant cytoplasmic domain of L1/NILE is phosphorylated on tyrosine by the Eph kinase Cek5. To determine whether NILE/L1 can be phosphorylated on tyrosine by Cek5 in vivo, we used the rat glioblastoma cell line B28 stably transfected with NILE/L1. Additional transient transfection with Cek5 cDNA led to expression of Cek5 in its phosphorylated, presumably activated form. Biochemical analysis of NILE/L1 by immunoprecipitation and immunoblotting with anti-phosphotyrosine antibodies revealed tyrosine phosphorylation of NILE/L1 in Cek5 transfected cells, but not in control transfectants. As NILE/L1 and Eph kinases co-localise in certain regions of the brain, such as the molecular layer of the cerebellum, we hypothesise that crosstalk between NILE/L1 and Eph kinases also occurs in vivo and alters phosphorylation-dependent functional properties of NILE/L1.
Funded by NIH, FWF-Austria and Schweizerischer Nationalfonds.

584.3

ELF1 SPECIFICALLY REPELS TEMPORAL RETINAL AXONS IN VITRO.
GC Friedman*[1], T McLaughlin[1], MJ Hansen[2], M Nakamoto[2], DDM O'Leary[1], JG Flanagan[2]. [1] The Salk Institute, La Jolla, CA; [2]Harvard Med Sch, Boston, MA.

Elf1, a ligand for the Eph receptor tyrosine kinase Mek4, is expressed in a gradient along the rostrocaudal axis of the chick optic tectum during formation of the retinotectal map, being high caudally and low rostrally. Mek4 is expressed in a complementary gradient along the temporal/nasal axis of the retina, suggesting that the interaction of Elf1 and Mek4 is involved in targeting of retinal axons to their appropriate termination zones in the tectum.

We have used the membrane stripe assay to investigate the response of retinal axons to membranes containing Elf1. Membranes from 293T cells transfected with an Elf1 expression construct and untransfected 293T cells were pooled 2 days after transfection, and membrane fractions were collected from each. In second set of experiments tectal membranes were collected from E10 embryos which had been infected at st. 11 and st. 14 with a replication competent retrovirus containing the Elf1 cDNA. Exogenous expression of Elf1 was confirmed with a Mek4-AP fusion protein; transfected 293T cells and infected rostral tecta had high levels of Mek4-AP staining which was absent in the untransfected 293T cells and uninfected rostral tectum. Alternating lanes of transfected and untransfected 293T cell membranes were prepared and explants from temporal or nasal retina were laid down perpendicular to the lanes. Temporal axons showed a strong growth preference for the untransfected lanes, whereas nasal axons showed no preference. Temporal axons also showed a preference for membranes from uninfected rostral tecta versus Elf1 infected rostral membranes, whereas nasal axons again showed no preference. In addition, temporal axons grew well but showed no preference when given a choice between membranes from Elf1 infected rostral tecta and normal caudal tecta, indicating that Elf1 acts as a repulsive molecule. These results support the hypothesis that Elf1 can regulate the topographic targeting of retinal axons in vivo. (Supported by R01 EY07025, R01 HD29417, F32 EY06550)

584.4

CONTROL OF TOPOGRAPHIC RETINAL AXON MAPPING BY ELF-1
M. Nakamoto, H-J. Cheng, A.D. Bergemann, M.J. Hansen, D. Van Vactor*, C.H. Yoon, and J.G. Flanagan. Dept. of Cell Biology, Harvard Medical School, Boston, MA 02115.

Topographically organized patterns of neuronal connectivity are found throughout the nervous system. The retinotectal projection is a particularly well-studied system, in which temporal retinal axons project to anterior tectum and nasal retinal axons to posterior tectum. As first proposed by Sperry in the 1940s, these topographic maps are believed to be established by complementary labels in gradients across pre- and post-synaptic fields. However, molecular identification of the position-specific labels had long been an elusive goal.

Recently, we cloned ELF-1 (Eph ligand family-1), detected as a ligand for Eph family receptor tyrosine kinases, by a novel method using a soluble receptor with an alkaline phosphatase tag to detect the ligand in embryos. We find matching gradients in RNA expression for ELF-1 in the tectum, and its receptor Mek4 in the retina. Ligand-receptor interactions detected with alkaline phosphatase fusions of ELF-1 and Mek4 also reveal gradients, and provide direct evidence for molecular complementarity of gradients in reciprocal fields. To address the function of ELF-1 in vivo, we altered the expression pattern of ELF-1 in the developing tectum using retrovirus vector. In those embryos, patches of ectopic ELF-1 expression are superimposed on the endogenous anterior-posterior gradient. Temporal retinal axons avoid the ectopic ELF-1 patches and project to positions more anterior than the topographically normal locations, while nasal axons do not show obvious abnormality. These results indicate that ELF-1 and Mek4 could be topographic mapping molecules of the type predicted by Sperry.

ELF-1 is a member of Eph ligand family, which consists of at least seven members, and current experiments indicate other Eph ligands have comparable roles in establishing the spatial pattern of the nervous system.

(Supported by HD29417 and DK45580)

584.5

NT-3 and trkC expression patterns suggest their role in the development of corticospinal axon projections to the brainstem and spinal cord. K.T. Yee,* and D.D.M. O'Leary Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

Previous work has demonstrated that layer 5 neurons innervate midbrain, hindbrain and spinal target sites by collateral branches that develop *de novo* from primary corticospinal axons. Unidentified target-derived diffusible tropic activities appear to regulate this innervation process. Recent evidence has shown that the neurotrophic factor NT-3 can enhance corticospinal axon sprouting in the spinal cord during development or following lesion and can induce turning of cortical neurites *in vitro*. Given these findings, we were interested in examining whether NT-3 may be involved in mediating target innervation in the corticospinal pathway during normal development.

To address this, we have assessed the expression of *NT-3* and the distribution of its primary receptor, trkC, within the developing corticospinal pathway in rats using in situ hybridiztion for *NT-3* mRNA (gifts from W. D. Snider and K.-F. Lee) and immunohistochemistry against trkC (Santa Cruz Biotech., Inc.). At postnatal day 1, *NT-3* mRNA is expressed by several corticospinal target areas (eg., superior colliculus, dorsal column nuclei and spinal intermediate gray) and trkC is expressed along the corticospinal tract and by layer V cortical neurons. A proportion of the trkC expressing cells could be labeled following injections into the pyramidal decussation indicating that at least some corticospinal neurons are trkC positive. Since the spatial and temporal expression of *NT-3* and its primary receptor correlates with corticospinal target innervation, it is possible that NT-3 may play a role in directing collateral extension into certain target regions of corticospinal axons and/or may be important for their maintenance into maturity.

Supported by NS 31249 and Spinal Cord Research Foundation.

584.6

EFFECTS OF NEUROTROPHINS ON RETINAL NEURONAL OUTGROWTH. J. Atkinson*, M.K. Panni and R.D. Lund. Dept. of Pathology, Inst. of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, UK.

Developing retinal axons follow a stereotypical course towards subcortical visual centres, which on arrival they must recognise. Transplantation studies suggest that local substrate cues close to the surface of the brainstem and diffusible factors emanating from the target region are important. One group of diffusible factors is the neurotrophin family, whose receptors have been found to be expressed in both the developing as well as the adult visual system.

In this study, both brain derived neurotrophic factor (BDNF), and neurotrophin 4 (NT4) were shown to promote neurite outgrowth from embryonic mouse retinal explants (E12-14) grown on laminin *in vitro*, whereas neurotrophin 3 (NT3) showed no neurite promoting activity. When cultured in the presence of an antimitotic agent such as cytosine arabinoside, outgrowth was unaffected in BDNF-containing cultures but was significantly diminished in NT4-containing cultures, suggesting a possible role for glial cells in NT4-promoted retinal outgrowth.

We have cotransplanted embryonic mouse retina (E12-14) with specific neurotrophin producing fibroblasts into the cortex of neonatal rats in order to assess a role of these molecules in developing retinal ganglion cells. Transplanted retinal ganglion cells showed directed outgrowth towards BDNF producing fibroblasts but not towards NT3 and NT4 producing fibroblasts. These results indicate that NT3 does not seem to promote retinal outgrowth, NT4-induced outgrowth is likely to be dependent on the environment whereas BDNF is equally effective *in vitro* and *in vivo* in promoting and guiding developing retinal axons.

We would like to thank Dr. F.H.Gage for providing the BDNF producing fibroblasts and Dr. E.Arenas for providing the NT3 and NT4 producing fibroblasts. This work was supported by Action Research and MRC, UK.

584.7

THE NEUROTROPHINS NT-3, BDNF AND NT-4, BUT NOT NGF, ENHANCE NEURITE TURNING IN AN APPLIED ELECTRIC FIELD. L. Sangster, R. Stewart, R. Zietlow and C.D. McCaig*. Department of Biomedical Sciences, University of Aberdeen, Aberdeen, Scotland, AB9 1AS.

Endogenous electric fields exist in embryos and their disruption results in abnormal neuronal development. *In vitro* applied electric fields affect the direction of neurite growth. Neurotrophins also influence nerve growth, both *in vivo* and *in vitro*. Electric fields and neurotrophins coexist in embryos thus interactions may occur. We have investigated whether or not neurotrophins can alter the effects of an applied electric field on neurite growth.

A d.c. electric field (150mV/mm) applied for 5 hours affected the growth of disaggregated cells from the neural tube of stage 20 *Xenopus laevis*. Neurites responded to the field by turning to grow cathodally. Exposure of neurites to neurotrophin 3 (NT-3; 50ng/ml), brain-derived neurotrophic factor (BDNF; 100ng/ml) or neurotrophin 4 (NT-4, 100ng/ml) lowered the threshold field strength necessary to elicit a turning response to 100mV/mm. Only BDNF enhanced cathodal orientation at 150mV/mm, with neurites turning three times as far as in the field alone. Concomitant exposure of neurites to BDNF and NT-3 did not enhance the effect of either alone. Neurite turning did not occur in a field of 100mV/mm in the presence of 50ng/ml nerve growth factor (NGF).

Xenopus growth cones release acetylcholine (ACh) spontaneously. This is enhanced by both NT-3 and BDNF. Nicotinic ACh receptor antagonists abolish field-induced cathodal turning, thus ACh release may be crucial for this response. These neurotrophins may enhance cathodal turning because they enhance ACh release.

Interactions *in vivo*, where neurotrophins and electric fields coexist, would increase the efficacy of endogenous electric fields as modulators of nerve growth.

Supported by the Wellcome Trust.

584.8

NONRECEPTOR TYROSINE KINASES pp60$^{C\text{-}SRC}$ AND p59^{FYN} AS MEDIATORS OF AXONAL GUIDANCE IN THE DEVELOPING OLFACTORY PATHWAY. W.R. Morse, J.G. Whitesides, P. Morell*, A.-S. LaMantia and P.F. Maness. Dept. Biochemistry, Univ. North Carolina Sch. of Med., Chapel Hill, NC 27599 & Dept. Neurobiology, Duke University Sch. of Med., Durham, NC 27710.

pp60$^{c\text{-}src}$ and p59^{fyn} are *src*-family nonreceptor tyrosine kinases that are essential components of L1- and NCAM-mediated signaling pathways leading to neurite outgrowth. Immunofluorescent localization of pp60$^{c\text{-}src}$, p59^{fyn} and the neural cell adhesion molecules NCAM and L1 at embryonic day 11.5 in the mouse shows their coexpression along nascent olfactory axons in the lateral cranial mesenchyme. We have examined the behavior of NCAM-immunoreactive olfactory axons in *fyn*-minus and *src/fyn*-minus mice as they exit the olfactory epithelium, fasciculate into a single nerve and project toward the forebrain. Mutant mice display altered axonal trajectories, as determined by the direction of growth and number of single axons and fascicles exiting the epithelium. These axons may therefore be defective in some mechanism of axon guidance or fasciculation. Nonetheless, mutant olfactory axons do ultimately reach the forebrain vesicle. Defects in axonal trajectories in *src/fyn* doubly null mutant mice are somewhat more severe than in *fyn*-minus mice, suggesting that *src* and *fyn* can partially compensate for one another during olfactory axon outgrowth. We are currently evaluating L1 and NCAM-mediated fasciculation of axons from olfactory explants *in vitro* as a potential mechanism requiring *src* and *fyn* tyrosine kinases. Our current *in vivo* observations suggest that *src* and *fyn* contribute to axon guidance during initial assembly of the olfactory pathway. This work was supported by NIH grant NS26620.

584.9

OVEREXPRESSION OF *C-SRC* AND *N-SRC* IN THE DEVELOPING *XENOPUS* RETINA DIFFERENTIALLY IMPAIRS AXONOGENESIS T. L. Worley* and C. E. Holt. University of California, San Diego, Dept. of Biology 0366, La Jolla, CA 92093.

To study the role of the cytoplasmic tyrosine kinases *c-src* and its neuronal splice-form *n-src* in the development of the vertebrate retina and retinal projection, *Xenopus* retinal precursors were transfected *in vivo* with constructs expressing pp60$^{c\text{-}src}$, pp60$^{n\text{-}src}$, or constitutively active mutants of *c-src* and *n-src*. The phosphotyrosine labeling intensity of all transfected retinal cells was significantly increased compared to untransfected cells, (indicating that the constructs were enzymatically active), and was greatest in cells expressing deregulated *n-src*. A comparison of the distribution of transfected retinal cell types showed that the expression of either the *c-src* or *n-src* deregulated constructs inhibited photoreceptor differentiation to similar degrees, but did not influence other retinal cell fate decisions. In contrast, the extension of axons from transfected retinal ganglion cell and forebrain neurons was severely impaired in cells expressing deregulated *c-src*, and only mildly or not impaired in neurons expressing deregulated *n-src*. Transfected retinal axons that did elongate were observed in whole-mount preparations to correctly navigate through the contralateral optic tract to the optic tectum. These results suggest that *c-src* and *n-src* may play a similar role in photoreceptor differentiation, however; the differences observed between *c-src* and *n-src* expressing cells in the intensity of phosphotyrosine labeling and the ability of transfected neurons to extend axons indicates that *c-src* and *n-src* also have distinct activites *in vivo*. This work was supported by NIH #NS23780 (C.E.H.).

584.10

POTENTIAL ROLES OF NETRIN-1 DURING AXONOGENISIS IN THE ZEBRAFISH EMBRYO. J. D. Lauderdale*, M.C. Halloran, N. M. Davis, and J. Y. Kuwada. Dept. Biology, Univ. Michigan, Ann Arbor, MI 48109-1048

During embryogenesis, pioneer neurons project growth cones that follow stereotyped, cell-specific pathways to reach specific, often distant, targets. These growth cones often follow extracellular directional cues. Netrin may be one such guidance cue. Netrins are secreted, laminin-related molecules. Netrin/UNC-6 acts as a bifunctional guidance cue for developing axons in the nematode (Hedgecock et al. 1990, Neuron 4:61; Wadsworth et al., 1996, Neuron 16:35). In vertebrates, explant studies have shown that chick netrin-1 can act as a chemoattractant for ventrally directed commissural axons (Serafini et al., 1994, Cell 78: 409; Kennedy et al., 1994, Cell 78:425; Shirasaki et al., 1995, Neuron 14:961) and as a chemorepellent for dorsally directed trochlear motor axons (Colamarino and Tessier-Lavigne, 1995, Cell 81:621). These data suggest that netrin-1 may act as a bifunctional guidance cue for developing axons in the embryonic vertebrate nervous system. In order to elucidate the *in vivo* function of netrin in vertebrates, we cloned a zebrafish homolog of chick *netrin-1* and examined the relationship of *netrin* mRNA expression to identified growth cones and axon tracts during early axonogenisis in the zebrafish embryo. In the brain, epiphysial growth cones project ventrally along a dorsoventral stripe of cells expressing netrin in the diencephalon. In contrast, axons which form the tract of the posterior commissure, including the ventrally directed nucPC axons, extend in a region of the diencephalon bordered by cells expressing netrin. Spinal cord commissural axons extend ventrally along cells expressing netrin. In the somites, the muscle pioneer cells, an intermediate target for the primary motor neurons, express netrin. These data suggest that netrin may be involved in guiding several different types of growth cones. We are currently manipulating netrin expression *in vivo* and examining which growth cones respond to ectopically expressed netrin. Supported by NIH-NRSA (J.D.L., M.C.H.), NSF-RTG (N.M.D.), and NINDS (J.Y.K.).

584.11

GENETIC ANALYSIS OF *Netrin* GENES IN DROSOPHILA: NETRIN-A AND NETRIN-B FUNCTION TO GUIDE COMMISSURAL AXONS IN THE CNS AND MOTOR AXONS IN THE PERIPHERY. Jennifer L. Doyle, Kevin J. Mitchell, Barry S. Dickson, and Corey S. Goodman*. HHMI, Dept. of Molec. and Cell Biology. Univ.of California, Berkeley 94720.

The vertebrate netrins (netrin-1 and 2) and their *C. elegans* homologue UNC-6 are bifunctional, chemotropic guidance molecules that are expressed at the ventral midline of either the spinal cord or the whole animal, respectively. In collaboration with the Tessier-Lavigne lab, we cloned two tandem *Netrin* genes in *Drosophila* (*D-netrinA* and *D-netrinB*). Both *Netrin* genes are expressed at the midline of the developing CNS, although each is also expressed by different subsets of lateral neurons. In addition, both *Netrin* genes are expressed in peripheral tissues in different subsets of muscles and patches of epidermis. We generated a small deficiency which deletes both *Netrin* genes. In embryos doubly mutant for both *Netrin* genes, the commissures are partially missing or thinner. We have been able to rescue this double mutant phenotype by expressing either Netrin-A or Netrin-B along the midline as driven by the *slit* promoter, suggesting that the function of the two genes is redundant at the midline. In the double mutant we also find striking defects in the projections of motor axons; in particular, the distal path of the intersegmental nerve (ISN) is aberrant. In order to further test the functions of the netrins, we ectopically expressed both genes using the GAL-4/UAS system of tissue-specific transcriptional activation. Pan-neural expression of either gene causes severe disruption of both CNS commissural and longitudinal tracts. Ectopic expression throughout the developing somatic musculature results in misrouting of the ISN. This genetic analysis suggests that the two Netrins function as attractants and repellents during axon guidance in both the CNS and periphery in Drosophila. Supported by NIH/NRSA # T32-GMO7048-21 to JLD and NIH/NINDS #5 R01 NS18366 to CSG.

584.12

LEECH NETRIN IS EXPRESSED IN SPECIFIC CENTRAL NEURONS AND VENTRAL MUSCLE CELLS. G. O. Aisemberg*, W.-B. Gan, V. Y. Wong, T. R. Gershon and E. R. Macagno. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

A member of the netrin family of axonal guidance proteins was cloned and characterized in the leech *Hirudo medicinalis*, an organism in which neuronal function and development can be addressed at the level of single, identified neurons. Leech netrin (Hm-netrin) mRNA was detected in both embryos and adults by *in situ* hybridization, in a specific subset of central neurons of the midbody, beginning at the early period of gangliogenesis. These Hm-netrin-producing neurons include the Bipolar cells, a pair of transient neurons that may be involved in marking pathways in the longitudinal connective nerves. Hm-netrin is also detected in a band of ventral muscle cells segmentally repeated along the midbody.

To further characterize this extracellular protein in the leech system, a polyclonal antibody against *E. coli*-expressed Hm-netrin was raised and affinity-purified. This antibody was used to localize the Hm-netrin protein, a putative diffusible factor, in the leech embryo.

Funded by NIH Grant #NS-34545.

584.13

IMMUNOHISTOCHEMICAL LOCALIZATION OF NETRIN-1 EXPRESSION IN THE EMBRYONIC CHICK NERVOUS SYSTEM. A.J. MacLennan*, D.L. McLaurin, L. Marks, E.N. Vinson, M. Pfeifer, S. Szulc, M.B. Heaton and N. Lee. Department of Neuroscience, Brain Institute, University of Florida, Gainesville, FL 32610-0244

Evidence suggests that netrin-1 participates in axon guidance mechanisms, particularly those which determine whether developing axons grow toward or away from the floor plate. Explanted, embryonic, spinal cord floor plate releases a substance(s) that acts as a chemoattractant for commissural spinal axons and a chemorepellent for trochlear motor axons. Cells engineered to express netrin-1 mimic both of these effects. Moreover, netrin-1 mRNA is expressed in the floor plate *in vivo* during stages of development in which these and many other axons are extending. We prepared anti-netrin-1 polyclonal antisera to study netrin-1 protein expression in the embryonic chick nervous system. Netrin-1 immunoreactivity was detected in several regions including: 1) the floor plate of the brain and spinal cord, 2) the retina, and 3) particular pathways travelled by extending axons. Notably, netrin-1 immunoreactivity was not detected in the spinal cord floor plate until after the arrival of the first commissural axons. Supported by PHS grants DA07244 to A.J.M. and AA09128 to M.B.H.

584.14

CHANGE OF GROWTH CONE RESPONSIVENESS TO FLOOR PLATE-DERIVED CHEMOATTRACTANT AFTER CROSSING THE FLOOR PLATE. R. Katsumata, R. Shirasaki, N. Yamamoto* and F. Murakami. Dept. Biophys. Engineering, Fac. Engineering. Sci., Osaka University, Toyonaka, Osaka 560, Japan.

A variety of ventrally decussating commissural axons in the vertebrate central nervous system are guided toward the ventral midline by floor plate (FP)-derived chemoattractant(s) (Shirasaki et al., Neuron 14, 1995; Tamada et al., Neuron 14, 1995). If the growth cones of these axons follow a concentration gradient of chemoattractant, one would expect them to stall at the FP where chemoattractant concentration is maximal. However, during development the axons continue to grow past the FP. One possible explanation for this is a change of the growth cone responsiveness to FP-derived chemoattractant during migration through the FP. A prediction from this theory is that commissural axons would show no reoriented growth toward the FP once having crossed it. Here we tested this by culturing rhombomere-1 segment from embryonic day 13 rat for 2 days in collagen gel, either in the absence or presence of an ectopic FP explant placed on one side. In control preparations, commissural axons grew beyond the midline FP from both sides. When an ectopic FP explant was cultured next to the strip on one side, the axons on that side showed reoriented growth toward the explant, demonstrating chemoattractive activity of the ectopic FP toward the ipsilateral axons. However, the axons growing from the opposite side ignored the ectopic FP. When the midline FP was removed from the strip, axons growing from the contralateral side showed reoriented growth toward the ectopic FP. Together, these results suggest that growth cones of commissural axons change their responsiveness to FP-derived chemoattractant during their migration through the FP. Such a change may account for why commissural axons are not detained at the ventral midline FP *in vivo*. *(Supported by Grant-in-Aid from the Monbusho, Japan.)*

584.15

NETRIN DEPENDENT- AND INDEPENDENT-FLOOR PLATE CHEMOATTRACTION AND CHEMOREPULSION OF DEVELOPING AXONS IN THE MAMMALIAN BRAIN. R. Shirasaki[1], C. Mirzayan[2], M. Tessier-Lavigne[2] and F. Murakami[1,*]. [1]Dept. Biophys. Engineering, Fac. Engineering Science., Osaka Univ., Toyonaka, Osaka 560, Japan. [2]Howard Hughes Medical Inst., Dept. Anatomy, UCSF, CA 94143-0452.

Previous studies by our laboratories and others have shown that the floor plate (FP) attracts spinal cord, metencephalic (Met), and myelencephalic (My) alar plate (AP) axons, but repels mesencephalic (Mes) basal plate (BP) and AP axons in the rat embryo (Nature 336, 1988; Cell 81, 1995; Neuron 14, 1995; Neuron 14, 1995). They have also shown that netrin-1 attracts spinal cord and Met AP axons and repels trochlear motor axons (Cell 78, 1994; Neuron 14, 1995; Cell 81, 1995), but molecules responsible for chemoattraction and chemorepulsion of other types of axons remain largely unknown. Here we examined whether netrin-1 can mimic FP chemotropic activities toward My and Mes axons. Aggregates of netrin-1-secreting COS cells attracted both MyAP and MesAP axons in the same manner as FP explants. These axons expressed the TAG-1 glycoprotein in vitro. Together with our previous findings that Mes, My and Met commissural axons express TAG-1, these results suggest that netrin-mediated chemoattraction operates for commissural axon guidance at all these levels of the brain. Regarding FP chemorepulsion, we found that FP at all axial levels repels two types of MesBP axons: tyrosine hydroxylase-positive axons and dorsally-directed axons in the rostral MesBP (likely those that form the posterior commissure). Neither of these classes of axons was repelled by recombinant netrin-1 secreted by aggregates of transfected 293 cells, under conditions where trochlear motor axons were repelled. Together, these results suggest that, although netrin-1 can function as a common chemoattractant for ventrally-decussating CNS axons, FP chemorepulsion of MesBP axons may involve diffusible molecules other than netrin-1. *(Supported by Grant-in-Aid from the Monbusho and the Howard Hughes Medical Institute.)*

584.16

GRADED EXPRESSION OF NETRIN PROTEIN IN THE EMBRYONIC SPINAL CORD. T.E. Kennedy*[1], L. Hinck[1], S. Colamarino[1], C. Mirzayan[1], S. Faynboym[1], W. Marshall[2], M. Tessier-Lavigne[1]. [1]Howard Hughes Medical Institute, University of California, San Francisco, [1]Depts. of Anatomy, [2]Biochemistry and Biophysics, SF, CA, 94143.

Gradients of molecular cues have been proposed to guide growing axons during neural development. In the embryonic spinal cord, commissural axons pioneer a circumferential pathway to the floor plate at the ventral midline. Floor plate cells secrete a factor(s) that promotes the outgrowth of these axons and attracts them *in vitro*. Two proteins purified from embryonic chick brain, netrin-1 and netrin-2, possess outgrowth promoting and chemotropic activity like floor plate cells. In the chick, netrin-1 mRNA is expressed at high levels in the floor plate region, whereas netrin-2 mRNA is expressed more widely in the ventral spinal cord but is absent from the floor plate. We have raised antibodies that recognize netrin-1 and netrin-2. These antibodies reveal a graded distribution of netrin protein in the embryonic spinal cord during the initial period of commissural axon extension. The antibodies also block the ability of floor plate cells to promote the outgrowth of commissural axons *in vitro*. These results support the hypothesis that a gradient of netrin protein directs the growth of commissural axons to the ventral midline of the developing spinal cord.

Supported by the NIH, the Howard Hughes Medical Institute, and the Spinal Cord Research Foundation.

584.17

***DELETED IN COLORECTAL CARCINOMAS (DCC)* ENCODES A NETRIN RECEPTOR.** K. Keino-Masu[1,2], M. Masu[1], E. D. Leonardo[1], L. Hinck[1], and M. Tessier-Lavigne[1*]. [1]Howard Hughes Medical Institute, Dept. of Anatomy, University of California, San Francisco, CA 94143-0452. [2]Dept. of Physiology, National Defense Medical College, Saitama, Japan.

Floor plate cells at the ventral midline of the developing spinal cord express a chemotropic factor, netrin-1, which promotes the outgrowth of commissural axons and attracts these axons *in vitro*. Netrin-1 is a homologue of the *C. elegans* protein UNC-6, which is required for circumferential migrations of axons and cells in the nematode. To investigate the mechanisms through which netrin-1 produces its actions, we analyzed two genes, *deleted in colorectal carcinomas* (*DCC*) and *neogenin*, which are vertebrate homologues of *unc-40*, a gene that is involved in *unc-6* dependent migrations in *C. elegans*. These genes encode transmembrane proteins that are members of the immunoglobulin gene superfamily. We have found that *DCC* is abundantly expressed in commissural neurons in the developing rat spinal cord, and that its protein product is expressed on commissural axons and binds netrin-1. Moreover, an antibody against the extracellular portion of DCC blocks netrin-dependent outgrowth of commissural axons *in vitro*, without effect on netrin-independent outgrowth. These results implicate DCC as a receptor or a component of a receptor that mediates attraction by netrin-1.

Supported by the Howard Hughes Medical Institute, the American Paralysis association, and Jane Coffin Childs fellowship.

584.18

VERTEBRATE HOMOLOGS OF *C. ELEGANS* UNC-5 ARE CANDIDATE NETRIN RECEPTORS. L. Hinck[1], E. D. Leonardo[1], M. Masu[1], K. Keino-Masu[1,2], T. Serafini[1*] and M. Tessier-Lavigne[1]. [1]Howard Hughes Medical Institute, Dept. of Anatomy, University of California, San Francisco, 94143. [2]Dept. of Physiology, National Defense Medical College, Saitama, Japan.

Growth cones are guided to their targets in the embryonic nervous system in part by diffusible attractants and repellents. Netrin-1 is a diffusible factor which acts bifunctionally to attract and to repel different classes of axons in vertebrates. Genetic analysis in *C. elegans* has identified a netrin-1 homolog, UNC-6, which functions in axon guidance, and a transmembrane protein, UNC-5, which is a candidate receptor for repulsive actions of UNC-6. We report the identification of two vertebrate homologs of UNC-5, termed UNC5H-1 and UNC5H-2, which, like UNC-5, possess in their extracellular domains two immunoglobulin-like domains and two thrombospondin type-1 repeats. *In situ* hybridization analysis reveals that *Unc5h-1* mRNA is expressed in the embryonic spinal cord, thalamus and cortex, whereas *Unc5h-2* mRNA is expressed in developing sensory structures including the optic cup, otic vesicle and nasal epithelium, as well as in restricted domains in the developing midbrain and diencephalon. Expression of the two UNC-5 homologs was also detected by Northern analysis in the adult, in both neural and non-neural tissues. Transfected cells expressing UNC5H-2 bind netrin-1, supporting the hypothesis that UNC5H-2 is a netrin receptor.

Supported by the Howard Hughes Medical Institute, the American Paralysis Association and a Jane Coffin Childs fellowship.

585.1

ANTIBODIES RAISED TO COLLAPSIN-1 BLOCK A SENSORY AXON REPELLENT ACTIVITY SECRETED FROM VENTRAL SPINAL CORD EXPLANTS. Iain Shepherd*, Yuling Luo and Jonathan Raper. Department of Neuroscience, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104

During embryogenesis different classes of DRG primary sensory neurons extend central projections to specific dorso-ventral locations in the developing spinal cord. Between E3.5 and E8.5 the pattern of projection of muscle and cutaneous central sensory afferents is identical. Initially these afferents are restricted to the dorsal funculae and then subsequently they are restricted to the dorsal cord gray matter. Only at E8.5 do muscle afferents begin to diverge from cutaneous afferents and project into ventral cord gray matter.

Previous studies have shown that ventral spinal cord explants secrete a sensory neurite repellent factor. To test whether this ventral cord repellent activity is due to collapsin-1 we have raised rabbit antibodies to recombinant chick collapsin-1 protein. These antibodies i) recognize collapsin-1 on western blots ii) block collapsin-1 function in an in vitro growth cone collapse assay iii) block the ventral spinal cord sensory neurite repellent activity.

We have also carried out a series of in vitro experiments investigating the sensitivity of muscle and cutaneous sensory growth cones to collapsin-1. We have found that growth cones of chick E7 DRG neurites are equally sensitive to collapsin-1 when cultured in NT-3 or NGF supplemented media. However in E10 DRG cultures NT-3 dependent muscle sensory neurite growth cones are partially insensitive to collapsin-1.

These results support the hypothesis that collapsin-1 helps confine central sensory afferents to the dorsal cord during early stages of embryogenesis. We suggest that initially all central sensory afferents are collapsin-1 sensitive. Only at later stages, coinciding with the onset NT-3 dependence, do muscle sensory afferents become insensitive to collapsin-1 allowing them to project into ventral cord.

This work was sponsored by grants from the NINDS and NINCDS to JAR.

585.2

STRUCTURE-FUNCTION ANALYSIS OF THE REPELLENT AXON GUIDANCE MOLECULE COLLAPSIN-1. Adam M. Koppel* and Jonathan A. Raper. Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Axon pathfinding during development depends on both attractive and repulsive signaling cues that act upon migrating growth cones. Collapsin-1, a chick brain glycoprotein, could be such a repulsive guidance cue. Collapsin-1 induces the collapse and paralysis of sensory, but not retinal, growth cones in an *in vitro* collapse assay.

The protein sequence of collapsin-1 predicts several structural domains, including an amino-terminal signal sequence, a semaphorin domain followed by a C-2 type immunoglobulin loop, and a carboxy-terminal basic (positively charged) region. Another collapsin family member, collapsin-2, has identical structural domains but does not induce collapse of sensory neuronal growth cones in our functional assay.

To study the functional roles of these putative structural domains, several deletion and chimeric (collapsin-1—collapsin-2) mutants of collapsin-1 were constructed with a carboxy terminal *myc* tag, cloned into a mammalian expression vector, and expressed in a mammalian cell line. These muatant proteins, detected on western blots with anti-*myc* and anti-collapsin-1 antibodies, were assayed for their collapsing activity.

We found that 1) the carboxy-terminal basic region is not essential for activity; 2) the immunoglobulin loop is not essential for activity; 3) the collapsin-1 sequence at the amino-terminal end of the semaphorin domain is necessary for activity.

Additional chimeric constructs are being produced to further define the regions of collapsin-1 sequence required for its activity and specificity.

This work was sponsored by grants from the NINDS and NINCDS to JAR.

585.3

IDENTIFICATION AND CHARACTERIZATION OF CHICKEN COLLAPSINS 3, 4 AND 5. Leonard Feiner, Yuling Luo*, and Jonathan A. Raper. Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

In order to achieve the exquisitely complex connectivity found in the nervous system, the axons of newly born neurons navigate unerringly to their appropriate targets. This feat is accomplished by growth cones which respond to cues in the local environment which can be permissive, attractive or repulsive. Collapsin-1 is a glycoprotein that appears to be a repulsive cue for the growth cones of dorsal root ganglion cells but not retinal ganglion cells. We have cloned the entire open reading frames of three related proteins, collapsin-3, collapsin-4 and collapsin-5 by screening an E10 chicken library with partial sequences isolated by PCR. Collapsin-3 is the chicken homolog of mouse semaphorin E, while collapsin-4 and collapsin-5 are novel family members. All of these putative proteins contain a N-terminal semaphorin domain followed by a immunoglobulin domain. Collapsin-3 and collapsin-5 appear to be secreted proteins while collapsin-4 contains a transmembrane region followed by a 100 amino acid C-terminal region with no known homology.

We have begun to study the patterns of collapsin-3 mRNA distribution by in situ hybridization in both whole mounts and sections of chicken embryos. Collapsin-3 is expressed in dynamic patterns in the spinal cord and hindbrain as well as in non CNS tissues such as cardiac and renal primordia. Within the spinal cord, collapsin-3 is initially expressed during E3 and E4 in the roof plate, floor plate and notochord. By E6, spinal cord expression becomes restricted to a medial subpopulation of motor neurons. In the hindbrain, expression is found in the cranial ganglia of nerves V and VII and in their exit points in rhombomeres 2 and 4. We are now testing recombinant collapsin-3, 4 and 5 for collapsing activity on a variety of growth cones in culture.

This work was sponsored by grants from the NINDS and NINCDS to JAR.

585.4

A COLLAPSIN-1 BINDING PROTEIN IS EXPRESSED ON BOTH THE GROWTH CONES AND AXONS OF DRG. Hiroaki Kobayashi, Shigeru Saito*, Adam M. Koppel, and Jonathan A. Raper. Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Collapsin-1 (coll-1) is a secreted glycoprotein that is expressed in the nervous system during development and is thought to act as a repellent guidance cue for growth cones. All cultured E7 DRG growth cones collapse in response to coll-1. At E7 *in vivo*, coll-1 mRNA is expressed in the dorsal gray matter of the spinal cord. It could help to confine DRG axons to the dorsal funiculi and prevent premature entry of DRG axon collaterals into the cord. The receptor for coll-1, although not yet identified, should be expressed on coll-1 sensitive DRG growth cones.

To investigate the distribution of coll-1 receptors *in vivo*, a recombinant collapsin-1-alkaline phosphatase fusion protein (Coll-1-AP) was made to use as a probe. To test its specificity, DRG and retinal explants were incubated with Coll-1-AP. AP signal was observed along the entire length of DRG axons including the growth cones. Retinal growth cones do not respond to coll-1, and no staining was detected on retinal axons or growth cones. These observations suggest that the Coll-1-AP probe detects a cell surface coll-1 binding protein that could be a receptor. Specificity was further shown by the fact that excess unlabeled coll-1 competes with the binding of Coll-1-AP to DRG axons. The Coll-1-AP construct may be useful in identifying a coll-1-receptor.

This work was sponsored by grants from the NINDS and NINCDS to JAR.

585.5

IDENTIFICATION OF THE HOMOLOGUE OF CHICK COLLAPSIN-2 IN THE MOUSE. Michael J. Renzi and Jonathan A. Raper*. Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

The growth cone responds to a number of guidance cues during its complicated trek through the developing nervous system. Both attractive and repulsive in nature, these guidance cues help to assure that the appropriate connections are made between neurons and their targets. Collapsin-1 is a diffusible glycoprotein that has been shown to collapse sensory growth cones in an *in vitro* assay, suggesting that it acts as a repellent. It is a member of the semaphorin/collapsin family of proteins. Collapsin-2 is 50% identical to Collapsin-1 and contains a semaphorin domain, an immunoglobulin domain, and a carboxy-terminal basic region.

We have identified a partial mouse cDNA 85% homologous to chick collapsin-2. The 3' portion of this cDNA was isolated from an E10 mouse cDNA library. PCR was performed on cDNA from the same age mice to identify a clone which includes the 5' region and overlaps the library clone. Together these two clones comprise 93% of the expected mouse collapsin-2 coding sequence. We are currently probing sections with a digoxigenin labeled RNA probe to determine the distribution of mouse collapsin-2 RNA during development. We are also isolating genomic clones in an effort to further characterize this molecule.

This work was sponsored by grants from the NINDS and NINCDS to JAR.

585.6

COLLAPSIN-1 DELAYS THE GROWTH OF SENSORY AFFERENTS INTO THE DORSAL SPINAL CORD. K. Sharma*, Y. Luo, J. Raper, and E. Frank, Depts. of Neurobiology, Univ. Pitt. Med. Sch., Pittsburgh, PA 15261 and Univ. Penn. Med. Sch., Philadelphia, PA 19104.

During development, sensory axons from DRG grow to the spinal cord and project rostro-caudally within the future dorsolateral funiculus for 1 - 2 days before they begin to sprout collaterals into the dorsal horn. What prevents these axons from growing into the dorsal horn prematurely, during this waiting period? Recently, collapsin-1 mRNA was found not only in the ventral cord, but also dorsally, near the dorsal root entry zone, at stages shortly before and during the waiting period (st 25 - 31 in chick embryos). Because collapsin-1 can block the growth of sensory axons in culture, we tested if it could delay the normal ingrowth of sensory axons when applied exogenously at the end of the waiting period.

Spinal cord explants with attached DRG were made from st 30 chick embryos, embedded in agarose, and kept in culture for 2 days. At st 30, no afferents have grown into the dorsal horn, but after 2 days in culture extensive ingrowth has occurred. Muscle spindle afferents, revealed by staining for trkC, project ventrally through the medial dorsal horn while trkA-positive afferents arborize in the lateral dorsal horn. Injection of recombinant collapsin-1 into the ventral horn at the time of culture blocked the growth of both trkA+ and trkC+ sensory collaterals into the dorsal horn for the 2 day culture period. Thus if the collapsin-1 mRNA seen in dorsal cells reflects the presence of functional protein, it could explain why sensory afferents wait.

Although the inhibitory effects of collapsin-1 on trkA+ axons was expected, we were surprised to find that trkC+ axons were similarly affected. Earlier studies reported that sensory neurons grown in NT-3, which would select for trkC+ cells, were not inhibited by semaphorin-3, the mammalian homolog of collapsin-1. A possible explanation is that at earlier stages, during the waiting period, all afferents are inhibited, but then trkC+ afferents selectively lose their sensitivity to collapsin-1.

Supported by NINDS and NICHD to EF and JR.

585.7

COLLAPSIN-1 SPECIFICALLY ARRESTS CEREBELLAR MOSSY FIBER GROWTH CONES IN *VITRO*. D.H. Baird*, S. Rabacchi, B. Kruk, J.M. Solowska. Dept. of Neurobiology and Anatomy, Medical College of Pennsylvania and Hahnemann Univ., Philadelphia, PA 19129.

Cerebellar granule neurons are known to specifically arrest the extension of their mossy fiber afferents (the "stop-growing signal"), but the molecular basis of this effect is unknown. To determine if members of the collapsin/semaphorin family of proteins might contribute to this growth arrest, we applied purified, recombinant collapsin proteins to cultured mossy fiber growth cones from the basilar pontine nuclei of new born mice. Pontine mossy fibers innervate the cerebellar cortex postnatally during the period when granule neurons and Purkinje cells are maturing. When added to the culture medium at 1-5 ng/ml (final), collapsin-1 interrupted neurite elongation and collapsed growth cone lamellae within 1 min. Filopodia persisted, but became static. Theses effects were observed in 15/19 growth cones that extended rapidly prior to collapsin application. Neurite retraction was observed in some case. Sometimes growth cones recovered and advanced after collapsin-1 application, but recovery was not seen until at least 2 h post collapsin-1, and recovery was faster at lower concentrations. Collapsin-2 at an effective concentration twice that of collapsin-1 had little or no effect on growth cone motility in 17 rapidly advancing growth cones. In a second type of assay, collapsins or control materials were applied to cultured mossy fibers for 40 m. The cultures were fixed, and growth cone areas were measured. Collapsin-1 at about 5 ng/ml reduced growth cone areas by 40-50%, a highly significant result. These results indicate that collapsin-1 might play a role in the pathfinding and targeting of mossy fibers, including the stop-growing signal. Collapsins were generously provided by Y. Luo and J. Raper. Supported by NS33214 and HD07467.

585.8

CLONING AND ANALYSIS OF THREE ZEBRAFISH SEMAPHORINS. M.C. Halloran*, C.S. Yee, S.M. Severance, and J.Y. Kuwada, Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109

The semaphorin/collapsin gene family encodes putative repulsive axon guidance molecules, including both secreted and transmembrane proteins, all of which share a conserved sema domain of about 500 amino acids (Kolodkin, 1996, *Trends Cell Biol.* **6**:15). Several members of this family have been identified in chicken (Luo et al.,1995, *Neuron* **13**:1131), mouse (Messersmith et al., 1995, *Neuron* **14**:949; Puschel et al., 1995, *Neuron* **14**:941), human (Messersmith et al., 1995), and insects (Kolodkin et al., 1993, *Cell* **75**:1389). Chick collapsin-1 and mouse semaD inhibit specific axon outgrowth *in vitro*, however no function or activity has been described for the other vertebrate semaphorins. As a first step toward elucidating their *in vivo* function, we have initiated the cloning and analysis of these genes in the zebrafish. A zebrafish homolog to chick collapsin-1 has been characterized previously (Yee et al., 1995 *Soc. Neurosci. Abstr.* **21**:293), and we have now isolated three additional zebrafish semaphorins. SemaZ-VI shares 71% amino acid identity in the sema domain to chick collapsin-2. Whole mount *in situ* hybridization shows semaZ-VI expression in the floor plate underlying the midbrain and the posterior hindbrain, in two regions of the diencephalon, in the hindbrain, in sensory ganglia, and in the notochord. In the *cyclops* mutant, semaZ-VI expression in the floor plate and diencephalon is absent, while expression in other structures is unaffected. SemaZ-V shares 53% amino acid identity to chick collapsin-5 in the sema domain, and is expressed in the developing somites and in a midbrain nucleus. SemaZ-IV encodes a transmembrane protein sharing 44% amino acid identity with mouse semaC. It is expressed in the dorsal spinal cord and hindbrain, in a subset of cells in the ventral spinal cord, in the branchial arches, and in the tectum. The unique and specific expression patterns of these genes, and their identity as semaphorin family members suggests they play important roles during zebrafish development. Supported by NIH-NRSA (M.C.H.), NSF-RTG (S.M.S) and NINDS (J.Y.K.).

585.9

GENETIC ANALYSIS OF DROSOPHILA SEMAPHORIN I FUNCTION DURING GROWTH CONE GUIDANCE.
H-H. Yu, H. Araj, S. Ralls, and A.L. Kolodkin*, Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205

The semaphorins are a large family of related secreted and transmembrane proteins, several members of which are likely to function during neurodevelopment to inhibit or repel neuronal growth cones. These include the secreted proteins Semaphorin III in vertebrates and Drosophila semaphorin II, and the transmembrane protein Grasshopper Semaphorin I. Functional analysis of these semaphorins has thus far addressed the role they play in the generation of peripheral neuronal pathways, however the selective expression of these and other semaphorins in the central nervous system (CNS) suggests that semaphorins may also play a role in the establishment of central pathways.

We have undertaken a functional analysis of the transmembrane semaphorin D-sema I during embryonic neuronal development to address semaphorin function in CNS development. D-sema I shares with other semaphorins a 500 amino acid extracellular semaphorin domain, contains a single transmembrane domain, and has a small cytoplasmic domain. D-sema I is expressed in the CNS at the time when axon pathways begin forming and continues throughout embryogenesis, suggesting a role for D-sema I in the generation of CNS pathways. To gain insight into the role played by D-sema I in this process, we have taken a genetic approach, examining loss-of-function mutations in *d-sema I* for defects in selective fasciculation. We have also used transgenic strategies to complement these studies by ectopically expressing D-sema I on a variety of embryonic tissues. These studies will allow us to determine the role played by this transmembrane semaphorin in embryonic growth cone guidance.

Supported by grants from the Klingenstein, McKnight, and Whitehall foundations.

585.10

CLONING AND EXPRESSION OF TWO NOVEL MURINE SEMAPHORIN FAMILY MEMBERS L. Zhou*S.I. Lentz , F.A. White, D.E. Wright, and W.D. Snider, Dept. of Neurology, CSNSI, Washington Univ., St. Louis, MO, 63110.

Semaphorins are a very large family of molecules found in both vertebrates and invertebrates, the prototypical members of which are thought to be important for axon guidance. All family members have a characteristic 500 amino acid (AA) extracellular "semaphorin" domain which has a novel molecular structure. Most reported chick and murine homologues contain an IgG domain C-terminal to the Sema domain. At the N-terminal region these molecules contain a signal sequence and are thought to be secreted. We have previously described SemaX (here called Sema9), a novel Sema family member with 30-40% identity at the AA level in the Sema domain to previously published mouse homologues. We now report full length sequence of this new Sema family member. Sema9 shares considerable homology to Sema1 (SemaIII, SemaD) near the 3' end but diverges at the 5' end. Importantly, Sema9 has a molecular structure different than most vertebrate Semas in that it contains a transmembrane domain. We also now report partial sequence for Sema10, another novel murine Sema with approximately 25% identity at the AA level to other Sema family members.

In order to gain insights into potential functions of Sema9 and Sema10, we have compared expression of these molecules to other Semas in multiple regions of the developing and mature nervous system. Sema9 mRNA is widely expressed in the periphery (E9.5-PN-1) in patterns clearly distinct from Sema1 and Sema5 (SemaE). Interestingly, Sema9 is intensely expressed by non-neuronal cells as well as neurons in both dorsal root ganglion and spinal cord. Within the CNS, Sema9 has distinctly different expression patterns in several regions compared to Sema1 and Sema5. Sema10 also has novel expression patterns. In particular, Sema10 exhibits expression in peripheral and optic nerves and diffuse expression in spinal cord and sensory ganglia at embryonic stages. In addition, Sema10 is expressed in specific CNS cranial nuclei during development. Our findings of two additional Sema family members with expression patterns clearly distinct from any previously reported members underscore the diversity and complexity of this family of molecules in mammals. Supported by NIH.

585.11

SEMAPHORIN EXPRESSION IN THE DEVELOPING MAMMALIAN BRAIN.
I. Skaliora*[1], H. Betz[2] and A.W. Püschel[3]. [1]University Laboratory of Physiology Oxford OX1 3PT, UK and [2]Max Planck Institute for Brain Research, Frankfurt 60528, Germany.

During development, many axons have to navigate over considerable distances and through a variety of cellular environments in order to reach their appropriate targets. In this endeavour, they are guided by a combination of attractive and, as is becoming increasingly apparent, repulsive cues. We have examined the expression of five members of the semaphorin family of proteins, which have been implicated in several aspects of inhibitory or repulsive guidance mechanisms.

To determine the patterns of semaphorin expression during development of the thalamocortical pathway, we performed in situ hybridizations on coronal and sagittal sections from rat brains between E15 and P7. The tissue was embedded in paraffin, cut at 10µm and processed using ^{35}S-labelled RNA probes specific for semaphorins B, C, D, F and G, as described previously (Püschel et al., 1992).

All five semaphorins were detected during this developmental period and each manifested unique expression domains, examples of which are given below. SemD was expressed in the basal telencephalon from the earliest ages examined (E15-E17) at a time when thalamocortical and early corticofugal fibers coestablish the internal capsule (IC). It was also transiently expressed in the thalamic reticular nucleus (E17-P2) and the PRN/IC region (E17-P0). Transcripts for semB, C, F and G were detected in the developing cortical mantle throughout embryonic development in complementary and partially overlapping patterns - but *excluding* the intermediate zone and accumulating WM, through which many long-range axons are growing. In contrast, during *postnatal* development, the WM was heavily labelled with probes for semF and G. In the subplate, four out the five semaphorins were detected, and each followed a distinct timecourse of expression. Taken together these results document a high degree of specificity in the spatial and temporal expression patterns of semaphorins and support their possible instructive role in axon elongation, branching and synapse formation.

Supported by the MRC and DFG (SFB 269 and grant Pu102/4-1)

585.12

A STUDY ON A NOVEL MEMBER OF MOUSE SEMAPHORIN GENE FAMILY. S. INAGAKI[1], T. FURUYAMA[2], N. MIYAZAKI[1], S. SHIMADA[2], K. INOUE, Y. MINAMI, MASAYA TOHYAMA[2], [1]Dept. of Neurobiol., School of Allied Health Sci., [2]Dept. Anatomy & Neuroscience, Faculty of Medicine, Osaka University, Suita-shi, Osaka, 565, Japan.

Invertebrate semaphorin I (Sema I) and its related proteins, chick collapsin and mouse Sema III contribute to the axon guidance by their repellent actions. We have identified a novel member of the semaphorin gene family (M-Sema F) from the mouse brain. This member encodes a Sema domain which is similar among Sema I - III followed by a single putative immunoglobulin-like domain, a transmembrane domain, and a proline-rich intracellular domain. In situ hybridization histochemistry displayed the expression of the mRNA from early to late embryos throughout the brain and spinal cord. But the expression decrease as animals grow and was restricted to the forebrain, tectum and several brain regions, and only weak expression was seen in the adult. The expression was detected in the embryo In addition to the nervous tissue, some other tissues such as the lung and kidny. These suggest that this molecule is a novel transmembrane member of the semaphorin gene family of the vertebrate which may function in forming the neuronal network during development, although it is not known whether this molecule functions in the same fasion in the nervous system as Sema III functions as repulsive guidance cues to sensory nerve.

585.13

MOLECULAR CLONING AND CHARACTERIZATION OF NOVEL SEMAPHORINS

T. Kimura, S. Tojo* and K. Kikuchi Sumitomo Pharmaceuticals Research Center, Konohana, Osaka 554, Japan

Semaphorins are large family of proteins implicated in neuronal guidance during development. Although more than ten semaphorin genes have been cloned, only a few have been functionally analyzed. Northern analysis, however, has shown that some semaphorins are expressed in neonatal or matured animals as well as embryos(Neuron 14,941-948(1995), FEBS Lett. 370,269-272(1995), and our unpublished data). This prompted us to study function of semaphorins in matured animal. Our computer analysis identified more than twenty semaphorin-related sequences in EST database of infant or adult brain cDNA library. Using the sequence data, we cloned three new members of semaphorin genes from adult rat cDNA library(*semaW, Y, Z*). They were expressed mainly in adult. And the expression pattern was very characteristic in adult rat. That is, *sema*W was expressed in cerebrum, cerebellum and lung, *sema*Y in cerebellum and muscle, and *semaZ* in cerebrum, cerebellum and spinal cord. These results suggest that the semaphorins would, if any, have some function other than axon guidance in adult nervous system, in which neural network has been established. We are presently analyzing expression distribution of these semaphorins in more detail by in situ hybridization and immunohistochemistry.

Supported by Sumitomo Pharmaceuticals

585.14

MEMBRANE-ASSOCIATED MOLECULES REGULATE THE FORMATION OF LAYER-SPECIFIC CORTICAL CIRCUITS. V. Castellani and J. Bolz *, INSERM U.371 Cerveau et Vision, 69500 Bron, France.

Axon collaterals of pyramidal cells make stereotyped connections within some of the 6 cortical layers. In our previous experiments we examined the branch formation of axons extending from cortical explants prepared from E16 rats (which contain neurons destined for layer 6) on membrane substrates prepared either from layer 6, or from layer 5, or from layers 1-4 of young postnatal rats. We observed that axons emitted more collaterals on membranes from their target layers (layer 6 and layers 1-4) than on membranes from layer 5, their non-target layer (Castellani et Bolz, Neurosci. Abstr. 1995). We now examined the behavior of axons from cells in the superficial cortical layers. For this we prepared explants of the ventricular zone at E19, the time at which cells of the deep cortical layers have migrated out into the cortical plate. Axons of cells destined for layers 2/3 branched about 2 times more often on membranes from layer 5 or from layers 1-4 than on membranes from layer 6. Our finding that layers 2/3 cells exhibit many axonal branches on membranes from layer 5, a target layer, as well as on membranes from layers 1-4, a mixture of target and non-target layers for this set of axons, suggests the existence of sprouting factors in the target layers. To address this issue further, for layer 6 cells we mixed membranes from their target and non-target layers. We observed an equal amount of axon collaterals on the mixed membrane substrates as on membranes from the target layers alone. In addition, heat-inactivation strongly decreased axonal branching on target layers, but had no effect on non-target layers. This suggests that membrane-associated molecules restricted to individual layers induce collateral branch formation of specific sets of axons. Moreover, cortical axons in vitro express their layer-specific branching behavior at very early developmental stages, before the neurons have migrated into their final position in the cortex. (Supported by INSERM).

585.15

DO SEMAPHORINS ACT AS GUIDANCE MOLECULES FOR AFFERENT AND EFFERENT PROJECTIONS IN THE DEVELOPING CEREBRAL CORTEX ? D. Bagnard[1], H. Betz[2], M. Götz*[1], A.W. Püschel[2] and J. Bolz[1]. [1]INSERM U.371 Cerveau et Vision, 69500 Bron, France; [2]Max-Planck-Institut für Hirnforschung, Abt. Neurochemie, 60528 Frankfurt, Germany.

Previous tissue culture studies provided evidence for the existence of diffusible and membrane-associated guidance factors which control the formation of reciprocal connections between thalamus and cortex during corticogenesis (for review see Bolz et al., TINS 16, 310, 1993). The molecular nature of these factors is not known. In the developing spinal cord it has been shown that members of the semaphorin gene family act as repellent signals during the navigation of sensory and motor axons towards their peripheral targets. Based on their homology to chicken collapsin and grasshopper semaphorin I several distinct cDNAs (*semA - semG*) have been cloned from mouse cDNA libraries (Püschel et al., Neuron 14, 941, 1995). We are studying whether semaphorins might also exert an effect on growing thalamic and cortical axons. For this we employ various in vitro systems to examine the influence of soluble and membrane-bound semaphorins on growth cone navigation and axon elongation. Application of conditioned medium from 293 cells transfected with Sem D caused a collapse in 54% of cortical growth cones and 51% of thalamic growth cones. In contrast, after addition of medium from non-transfected 293 cells or from 293 cells transfected with Sem E, only 20-24% of cortical and 9-23% of thalamic growth cones were in a collapsed state. In cocultures with aggregates of 293 cells transfected with a Sem D expression vector and either thalamic or cortical explants in a 3 dimensional plasma matrix, axons extending from the explants were deflected from the cell aggregates. These results suggest that at least one member of the semaphorin gene family can orient the growth of thalamic and cortical axons. (Supported by INSERM and DFG grant Pu102/4-1 to A.W.P.).

585.16

REPULSION OF SPINAL COMMISSURAL AXONS BY A DIFFUSIBLE FACTOR FROM THE ROOF PLATE A. Augsburger, A. Schuchardt, S. Hoskins and J. Dodd* Dept. Physiology & Cellular Biophysics, Columbia University, New York, NY 10032.

The earliest spinal commissural (C) neurons in the rat differentiate close to the roof plate (RP) at the dorsal midline of the spinal cord. C axons extend in a ventral direction, away from the RP. Upon reaching a point approximately half way down the dorsoventral axis of the spinal cord, C axons alter their trajectory and project ventro-medially towards the floor plate (FP), apparently under the influence of diffusible chemoattractants, the netrins. Because the initial ventral extension of C axons occurs in the absence of FP-derived signals, we have examined the possibility that the direction of initial axon growth is influenced by repellant signals from the RP. To test this we have assayed the effects of RP tissue on the growth of C axons. Explants of dorsal neural tube containing C neurons were grown in collagen gel in the presence of netrin-1 or FP-conditioned medium, under which conditions C axons, identified by TAG-1 expression, projected into the collagen gel. When E11 or E13 RP was positioned ~150 μm from the ventral edge of the dorsal explant ventrally directed C axons stopped growing or altered their direction of extension, to avoid the RP explant. RP tissue repels C axons but not several other classes of central and peripheral axons. The RP-derived repellant activity is not mimicked by several other tissues of neural and non-neural origin. These studies suggest that the dorsoventral trajectory of C axons in the spinal cord is controlled initially by a RP-derived repellant and subsequently by a FP-derived chemoattractant.

NIH: NS 27113 to JD. NIH: T32-AG00189 (AS).

FORMATION AND SPECIFICITY OF SYNAPSES V

586.1

ROLE OF HB-GAM (HEPARIN-BINDING GROWTH-ASSOCIATED MOLECULE) IN PROLIFERATION ARREST AND ITS ASSOCIATION WITH THE DIFFERENTIATING NEUROMUSCULAR SYSTEM. E.Szabat, L. Ahtee* and H.Rauvala. Lab. of Molecular Neurobiology, Institute of Biotechnology, University of Helsinki, Finland

HB-GAM is a secretory, extracellular matrix-associated protein that was isolated be sreening for proteins that enhance neurite outgrowth in perinatal rat brain neurons. In the present study we have investigated the possible role of HB-GAM in cell proliferation in the developing limb of the rat. Exogenously added recombinant HB-GAM was found to inhibit the proliferation of mesenchymal and epithelial cells in cultured limb buds, as demonstrated by bromodeoxyuridine incorporation and by staining for proliferating cell nuclear antigen. The inhibitory effect of HB-GAM on cell proliferation was reversed by heparin, suggesting that HB-GAM may bind to heparin-type carbohydrate epitope that is required for cell proliferation in the developing limb. In agreement with the putative role in proliferation arrest, endogenous HB-GAM of the developing limb was found to be expressed in a proximal-to-distal pattern. Furthermore, HB-GAM was associated with the muscle surface as demonstrated in double-immunostaining of HB-GAM with desmin and myosin heavy chain proteins. Coinciding with the onset of synapse formation, HB-GAM was found in patches on the muscle cell surface in close proximity to nicotinic acetylcholine receptor clusters.

Academy of Finland and S.Juselius Foundation

586.2

PROTEIN AND GENE EXPRESSION FOR TRANSGLUTAMINASE (TG) IN DEVELOPING MOUSE MUSCLE. E. J. Gregory, D. L. Steigerwalt, M. N. Zoubine, B. A. Citron, B. W. Festoff* Neurobiology Research Lab, VA Medical Center, Kansas City, MO: and Department of Neurology, University of Kansas Medical Center, Kansas City, KS

TG catalyzes the Ca^{++} dependent cross-linking of proteins via the γ-carboxamide group of glutamine residues and the ε-amino group of lysine. TG is important in neural development, having a biphasic increase in activity: the first embryonic then postnatal, coincident with neural apoptosis. TG is implicated in formation of the apoptotic body, but it also has a role in tissue stabilization, since the resulting isopeptide after cross-linking is stable and resistant to proteolysis. Our model of synapse formation and maintenance at the neuromuscular junction (NMJ) led us to study clotting cascade components and related molecules in development. In this study, we analyzed protein (cytosolic and particulate fractions) and gene expression of TG in developing murine skeletal muscle from embryonic through postnatal times. TG activity was determined by the incorporation of radioactive putrescine into dimethylcasein. RT-PCR was performed using synthesized 3' and 5' oligonucleotide primers based on known TG sequence. Cytosolic TG specific activity increased significantly from E14 (383±8 pmol/min/mg) to B (2340±500) and remained elevated until P7 when it decreased rapidly by P10, remaining low through P30. Particulate TG also increased significantly from E14, but with a much shorter duration, decreasing just prior to birth and remained constant through P30. RT-PCR showed increased TG mRNA levels from birth to P15 with lower levels at P30. Peak cytosolic TG activity coincides with NMJ formation and previous work in this lab led us to hypothesize a role for TG in synapse stabilization. Murine polyneuronal synapse elimination occurs from birth to P15, coinciding with the dramatic decrease in cytosolic TG, when its role in synapse stabilization and other cellular stabilization processes may be diminished. Supported by the Marion Merrell Dow Foundation/SEP and the Medical Research Service of the Dept. of Veterans Affairs.

586.3

EVIDENCE THAT S-LAMININ/LAMININ-β2 HAS A DIRECT ROLE IN SYNAPTIC REGULATION OF SCHWANN CELLS *IN VIVO* AT THE NEUROMUSCULAR JUNCTION. Bruce L. Patton* and Joshua R. Sanes. Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

The synaptic basal lamina (BL) of the vertebrate neuromuscular junction promotes pre- and postsynaptic differentiation and contains specialized components. *In vitro*, recombinant forms of one such component, S-laminin/laminin-β2, cause neurites extending from motoneurons to stop and differentiate into presynaptic terminals. In "knockout" mice lacking laminin-β2, nerve terminal differentiation is impaired, consistent with *in vitro* results (Noakes et al., 1995, Nature 374:258). In addition, Schwann cell processes, which normally cap nerve terminals, invade the synaptic cleft in the laminin-β2 mutant thus enwrapping presynaptic terminals. Our initial interpretation of the aberrant Schwann cell behavior was that Schwann cells were reacting to defective nerve terminals. This hypothesis is consistent with some neuromuscular pathologies in which nerve terminals become enwrapped by the terminal Schwann cell. However, we later found that recombinant laminin-β2 inhibited the migration of Schwann cells *in vitro* (Patton et al., 1995, Soc. Neurosci. Abst. 323.3). This suggested that one function of laminin-β2 *in vivo* is to actively inhibit Schwann cell invasion of the synaptic cleft. To test this idea, we examined denervated control and laminin-β2 mutant muscles by EM. As expected, Schwann cells remained at synaptic sites after the axon had degenerated. However, in controls, only 5% of the synaptic BL was directly contacted (<70 nm) by a Schwann cell process. In contrast, close contact was increased 10-fold in mutants, averaging 50% of the total cleft. Thus laminin-β2 appears to inhibit Schwann cells from establishing intimate contact with the synaptic BL *even in the absence of axons*. Interestingly, in ganglia and spinal cord as well as in muscle, pathologic detatchment of synapses involves enwrapping of nerve terminals by their associated glial cells. The usual interpretation is that deprived nerve terminals first withdraw from their postsynaptic contact and are secondarily enwrapped. Our results raise the possibility that in some cases a change in postsynaptic structure regulating glial cell behavior is more directly responsible. (Supported by NIH.)

586.4

RAT NEUROMUSCULAR JUNCTIONS GAIN TERMINAL SCHWANN CELLS DURING POSTNATAL DEVELOPMENT. F.M. Love* and W.J. Thompson. Department of Zoology, The University of Texas, Austin, TX 78712.

Rat neuromuscular junctions undergo synaptic rearrangements in early postnatal development leading to the innervation of each muscle fiber by a single motor neuron. Recent experiments have suggested that terminal Schwann cells (SCs) influence the growth of nerve terminals and the innervation of endplates. We have, therefore, examined how the number of terminal SCs at neuromuscular junctions changes between birth and adulthood. SCs were labelled with antibodies to S100, nerve terminals and axons were labelled with antibodies to neurofilament and synaptophysin, and nuclei were labelled with DAPI. The number and position of SC nuclei were determined by the colocalization of DAPI and S100 labels. All nerve terminals were covered by SC processes at all ages examined. However, at birth, SC somata were present over only about one-half of the endplates in soleus and two-thirds of those in EDL. In most cases where a SC soma was absent from the terminal, one was present along the preterminal axon. The SC processes which cover the terminal, therefore, are extensions of these preterminal SCs. After postnatal day 7, all junctions had overlying SC somata. During the period from birth to adulthood, the average number of terminal SC somata increased from 0.4 to 2.6 in soleus and from 0.9 to 3.4 in EDL. As the number of SC nuclei atop the terminals increased, the number along the preterminal axons declined. Taken together, these observations suggest that during development SCs migrate along preterminal axons to cover the nerve terminals. Moreover, as the number of SCs at an endplate increases, the proportion of the endplate area occupied by an individual SC decreases. This change in the number and morphology of terminal SCs shows that SCs and nerve terminals rearrange their contacts at the developing neuromuscular junction.

This research was supported by a grant from NSF.

586.5

THE SCHWANN CELLS AND THE NEUROMUSCULAR SYSTEM. J. Koenig*, J. Chapron, S. de la Porte, K.X. Zhang, G. Escher[a] and H. Cheneiweiss[a°]. University Bordeaux II- CNRS URA 1226 - France. [a]Insutut Anatomie Lausanne - Suisse. [°]INSERM U114, Paris -France.

At the neuromuscular junction (NMJ), nerve terminal, Schwann cell and muscle cell are juxtaposed during cell differentiation and synaptogenesis. We investigated in vitro the influences of Schwann cells (S) on motoneurons (MN) outgrowth and synaptogenesis.

For MN outgrowth: when motoneurons were cultured for 48 h in presence of S or conditioned medium (CM) by S, the axon outgrowth increases by 16-fold, that as to be compared to the 3-4-fold increase produced by basal lamina components. We will compare the effect of GDNF (glial cell line derived neurotrophic factor) and CM by S cells on the axon outgrowth.

For synaptogenesis, we analyzed the appearance and maturation of synaptic markers: i) When Schwann cells or CM were added to muscle-nerve co-cultures, we observed a modest increase in the number of membranes acetylcholine receptor (AChR) (10%), but a large increase of AChRs clusters (85% with S and 40-50% with CM). Thus, the Schwann cells release factor(s) which induce AChRs clustering, 2) Synaptic maturation results in a switch of synaptic cholinesterase from AChE-BChE complex to AChE. S are involved in this phenomena. Indeed, addition of S or CM to muscle-neuron co-cultures induced a regression of BChE, as in adult NMJ. This effect is inhibited by the 6.17 antibody which recognizes a molecule synthetized by S. Thus, in addition to the neuronal factors CGRP, ARIA/heregulin, agrin, which control the synthesis, maturation and accumulation of AChRs, Schwann cells synthesize factor(s) involved in maturation of synaptic cholinesterases.

By acting on motor-axon outgrowth, AChRs clustering and synaptic cholinesterases maturation, Schwann cells play a key role in the neuromuscular system.

586.6

SCHWANN CELLS AT THE NEUROMUSCULAR JUNCTION AND THEIR ROLE IN THE MAINTENANCE OF SYNAPTIC INTEGRITY. J.T. Trachtenberg* and W.J. Thompson. Department of Zoology, The University of Texas, Austin, Texas 78712.

Schwann cells (SCs) at neuromuscular junctions (nmjs) behave during nerve sprouting in a manner that suggests that these cells play a major role in inducing and guiding nerve growth. The role of SCs at unperturbed nmjs, however, is largely unknown. We have begun to examine the influence of terminal SCs on the maintenance of synaptic integrity at nmjs by disturbing the morphology and position of these cells. To induce changes in terminal SC morphology and position, we have transplanted foreign nerves over the surface of normally innervated soleus muscles. In these preparations, SCs growing from the transplant interact with terminal SCs at nmjs, resulting in the morphological disruption of affected synapses manifest as a smaller and fragmented nerve terminal arborization. Muscles were stained with antibodies to neurofilament and synaptophysin to label axons and nerve terminals and either anti-S100 to label SCs or bungarotoxin to label acetylcholine receptors (AChRs). In muscles in which the SCs and the nerve terminals were stained, we were able to identify remodeled endplates at which the SCs growing out of the transplant had interacted with and induced the migration of the terminal SCs. In preparations employing bungarotoxin we were able to identify remodeled endplates in which portions of the AChR plaque were no longer covered by a nerve terminal. Some of these deserted AChR patches were fragmented in appearance, suggesting that these receptors were being lost. These data suggest that the integrity of an endplate is dependent on the maintenance of its terminal SCs and that the displacement of terminal SCs or their processes is followed by the loss of the underlying nerve terminal, resulting in the presence of bare patches of AChRs. These results may provide a mechanism by which transplanted, foreign nerves compete with the original nerve for the innervation of existing synaptic sites (Bixby & Van Essen, *Nature* 282: 726-728). This work was supported by a grant from the NIH.

586.7

Agrin Function is Required for Pre- and Postsynaptic Differentiation at Neuromuscular Junctions *in vitro*. J.L. Bixby*, M.A. Rüegg[‡] and J.A. Campagna. Dept. Mol. & Cell. Pharm., Univ. Of Miami, Miami, FL 33101 and [‡]Dept. of Pharm., Biozentrum, Basel, Switzerland.

Agrin is a heparin sulfate proteoglycan; multiple isoforms are synthesized by both muscle and nerve. Neural agrin is likely to induce aggregation of acetylcholine receptors (AChRs) and other postsynaptic proteins during synapse formation at the neuromuscular junction (NMJ) (Reist *et al.*, Neuron 8, 865; Bowe and Fallon, Ann. Rev. Neurosc. 18, 443). We have previously shown that either neural or muscle agrin, when heterologously expressed, has those properties predicted for a motoneuronal "stop signal" (Campagna *et al.*, Neuron 15, 1371, 1995). To test the hypothesis that agrin's activities on motoneurons are important for presynaptic differentiation *in vitro*, we used an agrin antibody to disrupt agrin function in chick ciliary ganglion (CG) neuron/myotube cocultures (e.g. Bixby, J. Neurobiol. 26, 262, 1995). In cocultures grown in the presence of 200 μg/ml anti-agrin IgG, both AChR clustering and clustering of the synaptic vesicle protein synaptotagmin (syt) were inhibited. Syt clustering was still abrogated in the presence of 100μg/ml blocking antibody, while the postsynaptic clustering of AChRs, heparan sulfate proteoglycan, and s-laminin was retained. These results demonstrate that agrin function is required for clustering of postsynaptic proteins at nerve-muscle contacts *in vitro*. Coupled with the observations that agrin can be sufficient to induce syt clustering, our results also suggest that agrin is directly involved in the induction of presynaptic differentiation, at least *in vitro*. Supported by NSF grant IBN 9309526.

586.8

FORMATION AND ELIMINATION OF NEUROMUSCULAR JUNCTIONS DIRECTLY OBSERVED IN LIVING FROGS (*XENOPUS LAEVIS*). Albert A. Herrera,* Neurobiology Program, Biological Sciences, University of Southern California, Los Angeles, CA 90089-2520.

The pectoral muscle (supracoracoideus) of early postmetamorphic *Xenopus laevis* is being used as a preparation in which myogenesis and the formation, elimination, and maturation of neuromuscular junctions can be directly observed and manipulated. There are about 800 fibers in the adult muscle, most of which are innervated at two widely separated sites. Starting at a stage when only 10% of these fibers have developed, muscles are surgically exposed and stained with the vital fluorescent dye RH414 and other synapse-specific markers. *In vivo* observation of identified cells repeated at 1-4 week intervals reveals the following. 1) Fibers are innervated as soon as they appear, with well-formed synaptic contacts seen on fibers that are still enlarging by myoblast fusion. 2) One or more additional junctions is added soon after the first junction, usually at a distant location. 3) New junctions are formed by nodal or terminal sprouts that arise from axons supplying nearby fibers. 4) Elimination of focal polyneuronal innervation is seen as regression of established synapses in junctions that are apparently multiply innervated. 5) Following such synapse elimination, surviving nerve terminals in the same area enlarge by extension of growth cones, but abandoned synaptic sites are not reoccupied. 6) Growth in muscle fiber and body size is accompanied by the elaboration of many new junctional branches, with synaptic size increasing 20-50 fold. 7) At the earliest stages, each muscle has the full complement of 2-3 muscle spindles.

A variety of molecular probes and focal lesions are being used to study these processes in identified, repeatedly observed junctions and to test hypotheses for underlying mechanisms. Supported by the NIH (NS24805).

586.9

NEUROMUSCULAR JUNCTION FORMATION DURING NORMAL DEVELOPMENT: A REFLECTED LIGHT CONFOCAL STUDY OF THE POSTSYNAPTIC MEMBRANE. M.J.Marques, C.C.Nelson and J.W.Lichtman*, Dept of Anatomy & Neurobiol.,Washington Univ. Med. Sch., Saint Louis, MO 63110.

Developmental changes at the neuromuscular junction were studied by making reflected light confocal reconstructions of the acetylcholine receptor rich postsynaptic membrane. Sternomastoid muscles from embryonic day 17 (E17), postnatal day 2 to 21 (P2 to P21) and adult mice were fixed and their AChRs stained with α-bungarotoxin-biotin-avidin-HRP. Using a confocal microscope (Noran,Odyssey) the muscles were observed under epi-illumination with a 100X, 1.4NA oil objective. The structural findings obtained with this technique were superior to those we obtained with fluorescenctly tagged α-bungarotoxin also using confocal microscopy. Because the HRP reaction product at the postsynaptic membrane shows specular reflectance, only light coming from the relatively untilted regions of the membrane is collected by the objective. Thus, junctional folds appear as dark bands whereas the tops of the folds are bright. Typically, in adults, the folds run parallel to each other and roughly orthoganol to the long axis of the primary synaptic cleft. Much flatter endplates with no clefts were typical for the earliest ages (E19 to P2), where the postjunctional membrane shows a mosaic of wide irregularly distributed receptor areas. Beginning at P4, receptor regions with different heights were observed within the same endplate, nascent folds were seen in the deeper areas while loose receptor spots irregularly distributed and less reflective were seen at the more superficial areas. From our studies we conclude that the 3D topography of the neuromuscular junction is being established at the same time the distribution of AChRs is undergoing major changes. Reflected light confocal microscopy thus seems to be a useful approach for the study of the topography of the postsynaptic membrane.

Supported by grants from FAPESP and the NIH.

586.10

ULTRASTRUCTURE OF THE DEVELOPING NEUROMUSCULAR JUNCTION IN DROSOPHILA EMBRYOS. M.B.Rheuben*, M.Yoshihara, and Y.Kidokoro, Dept. of Anatomy, Michigan State Univ., E. Lansing, MI 48824, and Inst. for Behavioral Science, Gunma Univ. Sch. of Med., Maebashi 371, Japan.

The development of neuromuscular junctions on embryonic abdominal muscle fibers 1 and 9 was examined using scanning and transmission electron microscopy. Around 16.5 hrs AEL, during a stage designated as the "prevaricosity period" from confocal observations (Yoshihara *et al*, in preparation), the axons swell at the nerve entry point (NEP) on the muscle fiber, and partial retraction of the long, exploring filopodia occurs. Contacts of an axon terminal with the muscle fiber in this region include synaptic components: a flat apposition with the muscle fiber membrane and a presynaptic dense body surrounded by a cluster of vesicles. Postsynaptic folds of the muscle are lacking. Peripheral glial processes end within the NEP. The first, smaller than normal, miniature endplate currents are observed during this period (Kidokoro and Nishikawa, 1994). Around 19 hrs AEL the enlarged regions of the axons of the "prevaricosity" condense, and constrictions divide them into several varicosities at the NEP. Each varicosity might contain 4 to 9 well-formed active zones. In Type I junctions, the postsynaptic membrane becomes more obviously electron dense, and cavities form beneath it, separating a thin layer of muscle close to the terminal. These thin layers, as well as outgrowths from the muscle fiber, form the subsynaptic reticulum, which, by hatching, only consists of one or two lamellae in Type I varicosities. By 16 hrs multiple axon types, having vesicles with differing structures, are present on the dorsal muscles, and by hatching both Type I and Type II varicosities can be distinguished. Supported by grants from M.S.U. AURIG (MBR), Yamada Science Foundation (YK), and the Ministry of Education, Science, and Culture of Japan (YK and MY)

586.11

DEVELOPMENTAL REGULATION OF β-AMYLOID PROTEIN PRECURSOR DURING NEUROMUSCULAR SYNAPTOGENESIS. M. Akaaboune*, B. Allinquant, P. Mailleux and D. Hantaï. INSERM U.153 and CNRS URA 1414, F-75005 Paris.

β-amyloid protein precursor (APP) is a transmembrane glycoprotein present in numerous tissues and especially in the central and peripheral nervous system. In this work we have examined the expression of APP in the developing skeletal muscle of the mouse using Western blotting and immunocytochemistry with confocal microscopy. Using anti-APP polyclonal antibodies directed against different domains of the molecule, we have found that APP isoforms are present in increasing amounts from embryonic day 16 to postnatal day 5 and then decrease progressively to reach low levels observed in the adult. An antibody against the 695 isoform of APP detected a 94 kDa band that appeared in the adult skeletal muscle, whereas isoforms of higher molecular weights were decreased. Immunocytochemistry revealed that APP was localized in the cytoplasm of myotubes at embryonic day 16 and became gradually concentrated at the neuromuscular synapse from birth to adulthood. Moreover, confocal laser microscopy analysis showed that APP became increasingly colocalized with acetylcholinc receptor from birth onward. Taken together, these results show that APP is regulated as neuromuscular development proceeds and suggest a role for APP in synaptogenesis. This work was supported by AFM.

586.12

Regulation of Synaptotagmin Gene Expression During Neuromuscular Synaptogenesis in vivo. J.A. Campagna*, David Prevette‡, R.W. Oppenheim‡, J.L. Bixby. Dept. of Mol. & Cell. Pharm., Univ. Of Miami, Miami, FL 33101 and ‡Neurobiology & Anatomy, Bowman Gray School of Med., Winston-Salem, N.C., 27157.

During neuromuscular synaptogenesis, signals from motor neurons regulate the synthesis and the assembly of postsynaptic elements. Similarly, it has been hypothesized that signals from target muscles regulate synthesis and assembly of presynaptic components in motor neurons. The synaptotagmins (syts), a family of synaptic vesicle proteins, are differentially regulated during development of peripheral motor neurons, and upregulation of syt I mRNA is temporally correlated with target innervation (Lou and Bixby, Mol. Cell. Neurosci. 6, 252, 1995). To test the generality of these observations, and to test the hypothesis that upregulation of syt mRNA is causally related to target contact, we examined the expression of syt I and syt II mRNAs in motor neurons of the chick lumbar spinal cord using in situ hybridization. Ventral horn motor neurons upregulate syt I mRNA at the the time when axons are making contact with peripheral targets (E5-E6). Syt I mRNA is downregulated after synaptogenesis (E15-E20), and syt II mRNA is upregulated. Unilateral limb removal (Oppenheim et al., J. Comp. Neur., 177, 87) largely prevents the early increase in ipsilateral syt I gene expression. These results suggest that target contact induces the developmental upregulation of syt I in spinal motor neurons , and that a later stage of synaptic maturation results in changes in syt isoform expression. Supported by NSF grant IBN 9309526.

586.13

RECONSTITUTION OF AVIAN ACETYLCHOLINESTERASE ON TO THE FROG NEUROMUSCULAR SYNAPSE. Susana G. Rossi, Richard L. Rotundo, and Lili Anglister*, Depts. of Cell Biology and Anatomy, Univ. of Miami Sch. of Med. Miami, FL 33101, and Anatomy and Cell Biology, Hebrew Univ. Hadassah Med. Sch. Jerusalem, Israel.

The highly organized pattern of acetylcholinesterase (AChE) molecules at the neuromuscular junction (NMJ) suggests the existence of specific binding sites for their precise localization. The predominant AChE isoform localized at NMJs is the asymmetric A12-AChE which is tightly linked to the extracellular matrix via its collagen tail. To test the hypothesis that a specific array of target molecules exists for directing attachment of newly synthesized enzyme, we "transplanted" immunoaffinity purified globular (G4/G2) and asymmetric (A12) AChE forms from quail muscle cultures to frog (*Rana pipiens*) NMJs. Frozen sections of adult frog muscles, or empty basal lamina sheaths of the muscles, were incubated overnight with purified AChE forms under conditions that allowed a gradual decrease of ionic strength. Frog NMJs were localized using Rh-αBTX and the quail AChE was localized using avian-specific mAb 1A2. There was no detectable binding above background levels of the globular avian G4/G2 AChE to frog NMJs, whereas more than 80% of the frog NMJs bound the quail enzyme when incubated with A12-AChE form. These results indicate that specific binding sites for A12-AChE exist at the NMJ and are organized to form a "molecular parking lot" in which the AChE molecules can be inserted and removed as required. Supported by grants from the NIH to R.L.R. and Israel Acad.of Sci. to L.A.

586.14

MUSCARINIC RECEPTOR-DEPENDENT DAG PRODUCTION IN SKELETAL MUSCLE REQUIRES Ca^{++} INFLUX THROUGH VSCCs Rosely O. Godinho and Richard L. Rotundo*, Department of Cell Biology and Anatomy, Univ. of Miami Sch. of Med. Miami, FL 33101.

The synthesis and assembly of acetylcholinesterase (AChE) requires Ca^{++} influx through L type channels and diacylglycerol (DAG)-dependent PKC activation. Previous experiments from our lab showed that DAG production in skeletal muscle can be stimulated through muscarinic receptors (mAChR). To study the interaction between membrane depolarization/Ca^{++} influx and mAChR activation, we examined the effects of Ca^{++} channel agonist (Bay-K 8644) and antagonist (nifedipine) on DAG production induced by mAChR activation. Electrical stimulation of myotubes resulted in an 80% increase in DAG. This effect was mimicked by 1 μM veratridine and 50 nM Bay-K, but inhibited by 10 μM nifedipine. Stimulation of mAChR with Oxotremorine-M produced a 750% increase in DAG that was blocked by nifedipine. Our results indicate that both membrane depolarization and mAChR activation are necessary to increase DAG production in cultured myotubes. Immunofluorescence studies using anti-rat mAChR antibodies showed that M1 mAChRs are present on cultured myotubes as well as restricted to the neuromuscular junction of adult skeletal muscle where they co-localize with nAChRs. We suggest that concurrent activation of both nAChRs and mAChRs at the neuromuscular junction are involved in DAG production and PKC activation leading to the regulation of AChE synthesis and assembly. Supported by the FAPESP and NIH/FOGARTY (ROG) and NIH (RLR).

586.15

LOCALIZED MEMBRANE DEPOLARIZATION REGULATES ACETYLCHOLINESTERASE mRNA LEVELS IN MYOTUBES.
A.E. Vazquez*, S.G. Rossi and R.L. Rotundo. Dept. of Cell Bio. and Anat.,Univ. of Miami Sch. of Med., Miami, Fl. 33136.

In skeletal muscle, acetylcholinesterase (AChE) protein as well as mRNA are highly concentrated at neuromuscular junctions. To study the mechanisms responsible for the local regulation of expression, we examined the effects of localized membrane depolarization or sodium channel block on the levels of AChE mRNA in quail muscle cultures. RNase protection experiments indicate that 5 μM tetrodotoxin increases the AChE mRNA levels while treatment with 20 nM scorpion venom or 1-10 μM veratridine decrease AChE mRNA levels with respect to control. Localization of AChE mRNA by in situ hybridization allows identification of individual nuclei expressing the AChE gene (AChE mRNA+ nuclei). Tetrodotoxin increases the number of AChE mRNA+ nuclei, whereas scorpion venom treatment decreases the number of AChE mRNA+ nuclei relative to untreated controls. Detection of AChE mRNA+ nuclei in experiments where only a portion of the muscle fibers were exposed to the drugs, while the other portion was maintained in normal medium, show that each nuclei responds to signals originating specifically on the overlying region of the plasma membrane. These studies suggest that localized membrane depolarization can be responsible for regulating AChE mRNA expression in the underlying nuclei. Supported by grant from the NIH to R.L.R.

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS VIII

587.1

NEUROFIBROMIN NEGATIVELY REGULATES NEUROTROPHIN SIGNALLING IN EMBRYONIC SENSORY NEURONS. K. S. Vogel, M. El-Afandi, L. Klesse, and L. F. Parada*. Center for Developmental Biology, U.T. Southwestern Medical Center, Dallas, TX.

Neurofibromin, the product of the neurofibromatosis type 1 (NF1) gene, negatively regulates p21 ras signalling through its GTPase-activating protein (GAP) domain. To understand how neurons acquire dependence on neurotrophins that signal through trk receptors and the ras pathway, we compared survival of sensory and sympathetic neurons isolated from NF1 mutant and wild-type mouse embryos. We have shown that neurons that lack neurofibromin survive in the absence of neurotrophins and that mutant neurons isolated prior to target contact fail to acquire neurotrophin dependence in vitro. To determine whether neurofibromin acts as a negative regulator of p21 ras in embryonic neurons, we introduced Fab fragments of a function-blocking anti-ras antibody into NF1 mutant and wild-type trigeminal and DRG neurons. Anti-ras antibodies block the survival of both NF1 mutant and wild-type sensory neurons; these neurons can be rescued by NGF. In the presence of anti-ras Fab fragments, NF1 mutant neurons are ten times more sensitive to the rescue effects of NGF than are wild-type neurons. Our results are consistent with a model wherein p21 ras signalling may occur constitutively in the absence of neurofibromin in embryonic sensory neurons. To explore further the role of neurofibromin in neurotrophin signalling, we are examining the survival of neurons isolated from embryos mutant for both neurofibromin and trk receptor or neurotrophin genes. Supported by NIH Grant# 1-R01-NS34296-01.

587.2

A PROPOSED ROLE FOR NARIA IN SKELETAL MUSCLE DEVELOPMENT. T. Mason*, Y. Kuo, X. Yang and L. Role. Department of Neurology; Center for Neurobiology and Behavior, Columbia University, P&S, 722 W. 168th St. NY, NY 10032

The neuregulins represent a family of related proteins which are potential ligands for erbB3 and erbB4. They exist in multiple isoforms, sharing a common epidermal growth factor (EGF) domain. Neuregulins function to activate acetylcholine receptor gene (AChR) expression and to induce Na^+ channel formation in cultured chick muscle (Fischbach and colleagues; *Cell*, 72: 801; *JNeurosci*, 13: 2118). Recently, we have described a novel neuregulin splice variant which lacks an immunoglobulin-like domain in its N-terminus, termed nARIA (unpublished work of Y.K., L.R. and X.Y.). PCR, Northern, and *in situ* hybridization demonstrate that nARIA is the predominant form of neuregulin expressed in both visceral and somatic motoneurons early in synaptogenesis (X.Y., Y.K. , L.R.; in prep.).

Using a nARIA isoform-specific antibody, protein can be detected in nerve fibers within skeletal muscle. To investigate a potential role of nARIA in nerve-muscle interactions, we have examined the effects of recombinant nARIA on a mouse cell line, Sol8. Sol8 cells provide a system in which one can study the maturation of skeletal muscle, including the transition from myoblasts to myotubes and the expression of mature, epsilon subunit-containing AChRs. This transition is associated with serial changes in mRNA expression, involving AChR subunits and myogenic transcription factors, among others. Treatment of undifferentiated Sol8 cells with recombinant nARIA and analysis of mRNA by Northern blot reveals changes in the levels of several AChR subunit mRNAs. These results suggest that nARIA may play an important role in the early development of vertebrate skeletal muscle. (Supported NS29071 to L.R.; NSAD to Neurol).

587.3

REGULATION OF MuSK EXPRESSION IN SKELETAL MUSCLE DURING DEVELOPMENT AND AFTER INJURY.
D. C. Bowen, J. S. Park, G. D. Yancopoulos, R. M. Lindsay, D .J. Glass, P. S. DiStefano* Regeneron Pharmaceuticals, Tarrytown, NY.

The receptor tyrosine kinase, MuSK (Muscle Specific Kinase), has recently been shown to be a required component of the signaling receptor complex for agrin and to be necessary for neuromuscular junction (NMJ) formation *in vivo*. To understand better the mechanism by which MuSK activation leads to NMJ formation, we are investigating the regulation of MuSK expression during development in the embryo and during atrophy in the adult. We find that MuSK is expressed in skeletal muscle early in development and becomes concentrated at the motor endplate by birth. In the adult, full-length MuSK is precisely co-localized with acetylcholine receptors (AChRs) while *MuSK* mRNA is expressed exclusively by subsynaptic nuclei. Although substantial MuSK immunoreactivity remains concentrated at the motor endplate after denervation or limb immobilization, *MuSK* mRNA becomes expressed throughout the myofiber under these conditions. Finally, we have examined the distribution of MuSK on the surface of cultured myotubes and find that after treatment with agrin, MuSK becomes concentrated at the site of AChR clusters. Experiments are currently underway to determine the relationship between the expression of full-length MuSK and that of other components of the NMJ.
Research supported by Regeneron Pharmaceuticals, Inc.

587.4

THE RECEPTOR TYROSINE KINASE, MuSK, IS REQUIRED FOR NEUROMUSCULAR JUNCTION FORMATION IN VIVO, AND IS A COMPONENT OF A RECEPTOR COMPLEX FOR AGRIN.
D. J. Glass, T. M. DeChiara, D. C. Bowen, T. N. Stitt, C. Radziejewski, J.-A. Bruno, T. E. Ryan, D. R. Gies, S. Shah, S.J. Burden[2], P. S. DiStefano, D. M. Valenzuela, and G. D. Yancopoulos*. Regeneron Pharmaceuticals, Tarrytown NY; [2]Skirball Institute, NYU, NYC, NY

Formation of the neuromuscular junction requires a series of inductive interactions between growing motor axons and differentiating muscle cells, culminating in the precise juxtaposition of a highly specialized nerve terminal with a complex molecular structure on the postsynaptic muscle surface. The receptors and signaling pathways mediating these inductive interactions have not yet been characterized. We have isolated a novel receptor tyrosine kinase, MuSK, (Muscle Specific Kinase), which is localized at the motor endplate in skeletal muscle, and which is up-regulated upon denervation. We have further generated mice with a targeted disruption of the gene encoding MuSK. Neuromuscular junctions do not form in these mice, suggesting a failure in the induction of synapse formation. Agrin is a nerve-derived factor that can induce molecular reorganizations at the motor endplate, but the mechanism of action of agrin remains poorly understood. We demonstrate that: myotubes obtained from MuSK(-/-) mice fail to cluster AChRs in response to agrin stimulation; agrin acts via a receptor complex that includes and requires MuSK; and that agrin-induced activation of MuSK additionally necessitates a myotube-specific accessory component (MASC).
Research supported by Regeneron Pharmaceuticals, Inc.

587.5

AGRIN 4,8 STIMULATES TYROSINE PHOSPHORYLATION OF MuSK IN DENERVATED SKELETAL MUSCLE IN VIVO. P.S. DiStefano, T.N. Stitt, T. Daly, G. Elove, D.M. Valenzuela, G.D. Yancopoulos, R.M. Lindsay*, D.J. Glass. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

We have previously demonstrated that the receptor tyrosine kinase, MuSK, is up-regulated in skeletal muscle as a result of denervation or limb immobilization. Furthermore, MuSK is a signal transducing molecule necessary for the acetylcholine receptor-clustering actions of agrin. To determine whether agrin could activate MuSK *in vivo* and potentially affect the integrity of skeletal muscle, purified soluble recombinant human agrin isoforms (agrin 4,8 and agrin 4,0) were injected into rats with unilateral hindlimb denervation. Agrin was infused either into the femoral artery or into the jugular vein and muscles were assayed for tyrosine phosphorylation of MuSK by Western blot techniques. Intra-arterial infusions of agrin 4,8, but not agrin 4,0 (3 mg/kg) caused a time-dependent phosphorylation of MuSK, peaking at 2 hr. The response was only observed in denervated muscles. Intravenous injections (3-15 mg/kg) resulted in MuSK phosphorylation only in denervated muscles. Pharmacokinetic analyses using ^{125}I-agrin isoforms showed a rapid initial clearance and an elimination half life of ~3 hr for agrin 4,8. The results illustrate the selective responsiveness of denervated skeletal muscle to agrin.

Research supported by Regeneron Pharmaceuticals, Inc.

587.6

EXPRESSION PATTERNS OF BONE MORPHOGENETIC FACTOR RECEPTOR mRNAs IN THE DEVELOPING AND ADULT RAT BRAIN

S. Söderström, H. Bengtsson and T. Ebendal*. Department of Developmental Neuroscience, Uppsala University, Biomedical Center, Box 587, S-751 23 Uppsala, Sweden.

In addition to neurotrophins, members of the superfamily of transforming growth factors(TGF)-β, such as activins, bone morphogenetic factors (BMPs) and glial cell line-derived neurotrophic factor (GDNF), have been suggested to regulate developing neurons. Human recombinant GDNF stimulates survival and fibre outgrowth in chicken embryo sympathetic and ciliary ganglionic neurons. Molecular cloning of serine-threonine protein kinase receptors from peripheral ganglia and retina of the chicken embryo shows the presence of several different types II and I receptors likely to mediate neurotrophic effects. In situ studies have shown that the type II and type I receptors in developing and adult rat brain are expressed in different, developmentally regulated patterns. Only partial overlap in distribution was found among type II (ActR-II, BMPR-II) and type I receptors (ActR-I, ActR-IB, BMPR-IA, BMPR-IB and TβR-I), suggesting that complex interactions between ligands and receptors may mediate trophic signals in CNS. Preliminary in situ results show that some ligands among activins, BMPs and the growth/differentiation factors (GDFs) known to bind and activate serine/threonine kinase receptors, are expressed in the brain parenchyma, whereas other ligands appear to be expressed mainly in meninges and the vasculature of CNS.

Supported by the Swedish Natural Science Research Council (B-BU 4024-317) and Swedish Brain Society.

587.7

CALBINDIN-D_{28k} AND PARVALBUMIN LOCALIZATION IN HIPPOCAMPAL ORGANOTYPIC CULTURES. M.E. Morris*, S. Hefft, D. Muller and K.G. Baimbridge. Dept. of Pharmacology, Centre Médical Universitaire, Université de Genève, CH-1211 Genève 4 and Dept. of Physiology, University of British Columbia, Vancouver BC, Canada V6T 1Z1.

In order to assess the presence, distribution and potential function of calbindin-D_{28k} (CaBP) and parvalbumin (PV) immunohistochemical staining of hippocampal organotypic cultures was carried out at different stages of development and correlated with electrophysiological recording in slices from 7 and 12 day postnatal rats (P7 & P12), cultured for 7 to 28 days (D7-28). At P7-D7 there is significant CaBP staining in dentate granule cells, mossy fiber projections, reactive astrocytes in the dentate and edges of the slice, and interneurons and pyramidal cells of CA1. PV staining is remarkably strong in pyramidal cell layers and weaker in the dentate. At P7-D8-14 CaBP staining of interneurons and superficial neurons of the more clearly defined pyramidal cell layers becomes prominent, with greater density in ventral cf. dorsal hippocampus; staining for PV remains strong. For P7-D21-28 and P12-D18 cultures CaBP and PV staining is present but attenuated. Exposure of P7-D7 slices to bFGF (50 ng/ml) during culture markedly depressed PV but spared CaBP expression. In both P7-D7 and D8-14 cultures paired pulse facilitation and inhibition, and LTP and LTD of extracellular CA1 EPSPs could be observed. Although expression of CaBP resembles *in vivo* development, that of PV is considerably enhanced. The early presence and distribution of CaBP and PV in interneurons correlates with evidence for the excitatory role of GABA-ergic neurons in synaptogenesis and suggests that the Ca-binding proteins may be playing a significant role in buffering and translocation of Ca^{2+}.

Supported by MRC of Canada, NCE, & Swiss National Research Foundation.

587.8

DEVELOPMENTAL REGULATION OF CILIARY NEUROTROPHIC FACTOR RECEPTOR IN EMBRYONIC CHICK HEART. X. Wang and S. W. Halvorsen*. Dept. Biochemical Pharmacology, SUNY at Buffalo, Buffalo, NY 14260.

Ciliary neurotrophic factor (CNTF)/growth promoting activity (GPA) are members of the neurokine family of factors that have extensive effects on a wide variety of neuronal and glial populations. Yet, outside of the nervous system there is a limited response to CNTF due to the low level of expression of the CNTF receptor α subunit. In the peripheral nervous system chick parasympathetic neurons are exquisitely sensitive to the neurotrophic effects of CNTF. We have begun to examine the interaction of avian parasympathetic neurons with their targets of innervation. Surprisingly, we found, using Northern blot analysis, that the embryonic chick heart expresses significant levels of mRNA for the CNTF receptor α subunit. The cardiac mRNA is the same size as that detected in chick embryonic ciliary ganglia and brain (~2.7kb) and was seen as early as embryonic day 3 (e3). The levels of expression were 2 to 3-fold greater in atria than in ventricles at e15. Further, we found that the expression of CNTF receptor mRNA was developmentally regulated in both atria and ventricles, with levels at e11-13 as much as 4 to 5-fold greater than those at e3. E11-13 is a critical period in cardiac development when functional parasympathetic synaptic activity begins to appear suggesting that cell-cell interactions may regulate CNTF receptors. The relative contribution from cardiomyocytes compared with cardiac ganglion neurons to this receptor signal has not been determined. In conclusion, the finding that the regulation of CNTF receptors occurs at a time of synaptogenesis provide a means for using this chick model for elaborating the molecular mechanisms of controlling neurokine responses and understanding their physiological role in a defined cell population.

(Supported by grants NIH R55-NS30232 and AHA-NYS Affiliate #9400021)

587.9

EXPRESSION OF CNTF RECEPTOR α IN RAT BRAIN NEUROEPITHELIUM CLOSELY CORRELATES WITH NEUROGENESIS. M.-Y. Lee, H.-D. Hofmann, B. Heimrich* and M. Kirsch, Catholic University Medical College, Seoul, Korea and Institute of Anatomy I, Freiburg, Germany.

The involvement of CNTF receptor α in the regulation of central nervous system development is suggested by several lines of evidence including its early expression in the proliferating neuroepithelium. To investigate the relation of CNTFRα expression to the proliferation and differentiation of neuronal precursor cells we have studied the distribution of receptor mRNA in proliferative zones of the embryonic and postnatal rat brain by non-radioactive in situ hybridization.

At embryonic day 16 (E16) the entire neuroepithelium surrounding the ventricles was strongly labeled. During further development expression of CNTFRα became increasingly restricted to specific areas of the ventricular zone. CNTFRα transcripts were never detected in other proliferation zones (subventricular zone, cerebellar external germinal layer) or in areas of precursor cell migration. In the neuroepithelium of the lateral ventricles CNTFRα expression started to cease around birth and only a small area close to the olfactory ventricle remained labeled until P14. In the third ventricle expression was restricted to the most dorsal habenular neuroepithelium at P3. CNTFR expression in the neuroepithelium of the fourth ventricle ceased already at late embryonic stages except at the edge of the lateral recess. The aqueductal neuroepithelium also showed a very distinct expression pattern and the most dorsal part of the tectal recess remained labeled until P10. Thus, the temporal and spatial distribution of CNTFRα mRNA in the neuroepithelium closely correlated with previously described patterns of persisting neurogenesis in the neuroepithelium itself and in the neighbouring subventricular zone. This was comfirmed by comparing CNTFRα in situ hybridization with BrdU immunocytochemistry. Our results suggest that CNTFRα plays a role in the regulation of neurogenesis in the ventricular and subventricular zone, although it is not generally expressed in proliferating neuronal progenitors.

Supported by DFG: SFB 505/A4.

587.10

HIGH POTASSIUM INCREASES SURVIVAL MOTONEURONS FROM CHICK EMBRYO SPINAL CORD THROUGH A CALMODULIN DEPENDENT MECHANISM. J.X. Comella*, R.M. Soler, J. Egea, E. Giné, C. Sanz-Rodriguez. Unit of Molecular Neurobiology, Dept. Ciències Mèdiques Bàsiques, Univ. Lleida, E-25198 LLEIDA, Spain.

During development most neuronal populations undergo a process usually referred to as naturally occurring cell death. For motoneurons (MTNs) of the lumbar spinal cord of chick embryo, this process takes place in a well defined period of time, between embryonic days 6 and 10. For their survival, neuronal populations depend on the availability of specific neurotrophic factors derived from their innervation target tissue. It is known that an increase in the intracellular calcium levels allows to maintain cultures of neuronal populations in spite of the absence of neurotrophic factor. This can be achieved by increasing the extracellular potassium concentration that in turn depolarizes the cytoplasmic membrane and voltage-dependent calcium channels open. We have found that the same phenomenon can be induced in MTNs by adding 30 mM potassium to the culture media. Survival is dependent on extracellular calcium since addition of the calcium chelator EGTA blocks the potassium effect. By using FURA-2 intracellular calcium measurements it can be demonstrated that potassium increases chronically the concentration of calcium from a resting value of ca. 50 nM to 150 nM value in depolarized MTNs. We have observed that calcium is able to specifically activate the ras/MAPK pathway and this activation is mediated in some point by calmodulin since specific inhibitors (W13) but not the inactive chemically-related analogue (W12) blocks, both survival effects and activation of the ras/MAPK pathway.

Supported by FIS 94/1576, Ajuntament de Lleida, Telemarató de TV3 66/95, Grups de recerca consolidats (GC) and E.U.'s Biomed-2.

587.11

REK-4 RECEPTOR EXPRESSION IS DEVELOPMENTALLY REGULATED IN THE RODENT FOREBRAIN. R.M. Cassidy*, P.L. Holst[1], G.M. Fox[1], and L.F. Kromer. Dept. of Cell Biology & Interdisciplinary Program in Neurosci., Georgetown Univ. Med. Ctr., Washington, DC 20007 and [1]Amgen, Inc., Thousand Oaks, CA 91320

Recently Cek-4 & 5, members of the Eph-related tyrosine kinase receptor subfamily, have been implicated in the development of topographic retinotectal projections. In the present study, *in situ* hybridization procedures were used to evaluate the embryonic (E18), postnatal (P0, P3, P14), and adult expression of a rat homologue of the Cek-4 receptor, Rek-4. At E18 there is extensive expression of Rek-4 in neuroepithelium within the ventricular and subventricular zones of the lateral ventricles. Migrating neuroblasts and neuroblasts within the cortical plate and developing pyramidal cell layers of the hippocampus, piriform, and insular cortices also exhibit a high hybridization signal. At birth and during early development (P0-3), there is continued expression of Rek-4 in the germinal zones, in hippocampal and dentate granule cells, and within specific neocortical lamina. By P14 there is a global reduction in Rek-4 expression in all forebrain regions with heavily labeled neurons most frequently observed in the piriform cortex and hippocampus. In the adult, Rek-4 expression is uniformly reduced in all forebrain areas, including the hippocampus. These observations suggest that Rek-4 mRNA is expressed at high levels in many forebrain regions during neurogenesis, neuronal migration, and axonal growth, with a rapid postnatal down-regulation to very low levels in the adult. Supported by Amgen grant #942927 and NIH grant T32HD07549.

587.12

FLOOR PLATE NEUROEPITHELIUM HAS BINDING SITES FOR GROWTH REGULATOR, VASOACTIVE INTESTINAL PEPTIDE. I. M. Hill*, P. Gressens, G. W. Glazner and D. E. Brenneman. Lab. of Dev. Neurobiol. NICHD, NIH, Bethesda, MD 20892; Service de Neuropediatrie, Hopital Robert-Debre, Paris, France.

The trophic actions of VIP in CNS cultures occur indirectly through the vasoactive intestinal peptide (VIP)-induced release of several glial factors, including activity dependent neurotrophic factor (ADNF). In the mouse, VIP is an important regulator of growth during embryonic days (E) 9-11. Since VIP binding sites are restricted to the nervous system during this period, the VIP-induced coordinated growth of brain and body suggests that the growth-regulatory functions of VIP are indirect. Furthermore, VIP-stimulated growth has been shown to occur through the action of the endogenous factor, ADNF. Here we describe the regional localization of VIP binding sites in the neural tube of the E9 mouse embryo examined with ^{125}I-VIP and *in vitro* autoradiography. Moderately dense VIP binding was seen throughout the neural tube. High densities of VIP binding were seen along the floor plate and ventral midline neuroepithelium and were especially evident in the midbrain, hindbrain and spinal cord. The high density of VIP binding sites along the extent of the floor plate of the neuraxis, the center governing tissue organization during this period of development, suggests that VIP regulates the release of diffusible signals that are secreted from this region and coordinate early morphogenic events. The floor plate is composed exclusively of macroglia in the rat. This is consistent with the hypothesis that VIP-regulated growth is mediated through ADNF and other factors released from the floor plate glial cells.

587.13

BRIEF EXPOSURE TO NEUROTROPHINS INCREASES THE CHOLINE ACETYLTRANSFERASE ACTIVITY OF CULTURED SEPTAL NEURONS IN A CALCIUM- DEPENDENT MANNER. D. Nonner, E.F. Barrett* and J.N. Barrett. Dept. Physiology & Biophysics. Univ. Miami Med. School, P.O. Box 016430, Miami FL 33101.

Previous studies have shown that week-long exposures to nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF) increase the activity of choline acetyltransferase (ChAT) in basal forebrain cholinergic neurons. Since in other cells neurotrophins (NTs) cause phosphorylation of their trk receptors within a few minutes, we tested whether brief exposure to NTs might be sufficient to elevate ChAT activity.

Embryonic (E15) rat septal neurons grown for 7 days in medium lacking exogenous neurotrophins were exposed to NGF, BDNF, NT-3 or NT-4 (each at 100 ng/ml) for 15-60 min, after which NTs were washed out and replaced with control medium. After 12 hr ChAT activities in NT-treated cultures were $\geq$50% greater than those in sister cultures never exposed to NT, and the increase persisted for at least 24 hr. This NT-induced increase in ChAT activity was not mediated by increased neuronal survival, but did require the presence of extracellular Ca^{2+} during NT exposure. Consistent with a role for Ca^{2+} in this response, NT exposure produced a transient increase in intracellular $[Ca^{2+}]_{in}$ a subpopulation of fura-2-filled septal neurons. The NT-induced increase in ChAT activity was blocked by the tyrosine kinase inhibitors K252a (2 μM), K252b (5 μM) and genistein (20 μM).

These results suggest that brief exposure to NTs can produce prolonged changes in ChAT activity, and that the signalling pathway requires Ca^{2+} and kinase activity of trk receptors. Supported by NS 12207. Neurotrophins kindly supplied by Regeneron Pharmaceuticals.

587.14

GESTATIONAL METHYLMERCURY EXPOSURE ALTERS NEUROTROPHIN- AND NEUROTRANSMITTER- STIMULATED PHOSPHOINOSITIDE (PI) HYDROLYSIS IN NEONATAL RATS. S. Barone, Jr.*, T.M. Freudenrich and W.R. Mundy. Neurotoxicology Division, NHEERL, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Neurotrophin-induced signal transduction mediated through the *trk* receptors has been implicated in neuronal growth, differentiation, and survival. In this study we have examined changes in the ontogeny of agonist-stimulated PI hydrolysis as a possible mechanism for the neuropathology resulting from exposure to the developmental neurotoxicant, methylmercury (CH_3Hg). Long-Evans dams were dosed p.o. on gestational days 6-15 with 0 or 2 mg/kg CH_3Hg dissolved in saline. Pups were sacrificed and regional dissections were performed on postnatal days (PND) 1, 4, 10, 14, and 21. Agonist-stimulated PI hydrolysis was measured as the accumulation of inositol phosphates (IP) in brain slices from cortex and cerebellum. In control slices (PND 4), the neurotrophins NT-3 and BDNF (1-1000 ng/ml) stimulated IP accumulation in a concentration-dependent manner with an EC_{50} of 400 ng/ml and a maximum stimulation of 250% of basal. NGF was effective only in the cortex with maximum stimulation of 150%. Carbachol-, norepinephrine-, and neurotrophin-stimulated PI hydrolysis was greatest on PND 1-4, and decreased with age in both brain regions. Neurotrophins did not stimulate IP accumulation above basal levels after PND 10. CH_3Hg had no effect on Norepinephrine-, NT-3-, or BDNF-stimulated IP accumulation at any age in the cerebellum. In the cortex, carbachol-stimulated IP accumulation was decreased in PND 1 compared to controls. NT-3-stimulated IP accumulation was decreased on PND 1, and increased on PND 14 compared to controls. NGF-stimulated IP accumulation, which was not above basal levels in control animals on PND 14-21, was increased on PND 10-21 in CH_3Hg exposed rats. These results suggest that gestational exposure to CH_3Hg alters the signal transduction cascade associated with neurotransmitter and neurotrophin receptors in the cortex.

587.15

QUANTITATIVE ANALYSIS OF THE INFLUENCE OF NEUROTROPHINS ON THE CELL-CYCLE PARAMETERS OF MOUSE CORTICAL PRECURSOR CELLS. C. Dehay, A. Shering V. Cortay ,P. Savatier*° and H. Kennedy. INSERM U371, 18 avenue du Doyen Lépine 69675 Bron, FRANCE and °Ecole Normale Supérieure, 46 allée d'Italie, 69364 Lyon Cedex 07 FRANCE.

The influence of neurotrophins BDNF, bFGF, EGF, NT3 and NT4 was studied in dissociated cell cultures of E14 embryonic cortical precursors. Proliferation and cell cycle parameters were studied by means of BrdU incorporation by cells in S phase following a 3 hours exposure. The total cycling population (i.e. the growth fraction: GF) was revealed by PCNA (proliferating cell nuclear antigen) immunostaining. The cell-cycle parameters were estimated (1) with FACS analysis which when using BrDU and propidium iodide double labelling allows estimation of the absolute duration of the different phases of the cell-cycle and (2) with immunocytochemistry by means of the labelling index (LI) calculation. The LI corresponds to the proportion of BrdU positive cells (ie cells in S phase) with respect to the total cycling population (PCNA imunoreactive cells). Variations of the LI value reflect modifications of the cell-cycle kinetics so that low values of LI reflect low rates of division due to long cell-cycle duration whereas high LI values indicate fast rates of cell division with shorter durations of cell cycle time.We found that in presence of bFGF (50ng/ml during 48 hours), there is an increased proliferation of mouse cortical precursors. This is accompanied by higher values of LI and GF indicating that bFGF promotes cortical proliferation by shortening the cell cycle and by influencing the mode of division. The increase in GF indicates that bFGF inhibits differentiation by inducing the precursors to retain their proliferative abilities. Similar but less pronounced effects were obtained when EGF (50ng/ml) was added to the culture medium for 48 hours. NT3, NT4 and BDNF failed to influence cortical cell proliferation.

Supported by HFSP grant RG-55/94B and INSERM poste vert (A.S).

587.16

IMMUNOREACTIVITY OF HIGH-AFFINITY NEUROTROPHIN RECEPTORS (TRK-A, TRK-B AND TRK-C) IN CAT VISUAL CORTEX DURING POSTNATAL DEVELOPMENT. Q. Gu*, Y. Li and M. Cynader. Dept. of Ophthalmology, Univ. of British Columbia, Vancouver, BC, Canada V5Z 3N9.

Recently, it has been shown that neurotrophins are involved in visual cortex development and activity-dependent plasticity. To further explore the role of neurotrophins in the developing visual cortex, we investigated the localization and distribution of the high-affinity neurotrophin receptors trkA, trkB and trkC in the primary visual cortex of cats aged at 2, 4, 6, or 12 weeks, and in adult animals. TrkA, trkB, and trkC, were visualized in cortical sections with specific polyclonal antibodies using immunocytochemical techniques. Each of these high-affinity neurotrophin receptors showed unique and selective laminar distribution patterns, and the immunoreactivity associated with all three receptors declined during postnatal development. At all ages examined, trkB-related immunoreactivity was always stronger than that associated with trkA and trkC. While trkB-related immunoreactivity was found to be still abundant in adult visual cortex, immunoreactivity associated with trkA and trkC was reduced to a minimum at this age. Immunoreaction product for all three receptors was primarily associated with neuronal somata and dendrites, especially those of pyramidal neurons. However, immunoreactivity related to trkB also was found within glial cells at 6 weeks of age, and immunoreactivity of trkC was mostly localized in axons in adult visual cortex. In conclusion, since trkA, trkB and trkC are the primary targets of different neurotrophin family members, our results suggest that each neurotrophin family member may play a specific role in the development and maintenance of neuronal structures in the visual cortex.

Supported by BCHRF and NCE of Canada

587.17

TrkB-LIKE IMMUNOREACTIVITY IS NOT EVIDENT ON GENICULOCORTICAL AFFERENTS IN LAYER IV OF KITTEN VISUAL CORTEX. M.A. Silver*[1], M.J. Radeke[2], S.C. Feinstein[2], and M.P. Stryker[1]. [1]W.M. Keck Center for Integrative Neuroscience and Neuroscience Graduate Program, Univ. of California, San Francisco, CA 94143; and [2]Neuroscience Research Institute and Dept. of Molecular, Cellular, and Developmental Biology, Univ. of California, Santa Barbara 93106.

Delivery of neurotrophic factor ligands of TrkB locally to primary visual cortex has been shown to have effects both on geniculocortical afferents, causing desegregation of ocular dominance columns (NT-4/5 and BDNF, Cabelli, et al., Science, 1995), and on geniculate cell bodies, preventing changes in size following monocular deprivation (NT-4/5, Riddle, et al., Nature, 1995). These results suggest that the terminal branches of geniculocortical afferents in layer IV of the cortex may express TrkB, the high affinity receptor for NT-4/5 and BDNF.

To investigate this possibility, we have labeled geniculocortical afferents by injections of the anterograde neuronal tracer Phaseolus vulgaris leucoagglutinin (Pha-L) into the dLGN of 28 day old kittens. After a 12 day survival, brain regions of interest were processed for double label immunofluorescence using an antibody to Pha-L and either an antibody raised against amino acids 23-36 of the extracellular domain of the TrkB protein (anti-TrkB23) or one directed against amino acids 606-619 of the tyrosine kinase domain of the full length isoform (anti-TrkB.FL).

TrkB-like immunoreactivity was present in many cortical neurons and in the cortical neuropil both in punctate and diffuse distributions. It was also observed in the medial septal nucleus and the diagonal band of Broca. The geniculocortical afferents in layer IV of the cortex were not labeled above the background of the cortical neuropil, even though TrkB protein has been reported to be present on neurons within the LGN (Cabelli, et al., Soc. Neurosci. Abs., 1994). Neither punctate nor diffuse TrkB-like immunoreactivity was specifically associated with these afferents. These results suggest that the effects of NT-4/5 and BDNF on development and plasticity of geniculocortical afferents are probably not due to their direct actions on these afferents. Supported by NIH EY02874, NIH EY10739, and HHMI.

587.18

TRKB RECEPTORS LOCALIZE TO PATCHES IN DEVELOPING STRIATUM. L.C. Costantini*, S.C. Feinstein, M.J. Radeke, and A. Snyder-Keller, Wadsworth Center, NYS Dept. of Health, Albany, NY, and Neuroscience Research Institute, U.C. Santa Barbara, Santa Barbara, CA.

The compartmentalized expression of neurotransmitters and neuropeptides in developing striatum begins prenatally at embryonic day 19 (E19), when dopamine (DA) afferents cluster into patches which coincide with patches of substance P (SP)-expressing striatal neurons. To investigate the role of growth factors in the initial formation of striatal patch vs. matrix, we examined the spatial and temporal expression of trkB receptors using immunohistochemistry. Polyclonal antibodies against the C-terminus (Santa Cruz; residues 794-808), or specific for the tyrosine kinase domain (residues 606-619), revealed dense patches of immunoreactivity in the developing striatum: trkB+ cells and fibers were localized to patches beginning at E19, and co-localized with DA fibers and SP+ neurons. This patchy distribution persisted through postnatal day 10, then became more uniform, with the exception of a small population of densely-labeled cells. Antibodies raised against the extracellular domain (residues 23-36), which recognize all known isoforms of trkB, showed high expression of trkB in fiber bundles coursing through the immature striatum. Glutamate receptors (GluR1 and GluR2/3 AMPA-type and NMDA-type) were also clustered in developing striatum, co-localizing with patches of trkB+ and SP+ cells, and DA fibers. A concentration of these receptors on neurons of the developing patches may indicate a convergence of growth factors and afferent innervation onto future patch cells that may regulate the phenotypic differentiation of patch vs. matrix cells of the striatum. Specific effects of perinatal manipulation of afferents on the development of these receptors are currently being investigated. Supported by MH 46577 (A.S.K.) and EY 10739 (S.C.F.).

587.19

IN VITRO EXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTORS IN THE DEVELOPING CEREBRAL CORTEX D.McLaughlin*, J.A.Clausen and D.J.Price, Dept. of Physiol., Univ. Med. Sch., Teviot Place, Edinburgh EH8 9AG, UK.

There are et least nine fibroblast growth factors (FGF1-9) and four FGF receptors (FGFR1-4). FGF-2 is expressed by the neuroepithelium of the developing telencephalon and promotes the proliferation and migration of cortical cells. It may maintain cortical cells in an immature state until neurotrophin 3 (NT-3) initiates cell differentiation. Later, FGF-2 may promote the survival of cortical and/or subplate neurons.

In this study we first examined the distributions of FGFR1,2 in the developing cortex in vivo. Immunohistochemistry revealed the presence of these receptors throughout the wall of the developing telencephalon, from ventricular zone to cortical plate, with enhanced expression in the subplate. We then dissociated the developing cortex, and studied expression of FGFR1,2 in vitro. We added a range of neurotrophic factors to dissociated embryonic day 15 cortex. FGF-1 and FGF-2 (100pg/ml), NT-3 and NT-4/5 (10ng/ml) maintained FGFR1,2 expression in vitro.

We conclude that FGFR1,2 may be involved at several stages of cortical development, and that the level of expression of the receptors may be influenced by growth factors including the neurotrophins and the FGFs themselves.

Supported by The Wellcome Trust.

587.20

EXPRESSION AND LOCALISATION OF THE FOUR FIBROBLAST GROWTH FACTOR RECEPTOR TYPES IN THE HUMAN FETAL MUSCLE AND HEART.

M.G. Ennas, L. Balaci, V. Sogos, S. Laconi, M. Presta and F. Gremo*
Medical School, Cagliari, Brescia (Italy)

Basic fibroblast growth factor (bFGF) has been shown to stimulate proliferation and to depress differentiation of myogenic cells. It exerts its action through stimulation of high and low affinity receptors, whose presence however has never been extensively investigated in the human immature muscle. Thus, we studied the expression and localisation of the four types of high affinity, tyrosine-kinase FGF receptors (FGFRs) and of low affinity FGF binding molecules perlecam and syndecam in the embryonic and fetal skeletal and heart muscle. In brief, samples were dissected out from 8-18 week old human embryos and tissues processed for Northern and Western blot analyses as well as for immunocytochemistry. Results showed that in the developing muscle mRNAs for FGFR1, FGFR3 and FGFR4 were expressed, but a progressive decrease in their content paralleled fetal maturation. In accord with it, in the human adult muscle no specific mRNA was detected. Double staining with antibodies directed against the different FGFRs and against markers of myotubes/myofibers maturation confirmed a relative abundance of FGFR3 and FGFR4 in more immature myotubes. Moreover, a different distribution of the two receptors within fibres was observed. In adult muscle, only a few FGFRs were present associated to the connective sheath. Immunostaining of samples of dystrophic muscles suggested an increase of specific FGFRs in regenerating tissue.

(Supported by Telethon-Italy and Regione Autonoma Sardegna Grants to F.G.; L. Balaci was a recipient of a Telethon fellowship).

NEURONAL DEATH: OXIDATIVE STRESS

588.1

CHARACTERIZATION OF APOPTOTIC PROTEASE ACTIVITY IN ISCHEMIC BRAIN TISSUE BY TWO-DIMENSIONAL PROTEIN ELECTROPHORESIS. S. Massa* and M.W. Sganga, Dept. of Neurology, Veterans Administration Medical Center and University of California, San Francisco 94121.

Activation of specific proteases plays a major role in a cell's comittment to undergo efficient self-destruction after sub-necrotic injury. We have used in vivo and in vitro models of such cellular injury combined with large format two-dimensional electrophoresis to examine the post translational processing and proteolytic degradation of neuronal proteins important to the apoptotic program. Proteins were extracted from primary cultured neurons metabolically labelled with 35-S-methionine. These normal proteins were then exposed to enzymes generated by either cultured neurons undergoing potassium shift-induced apoptosis or cortical and hippocampal tissue from gerbils which had experienced global ischemia. Two-dimensional analysis revealed several major and minor protein components which were degraded by activated proteases or otherwise modified post-translationally. Studies using specific inhibitors have identified several of the processing events to be dependent on ICE-related proteases in particular. Further identification of cell death-specific proteases, modifiers and their substrates will provide targets for therapy which may decrease the extent of neuronal injury caused by stroke-related ischemia. Supported by grants from UCSF and the VA.

588.2

PRETREATMENT WITH THE PLATELET - DERIVED GROWTH FACTOR - BB AMELIORATES THE DELAYED NEURONAL DEATH FOLLOWING FOREBRAIN ISCHEMIA IN RATS. K. Iihara, T. Tsukahara*, M. Sakata, H. Yanamoto, T. Taniguchi, and N. Hashimoto. Dept. of Cerebrovascular Surg., National Cardiovascular Center, Osaka 565 ; Dept. of Neurosurg., Maizuru Municipal Hospital, Kyoto 625 ; Dept. of Neurobiology, Kyoto Pharmaceutical Univ., Kyoto 607: Japan

Our previous study demonstrated that ischemia induces the expression of the platelet-derived growth factor (PDGF) -B chain, but not -A chain, first in neurons and later in brain macrophages in both mRNA and protein levels after focal ischemia in rat brain (Iihara et al., J CBF Metab 14, 818, 1994). Recently, we have shown the induction of PDGF β-receptor in focal ischemia and suggested the importance of the coordinate expression of the B chain and the ß-receptor in the neuronal survival, glial response and accumulation of the bain macrophages in the ischemic brain (Iihara et al., J CBF Metab 16, 1996 in press). Here we demonstrated that pretreatment with PDGF-BB, but not-AA, ameliorates the delayed neuronal death (DND) following forebrain ischemia in rats. Intraventricular cannula connected to osmotic minipump was placed using stereotaxic apparatus on male Wistar rats two days before the operation. Rats subjected to ischemia were divided into subgroups with infusion of 2µg of ; a) PDGF-AA, b) PDGF-BB, c) albumin, (n=7) respectively. Forebrain ischemia was induced on Wistar rats by transient occlusion of bilateral carotid arteries together with lowering the blood pressure to 50 mmHg. On day 7 post ischemia, rats were sacrificed and the number of survived CA1 pyramidal neurons was counted on histological specimen. Pretreatment with PDGF-BB significantly increased the number of survived CA1 subfield ($P<0.001$) as compared with albumin group. In contrast, PDGF-AA showed no protective effect on the DND. This suggests that PDGF-BB, but not PDGF-AA, has neuroprotective action in the ischemic brain .

Table. Survived Neurons in the CA1 (cell counts/mm) ($P<0.001$)

normal	ischemia	vehicle	PDGF-AA	PDGF-BB
401±23	23±5	53±6	46±7	252±79*

This work was supported by a Grant-in-Aid for Scientfic Research from the Ministry for Education, Science and Culture of Japan.

588.3

CLONING OF A SERINE PROTEINASE INHIBITOR FROM THE BOVINE BRAIN AND ITS CHARACTERIZATION. M. Nishibori*, N. Nakaya, M. Kawabata, N. Adachi and K. Saeki. Dept. of Pharmacology, Okayama Univ. Med. School. Okayama 700, JAPAN.

A growing evidence suggests that serine proteinases such as thrombin and tissue-type plasminogen activator may be involved in the processes of neuronal degeneration, repair and cell death. Serine proteinase inhibitor (serpin) is one of the counter molecules of the action of proteinase. Previously, we purified a novel serpin B-43 from the bovine brain. In the present study, we cloned B-43 cDNA and investigated the biochemical feature of the recombinant protein. Moreover, we examined the effect of transient ischemia on the expression of B-43-like immunoreactivity in the rat hippocampus. The bovine B-43 cDNA was cloned from bovine brain cDNA library. The length of the cDNA was 1338 bp. It encoded 378 amino acids, and the mW of the protein was estimated to be 42.6 kDa. which is consistent with that of the native B-43 purified from the bovine brain. A homology search revealed that B-43 belongs to the ovalbumin branch of the serpin superfamily. The sequence of the reactive site of B-43 was estimated to be Arg-Cys on the basis of the alignment of the amino acid sequence of B-43 to some members of the serpin superfamily. B-43 was the most homologous to PI-6/mSP-3. Northern blot analysis showed that the size of the transcript was 1.4 kb and that there was marked variation in the expression of B-43 among the brain regions. [^{35}S]-labeled B-43 protein synthesized in vitro formed complexes with thrombin, trypsin and α-chymotrypsin, but not with urokinase or plasmin. After transient forebrain ischemia induced by four vessel occlusion, the expression of B-43 was increased in the pyramidal cells of CA1-CA3 in the rat hippocampus. These results suggest that B-43 might be involved in the processes of neuronal degeneration through the inhibition of serine proteinases in the brain.

588.4

TISSUE INHIBITOR OF METALLOPROTEASES 1 (TIMP-1) IS AN IMMEDIATE EARLY GENE UPREGULATED IN EPILEPSY AND ISCHEMIA S. Rivera*, S. Timsit, E. Tremblay, E. Prats, P. Quaghi, Y. Ben-Ari and M. Khrestchatisky. Université René Descartes, Paris V. INSERM U-29, 123 Bld. Port Royal, 75014 Paris, France.

TIMP-1 is a secreted glycoprotein of the extracellular matrix (ECM) that inhibits type IV collagenases which have been largely implicated in the destruction of matrix leading to tissue degeneration. We have studied by *in situ* hybridization and immunohistochemistry the expression of TIMP-1 in the brain of epileptic and ischemic rats using kainate (KA) intraperitoneal (ip) and intraamygdalar (ia) injections and the 4-vessel occlusion model of global ischemia. An early (1 h) transient increase in mRNA expression occurred in the granule neurons of the dentate gyrus in both epileptic and ischemic hippocampi. Such increase was not blocked by pretreatment with cycloheximide, which in contrast inhibited Fos protein induction in the same animals. The increase in mRNA levels, that peaked around 4-8 h, was followed by an increase of TIMP-1 protein in the cell bodies of the dentate gyrus granule cells and in their axons and dendrites. At later time points (16-72 h), in epileptic animals, astrocytes also exhibited a dramatic induction of TIMP-1 mRNA levels. TIMP-1 mRNA was particularly high at 72 h in the vulnerable CA3 and CA1 hippocampal subfields of KA ia-injected and ischemic animals respectively. In conclusion, TIMP-1 is regulated by neuronal activity, behaves like an immediate early gene, is expressed by neurons and astroglia, and may be related to excitotoxic neuronal death. Ongoing *in vitro* studies using TIMP-1 antisense oligonucleotides in cultured neurons should provide information on the potential role of TIMP-1 in neuronal protection.
S.R. was supported by a Biomed fellowship from the EU.

588.5

CYCLIN D1 IS UPREGULATED IN REGIONS OF NEURONAL DEATH DURING ISCHEMIA, EPILEPSY AND IN A DEVELOPMENTAL MODEL OF NEURONAL DEATH. S. Timsit, S. Rivera, N. Chevassus Au Louis, E. Tremblay,* F. Guischard, P. Quaghi, Y. Ben-Ari and M. Khrestchatisky. Université René Descartes, ParisV, INSERM U-29, 123, Bd Port-Royal, 75014 Paris France.

Cyclins D play a key role in the G1 phase of the cell cycle. They have also been involved in programmed cell death in cultured neurons. To investigate the potential role of cyclins D in selective and delayed cell death we have studied the expression of cyclins D1, D2, D3 in two well known models of in vivo pathological neuronal death: transient global ischemia and intra-amygdaloid kainate injections causing status epilepticus. In situ hybridization with cyclin D1, D2 and D3 probes revealed a specific upregulation of cyclin D1 in regions of ischemia-induced neuronal death as assessed by TUNEL method: namely the hilus at 48 hours, followed by the CA1 region of the hippocampus at 72 hours and the cortex at 96 hours. Cyclin D1 was also upregulated in the CA3 region starting 16 hours after kainate injection. Parallel induction of cyclin D1 was observed by immunohistochemistry in ischemia. Neither cyclin D2, nor cyclin D3 mRNA were increased in the hippocampus of ischemic or epileptic rats. The cyclin D1 upregulation in regions of neuronal death was not restricted to the hippocampus. Furthermore, cyclin D1 expression was also observed postnatally in visual diencephalic regions after experimental lesion of the occipital cortex. Our data show for the first time that cyclin D1 is upregulated *in vivo* in regions of neuronal death in a variety of structures and regardless of the model utilized. This work was supported by INSERM

588.6

Apoptosis Depends on Severity and Duration of Hypoxia in Cultured Neocortical Neurons Kenneth Banasiak, Alia Bazzy-Assad* and Gabriel G. Haddad. Department of Pediatrics, Section of Respiratory Medicine, Yale University School of Medicine, New Haven, CT 06520 Various animal models of cerebral hypoxia-ischemia have shown evidence of neuronal apoptosis. However, it is not clear as to whether neuronal apoptosis is the direct result of hypoxia or whether other substances released during *in vivo* hypoxia-ischemia mediate this process. We hypothesized that hypoxia can directly induce neuronal apoptosis and that the duration and severity of hypoxia may alter the time course of apoptosis. Since apoptosis implies a genetically controlled mechanism, we examined the time course of expression of bcl-2 and p53 proteins (genes implicated in apoptosis and cell cycle control) in neurons cultured under various hypoxic conditions. Rat neocortical neurons were cultured under conditions of 21% O_2/ 5% CO_2 for 7 to 10 days. After this period, culture plates were placed in an incubator with levels set at 0.1%, 1%, and 3% O_2 with 5%CO_2. Plates were removed at 24 hour intervals for TUNEL stain, immunofluorescence staining for p53 and bcl-2, and protein extraction for Western blotting of p53 and bcl-2. When $\geq$ 70% of the neurons exhibited positive TUNEL staining, the remaining plates were collected for DNA extraction and gel electrophoresis to detect DNA fragmentation. Under all hypoxic conditions, positive TUNEL stain and DNA fragmentation were observed *in vitro*. However, these alterations were seen after a shorter period of hypoxia when the stress was more severe (0.1% O_2, ~48hrs; 1% O_2, ~72hrs; 3% O_2, ~72 hrs). Time dependent increases in p53 and bcl-2 proteins were also observed on immunofluorescence staining and Western blot. These increases followed the same pattern as that of TUNEL staining and DNA fragmentation. We conclude that hypoxia can directly induce apoptosis in cultured rat neocortical neurons. The duration and severity of hypoxic exposure appears to affect the time course of neuronal apoptosis. In addition, there appears to be an increase in p53 and bcl-2 proteins in hypoxia-induced neuronal apoptosis. These gene products have been implicated in cell cycle control and alteration of there expression suggests a link between hypoxia-induced apoptosis and cell cycle control.
NIH #HD32573

588.7

ANTIOXIDANTS REDUCE DELAYED RETINAL CELL DEATH INDUCED BY ISCHEMIA OR HYPOGLYCEMIA-LIKE CONDITIONS A. C. Rego and C. R. Oliveira* Center for Neurosciences of Coimbra, Department of Zoology and Faculty of Medicine, University of Coimbra, 3000 Coimbra, Portugal

Cell death upon ischemia is frequently associated with the alteration of cell function, involving an increased release of excitatory amino acids, the alteration of Ca^{2+} homeostasis and the reduction of cellular ATP. Our aim was to analyze the survival of retinal cells after incubation for a short period with hypoxia, hypoglycemia or ischaemia inducers, and further examine the effect of antioxidants. Co-cultures of retinal cells were incubated, for 15 min, in the presence of ascorbate/Fe^{2+} at pH 6.5 (oxidative stress), oligomycin and cyanide (hypoxia), iodoacetic acid (hypoglycemia) or in the presence of hypoxia and hypoglycemia inducers (ischemia). The viability of retinal cells was analyzed by determining the LDH leakage to the culture medium (in % of total), for up to 24 hours. The release of LDH in cells submitted to oxidative stress increased steadily up to 8 hours, with values of 11.86±1.45 %. LDH leakage after hypoglycemia-like conditions increased significantly from 4 hours to up to 10 hours, with values of 21.42±1.05 %, whereas LDH leakage after ischemia-like conditions increased significantly from 4 hours to up to 24 hours (42.83±3.39 %). Pre-incubation with 20 µM vitamine E or 10 µM idebenone completely prevented the cell death that occurred after oxidative stress. Cell death occurring after hypoglycemia was completely reduced for up to 10 hours in cells pre-treated with vitamine E, but only partially reduced upon pre-incubation with idebenone. Nevertheless, cell death following ischemia-like conditions (10-12 hours incubation) was only significantly reduced upon pre-incubation and maintenance of the antioxidants. These data indicate that the pattern of retinal cell death depends on the extent of metabolic depletion and that the antioxidants vitamine E and idebenone can reduce cell death, suggesting that free radical formation is involved in the intracellular events that culminate in cell death after hypoglycemia or ischemia conditions.
[This work was supported by JNICT (Portuguese Research Council) and the Human Capital Mobility Program (EU) Proposal nº ERB 4050 PL 932039]

588.8

MUSCARINIC RECEPTOR SUBTYPE DETERMINES VULNERABILITY TO OXIDATIVE STRESS IN COS-7 CELLS. J.G.Strain, and J.A. Joseph*. USDA-Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Previous research has suggested that there may be increased selective vulnerability (SV) to oxidative stress (OS) among central neuronal regions in aging, and that OS SV may be important in determining regional differences in neuronal aging. In an effort to assess whether one factor determining OS SV might involve qualitative/quantitative differences in receptor subtypes in various neuronal populations, we exposed COS-7 cells transfected with one of five muscarinic receptor subtypes (M_1-M_5 AChR) to low concentrations of H_2O_2 (0, 300 or 500 µM for 30 minutes in growth medium) and examined intracellular Ca^{2+} levels prior to and following 500 µM oxotremorine (oxo). Fluorescent imaging was used to visualize intracellular Ca^{2+} changes in individual Fura II-loaded cells. No consistent differences were observed in baseline (pre- oxo) Ca^{2+} levels or oxo-stimulated increases among the various MAChR subtypes either in the presence or absence of H_2O_2. However, following H_2O_2 exposure the number of cells responding to oxo, as well as the ability of the cells to clear excess Ca^{2+} (i.e., Ca^{2+} recovery time, Ca^{2+}RT) following oxo stimulation varied as a function of transfected MAChR subtype. The percent of cells showing the greatest decreases in responding to oxo were those transfected with the M_1 (500 µM H_2O_2, 30%) and M_2 (45 %) subtypes while $M_{3,4,}$ and $_5$ cells showed no significant decreases with H_2O_2. However, M_3 transfected cells showed decreases in Ca^{2+}RT following H_2O_2 (25-30%). Assessments are presently being carried out to discern the factors (e.g., signal transduction, G-protein coupling) that may determine OS SV among these (MAChR) and other (dopamine, D_1, D_2 etc.,) receptor subtypes. However, these findings suggest that receptor subtype differences in various neuronal populations may determine OS SV in various neuronal regions. These differences are expressed as selective regional declines in aging.
(Supported by USDA Intramural)

588.9

DOPAMINE TOXICITY AND THE PROTECTIVE EFFICACY OF RECOMBINANT ANTI-OXIDANT ENZYMES. S. Duffy and T.H. Murphy*, Dept. of Psychiatry, University of British Columbia, Vancouver, CANADA V6T 1Z3

Oxidation of dopamine(DA) results in formation of several pro-oxidant neurotoxins, including H_2O_2 and DA quinones, suggesting that aberrant DA oxidation may contribute to the degeneration of dopaminergic neurons observed in Parkinson's Disease. Conversely, up-regulation of antioxidant enzymes may provide protection. Thus, we are examining the mechanisms of DA toxicity in Human Embryonic Kidney (HEK 293) and N18 RE105 neuronal cell lines, and the possible protection conferred by transfecting these cells with plasmids encoding the antioxidant enzymes quinone reductase (QR) and glutathione S-transferase (GST) (resulting in overexpression). These enzymes may be particularly efficacious because of their possible role in DA quinone detoxification; moreover, QR and GST activity can be induced pharmacologically, making them possible targets for therapeutic intervention. DA (10-100 μM) produced a dose-dependent toxicity as revealed by lactate dehydrogenase and β-galactosidase assay (measures of toxicity in total cells and transfected cells respectively). In N18 cells, this effect was largely ameliorated by extracellular addition of the H_2O_2 metabolizing enzyme catalase or the iron chelator desferroxamine, but not monoamine oxidase inhibitors, indicating toxicity mediated by H_2O_2 (from DA auto-oxidation) with subsequent iron-dependent production of hydroxyl radicals ($OH^{\cdot}$). Transfection of QR and GST plasmids resulted in enhanced enzyme activities (3-7 fold); however, this overexpression did not provide significant protection against extracellular DA ($OH^{\cdot}$-mediated toxicity) in either cell line. To examine toxic effects of intracellular DA, HEK cells were transfected with a DA transporter cDNA. This greatly potentiated DA toxicity, indicating that intracellular DA accumulation is also deleterious. The mechanisms involved and effects of QR and GST overexpression on the toxicity of intracellularly accumulated DA remain to be determined. Supported by Heart and Stroke Foundation of BC and Yukon.

588.10

SELECTIVE DEGENERATION OF NEURONS and OLIGODENDROCYTES BY OXIDATIVE STRESS: INVOLVEMENT OF NF-kappa B. K.-Y. Park*, E.C. Park, I.-O. Jou, and B.J. Gwag Dept. of Pharmacology, Ajou Univ. School of Medicine, Suwon 442-749, Korea.

Cocultures of neurons and glia exposed to 10-50 μM ferrous chloride ($FeCl_2$) or 0.1-1 mM buthionine sulfoximine (BSO) undergo neuronal degeneration over 24 hr. The oxidative stress also induced death of oligodendrocytes in the absence of detectable astrocyte injury. The degeneration pattern of neurons and oligodendrocytes is similar to necrosis involving cell body swelling. This degeneration pattern correlates well with localization of NF-kB. Cytoplasm of neurons and oligodendrocytes were immunolabeled with antibodies specific to NF-kB. However, NF-kB immunoreactivity was barely detectable astrocytes. Considering the potential role and localization of NF-kB, it is possible that neurons and oligodendrocytes exposed to Fe^{2+} or BSO may degenerate through mechanisms involving activation of NF-kB. In support of this, $FeCl_2$ treatment induces translocation of NF-kB from cytoplasm to nucleus in cortical neurons and oligodendrocytes prior to death. Inclusion of 100 μM trolox, an antioxidant, or pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-kB, blocks nuclear translocation of NF-kB. In addition, trolox or PDTC blocks the free radical-induced swelling of cell bodies and cell death. In contrast, the same treatment with PDTC or trolox does not block excitotoxic neuronal death induced by exposure to 15 μM NMDA or 40 μM kainate. These results suggest that NF-kB activation is a critical step to the free radical-mediated cell death in central nervous system.

Supported by a graduate research fund from Ajou University School of Medicine.(ILJ and BJG)

588.11

NERVE GROWTH FACTOR POTENTIATES FREE RADICAL-MEDIATED NEURONAL NECROSIS IN STRIATAL CELL CULTURES. E.C. Park*, I.-O. Jou, and B.J. Gwag, Dept. of Pharmacology, Ajou Univ. School of Medicine, Suwon 442-749, Korea.

Extensive evidence supports that neurotrophins protect central neurons against various injuries including programmed cell death or apoptosis.

Recently, we reported that neurotrophins (e.g. BDNF, NT-3, and NT-4/5) potentiated certain forms of neuronal necrosis induced by exposure to NMDA or oxygen-glucose deprivation (Koh et al., 1995, Science 268:573-575). BDNF also increased neuronal necrosis induced by exposure to $FeCl_2$ or buthionine sulfoximine (BSO) in cortical cell cultures (Gwag et al., 1995, neuroreport, in press). However, NGF effect was not observed due to absence of functional receptors, trkA. The present study was performed to address effect of NGF on neuronal apoptosis and necrosis.

To test this, we prepared rat striatal cultures known to express trkA receptors and respond to NGF. Neuron-glia cocultures (DIV 13-15) exposed to 5 μM $FeCl_2$ or 100 μM BSO produced mild neuronal death 24 hr later. Pretreatment with 100 ng/mL NGF caused rapidly-evolving expansion of neuronal cell bodies about 4 hr following exposure to $FeCl_2$ or BSO, and markedly increased the resulting neuronal death observed 24 hr following the oxidative stress. This necrosis potentiation by NGF was prevented by inclusion of 1 μg/mL cycloheximide a protein synthesis inhibitor, as well as 100 μM trolox, an antioxidant, suggesting that NGF may potentiate free radical-induced neuronal necrosis through mechanisms involving synthesis of new proteins.

Neuron-rich striatal cultures (DIV 7) deprived of serum undergo neuronal apoptosis characterized by cell body shrinkage, chromatin condensation, and sensitivity to cycloheximide. However, concurrent treatment with NGF did not attenuate neuronal apoptosis induced by serum deprivation. Absence of neuroprotection by NGF against the neuronal apoptosis may be associated with expression profile of trkA, a functional NGF receptor in such an immature culture. This study provides evidence that NGF like other neurotrophins potentiates certain necrosis neuronal injury. Supported by a graduate research fund from Ajou University School of Medicine (ILJ and BJG).

588.12

SURVIVAL FACTOR-INSENSITIVE GENERATION OF REACTIVE OXYGEN SPECIES INDUCED BY NEURONAL APOPTOSIS IN CULTURED CELLS. **T. Satoh[1], N. Sakai[1], Y. Enokido[1], Y. Uchiyama[2] and H. Hatanaka[1]*; [1]Div. of Protein Biosynthesis, Institute for Protein Research, Osaka Univ., 3-2 Yamadaoka, [2]Dep. of Anatomy, Med. Sch. of Osaka Univ., 3-1 Yamadaoka, Suita, Osaka 565, Japan.**

ROS (Reactive oxygen species) play a key role in not only several pathological, but also physiological events in the nervous system. We performed confocal and flow cytometry with a ROS-specific fluorogen, 6-carboxy-2',7'-dichorodihydrofluorescein diacetate, di(acetoxymethyl ester) (C-DCDHF-DA) to investigate the involvement of ROS in neuronal apoptosis. For flow cymetry, the total number of events was 5,000 per sample. The samples were gated to obtain data from viable cells stained with C-DCDHF -DA (T. Satoh, *et al*, Neurosci. Lett. 201 (1995) 119-122). Serum deprivation decreased the viability to about 40 and 50% of that of PC12 cells and cortical neurons, respectively, incubated in the presence of serum. In the presence of NGF, cAMP analog and insulin, the number of viable PC12 cells recovered to the same level as the control. Bcl-2 blocked serum deprivation-induced apoptosis in PC12 cells. In rat cortical neurons, EGF completely prevented serum deprivation-induced apoptosis. In PC12 cells, serum deprivation significantly increased the level of ROS. However, NGF, cAMP analog, insulin and Bcl-2, which prevented apoptosis, did not affect the upregulation of ROS induced by serum deprivation. Also, in rat cortical neurons, serum deprivation increased the level of ROS. EGF, which prevented apoptosis, did not affect the increase of ROS. These data suggest that survival factors rescue the serum deprivation-induced apoptosis independently of ROS production.

588.13

HYDROXYNONENAL MAY MEDIATE APOPTOTIC NEURONAL DEATH INDUCED BY TROPHIC FACTOR WITHDRAWAL AND OXIDATIVE INSULTS. I. Kruman, Q. Guo, A. J. Bruce, D. E. Bredesen, W. R. Markesbery*, and M. P. Mattson. Sanders-Brown Research Center on Aging and Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536. La Jolla Cancer Res. Foundation, La Jolla, CA 92037.

Oxidative stress can induce apoptosis in a variety of cell systems. In PC12 cells withdrawal of trophic factor support (WTFS) and amyloid β-peptide (Aβ) induce apoptosis, and the cell death can be prevented or delayed by administration of antioxidants. We have found that WTFS and Aβ induce oxidative stress in PC12 cells and primary embryonic rat hippocampal neurons; the oxidative stress precedes neuronal death and antioxidants protect the neurons from death. 4-hydroxynonenal (HNE) is a relatively stable aldehydic product of lipid peroxidation that has been linked to several different disease processes including atherosclerosis and liver toxicity.

We report here that: (1) HNE can induce apoptosis of PC12 cells and hippocampal neurons in a concentration-dependent manner. (2) Aβ and other oxidative insults induce lipid peroxidation, HNE production, and conjugation of HNE to multiple cellular proteins. (3) Expression of Bcl-2 protects PC12 cells against apoptosis induced by HNE. High concentrations of HNE induce necrosis rather than apoptosis and this death is not prevented by Bcl-2. HNE may promote neuronal damage and death by impairing ion-motive ATPases and disrupting calcium homeostasis (see Mark et al., this meeting). Moreover, HNE may induce further oxidative stress in neurons by causing glutathione depletion. We have found that glutathione-ethyl ester protects cultured neurons against Aβ toxicity, WTFS, and HNE toxicity. A better understanding of the chemistry and cellular bioactivities of HNE may lead to the development of novel compounds that protect neurons from degeneration in the myriad of neurodegenerative conditions in which oxidative stress and apoptosis play roles. (supported by the NIH and the Alzheimer's Association).

588.14

NEUROPROTECTION OF ORGANOTYPIC SLICE CULTURES AGAINST OXIDATIVE STRESS-INDUCED CELL DEATH AS ASSESSED BY DIFFERENT VIABILITY ASSAYS. T. Skutella[1]*, A. Scheidereiter[1], R. Nitsch[1], and C. Behl[2], Humboldt University Clinic (Charite), Institute of Anatomy, D-10098 Berlin[1] and Max-Planck Institute of Psychiatry, Clinical Institute, D-80804 Munich[2], Germany.

The entorhinal-hippocampal system is one major target in neurodegenerative disorders such as Alzheimer's disease in which a role of oxidative stress-induced cell death has been implicated. Antioxidants such as vitamine E and ß-estradiol scavenge free radicals, prevent the peroxidation of membrane lipids and therefore may protect neurons against oxidative challenges. To study if these antioxidants do effectively protect hippocampal neurons maintained in their organotypic organization, hippocampal slice cultures from adolescent rats were employed. Using this cellular system, hydrogen-peroxide- (H_2O_2)-, glutamate- and ß-amyloid induced cell death as paradigms of oxidative stress-induced neurodegeneration were studied. Viability of neurons and glial cells in various hippocampal regions was determined using the fluorescent DNA dye propidium iodide. Early changes and impairments in mitochondrial activity were analysed using the MTT test. Furthermore lactate dehydrogenase release served as an indicator for cellular degeneration. A potent antioxidant effect in hippocampal tissue is reported.

Supported by the DFG: Ni 344/2-1 and SFB 507/C1

588.15

ESTROGEN PROTECTS AGAINST HYDROGEN PERIOXIDE TOXICITY IN DIFFERENTIATED NT-2 CELL CULTURES.
K.L. Rogers*, C.A. Singer, and D.M. Dorsa. Departments of Pharmacology and Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195 and the VA PSHCS, Seattle, WA 98108

Trophic effects of estrogen on neuronal survival and differentiation are well-described. We have noted neuroprotective effects of estrogen against glutamate toxicity in primary cortical cultures, and others have reported neuroprotection against serum deprivation and hypoglycemia in cell lines. We now present data addressing the mechanism of estrogen neuroprotection using the human NT-2 cell line terminally differentiated into a neuronal phenotype with retinoic acid treatment. Oxidative stress induced by hydrogen peroxide (H_2O_2) results in a dose- and time-dependent cell death of NT-2 neurons, quantified by measuring release of lactate dehydrogenase (LDH). Estrogen pretreatment decreases LDH release following H_2O_2 exposure. The proto-oncogene BCL-2 is thought to regulate cell survival and BCL-2 protein levels seen on immunoblots with the OM-11 antibody are upregulated with differentiation in the NT-2 cultures. Estrogen treatment results in further upregulation, suggesting that enhanced expression of BCL-2 is a possible component of estrogen neuroprotection. The cultures appear to express estrogen receptor, as demonstrated by immunoblotting with the H-222 estrogen receptor antibody. Since BCL-2 has been shown to reduce oxidative stress and prevent cell death following glutathione deprivation, these data suggest an estrogen regulation of BCL-2 could serve to reduce free radical-related toxicity. A possible direct antioxidant effect of the neurosteroid estrogen itself may also underlie its neuroprotective actions, in addition to estrogen regulation of genomic determinants of neuron survival. Supported by NARSAD Young Investigator Award to K.L.R., a Molecular Neurobiology Training Grant to C.A.S. and ADRC Grant AG05136 to D.M.D.

NEURONAL DEATH: EXCITOTOXICITY

589.1

BRAIN MATURITY AND SELECTIVE GLUTAMATE RECEPTOR SUBTYPE ACTIVATION INFLUENCE EXCITOTOXIC NEURONAL DEATH MORPHOLOGY ALONG AN APOPTOSIS-NECROSIS CONTINUUM.
C Portera-Cailliau*, DL Price, and LJ Martin. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Glutamate excitotoxicity is a clinically relevant process that results in selective neuronal death by mechanisms which remain unclear. Cell death has been traditionally classified as apoptotic or necrotic based on biochemical and morphological criteria. Necrotic morphologies and apoptotic DNA laddering can co-exist following excitotoxic and ischemic insults to the adult rat brain, suggesting that apoptosis and necrosis may not be mutually exclusive modes of cell death in the nervous system. We tested the hypothesis that neuronal maturity and selective activation of glutamate receptor subtypes influence the morphological phenotype of neuronal death *in vivo*, along an apoptosis-necrosis continuum. Neuronal death caused by excitotoxin injections into the newborn and adult rat forebrains was assessed with respect to degenerative neuronal morphologies and DNA fragmentation patterns. In both cases, death of neurons exhibiting a variety of morphologies was observed, but unequivocal apoptosis occurred only in the immature brain. Neuronal death in developing and adult brains was characterized ultrastructurally by highly ordered but distinct sequences of subcellular organelle abnormalities, and by similar DNA laddering patterns. In the adult brain, NMDA and non-NMDA receptor-mediated neurotoxicities approximated necrosis and apoptosis, respectively. We conclude that neuronal death phenotype varies along an apoptosis-necrosis continuum, rather than through two distinct, independent cell death pathways.
Supported by NIH grants AG05146, NS 20471, NS 10580.

589.2

THE ALIAMIDE PALMITOYLETHANOLAMIDE BUT NOT ANANDAMIDE PROTECTS CENTRAL NEURONS FROM EXCITOTOXIC INJURY IN A DELAYED POSTGLUTAMATE PARADIGM.
S.D. Skaper*, A. Buriani, R. Dal Toso, L. Petrelli, S. Romanello, L. Facci and A. Leon. Research*life* S.c.p.A., 31033 Castelfranco Veneto, Italy.

N-Acylethanolamides, like palmitoylethanolamide (PEA), and N-acylphosphatidylethanolamides accumulate in conditions involving tissue degenerative changes, including ischemia. Excitatory amino acid receptors are known to stimulate the synthesis of N-acylethanolamides and N-acylphosphatidylethanolamides in cultured central neurons. Interestingly, it has recently been demonstrated that mast cells express a peripheral-type cannabinoid receptor (CB2) that recognizes PEA (ALIAmides) and downmodulates activation of these cells in vitro (Facci et al., PNAS, 1995). Here we report that PEA, but not the cognate N-acylamide anandamide (the ethanolamide of arachidonic acid), protects cultured mouse cerebellar granule cells against glutamate toxicity in a delayed postglutamate paradigm. PEA reduced this injury in a concentration-dependent manner and was maximally effective when added 15 min after a 5-min glutamate challenge. Cannabinoids, which like PEA are functionally active at CB2 on mast cells, also reduced neuronal loss in this delayed post glutamate model. The PEA and cannabinoid neuroprotective effects were antagonized by the putative brain CB1 receptor agonist anandamide. Anandamide was also found to antagonize the functional effects of PEA and cannabinoids on mast cells (Facci *et al.*, PNAS. 1995). Cerebellar granule cells expressed CB1 and CB2 mRNA, and two cannabinoid binding sites were detected in cerebellar membranes. Activation of CB2-like receptors by endogenous N-acylethanolamides may serve to downmodulate deleterious cellular processes following pathological events or noxious stimuli in both the nervous and immune systems, thus the acronym autacoid local injury antagonism (ALIA) for this mechanism.

589.3

GLUTAMATE INDUCES THE FORMATION OF HIGH MOLECULAR WEIGHT DNA FRAGMENTS CHARACTERISTIC OF APOPTOSIS IN CEREBELLAR GRANULE CELLS.
R. E. Paulsen*, H. R. Holte, A.-B. Kolstø, and O. J. Marvik. Biotechnology Centre of Oslo, University of Oslo, N-0316 Oslo, Norway.

Glutamate induces cell death when released during ischemia in the brain *in vivo* or when added to cultured neurons *in vitro*. A slow component of the cell death is due to Ca^{2+} influx following activation of the NMDA subtype of glutamate receptors. Several observations suggest that Ca^{2+} may activate endonucleases, leading to the formation of high molecular weight DNA fragments characteristic of apoptosis.

We have investigated glutamate (100 µM)-induced cell death in cerebellar granule cells in culture. In our assay >95 % protection was observed after 24 hours with the noncompetitive NMDA receptor antagonist MK801 (1 µM), and partial (30 %) protection was observed with the non-NMDA receptor antagonist CNQX (1 µM). A time-dependent formation of two classes of high molecular weight DNA fragments (~50 and >680 kbp) was observed using pulse-field gel electrophoresis and quantitative detection of the fragmented DNA with DNA-specific antibodies. The highest level of >680 kpb fragments was seen at 8-12 hours, whereas ~50 kbp fragments increased steadily from 6 to 30 hours following glutamate treatment. MK801, but not CNQX, protected completely against the formation of these fragments.

This work was supported by grant no 111295/310 from the Norwegian Research Council.

589.4

GLUTAMATE KILLS DEVELOPING CEREBELLAR GRANULE CELLS BY OXIDATIVE STRESS INDEPENDENT OF RECEPTOR ACTIVATION.
A. Leon*, G. Marcolongo°, M. Fabris and S.D. Skaper. Research*life* S.c.p.A., Castelfranco Veneto 31033 and ° Monselice 35043, Italy.

Glutamate (Glu) is thought to serve as the major excitatory neurotransmitter in the CNS, stimulating both NMDA and non-NMDA receptors. While activation of NMDA receptors participates in a variety of physiological processes, excessive Glu receptor stimulation has been implicated as the proximate cause of neurodegeneration in several types of brain insults, including hypoxia, ischemia and trauma. During episodes of metabolic compromise, such as hypoxia and ischemia, extracellular Glu levels rise. It is not clear how acute excitation alone could account for the delayed and progressive neuronal degeneration observed in the pathologies in which Glu is implicated. Glu, by inhibiting cystine uptake, may cause neuronal death due to accumulation of cellular oxidants (Murphy *et al.*, FASEB J, 1990). Using a homogeneous population of mouse cerebellar granule neurons, we now show that Glu concentration-depenently (LC_{50}: 2 mM) produces cellular toxicity at a stage (36-48 hr in vitro) prior to development of an excitotoxic response. Glu cytotoxicity was preceeded by a loss of intracellular GSH levels, and was prevented by cystine and cell permeant GSH esters, but not by NMDA and non-NMDA receptor antagonists. A novel series of antioxidants (ALOX) were also potently cytoprotective. The Glu receptor subtype agonists NMDA, kainate and AMPA, which do not inhibit neuronal cystine uptake, were not neurotoxic. Inhibition of cystine uptake by elevated concentrations of extracellular Glu in chronic-progressive brain insults might limit the ability of neurons to maintain redox homeostasis. Therapies directed against free radical attack may be beneficial in prevention of that injury.

589.5

PROTECTIVE ROLE OF CALCIUM-BINDING PROTEINS IN DIFFERENTIATED NEUROBLASTOMA CELLS EXPOSED TO EXCITATORY AMINO ACIDS. B. Fellay, C. D'Orlando, B. Schwaller, V. Gotzos, B. Schneider and M.R. Celio*, Institute of Histology and General Embryology, University of Fribourg, CH-1705 Fribourg (Switzerland)

In some neurological diseases, injury to neurons is related to overstimulation of receptors for excitatory amino acids (EAA). This overstimulation may disturb the Ca^{2+} homeostasis leading to an abnormal prolonged increase of its intracellular concentration. Various reports claim a protective role of calcium-binding proteins (CaBPs) in these pathologies. We tested the capacity of the CaBPs calretinin (CR) and parvalbumin (PV) to buffer Ca^{2+} and to protect nerve cells. For this, we first transfected P19 mouse embryonal carcinoma cells with CR or PV cDNA. This cell type has the capability of differentiating into neuron-like structures expressing functional glutamate receptors upon exposure to retinoic acid. The presence of the CaBPs in cloned, transfected cells was verified by immunohistochemical staining and immunoblots. After EAA exposure, $[Ca^{2+}]_i$ measured using the ratiometric dye fura-2 showed an initial rise in both transfected and control P19 cells from 60 to 400 - 500 nM. $[Ca^{2+}]_i$ decreased in transfected cells to 150 - 200 nM within 5 min, while it remained at the initial high level in control cells. The survival rate of CR-transfected P19 cell clones, compared with that of control cells, was measured using the lactate dehydrogenase assay. The results indicate a protective effect of CaBPs within the first hour following EAA stimulation of P19 cells. This protection is transient and fails to rescue cells after prolonged stress. In a second set of experiments we transfected with CR the N18RE-105 neuroblastoma-embryonic retina hybrid cell line which also show a Ca^{2+}-dependent, delayed cell death induced by glutamate. Preliminary results confirm a protective role of this CaBP also in this experimental paradigm. Thus, CaBPs may help to delay cell death after EAA stimulation. *Supported by Swiss National Foundation 3100.36483.92.*

589.6

HEAT SHOCK DOES NOT PREVENT APOPTOSIS IN CULTURED CEREBELLAR GRANULE CELLS. K. Sato*, H. Saito, and N. Matsuki, Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, 113.

We have shown previously that heat shock attenuates serum deprivation-induced neuronal death via production of HSP70. But it remains uncertain whether heat shock is also protective to apoptosis. Differentiated cerebellar granule cells undergo apoptotic cell death when deprived of depolarizing concentration of extracellular potassium in culture. Here we investigated the effect of heat shock on low potassium-induced apoptosis and glutamate toxicity. Cerebellar granule cells isolated from 8-day-old postnatal rats were plated at the density of 4×10^5 cells/cm^2 and cultured in the presence of 25 mM of potassium and 10 μM of AraC. After 7 days *in vitro*, cells were heat shocked (43 °C 30 min) and exposed to low potassium medium (5 mM) or glutamate containing medium (1-1000 μM) 24 hr after the heat shock. Cell viability was evaluated by measuring LDH release and MTT assay after 24 hr. Heat shock significantly protect cultured cerebellar granule cells from glutamate toxicity but had no effect on low potassium-induced apoptosis. These results suggest that the heat shock does not prevent apoptotic neuronal death.

589.7

DEPRENYL PROTECTS DOPAMINE NEURONS FROM EXCITOTOXIC CELL DEATH. C. Mytilineou*, P.M. Radcliffe, E.T. Kokotos Leonardi, P. Werner and C.W. Olanow, Department of Neurology, Mt. Sinai Sch. of Medicine, New York, N.Y. 10029

L-Deprenyl (selegiline), a MAO B inhibitor, protects several types of neurons from axotomy or neurotoxins by a mechanism independent of MAO inhibition (Finnegan et al., 1990; Tatton and Greenwood, 1991; Ansari et al., 1993). In this study we examined the effect of deprenyl on dopamine (DA) neurons challenged according to a paradigm that results in excitotoxic cell death. Mesencephalic cultures from E14 rat embryos were subjected, beginning on the 5th day *in vitro*, to 4 daily complete changes of feeding medium. Complete medium change results in increased extracellular glutamate and neuronal death, which is prevented by blocking glutamate receptors (Driscoll et al., 1993). Medium changes in mesencephalic cultures caused a 41±7% loss of tyrosine hydroxylase-positive (TH+) neurons and 65±5% reduction in [^{3}H]DA uptake and these effects were completely prevented by the NMDA receptor blocker MK-801 (10μM). At a concentration of 50μM, deprenyl prevented the loss of DA neurons (surviving TH+ cells: 107±12 of control). The loss in [^{3}H]DA uptake was also attenuated by deprenyl (31±3% reduction; $p<0.001$ compared to controls). The protective effect of deprenyl was dose dependent between 0.5 and 50μM. Pargyline, a potent MAO inhibitor, had no effect suggesting that protection by deprenyl was independent of MAO inhibition. Deprenyl also protected DA neurons from direct exposure to NMDA. Exposure to 200μM NMDA for 30 min. caused 25±3% loss of TH+ neurons and 46±6% loss in [^{3}H]DA uptake. Deprenyl completely prevented the loss of TH+ neurons and protected from loss in DA uptake (17±1% reduction; $p<0.01$ compared to NMDA alone). Deprenyl had no inhibitory effect on NMDA receptor binding indicating that neuroprotection occurs at a point beyond the activation of glutamate receptors. Supported by NIH NS-30898, the Lowenstein Foundation and Somerset Pharmaceuticals.

589.8

KAINATE-INDUCED NEUROTOXICITY IS ATTENUATED IN SOD-TG MICE. H.Hirata* and J.L. Cadet, Molecular Neuropsychiatry Section, Neuroscience Branch, NIDA/NIH, P.O. Box 5180, Baltimore, MD 21224.

Peripheral administration of kainic acid (KA) can cause cell death in the hippocampus of rodents. This cell death is thought to involve oxidative stress and to occur through an apoptotic process because it involves DNA fragmentation. In the present study, we have begun to investigate KA-induced neurotoxicity in SOD-Tg mice. Low or medium dose (15 or 30 mg/kg, i.p.) of KA did not induce apoptotic cell death in the hippocampus in every group. However, a higher dose (45 mg/kg, i.p.) of KA killed 6 of 13 (46% wild-type mice, 2 of 9 (22%) heterozygous SOD-Tg mice but none of the homozygous SOD-Tg mice died. These results show that SOD-Tg mice are protected against the lethality of KA. These results suggest that superoxide radicals are involved in the toxic effects of the drug. Ongoing studies are evaluating if the SOD-Tg mice are also protected against apoptotic changes seen in the hippocampus of wild-type mice by using in situ nick translation technique.

589.9

APOPTOSIS OF HIPPOCAMPAL INTERNEURONS AFTER AMYGDALA KINDLED SEIZURES. L.X. Zhang*, X.L. Li, S.R.B. Weiss, R.M. Post and M.A. Smith. Biological Psychiatric Branch, NIMH, Bethesda, MD 20982.

Seizure induced neuronal damage may involve both excitotoxic and apoptotic (Programmed cell death) mechanisms. Apoptosis of neurons has been reported after kainate-induced seizures, but it is difficult to determine the role of seizure activity itself in seizure-associated cell death when convulsant drugs are used because damage that might be caused by seizure activity can't be distinguished from damage caused by direct neurotoxic action of the drugs. Therefore, in the present study, we used a kindled seizure model in which electrical stimulation of the basolateral amygdala induces cell loss most notably in the hilus of the dentate gyrus. To identify which cells might be undergoing apoptosis, we labeled the 3' end of DNA fragments with digoxigenin nucleotides using terminal transferase (ApopTag, Oncor). We found that the number of ApopTag-positive cells increased 41.5% after one kindled seizure and 95.9% after 20 seizures compared to sham controls. The ApopTag-labeled cells appeared to be mainly interneurons of the hippocampal formation. Preferential vulnerability of GABAergic interneurons is consistent with previous studies on seizure-induced cell loss. Our present results, coupled with our previous observation that the apoptosis-related protein, BAX, is induced in the hippocampus by seizures, suggest that apoptosis of GABAergic interneurons may lead to dysinhibition in the hippocampus and result in the increased seizure activity.

589.10

KAINIC ACID INJURY OF THE RAT HIPPOCAMPUS INDUCES ENHANCED EXPRESSION OF APOLIPOPROTEIN D IN NEURONS DESTINED FOR CELL DEATH

Wei Yi. Ong and Shutish C. Patel*
Department of Anatomy, National University of Singapore, Singapore and *Neurobiology Research Laboratory, VA Connecticut Healthcare System (Newington) & Department of Neurology, UCONN Health Center, Farmington, CT.

Apolipoprotein D (apo D), a member of the lipocalin superfamily of transporters for small hydrophobic ligands, has been implicated in the transport of cholesterol, progesterone, arachidonic acid and heme-related molecules such as bilirubin. ApoD is widely expressed in neural and peripheral tissues but its physiological role(s) are poorly understood. High levels of apoD expression have been found in the regenerating rat sciatic nerve, suggesting that it may play a role in the repair process during nerve injury. Here we report our findings on the expression of apoD in the rat hippocampus following kainic acid (KA)-induced excitotoxic injury. Using an anti-rat apoD antibody and biotin-avidin enhanced immunocytochemistry, we observed that in the normal rat brain, there was little apo D expression, restricted mainly to scattered astrocytes and to perivascular fibroblasts. In contrast to control rat brain, KA injected rats showed apoD immunoreactivity in the pyramidal neurons of the affected CA fields 24 and 48 hours after injection of the excitotoxin, at a time when there was no histological evidence of cell death. ApoD immunoreactivity peaked by day 3 coincident with neuronal cell death and declined thereafter reaching very low levels by day 7. Besides pyramidal neurons, apoD immunoreactivity was also observed in a small number of reactive glial cells in the affected CA fields, but not in the vascular compartments at any time point. We conclude that following excitotoxic injury with KA, apoD is induced in hippocampal pyramidal neurons destined for cell death. [Supported by grants from the National University of Singapore (RP920367) and the National Institutes of Health (NS34339)].

589.11

REGIONAL LOCALIZATION OF KAINIC ACID-INDUCED GELATINASE EXPRESSION. W. Zhang* and P. E. Gottschall, University of South Florida, Department of Pharmacology and Therapeutics, Tampa, FL 33612-4799

Evidence from *in vitro* studies implicate a role for matrix metalloproteinases (MMPs) in several neuronal functions, including differentiation during development, neurite extension, and possibly neuronal degeneration. Few reports have documented the expression of these enzymes in the brain *in vivo*. In this study, neuronal degeneration was induced in Sprague-Dawley rats by systemic administration of kainic acid (10 mg/kg). Rats were subsequently divided into convulsive and non-convulsive groups, after observing their behavior in reaction to the drug. Animals were sacrificed 6, 12, 24, 72, and 168 h (7 days) after injection of kainic acid. Gelatinase A (MMP-2) and gelatinase B (MMP-9) were extracted from brain regions in buffer containing 1% Triton-X-100, partially purified using gelatin-sepharose, and assayed by standard gelatin-zymography. Without treatment, both enzymatic activities were expressed differentially in various brain regions with highest levels in the hippocampus and the lowest in the cerebellum, although MMP-2 was expressed at 10-20-fold higher levels compared to MMP-9. In regions from convulsive rats, gelatinase B activity was dramatically elevated 12 h after injection (8.1-fold hippocampus, 7.7-fold diencephalon, 7.2-fold striatum, 5.7-fold frontal cortex, 5.5-fold cerebellum, 2.6-fold midbrain). Gelatinase A activity was induced more than 2-fold in hippocampus, diencephalon and striatum, to a lesser extent in frontal cortex and midbrain, and unchanged in cerebellum 72 h after injection. Neither gelatinase activity was altered in any brain region from non-convulsive rats. These data demonstrate that gelatinase A and B are differentially expressed with respect to time after kainate injection and suggests that they are regulated by convulsion-associated mechanisms and may play different roles in the response to kainate-induced neuronal degeneration. (supported by NIH AG12160)

589.12

ASTRESSIN, A NOVEL CRF ANTAGONIST, PROTECTS AGAINST EXCITOTOXIC CELL DEATH. H. L. Maecker*, A. Desai, S. M. Brooke, R. M. Sapolsky, J. Rivier, and W. Vale. Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020 and The Salk Institute, La Jolla, CA 92186-5800.

In the current study, the neuroprotective potential of a novel and potent CRF antagonist, Astressin, against kainic-acid induced excitotoxic seizures was measured. Initially, 22 control and 21 Astressin-treated rats were subjected to 10 mg/kg systemic kainic acid, i.p. Ten minutes prior to and 30 minutes following the insult, the rats were injected stereotactically with either saline or 25 ug Astressin into the lateral ventricle. Three days post kainic acid injection, rats were perfused, their brains fixed, sectioned, and stained for quantification of hippocampal lesions. Astressin-treated rats exhibited a highly significant reduction of total hippocampal damage, as well as a reduction in damage incurred in all hippocampal fields ($p<0.001$). Next, the neuroprotective potential of Astressin administration AFTER excitotoxic insult was assessed. Twelve control and twelve Astressin-treated rats were injected i.p. with 10mg/kg of kainic acid. Thirty minutes following the excitotoxic insult, the rats were stereotactically infused with either saline or 50 ug Astressin. Three days post-insult the rats were perfused and brains were prepared for lesion quantification. Astressin was again shown to have a significant neuroprotective effect ($p<0.001$), reducing total hippocampal damage by 70% . The magnitude of protection afforded by Astressin was greater than any reported protection provided by other CRF Antagonists to date. These findings confirm the protective potential of CRF Antagonist use even when administered after excitotoxin exposure. Further, this study suggests the therapeutic potential of the novel and potent antagonist, Astressin, against neurological insult.

Supported by the Adler Foundation, CT.

NEURONAL DEATH: CALCIUM AND POTASSIUM

590.1

DNA REPAIR AND APOPTOSIS AS RESPONSE TO GENOTOXICITY DURING NEURONAL DIFFERENTIATION. G. Brescia°, G. Assennato° and P. Corsi*. *Istituto di Fisiologia Umana, °Istituto di Medicina del Lavoro, Facoltà di Medicina e Chirurgia, 70124 Bari, Italy.

Recently attention has been focused on the role of genotoxic stress in apoptosis and in the age-related failure of several cellular activities.

With the primary objective of investigating on a possible age-related response to genotoxic stress, the DNA repair activity, the markers of cytotoxicity and the apoptosis have been evaluated in neuronal cortical cultures after UV exposure.

At 7, 14 and 21 days in vitro we measured the Unscheduled DNA Synthesis (UDS), LDH release and the number of apoptotic cells immuno-positive to the TUNEL reaction. Moreover the levels of the two proteins involved in the cellular responses to genotoxic stress, in apoptosis and in the DNA repair, namely p53 and GADD45, were also analyzed.

Our experimental findings suggest that decreased performance of DNA repair activity is associated to the degree of cellular differentiation and to ageing, and results in increased cytotoxicity and apoptosis.

C.N.R. grants "Progetto Invecchiamento : Gerontobiologia"

590.2

SUBCELLULAR DISTRIBUTION OF GAPDH IN ARA-C INDUCED CEREBELLAR GRANULE CELL APOPTOSIS

P.A. Saunders*,R. Ishitani@,E Chalecka-Franaszek and D.-M. Chuang Sect. Molecular Neurobiol., Biol. Psychiatry Branch, NIMH, NIH Bethesda MD 20892, @ Josai U., Sakado, Saitama 350-02, Japan.

We have discovered that cytosine arabinoside (AraC) induced apoptosis of cerebellar granule cells increases a 38 kd protein band on SDS-PAGE identified as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) EC 1.2.1.12 (Ishitani et al, this volume) Oligonucleotides antisense to GAPDH mRNA afford significant protection to freshly plated granule cells against AraC cytotoxicity, while randomized antisense oligonucleotides are ineffective. To prove unambiguously that the 38 kd band is GAPDH, we have examined Western blots from control and AraC treated granule cells with a monoclonal antibody against GAPDH. A single 38 kd immunoreactive band (ir-GAPDH) was observed to be increased in the 200,000 x g pellets of sonicated cells treated with AraC. We used differential centrifugation to find which subcellular components are affected. Treated and untreated cells were sonicated in 0.32 M sucrose and sequentially centrifuged at 1000, 20,000, and 200,000 x g to obtain crude nuclear, mitochondrial, microsomal, and cytosolic fractions. ir-GAPDH was strongly increased in the 1000 and 20,000 x g pellets, unchanged in the 200,000 x g pellets, and markedly decreased in the cytosol of AraC treated cells. The dehydrogenase activity of GAPDH did not increase in parallel to the immunoreactivity. These observations suggest that GAPDH levels change in specific organells during apoptosis for reasons which are separate from its function as a glycolytic enzyme. (supported by NIMH/NIH)

590.3

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) ANTISENSE OLIGODEOXYNUCLEOTIDES PROTECT AGAINST CYTOSINE ARABINOSIDE-INDUCED APOPTOSIS IN CULTURED CEREBELLAR NEURONS. R. Ishitani*[1] and D.-M. Chuang[2]. 1)Group on Cellular Neurobiology, Josai Univ., Saitama 350-02, Japan. 2)Sect. Molecular Neurobiol., BPB, NIMH, MD 20892, U.S.A.

Cytosine arabinoside (AraC) is a pyrimidine antimetabolite that kills proliferating cells by inhibiting DNA synthesis and also is an inducer of apoptosis. We recently reported that age-induced apoptotic cell death of cultured cerebellar granule cells (CGC) is directly associated with over-expression of a particulate 38-kDa protein, identified by us to be GAPDH. We here show that the AraC-induced neuronal death of immature CGC in culture is effectively delayed by actinomycin-D (Act-D), cycloheximide or aurintricarboxylic acid. Furthermore, two GAPDH antisense, but not their corresponding sense, oligodeoxyribonucleotides markedly arrested AraC-induced apoptosis. Prior to AraC-induced neuronal death, GAPDH mRNA levels increased by an approximately 2.5-fold and this mRNA accumulation was blocked by Act-D and the GAPDH antisense (but not sense) oligonucleotide. Thus, the present results show that GAPDH over-expression is involved in AraC-induced apoptosis of CGC.

590.4

OVER-EXPRESSION OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) IS INVOLVED IN LOW K^+-INDUCED APOPTOSIS, BUT NOT NECROSIS, OF CULTURED CEREBELLAR NEURONS. K. Sunaga[1], M. Tanaka[2], N. Katsube*[2], H. Aishita[2], D.-M. Chuang[3] and R. Ishitani[1]. 1)Group on Cellular Neurobiol., Josai Univ., Saitama 350-02, Japan. 2)Ono Pharmaceuticals, Osaka 541, Japan. 3)Sect. Molecular Neurobiol., BPB, NIMH, MD 20892, U.S.A.

D'Mello et al. have reported the induction of apoptosis in cerebellar granule cells (CGC) by lowering K^+ to 5 mM in culture medium. We found that exposure of CGC to low potassium (K^+) for 24 hr induced not only apoptosis but also necrotic damage. Treatments with actinomycin-D (Act-D) and GAPDH antisense (but not sense) oligodeoxyribonucleotides attenuated low K^+-induced neuronal death by approximately 50%. Morphological inspection revealed that, like Act-D, GAPDH antisense oligonucleotides preferentially blocked low K^+-induced apoptosis with little or no effect on necrotic damage. The levels of GAPDH mRNA and protein were markedly increased in a time-dependent manner following low K^+ exposure. This GAPDH mRNA and protein up-regulation was blocked by the GAPDH antisense oligonucleotide. These results suggest that GAPDH over-expression participates in low K^+-induced apoptosis of CGC.

590.5

NEUROPROTECTIVE EFFECTS OF LITHIUM IN RAT CEREBELLAR GRANULE CELLS. S. Nonaka[1,2], X.-M. Gao[1], N. Katsube[2] and D.-M. Chuang[1]*. 1)Section on Molecular Neurobiology, Biological Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892, U.S.A. 2)Minase Research Institute, Ono Pharmaceutical Co., Ltd., Mishima-Gun, Osaka 618, Japan.

Lithium is the most commonly used drug for the treatment of manic depressive illness. We have studied its neuroprotective actions against various insults in cultured cerebellar granule cells (CGC) of rats. We have shown that carbamazepine and phenytoin induce apoptosis of CGC by a NMDA reversible mechanism. Here we found that lithium (3-10 mM) dose-dependently protected against carbamazepine (200 μM) and pnenytoin (20 μM)-induced neurotoxicity assessed by morphological inspection, MTT metabolism and DNA fragmentation. These protective effects are not prevented by RNA and protein synthesis inhibitors or inclusion of myo-inositol, suggestive of a mechanism independent of inositol monophosphatase blockade. Lithium also significantly protected against CGC apoptosis induced by aging of the cultures. Moreover, lithium rescued death of CGC exposed to low KCl (5 mM), confirming a previous report (D'Mello et al., 1994). Additionally, we found that glutamate (100 μM) and sodium nitroprusside (SNP, 30 μM)-induced CGC death was associated DNA fragmentation. Lithium had marginal protection against glutamate toxicity but no effect on SNP toxicity. Thus, lithium has a wide spectrum of neuroprotective effects which may provide a new avenue to study the underlying therapeutic mechanism of this drug. Supported by NIMH.

590.6

ANTI-APOPTOTIC IMPACT OF ATP AND ADENOSINE IN THE CULTURED RAT CEREBELLAR GRANULE CELLS. H. Amino*, H. Saito and N. Nishiyama. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo. 113 Japan.

Recent data suggested that nucleosides and nucleotides act not only as neurotransmitters and/or neuromodulators but also as neurotrophic factors in the nervous system. Therefore, we studied the effects of exogenously applied ATP and adenosine (Ado) on neuronal apoptosis. Cultured cerebellar granule cells, isolated from 8-day-old postnatal rat and grown *in vitro* for 6-7 days in medium with depolarizing concentration of extracellular potassium (K^+, 25 mM), die by apoptosis when concentration of K^+ was lowered to 5 mM. Lactate dehydrogenase (LDH) assay revealed that approximately half of neurons died by exposure to low K^+ medium for 30 hours. Treatment of neurons with ATP (1-1000 μM) and Ado (0.1-100 μM) significantly prevented low K^+-induced apoptosis in a concentration-dependent manner. Both ATP (1000 μM) and Ado (100 μM) protected more than 90 % of neurons from death. In contrast, UTP (1-1000 μM) did not have such an effect. Moreover, ionotropic P_2-purinergic receptor agonist, α,β-methylene ATP (1-1000 μM) mimicked the anti-apoptotic effect of ATP. These results suggest that extracellular ATP and Ado protect neurons from apoptosis via the G-protein-coupled P_1- and ionotropic P_{2X}- and/or G-protein-coupled P_{2Y}-purinergic receptors and that they might be involved in the regulation of development of the nervous system and in the pathophysiology of neurodegenerative disorders.

590.7

ADENOSINE MODULATES NEURONAL SURVIVAL IN PURIFIED AVIAN RETINAL CULTURES. R.Paes-de-Carvalho* and G.A.Maia. Dep. Neurobiology, Federal Fluminense University, Niterói, RJ 24001-970, Brazil

Adenosine (ado) has been characterized as a neuroprotective substance released during hypoxia and ischemia. The effects of ado are believed to be mediated by the modulation of cyclic AMP levels and inhibition of excitatory neurotransmitter release induced by activation of specific cell surface receptors. Previous work showed specific ado uptake and release in cultured chick retinal neurons. Here we show that ado regulates the survival of chick embryo retinal neurons and photoreceptors grown in purified cultures. Dissociated cells from retinas of E8 embryos were diluted in medium 199 containing 1% Fetal calf serum, 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin, plated in dishes pre-treated with poli-l-ornithine (8X 10^5 cells/dish), and incubated at 37°C in 5%CO_2/95% air. The drugs were added after 18 hs of culture and mantained for 48 hs. In some cases, the medium was changed and the drugs re-added. After further incubation for 72 hs, the cells were fixed and their number estimated by counting 10 fields of 0.114 mm^2 using an inverted microscope. The change of medium induces a cell death of 48.5 ± 7% in these cultures, an effect that is reduced when the cultures are incubated with NBI (5µM), an ado uptake blocker (12.4 ± 0.4%), ado (100µM) plus EHNA (10µM), an ado deaminase inhibitor (0 ± 0.5%), or CGS21680 (20µM), an A_2 ado receptor agonist (28.7 ± 3.5%), but not by CHA (10µM), an A_1 receptor agonist (52 ± 6.4%). In cultures mantained without changing the medium, NBI promotes an increase in cell number of 26.2 ± 3% and in the number of photoreceptors of 47.1 ± 7.4%. The addition of ado deaminase (0.5U/ml) promotes a cell death of 40 ± 5%, an effect blocked by EHNA. The results suggest a role for extracellular ado on the regulation of neuronal survival and differentiation in the developing retina. Financial support: CNPq and CEG-UFF

590.8

CYCLIC AMP PREVENTS PROGRAMMED CELL DEATH OF CULTURED RAT CEREBELLAR GRANULE CELLS. J.Y. Chang*, J.Z Wang, and V.V. Korolev Dept. of Anatomy, Univ. Arkansas for Med. Sci. Little Rock, AR 72205

Cultured cerebellar granule cells undergo programmed cell death when they are deprived of depolarizing KCl. We have performed a series of experiments to correlate the structural and biochemical changes in this process of cell death. The change of total protein level, protein synthesis rate, RNA synthesis rate, and mitochondria activity during the first 48 hours of KCl removal were determined. Further experiments indicate that the cAMP analog CPT-cAMP can prevent the cell death in a dose-dependent manner, with the maximal effect seen a 500 µM. Approximately 70% of cells can be saved with this concentration of CPT-cAMP for at least 6 days. The attempt to plate down cerebellar granule cells in CPT-cAMP (without depolarizing KCl) for long term culture was unsuccessful. While CPT-cAMP significantly slow down initial cell death, the viability was similar to those in low KCl alone a week after plating. Pituitary adenylate cyclase activating polypeptide (PACAP) increased the intracellular cAMP levels of these cells in a time- and dose-dependent manner. This agent can prevent the decrease of cAMP and cell death induced by KCl withdrawal. These results suggest that PACAP could function as a neurotrophic factor for cerebellar granule cells in vivo. (Supported by NIH NS32253)

590.9

MODULATION OF APOPTOSIS AND REPAIR OF DNA DAMAGE IN NEURONS BY CYCLIC AMP G.T. Gobbel* and P.H. Chan CNS Injury and Edema Res. Center & Brain Tumor Res. Center, Dept. of Neurol. Surgery, Univ. of California, San Francisco, CA 94143

DNA damage may contribute to the neuronal injury that accompanies various neurological disorders, but little is known concerning how neurons respond to DNA damage. Rat cortical neurons die by apoptosis following DNA damage induced by x-rays and repair DNA damage more slowly than other cells such as astrocytes. Because cAMP can increase the rate of rejoining of DNA strand breaks in some cells, we tested the effect of cAMP on apoptosis and rejoining of DNA strand breaks in neurons following x-irradiation. Cortical neurons isolated from rat embryos at day 15-17 of gestation were treated with dibutyryl cAMP (0.25-1 mM) for 3 days and then irradiated with 4-8 Gy of x-rays. Treatment with cAMP did reduce the number of apoptotic cells following irradiation as detected by nuclear fragmentation using Hoechst dye. However, unlike astrocytes, cAMP treatment significantly decreased the rate of double-strand break rejoining following 32 Gy as measured by pulsed-field gel electrophoresis. The association between slower DNA repair and reduced apoptosis did not appear to be due to a reduction in ADP-ribosylation and sparing of NADH and ATP; treatment with 0.5-5 mM benzamide to inhibit ADP-ribosylation increased the number of neurons induced by radiation to undergo apoptosis. We conclude that cAMP does not reduce apoptosis by increasing the rate of DNA repair and that depletion of NADH and ATP due to ADP-ribosylation does not account for radiation-induced neuronal apoptosis. Supported by NIH Grant CA 13525.

590.10

Natriuretic Peptides Prevent the Death of Trophic Factor-Deprived PC12 Cells and Promote the Survival of Basal Forebrain Cholinergic Neurons. S.E. Farinelli*, W.J. Friedman and L.A. Greene. *Dept. of Pathology and Ctr. for Neurobiology and Behavior, Columbia University, New York, NY 10032,.*

We have shown previously that nitric oxide prevents the death of trophic factor-deprived PC12 cells and primary sympathetic neurons by increasing intracellular cGMP. Since natriuretic peptides exert their biological actions by activating a receptor-linked guanylate cyclase, we proceeded to test these peptides for their ability to protect PC12 cells following removal of trophic support. Both atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) at 10 nM promoted complete survival for at least one day following removal of serum from naive PC12 cells while only 35% of the untreated cells remained alive. These peptides also provided complete protection for three days to neuronally-differentiated PC12 cells following withdrawal of NGF in serum-free medium while only 40% of the untreated cells survived. C-type natriuretic peptide (CNP) also significantly increased survival in these paradigms. Addition of the cGMP phosphodiesterase inhibitor dipyridamole increased both the potency and the duration of the effects of the peptides, suggesting that the peptides act by increasing cGMP.

Since natriuretic peptide receptors are expressed in the brain during embryonic development, we investigated whether these peptides might exert a trophic effect on primary neurons cultured from various brain regions. Addition of 100 nM CNP at the time of plating to cultures of basal forebrain from E16 rats, resulted in a 50% increase in the number of cholinergic neurons surviving at one week.

Taken together, the data suggest that natriuretic peptides may provide trophic support for certain responsive neuronal populations during development of the CNS. (Supported by grants from the NINDS).

590.11

DOPAMINE-INDUCED CA^{2+} DISREGULATION IN PC-12 CELLS. J.G.Strain G.Cao* and J.A.Joseph. USDA-Human Nutrition Research Center on Aging at Tufts University, Boston, MA.

Previous research has suggested that the initial effects of free radical neurotoxic insult involve large increases in intracellular Ca^{2+} and ultimately, cell death. It has been postulated that the neurotoxic effects of DA may involve autooxidation and reactive oxygen species (ROS)generation. However, the exact role of DA in cellular Ca^{2+} disregulation has not been specified. Thus, PC-12 cells were exposed to 1 mM dopamine (DA) for 30 minutes in growth medium alone (control) or containing various concentrations of either the calcium channel antagonists(CCA) nifedipine or conotoxin or the antioxidants (ANTOX), Trolox or PBN. Fura-II and fluorescent imaging analyses were used to visualize intracellular ΔCa^{2+} in individual cells following depolarization with 30 mM KCl. Results showed that while initial Ca^{2+} levels in the cells and the rise in free intracellular Ca^{2+} following depolarization were not affected by DA, there was a significant decrease in the ability of the cells to clear excess Ca^{2+} following depolarization ($Ca^{2+}R$). Both PBN and conotoxin partially antagonized DA-induced $Ca^{2+}R$ deficits, but not Trolox or nifedipine, suggesting that ROS initiated by DA autooxidation can have significant cytotoxic effects on Ca^{2+} homeostasis, which are only selectively antagonized by ANTOX or CCA. (Supported by USDA Intramural)

590.12

PROMOTION OF NEURONAL CELL SURVIVAL BY 6R-TETRAHYDRO-BIOPTERIN. K. Koshimura*, J. Tanaka, Y. Murakami and Y. KATO. First Division, Department of Medicine, Shimane Medical University, Izumo, 693, Japan.

Recently, we have reported that 6R-tetrahydrobiopterin (6R-BH_4), a natural cofactor for aromatic L-amino acid hydroxylases and nitric oxide synthase (NOS), activates voltage-dependent Ca^{2+} channels in rat neurons independently of its cofactor activity. Several lines of evidence suggest that Ca^{2+} channel activation-induced increase in intracellular Ca^{2+} concentration enhances neuronal cell survival. In the present study, we investigated the effect of 6R-BH_4 on neuronal cell survival using differentiated PC12 cells. 6R-BH_4 activated Ca^{2+} channel activity as estimated by $^{45}Ca^{2+}$ uptake, which was sensitive to nicardipine (1 μM), an inhibitor for L-type Ca^{2+} channels, but resistant to α-methyl-p-tyrosine (α-MT, 1 mM), an inhibitor for tyrosine hydroxylase, and L-nitroarginine (LNA, 0.1 mM), an inhibitor for NOS. The effect of 6R-BH_4 on Ca^{2+} channels was abolished by Rp-cAMPs (1 mM), an inhibitor for protein kinase A, and mimicked by 8-bromo-cAMP (1 mM). After 5 day-culture in the absence of serum and NGF, viable cell number estimated by MTT assay was decreased to one fifth of the number of cells cultured in the presence of serum and NGF. Addition of KCl (50 mM) increased viable cell number cultured in the absence of serum and NGF. 6R-BH_4 (10^{-7}- $3x10^{-5}$ M) dose-relatedly increased viable cell number. The effect of 6R-BH_4 on cell survival was inhibited by nicardipine (1 μM) and Rp-cAMPs (1 mM) but persisted in the presence of α-MT (1 mM) and LNA (0.1 mM). Addition of 8-bromo-cAMP (1mM) increased number of viable cells cultured without serum and NGF. These results suggest that 6R-BH_4 promotes survival of differentiated PC12 cells by activating L-type Ca^{2+} channels via cAMP-protein kinase A pathway indepedently of its cofactor activity.

590.13

ELEVATED INTRACELLULAR Ca^{2+} UNDERLIES THE PRODUCTION OF 50-600 kbp DNA FRAGMENTS DURING APOPTOTIC ROD CELL DEATH. D.A. Fox*, A.T. Poblenz, M.L. Campbell and L. He. University of Houston, College Optometry and Dept. of Biochemistry, Houston, TX 77204.

Electron microscopy studies show apoptotic rod and bipolar cell death in rats exposed to lead during development. This was temporally correlated with the production of 50, 300 and 600 kbp DNA (HMW) fragments and elevated intracellular Ca^{2+} ($[Ca^{2+}]_i$). Therefore, in vitro studies examined the role of $[Ca^{2+}]_i$ in producing DNA condensation and genomic DNA fragmentation. Dark- or light-adapted rat retinas were incubated (15 min) in Ringer's buffer with 0-2.0 mM free Ca^{2+} with or without chemical agents that elevated $[Ca^{2+}]_i$: IBMX, dibutyryl cGMP, ouabain and/or Pb^{2+}. The occurrence of HMW fragments and ladders were examined using field-inversion and conventional gel electrophoresis, respectively. DNA condensation was viewed with acridine orange staining (AO). Retinas incubated in all buffers without Ca^{2+} exhibited a minimal amount of HMW fragmentation and apoptotic rods. In the presence of Ca^{2+}, HMW fragments were differentially and significantly enhanced with all agents while AO revealed the selective occurrence of and significant increase in the number of apoptotic rods. Ladders or necrosis were not observed. These results demonstrate that selective rod apoptosis can be produced in vitro by elevating $[Ca^{2+}]_i$ and that fragmentation of genomic DNA is a multi-step process potentiated by Ca^{2+}. These results suggest that Ca^{2+} overload may be responsible for the apoptotic rod (and bipolar) cell death seen during developmental lead exposure. Supported by NIH Grant ES03183.

590.14

CALCINEURIN INHIBITORS BLOCK APOPTOTIC SIGNAL TRANSDUCTION IN CEREBELLAR GRANULE CELLS. C. Galli, O. Meucci^, A. Mascia, M.T. Ciotti, Mercanti D., M. Hribal, L. Milazzo, R. Possenti*. Inst. of Neurobiology CNR, Via K. Marx 43, 00137 Rome, Italy. ^Dept. of Pharmacol. Physiol. Sciences, Univ. of Chicago, Chicago, Illinois 60637.

We have previously shown that the drop of intracellular calcium is the first step in the signal transduction of the apoptotic message triggered by lowering extracellular potassium in cerebellar granule cells (J. Neurosci,15, 501-508.1995). We have also described (Soc. Neurosci. Ab.793.9. 1995) the hyperphosphorylation of a 120 kDa acidic protein occurring in the first hours of the apoptotic process. In this report we demonstrate that the hyperphosphorylation is caused by the decrease of intracellular calcium. In order to identify the enzymes involved in the calcium signalling of apoptosis we screened a series of protein kinase and phosphatase inhibitors and found that type II pyrethroids, which are specific inhibitors of calcineurin, protect cerebellar granule cells from apoptosis. We found that both deltamethrin and cypermethrin abolished the observed hyperphosphorylation of p120. One related compound, allethrin, which lacks any inhibitory action on calcineurin, was completely ineffective in preventing apoptosis and the associated p120 hyperphosphorylation. Moreover we found that treatment with type II pyrethroids, but not with allethrin is able to restore physiological calcium levels in neurons deprived of high potassium. This effect was mediated by an increased influx of calcium from extracellular compartment.These findings suggest that pyrethroids by inhibiting calcineurin cause an increase of calcium which is sufficient to block the hyperphosphorylation of p120 and the following apoptotic cascade. Pyrethroids as deltamethrin or cypermethrin were also able to prevent apoptosis in neuronal PC12 deprived of NGF but not in naive PC12 deprived of serum suggesting that passage from a non-neuronal phenotype to a neuronal phenotype is accompanied by an involvement of calcineurin in apoptotic message.

590.15

USE DEPENDENT PURKINJE CELL DEATH CAN BE RETARDED BY A BATH APPLIED SHORT HEPARIN-LIKE PROTEIN. M. Sugimori* and R. Llinás, NYU Medical Center, Dept. Physiology & Neuroscience, 550 First Ave., NY, NY 10016.

Neuronal death, to include only individual dendritic branches, was reported for Purkinje cell dendritic trees in guinea pig cerebellar slices following glutamic acid electrophoresis. The results indicated that such events were generated by secondary release of calcium from intracellular stores, probably via the activation of an IP3 receptor, as they were blocked by the intracellular injection of heparin, an IP3 receptor blocker (Sugimori et al., Soc. Neurosci Abst. 21,793.10, 1995). Using Fura II, calcium imaging of Purkinje cell in a somatic patch clamping paradigm demonstrated that bath application of a short heparin-like protein (enoxaparin) which, as regular heparin may block the IP3 receptors, produced a reduction of the secondary release of intracellular calcium to glutamic acid iontophoresis. It also produced a partial reversal in the dendritic death march if the added following glutamate administration. In addition, the electrophysiological deterioration that accompanies increased [Ca]i was also markedly reduced. The results suggest that cell death, as related to increased intracellular calcium concentration following an acute excitotoxin events may be reduced by extracellulary applied IP3 blockers. Support: NINDS-NS13742, NIA-AG09480.

590.16

DANTROLENE INHIBITS GT1-7 NEURONAL DEATH INDUCED BY MULTIPLE AGENTS. H. Wei, W. Wei and D.C. Perry*. Dept. of Pharmacology, George Washington University, Washington D.C. 20037.

Dantrolene inhibits release of Ca^{2+} from the endoplasmic reticulum via ryanodine receptor channels. The ability of dantrolene to inhibit neuronal death induced by multiple agents was investigated in GT1-7 hypothalamic neurosecretory cells. Cells were treated with serum deprivation, ionomycin (3 μM), A23187 (4 μg/ml), thapsigargin (50 nM) or caffeine (10 mM), alone or after 30 min pretreatment with dantrolene (120 μM). Cell viability was determined by the trypan blue exclusion assay. Viability decreased after 24 treatment with serum deprivation (37%), ionomycin (47%), A23187 (57%), thapsigargin (61%) or caffeine (59%). With dantrolene pretreatment, cell viability significantly increased to 68%, 80%, 79%, 93% or 90% respectively ($P<0.01$ except A23187). Cells treated with thapsigargin or caffeine were demonstrated to die by an apoptotic mechanism. The effect of these agents on cytosolic Ca^{2+} ($[Ca^{2+}]_i$) in GT1-7 cells was studied using the flourescent calcium dye Fura-2/AM. Caffeine did not induce elevation of $[Ca^{2+}]_i$. Ionomycin, A23187 and thapsigargin all significantly increased $[Ca^{2+}]_i$, to 437%, 1035% and 231% of control, respectively ($P<0.05$). Pretreatment with 120 μM dantrolene for 30 min decreased the stimulated $[Ca^{2+}]_i$ levels to 366%, 539% and 169% of controls respectively ($P<0.05$ for A23187 and thapsigargin). These results demonstrate that dantrolene can inhibit GT1-7 cell death induced by multiple agents, but that its protective effects may not always be due to inhibition of increases in cytosolic Ca^{2+} concentrations. Supported by the Souer Stroke Fund.

590.17

ALTERED ER SIGNALING AS REVEALED BY ENHANCED EXPRESSION OF THE BIP/GRP78 GENE DURING PROGRAMMED NEURONAL CELL DEATH

T. Koike*, K. Nakayama, S.Tanaka, and C. Mitsui Graduate Program in Biological Sciences, Hokkaido University, Faculty of Science, Sapporo 060, Japan.

By utilizing neuronal PC12 cells as a model, we have searched genes up-regulated during their cell death, and found that expression of the BiP/GRP78 gene among the HSP70 family genes is markedly enhanced during neuronal death following NGF deprivation(Aoki et al.,submitted). The BiP/GRP 78 induction is suppressed under depolarizing conditions with elevated K^+ or by treatment with cAMP. The BiP induction also occurred in cultured rat SCG neurons following NGF deprivation. We have previously reported that SCG neurons treated with an excessive dose of the Ca^{2+} chelator BAPTA-AM, underwent apoptosis (Nakayama and Koike, 1995). Under these conditions, we found that the BiP /GRP78 gene expression is greatly induced. Thapsigargin (TG), an inhibitor for Ca^{2+} ATPase in ER also caused similar enhancement, suggesting that depletion of Ca^{2+} from ER stores plays a role in its induction. Moreover, fura-2 measurements indicated a decrease in basal $[Ca^{2+}]i$ levels during cell death following NGF withdrawal along with significant reduction of TG-induced Ca^{2+} release form ER stores. Since TG itself is not toxic to SCG neurons, it is unlikely that the BiP/GRP 78 induction itself causes neuronal death. We propose that an alteration in ER signaling occurs in parallel with other cell death cascades, leading to altered processing of secretory proteins from ER that are necessary for maintenance of synaptic integrity of neurons.

590.18

POTENTIATED NECROTIC DEATH BY NGF IN A GLUCOSE-DEFICIENT ENVIRONMENT.

J.H. Hong, S.H. Lee, H.J. Kang, K.M. No, S.Y. Chang and J.M. Chung*. Dept. of Biology, Ewha Womans University, Seoul, Korea 120-750.

It is widely believed that nerve growth factor (NGF) may serve as a therapeutic agent for many types of neuronal diseases. One of the mechanisms suggested to explain the protective role of NGF is that the trophic factor can prevent the increase of intracellular calcium (Ca) ions which might be responsible for neural death. To examine whether the Ca hypothesis works even under a pathological condition, we applied NGF to PC12 cultures deprived of glucose and examined the effect of NGF on cell death. Here NGF rather promoted cell death under a glucose-deprived condition. Changes in chromatin morphology and patterns of DNA fragmentation showed that cell death induced by NGF was necrotic. The NGF-induced necrotic death occurred in a manner dependent on extracellular Ca. Also nifedipine (1-10nM) and ryanodine (0.1-10nM) could partially block the cell death. All these suggest that NGF might potentiate cell death by increasing intracellular Ca in cells deprived of glucose. However, the coapplication of nifedipine and ryanodine could not block the death completely. It is, therefore, possible that NGF induces necrotic death via 2 separate pathways, a Ca-dependent and a Ca-independent ones, in a hypoglycemic condition. *(Supported by Ewha Research Grant to JMC)*

REGENERATION: FUNCTIONAL RECOVERY

591.1

TIME COURSE OF OPTIC NERVE REGENERATION IN THE LIZARD, *CTENOPHORUS ORNATUS*.

[1]N. Tran, [1]S.A. Dunlop*, [2]J.M. Papadimitriou and [1]L.D. Beazley. [1]Department of Zoology and [2]Department of Pathology, The University of Western Australia, Nedlands 6907, Australia.

We have recently shown in lizards that, as in frogs, the optic nerve can regenerate. In lizards at one year after optic nerve crush, optic axons had re-grown along essentially normal pathways to reach retino-recipient centres; approximately two thirds of retinal ganglion cells had survived axotomy, a proportion similar to that in frog. However, unlike frog in which visuomotor function is restored, lizards appeared blind on the operated side and failed to exhibit feeding behaviour or startle responses. This lack of visually elicited behaviour was found to be correlated with a failure of axons to form a retinotopic map within the optic tectum. In frog, regenerating axons reach the tectum within 3-4 weeks and visually evoked responses can be recorded shortly thereafter. Here, we have examined the time course of optic nerve regeneration in the lizard to determine whether there was a delay compared to frog; delayed innervation might have resulted in an inability of regenerating axons to re-establish a retinotopic map. Animals received unilateral optic nerve crush and at monthly intervals up to 1 year were perfused with 4% paraformaldehyde. Crystals of the carbocyanine dye DiI were placed across the entire optic disk. Brains were kept at 37°C for 6 weeks to allow for transport, embedded, sectioned coronally at 100μm and examined using a fluorescence microscope. At all stages examined, the crush site persisted macroscopically as a translucent region indicative of reduced myelination and the nerve between the eye and the crush site was heavily labelled. By one month, a minority of axons had crossed the crush site, reached the optic tectum and spread across it; the low proportion of regenerated axons allowed individual terminals to be identified. By 2 months, the projection to the visual centres was heavy and remained pronounced for the remainder of the time course examined. The time course of optic nerve regeneration in the lizard is thus similar to that in the frog as was the mis-routing of some axons at the optic chiasm; we observed and abnormally pronounced projection to the ipsilateral tectum and other minor nuclei as well as a transient retino-retinal projection. We conclude that the absence of a retinotopic map in lizards is due to factors other than a delayed regeneration of optic axons.

591.2

ABNORMAL SENSORY REINNERVATION OF MYSTACIAL GUARD HAIRS IN LONG TERM CHRONIC CONSTRICTIVE INJURY (CCI) OF THE INFRAORBITAL NERVE (ION) IN RATS.

B.L. Munger*[1], G. B. Bennett[2], & B. P. Vos[3]. [1]Dept. Anat. & Physiol., U. Tasmania, Hobart, Tas., Aust. 7005; [2]NAB, NIDR, NIH, Bethesda, MD 20809; [3]Lab. Anesth., Dept. Med., U. Antwerp, Belgium.

Experimentally produced painful peripheral neuropathies result from a variety of physical injuries to peripheral nerves following the initial description by Bennett and Xie of CCI in sciatic nerves of rats (Pain 33:87, 1988). The present study compares the early and long term effects of CCI to the ION (Vos et al, J. Neurosci. 14:2708, 1994) with lesions in the sciatic nerve (Munger et al, Exp. Neurol. 118:204, 1992). Rats were anaesthetised with Nembutal and two ligatures were placed around the ION with mild tension. The rats were killed with an overdose of Nembutal following behavioural observations at early postoperative days 1-15 and long term days 15-130. Sections of mystacial skin were fixed in formalin and serial paraffin sections stained with silver. The early pathology in the ION was identical to that seen in Bennett's model (Munger et al). The large diameter axons degenerated with preservation of many Aδ and C fibres. The animals at 130 days persisted in demonstrating behavioral evidence of abnormal sensibility of mystacial skin. The animals at 130 days had abnormal sensory reinnervation of guard hairs identical to rats that had ION's cut or crushed (Munger and Renehan, JCN 283:169, 1989). The normal palisade of lanceolate terminals was absent and only simple terminals resembling free nerve endings could be found. The abnormal sensory re-innervation may account for persistent (long term) behavioural changes consistent with abnormal sensibility of reinnervated mystacial skin (Vos et al 1994).

591.3

FUNCTIONAL GAP JUNCTIONS COUPLE MOTOR NEURONS IN DEVELOPMENT AND REINNERVATION.

R.J. Balice-Gordon*[1], A. Pereda[2], & M.J. Pinter[2]. [1]Dept. Neurosci., Univ. Penn. Sch. Med., & [2]Dept. Neurobiol. & Anat., Med. College of Penn., Philadelphia, PA.

During development and adult reinnervation, neuromuscular junctions undergo a period of activity dependent synaptic plasticity, resulting in the loss of multiple motor neuron inputs and continuing until a single input remains. We have found that motor neurons are coupled by gap junctions during these times. Dye fills in mouse spinal cord slices and immunostaining with connexin specific antibodies shows that from E14 to birth, ca. 80% of the motor neurons in the mouse ventral lumbar spinal cord are coupled by gap junctions. The percentage of coupled motor neurons declines rapidly after birth; by P10, as in adults, few if any dye coupled or immunopositive motor neurons are detected. From E10 to E14, ventral ventricular zone cells are dye coupled as well as immunopositive for connexins. We are using time lapse video microscopy to determine whether dye coupled neurons populate the same motor pools in the ventral horn.

Following nerve damage, intracellular dye fills and immunostaining show that gap junctional coupling among motor neurons is transiently re-expressed. In adult cat spinal cord 3 weeks after nerve damage, clusters of 4 to 6 motor neurons are labeled following Neurobiotin injection of a single identified motor neuron. Similar injections in normal adults yield single labeled motor neurons. In adult mouse spinal cord, motor neurons show peak connexin expression 2 to 3 weeks following nerve damage. Five to 6 weeks after damage, little if any expression is detected. Transient gap junctional coupling among developing and axotomized motor neurons may mediate electrical or metabolic signaling that is important for the formation and refinement of synaptic connections. This work was supported by grants from the NIH and Spinal Cord Research Foundation.

591.4

INCREASING THE SPECIFICITY OF REGENERATING MOTOR NERVES

C.M. Neumann, T.M. Brushart*, and T. Gordon*, Dept Pharmacol., Div. of Neurosci., Univ of Alberta, Edmonton, Canada T6G 2S2 and Depts of Orthopaedics and Neurology, Johns Hopkins Hosp., Baltimore, Maryland 21287*.

Using the rat femoral nerve model, we looked at the specificity of regenerating motor neurons. Although it has been shown that motor neurons in juvenile rats will preferentially reinnervate the motor rather than the sensory branch of the femoral nerve, there is little preferential motor reinnervation in the adult rat. We sought to determine whether the specificity of regenerating adult rat motor neurons could be induced by enhancing axonal growth by either 20 Hz stimulation, or direct current stimulation. Using adult Sprague Dawley rats, we cut the left femoral nerve, and then repaired it using microsurgical technique. The rats were subjected to one of three conditions: 1) normal cage activity, 2) 20 Hz stimulation, or 3) direct current stimulation for 2 weeks, 24 hrs/day. The motor neurons that regenerate axons through the motor and sensory branches of the repaired femoral nerves were retrogradely labelled by applying horseradish peroxidase to the motor branch of the femoral nerve, and fluorogold to the sensory branch. Rats were sacrificed by perfusion with 4% paraformaldehyde. The spinal cord was cryosectioned, and the labelled motor neurons were counted. We found that, while direct current did not enhance the specificity of regenerating motor axons for the motor branch at 2 weeks, 20 Hz stimulation significantly increased this specificity at 2 weeks (58% motor, 31% sensory $p<0.01$) as compared with normal cage activity (49% motor, 40% sensory $p>0.01$). We conclude that 20 Hz stimulation may be beneficial to functional recovery following a peripheral motor nerve injury by enhancing appropriate connectivity. Long term studies presently underway aim to determine whether the specificity will increase with time, and whether this is due to the pruning of misdirected sprouts from the sensory branch. Supported by AHFMR, Canadian MRC and NCE.

591.5

SPROUTING OF PRIMARY AFFERENT FIBERS AFTER SPINAL CORD TRANSECTION. N.R. Krenz* and L.C. Weaver. Neuroscience Program, Robarts Res. Inst. & University of Western Ontario, London, Ont., Canada.

Spinal cord injury above mid-thoracic levels often results in hyperreflexive sympathetic regulation of arterial pressure. Sprouting of primary afferent fibers into regions containing sympathetic preganglionic neurons (SPNs) or interneurons may be responsible for this autonomic dysreflexia. Myelinated and unmyelinated afferent fibers were assessed in control rats and rats 7 and 14 days after spinal cord transection (SCT) at the 6th thoracic segment. Myelinated afferents were identified by immunofluorescence for cholera toxin B (CTB), which was transported from muscle to the cord. In lumbar, but not thoracic cord, CTB-containing fibers were more dense in laminae III and IV at both 7 and 14 days after SCT, compared to controls. This sprouting likely contributes little to dysreflexia, because it occurs in lumbar regions that contain few SPNs, but not in the thoracic cord that contains the majority of SPNs. The unmyelinated fibers were immunolabelled for calcitonin gene-related peptide (CGRP). The area of CGRP fibers in laminae III and IV increased at all cord segments at 14 but not 7 days after SCT. Moreover, 14 days after SCT, CGRP fibers sprouted in dorsal root ganglia rostral and caudal to SCT. CGRP fibers around the SPNs increased above SCT and in lumbar segments at 14 but not 7 days after SCT. CGRP fibers around SPNs did not differ from controls in segments T6-13. In conclusion, unmyelinated primary afferent fibers sprout in the dorsal root ganglia and throughout thoracic and lumbar dorsal horn after SCT. The CRGP fibers that increased around the SPNs after SCT do not extend from the dorsal horn and probably are not primary afferents. Sprouting of CGRP fibers may contribute to autonomic dysreflexia. Support: MRC Canada.

591.6

REGROWTH AND REDIRECTION OF VASOPRESSIN- AND OXYTOCIN-CONTAINING FIBERS AFTER ABLATION OF THE NEUROHYPOPHYSEAL TRACT IN SHEEP. M.J. McKinley*, M.E. Giles and B.J. Oldfield. Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Australia.

An immunohistochemical study was made of brains of sheep in which the hypothalamo-neurohypophyseal tract (HNT) had been ablated. Two electrodes were implanted into the HNT of 6 adult Merino cross-breed ewes while they were under the influence of general anesthesia induced by intravenous Na thiopentone (19 mg/kg) and maintained by halothane/oxygen inhalation.The HNT was ablated by passage of radiofrequency current across the tips of the electrodes. After 1-4 months, these sheep, as well as 4 normal ewes, were killed by intravenous injection of Na pentobarbital (100 mg/kg). Immunohistochemistry was used to identify the presence of oxytocin and vasopressin in cells and fibers in the diencephalon of these animals. In all 6 sheep with HNT lesions, a dense fiber tract was observed in which oxytocin- and vasopressin-containing fibers extended medially, then rostrally from the supraoptic nucleus across the base of the brain and optic chiasm to the lamina terminalis. A particularly dense array of these oxytocin- and vasopressin-containing varicose fibers surrounded the blood vessels in the vascular plexus of the organum vasculosum of the lamina terminalis, where the blood-brain barrier is lacking due to the presence of fenestrated capillary endothelium. Such fibers were not observed in the normal sheep. Sheep with HNT lesions also exhibited many more fibers extending to the lateral walls of the third ventricle and contacting the cerebrospinal fluid (CSF) than did controls. These results show that after ablation of the HNT, axons from hypothalamic magnocellular neurons can grow to new sites in the brain where it is possible that oxytocin and vasopressin may be released into the blood-stream or CSF.

Supported by an Institute Block Grant to the Howard Florey Institute by the National Health and Medical Research Council of Australia.

591.7

COMPENSATORY NEUROGENESIS IN THE DENTATE GYRUS OF ADULT RATS. E. Gould* and P.Tanapat. Lab. of Neuroendocrinology, Rockefeller Univ., New York, NY 10021.

In general, replacement of neurons following cell death is not a feature of the adult mammalian brain. Our previous studies have shown that both naturally occurring developmental cell death, as well as adrenalectomy-induced cell death in adulthood, are accompanied by increased granule cell proliferation in the dentate gyrus (Gould, 1994). These findings suggest that granule cell death stimulates the production of new granule neurons in this system. In order to directly investigate this possibility, we used ^{3}H-thymidine autoradiography, immunohistochemistry for cell-specific markers and in situ DNA end labeling to examine the effects of lesioning the granule cell layer on the degeneration and proliferation of cells in the dentate gyrus of adult rats. Lesion of the granule cell layer with ibotenic acid resulted in a greater than sixfold increase in the number of ^{3}H-thymidine labeled cells in the dentate gyrus. The vast majority of these ^{3}H-thymidine labeled cells (~80%) possessed the morphology of immature granule neurons, i.e., medium-sized, round or oval cell bodies, and were not immunoreactive for vimentin, a marker of immature glia. In situ DNA end labeling demonstrated that the degenerating cells were distinct in morphology and location from the ^{3}H-thymidine labeled cells. These results suggest that signals produced by dying granule cells stimulate granule cell progenitors to proliferate and potentially repopulate the damaged area. Supported by a NARSAD Young Investigator Award and MH52423 to EG.

591.8

COMPENSATORY REINNERVATION FOLLOWING UNILATERAL LESION OF THE OLIVOCEREBELLAR TRACT IN THE RAT. M. Zagrebelsky, F. Rossi*, M.E. Schwab§, P. Strata. Dept. of Neuroscience, University of Turin, I-10125 Turin, Italy; §Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland.

Functional recovery following brain injury depends upon the formation of new topographically organised projections. We have studied the reorganisation of the olivocerebellar projection following deafferentation of a hemicerebellum by unilateral section of the inferior cerebellar peduncle in newborn rats (P1). The normal olivocerebellar system shows a very precise topographical organisation in which climbing fibre stripes and zebrin II defined Purkinje cell bands align. Within less than 24 hours following the lesion olivocerebellar fibres from the intact side give rise to sprouts that grow across the cerebellar midline in the deafferented hemicerebellum. There the transcommissural axons restore the original pattern of calcitonine gene-related peptide (CGRP)-positive climbing fibre stripes within a few days. Furthermore in the deafferented hemicerebellum of adult rats, lesioned at birth, the alignment between climbing fibre stripes and zebrin II defined Purkinje cell bands is fully and precisely restored. These results show that a high degree of target specificity is attained by this reinnervation process. The extent of reinnervation of the deafferented hemicerebellum gradually declines if the lesion is performed in older animals and it is absent after postnatal day ten (Sherrard and Bower,'86). This phenomenon parallels the progressive myelination of the white matter with the appearance of myelin-associated neurite growth inhibitors (Caroni and Schwab,'88). To establish whether the transcommissural reinnervation can be obtained also in older lesioned rats we neutralised the myelin-associated neurite growth inhibitors by the application of a specific blocking antibody (IN-1; Caroni and Schwab,'88). First results show that lesions at postnatal day 15 induce sprouting and transcommissural reinnervation of the deafferented hemicerebellum by climbing fibres if the activity of the myelin-associated neurite growth inhibitors is blocked. Supported by the European Science Foundation

591.9

FUNCTIONAL RECOVERY AFTER SEPARATION OF FASCICLES IN TUBULAR REPAIRS OF GAP INJURED NERVES . M.R. Wells*, U. Vaidya, B.H. Hallas, A. Bosak, S. Tierney, and P. Jakovina. N. Y. College of Osteopathic Medicine, Old Westbury, N.Y. 11568-8000

It has been suggested that tubular repairs of peripheral nerves with short gap injuries can produce a functional recovery similar to nerves repaired by direct suture of the proximal and distal stumps. In the direct suture repair, the appropriate matching of fascicles appears to be important in the eventual recovery of function. In the present study, we have compared functional recovery after gap injuries of the rat sciatic nerve which were repaired either with a single tube or two smaller tubes in which two major fascicles of the nerve were matched. Twenty female, Sprague-Dawley rats received 8 mm gap injuries of the sciatic nerve which were repaired with either a single silicone tube (2 mm I.D., n=10) or two smaller tubes (1 mm I.D., n=10). For the double tube repair, the proximal and distal ends of the major tibial fascicle was placed in one tube, while the remaining fascicles were sutured into the other tube. A group of three animals which received only a sciatic nerve crush at the level of repair was also included for comparison. Functional recovery was followed over time using the sciatic functional index (SFI) and hindlimb extension pressure over a period of nine weeks. Animals in which repairs were made with two tubes had a significantly better recovery over the course of the experiment (SFI, $p<0.04$) compared to animals in which nerves were repaired with a single tube. However, the recovery was clearly poorer than with crush injured nerves. The results demonstrate that fascicular matching can also improve the functional outcome of tubular nerve repairs. It may also be implied that inappropriate choices by regenerating axons may be detrimental to recovery in tubular repairs.

Supported by the New York College of Osteopathic Medicine.

591.10

GAIT ANALYSIS AS A MEASURE OF FUNCTIONAL RECOVERY AFTER NERVE GAP INJURIES. B.H. Hallas*, A. Bosak, U. Vaidya, S. Tierney, P. Jakovina, and M.R. Wells. N. Y. College of Osteopathic Medicine, Old Westbury N.Y.

The detection of functional recovery after gap injuries of the sciatic nerve is problematical since the commonly used sciatic functional index recovers poorly or not at all in this model. In the present experiment, we have examined the use of a gait analysis technique to detect functional deficits produced by nerve gap injuries and subsequent recovery. Sprague-Dawley rats received an 8 mm gap injury of the sciatic nerve repaired with a silicone tube or two tubes in which nerve fascicles were matched (see Wells, M.R. et al. in this volume). A group of animals which received only a sciatic nerve crush was also included for comparison. Gait patterns of the affected legs were measured using a Peak Performance computer system. Walking patterns were digitized using reflective markers placed on the hip, knee and ankle joints and on the foot. Initial parameters examined included stride length, vertical displacement of the foot and ankle, and range of motion of the ankle and knee. All of the parameters examined were significantly altered by the nerve injuries over the course of the experiment. The severity of deficits expressed generally increased with time before recovering. Measurements of stride length and vertical displacement of the foot and ankle appeared to recover to normal values by 9 weeks after injury, while the range of motion of the ankle and knee showed an incomplete recovery which differed with the type of nerve injury. Of the parameters measured, the ankle range of motion appeared to be the best indicator of recovery. The data suggest that the analysis of gait parameters may be a useful behavior-based technique for detecting deficits and functional recovery in nerve gap injury models.

Supported by the New York College of Osteopathic Medicine, and Quantitative Motion Analysis.

591.11

CHARACTERISTICS OF DYSFUNCTION FOLLOWING SPINAL INJURY IN LARVAL LAMPREYS. V. Pate, L. Guan, T. Kiemel, J. Presson*, A. H. Cohen, Dept. of Zoology, University of Maryland, College Park, MD 20742.

The larval lamprey is known to recover function after spinal injury. Axons regrow, reconnect and can restore some function within the context of locomotion. We show that although there is considerable regeneration, 27 weeks after lesioning there can also be significant dysfunction following spinal crush at the mid-body level. Dysfunction is defined as abnormal movement during swimming not seen in sham operated controls. We begin to characterize abnormalities from electromyograms recorded in the whole animal. We use two measures of axonal regeneration: correlated spike activity across the lesion site in the isolated spinal preparation, and conduction of electrical activity across the lesion in both rostral to caudal and caudal to rostral directions.

Most, but not all, animals display some dysfunction. The major form of dysfunction relate to initiation, termination or maintenance of locomotion either when induced by tail pinch or when spontaneously initiated. Some animals exhibit a pattern of inhibition between the rostral and caudal segments; when one is active, the other is not. In the extreme, one may immediately silence the other. There can also be problems in termination of swimming. with, for example, the caudal segments continuing until the rostral are stimulated by head strokes. There can also be problems in maintaining a unified frequency above and below the lesion. The swimming can have episodes of two clear frequencies of large amplitude bursting, which may or may not be accompanied by phase locked bursting. The two frequency bursting can be associated with significant evidence for regeneration of the intersegmental coordinating fibers, suggesting that the brain was driving the rostral and caudal segments at frequencies that the fibers were unable to coordinate. In most cases in which dysfunction was seen, there was clear evidence for regenerated fibers crossing the lesion site. Supported by NIH NS16803 to AHC; VP was supported by Howard Hughes Foundation.

591.12

REGENERATION AND RECOVERY OF SENSORY EVOKED LOCOMOTOR BEHAVIOR FOLLOWING CRUSH INJURY OF THE TRIGEMINAL NERVE IN LARVAL LAMPREY. K. Philbrick, J.L. Calton*, A.D. McClellan, Biol. Sciences, Univ. Of Missouri, Columbia, MO 65211.

In the present study, the right trigeminal nerve was crushed in larval lamprey (Petromyzon marinus), and animals were tested at weekly intervals for recovery of the trigeminal sensory-evoked escape response. After various recovery times (0-12 wks.), anatomical regeneration of sensory and motor components of the trigeminal nerve were examined by application of HRP to the rostral part of the head. In some cases, muscle recordings were used to determine if regeneration of the sensory component of the trigeminal nerve was functional.

Behavioral recovery. In normal animals, tactile stimulation of the head elicits an escape response consisting of head flexion away from the stimulus, followed by swimming. During the first week following nerve crush, tactile stimulation ipsilateral to the crush did not elicit escape responses. By 3 weeks post-crush (PC), head flexure and escape activity could be elicited in about 52% of the animals. By 7 weeks PC, all the nerve crushed animals responded normally to tactile stimulation ipsilateral to the crush.

Anatomical regeneration. In normal animals, HRP application to the head labels trigeminal motorneurons (nVm), axons of the descending tract (dV), some medullary dorsal cells (Dcm), and some spinal dorsal cells (Dcs). Following nerve crush, there was a gradual increase in labeling in nVm and dV ipsilateral to the crush with increasing recovery time. However, it appears that relatively few dV fibers are required for behavioral recovery, as recovery of this trigeminal component was only partial at 12 weeks PC.

In summary, sensory and motor components of the trigeminal nerve in lamprey regenerate following a crush injury. Behavioral recovery of the trigeminal sensory-evoked escape response is presumably mediated by restoration of synaptic connections between trigeminal afferents and brain neurons, such as reticulospinal neurons.

Supported by NIH Grant NS 29043 and APA Grant MB1-9108 awarded to A. D. M.

591.13

LOCOMOTOR PERFORMANCE IN ADULT *MONODELPHIS DOMESTICA* (S. AMERICAN OPOSSUM) FOLLOWING COMPLETE SPINAL TRANSECTION AT ONE WEEK POSTNATAL AGE. N.R. Saunders*, G.W. Knott, P Kitchener, K. Lomasney, J. G. Nicholls and T. Smith. Department of Anatomy & Physiology, University of Tasmania, GPO Box 252C, Hobart, 7001, Australia.

Neonatal *Monodelphis* have previously been shown to recover and develop apparently normal locomotor performance following complete spinal cord transection (Saunders *et al*, 1995, Clin. Exper. Pharm. & Physiol., 22, 518-526; Soc. Neurosci. 21, 506.2). The behavior of these animals when adult has now been assessed using standard tests (eg Kunkel-Bagden *et al*. 1993, Exper. Neurol. 119, 153-164) including those recommended by the American Paralysis Association (1990, Exper. Neurol. 107, 113-117): placing, climbing an inclined beam, runways (wide runways and grids) footprint analysis and swimming. Spinal cords of litters of 6 to 8 day old *Monodelphis* were crushed in anaesthetised animals under sterile conditions. In 12 operated animals killed 0-24h after crushing, the cord was shown to be completely severed; 10 animals from 3 litters survived to adulthood. These were compared with 5 unoperated littermates. Only small differences were found in some parts of the footprint analysis (printlength, $P<0.005$ and toe spread, $P<0.04$, were significantly smaller than for controls). There were no significant differences in the time to climb and errors made while climbing a 45° angle pole; similarly there were no significant differences in crossing a 1.5 cm or 3.5 cm grid. It is concluded that *Monodelphis* that have had their spinal cords severed in the first week of life, develop to a remarkably normal extent with respect to locomotor ability. Nerve impulse conduction across the site of injury and pathway tracing in spinal cords of the operated animals are currently being investigated.

Supported by the Australian Research Council.

591.14

ARE SUPRASPINAL AND PROPRIOSPINAL AXONS WHICH GROW THROUGH THE LESION AFTER TRANSECTION OF THE THORACIC CORD IN DEVELOPING OPOSSUMS MAINTAINED IN THE ADULT ANIMAL? X.M. Wang, D.M. Basso, J.C. Bresnahan, J. R. Terman and G.F. Martin*. Department of Cell Biology, Neurobiology and Anatomy, The Ohio State University, Columbus, Ohio 43210.

When the thoracic cord of the opossum, Didelphis virginiana, is transected at postnatal day (PD)5 and injections of Fast Blue (FB) are made caudal to the lesion 30-40 days later, labeled neurons are present rostral to the lesion in each of the supraspinal nuclei and spinal laminae labeled by comparable injections in unlesioned, age-matched controls. In the present study we asked whether axons which grow through the lesion during development are still present in the adult animal. The thoracic cord of 3 anesthetized pups was transected at PD5, as in the previous study, but they were maintained until adulthood before being anesthetized again and subjected to injections of FB caudal to the lesion site. After a 5-7 day survival, each animal was sacrificed and perfused so that its spinal cord and brain could be removed and sectioned for fluorescence microscopy. In all cases, neurons were labeled rostral to the lesion in each of the supraspinal and spinal areas labeled by comparable injections in non-lesioned, age-matched controls. Three additional cases lesioned at PD5 were subjected to injections of Fluoro-Ruby into either the red nucleus or the medullary reticular formation as adults. In all cases, labeled axons were present caudal to the lesion. We conclude that supraspinal and propriospinal axons which grow through lesions of the thoracic cord during early development in opossums are still present at maturity. Behavioral analyses of the animals used for this study suggested that use of the hindlimbs in locomotion was comparable to that in non-lesioned, age-matched controls. (Supported by NS-25095 and 10165).

591.15

OPOSSUMS DEVELOP SOME STEPPING AFTER SPINAL CORD TRANSECTION (TX) ON POSTNATAL DAY (PD) 8 BUT DEVELOP NORMAL LOCOMOTION AFTER TX ON PD5. D.M. Basso*, J.R. Terman, X.M. Wang, G.F. Martin, J.C. Bresnahan. Dept. Cell Biology, Neurobiology and Anatomy. The Ohio State University, Columbus, OH 43210.

Spinal cord (SC) TX made in PD5 opossums (*Didelphis virginiana*) results in reconstitution of the cord and growth of supraspinal axons through the lesion (Wang et al '96). Growth through the lesion is reduced when SC TX is made later in development. The motor efficacy of these regenerated and/or late growing axons is currently unknown. Thus, we tested the motor behavior of adult opossums with varying degrees of axonal growth through SC TX as compared to adults with TX.

Opossums had complete mid-thoracic TX as neonates on PD5 (n=6), 8 (n=7), 12 (n=2), 19-40 (n=10) or as adults (6 mo; n=3). Age-matched, unlesioned animals served as controls (n=3). All animals were tested at 6 mos. for open field loco-motion using the Basso, Beattie, Bresnahan Rating Scale, tree climbing, stair descent, and reflexes. All opossums lesioned as neonates were able to support their weight or step with the hindlimbs, whereas those with TX as adults demonstrated no locomotor recovery. Locomotion in the PD5 group (gp) was nearly normal (x±sd: 20± 0.84), not significantly different from controls (21 ± 0; $p>0.05$) and notably better than in those lesioned at PD8-40 (11±0.79; $p<0.001$). Retrograde labeling from below the TX in PD5 and 8 animals showed a decrease in number but not distribution of labeled neurons in the brainstem, indicating that the same brainstem nuclei supported axonal growth in both gps. This suggests that the development of normal locomotion after TX may not be due solely to plasticity of supraspinal systems. Enhanced locomotor performance for the PD5 vs 8 gps may be due to plasticity of ascending and/or propriospinal systems. (Supp. by NS10165, 25095 and APA BB2-9302).

591.16

COMPARISON OF CONDUCTION IN CHRONIC AND ACUTE CHEMICALLY-INDUCED DEMYELINATED SPINAL CORD AXONS. O. Honmou[1], K. Hashi[1], I. D. Duncan[2], and J. D. Kocsis[3]. [1]Dept. of Neurosurgery, Sapporo Med. Sch., Sapporo, Japan. 064, [2]Dept. of Medical Sciences, Sch. of Veterinary Med. Univ. Wisconsin, Madison, WI. 53706-1102. [3]Dept. of Neurology, Yale Med. Sch., New Haven, CT. 06510; and VAMC, West Haven, CT. 06516

The conduction properties of the chronically-demyelinated axons of *taiep* rats, acute chemically-induced demyelinated axon (ethidium bromide with X-irradiation; EB-X), and normal Wistar (control) rats were studied in *in vitro*. The *taiep* rat has a new inherited disorder where myelination is attempted, but there is progressive demyelination. Myelin is present at two months of age, but the myelin is lost by about 12 months. The oligodendrocytes in the *taiep* rat are characterized by accumulation of microtubles in their cytoplasm; it has been proposed that this microtuble defect in oligodendroctyes results in disruption of normal myelination. In contrast to the chronic demyelination mutant model, the EB-X model is used as an acute chemically-induced focal demyelination model which has a relatively punctate glial-free region of spinal white matter with demyelinated axons lasting for several weeks. The conduction velocity of axons in both the *taiep* and EB-X dorsal column axons was reduced. Although the *taiep* rat axons display refractory periods similar to that of control rats, the ability to sustain high frequency discharges was reduced. In contrast, axons from the EB-X rat had longer refractory periods and had a reduced ability to follow high frequency stimulation. Pharmacological and morphological results suggest differences in ion channel organization on the axonal membranes and differences in the extra-axonal environment in the *taiep* and EB-X rat spinal cords. These results suggest that unlike acutely demyelinated axons which display marked frequency-dependent conduction block, chronically demyelinated axons of the *taiep* rat spinal cord develop compensatory mechanisms to stabilize action potential conduction. Supported by NIH and the NMSS.

591.17

REGENERATION AND PROLIFERATION OF HIPPOCAMPAL NEURONS IN CULTURE, G.J. Brewer,* and M.S. Evans. Southern Illinois Univ. Sch. Med., Springfield, IL 62794.

Adult mammalian CNS neurons are thought to be terminally differentiated and postmitotic. We have recently developed techniques for isolation of neurons from any age adult rat hippocampus (Soc. Neurosci. Abs. 21:673.8). A density gradient is used to isolate spherical remnants of neurons in high yield from debris and inhibitory oligodendrocytes. Axons and dendrites regenerate in the majority of cells over several days in serum-free NeurobasalA/B27 medium. Neuronal identity is inferred from electrophysiology, immunoreactivity for neurofilament, MAP2, tau, neuron-specific enolase, and synaptic like vesicles by EM. Nestin is an intermediate filament protein known to be expressed in neuroepithelial stem cells (Lendahl etal.,Cell 60:585). Nestin immunoreactivity is detected in isolated embryonic hippocampal neurons which declines during several days of differentiation in culture. In contrast, isolated adult neurons are initially nestin-negative. During the course of regeneration in culture, adult neurons increase nestin immunoreactivity for a few days and then lose it. With 5 ng/ml FGF2, a fraction of these neuron-like cells begin to proliferate after 3 days in culture. Proliferation occurs in cells with short processes as judged by incorporation of BrdU, time lapse photography and total cell numbers. These studies suggest that adult CNS neurons retain large regenerative and proliferative potential, and that the brain environment is growth restrictive. (Alzheimer Association and NIA Pilot)

591.18

CULTURE OF VIABLE NEURONS FROM POSTMORTEM ADULT RATS, DQ McManus* and GJ Brewer. Southern Illinois Univ. Sch. of Med., Springfield, IL 62794

Live neurons from postmortem patients may help to better understand age related neurologic disease. To explore this possibility, adult rat brain tissue was dissected from in situ frontal cortex at 0, 1 and 2 hours after death. After anesthesia and decapitation, the skull was resected to expose and remove frontal cortex. Between time points the skull was replaced over the brain. The decapitated head with remaining brain was kept humidified in a covered container exposed to ambient air at approximately 21 °C. At the next time point the skull was lifted off, the frontal cortex sample was removed, and the head with remaining brain tissue was manipulated as described above. The brain tissue was processed for each time point as previously described using serum-free medium B27/Neurobasal, GJ Brewer, Soc. Neurosci. Abs. 21:673.8 (1995). Successful neuronal cultures were obtained at all three time points. Total isolated viable cells declined only 30% from freshly isolated (7,900 cells/mg tissue) to 2 hours postmortem (5,600 cells/mg tissue). After 5 days in culture, the yield of neurons dropped from 65% to 25% of total live cells isolated from 2 hours postmortem brain tissue. Cells with neuron-like morphologies by phase contrast were recognized by antibodies to neurofilament, tau, and MAP2. Selective GFAP staining was observed for other astrocytic cell types, but not for neuronal cell types. Neurons cultured from 1 and 2 hours postmortem brain appeared similar to freshly isolated neurons.
(Alzheimer's Association and Mr. & Mrs. Malan's research gift)

591.19

FUNCTIONAL CHARACTERIZATION OF ADULT RAT HIPPOCAMPAL NEURONS IN CULTURE, M. S. Evans* and G. J. Brewer. Southern Illinois Univ. Sch. of Med., Springfield, IL 62794.

We have recently developed methods for culturing neurons from adult rat hippocampus. In order to determine whether adult cultured neurons have normal electrical properties we have studied cultures from rats of three age groups: 1) embryonic, 2) 10 months, and 3) 35 months old. Cells were selected for study by their appearance in phase contrast optics. Neurons had a polarized morphology, usually with a large branching apical dendrite and small basal dendrites. In whole-cell recording the mean resting potentials were almost identical in the three age groups. All neurons had nonlinear current-voltage relationships, indicating the presence of voltage-sensitive ion channels. Most neurons had a strong voltage-sensitive inward current followed by voltage-sensitive outward currents, and fired a single action potential. Tetrodotoxin blocked the voltage-sensitive inward current. Cells with a glial morphology had linear current-voltage curves and did not fire action potentials. Our results indicate that adult cultured neurons have a clearly neuronal electrophysiological phenotype.

NINDS K08-NS01503

591.20

AN *IN VIVO* MICRODIALYSIS STUDY OF THE CORRELATION BETWEEN THE REGENERATION OF 5-HT FIBERS, THE RELEASE OF 5-HT, AND LOCOMOTION.
C. GERIN*, A.PRIVAT, INSERM U-336, C.C.106, Univ. Montpellier II, Pl. E. Bataillon, 34095 Montpellier Cedex05, France.
The release of 5-HT from raphe-spinal cord terminals in the ventral horn is correlated to locomotor activity (Gerin *et al.*, 1994; Gerin *et al.*, 1995).
In order to appreciate the functional parameters of regenerating serotonergic fibers, a microdialysis probe was stereotaxically implanted ipsilaterally in the ventral horn of hemilesioned rats. The concentration of 5-HT in the ventral horn was analyzed with an HPLC-ED system. The animals were submitted to motor test performances (inclined grasp, beam walk, and treadmill run). The microdialysis test session was divided into three parts: rest (90 min), treadmill run (60 min), post-exercise (90 min). Microdialysate samples were collected at fifteen minute intervals. The animals were sacrificed 40 days after permanent implantation of the probe and the spinal cord was sectioned for histological analyses (location of the probe, and extension of the lesion) and for immunocytochemical detection of the 5-HT and GFAP.
Motor performances and the time course of recovery were both correlated with the extension of the lesion. The locomotor scores are correlated to the concentration of 5-HT measured at rest on days 8, 16-20, 32-36. Thus, after a complete recovery of the locomotor function, 5-HT concentrations tend to be similar to those measured in control animals
Supported by IRME and AFM.

591.21

SKIN BLISTER METHOD TO STUDY REINNERVATION OF HUMAN EPIDERMIS
W.R. Kennedy, M. Nolano, C.K. Knox*, G. Wendelschafer-Crabb
University of MN, Minneapolis, MN 55455 and Minnesota Datametrics Corporation, St. Paul, MN 55126

Skin blisters of 2-3 mm are made on forearm skin by application of negative pressure. The blister roof is removed and stained immunohistochemically for the neuronal marker protein gene product 9.5 and other markers of epidermis and nerves. The roof, which contains keratinocytes and epidermal nerves, separates from the underlying dermis at the dermal-epidermal basement membrane. Nerves are counted by computer analysis of confocal images. Immunohistochemical staining of skin biopsies from the blister site and adjacent skin shows that the regenerated epidermis exceeds normal thickness by 4 days. Regenerating nerves with growth cones begin to enter the new epidermis on day 4. Nerves in skin surrounding the blister lean toward the blister and develop branches with growth cones. Some nerves entered the upper epidermal cell layers by day 9. Quantitation of regenerated nerves is possible from a second blister or from biopsy sections. The method will be useful for diagnosis or staging of polyneuropathy or local nerve injury, studying the effect of substances on nerve regeneration in a rapidly healing superficial wound and judging potential response to therapeutic trials.
Supported by NS 31397, Toray Industries, MN Medical Foundation

592.1

GDNF TRIGGERS THE OUTGROWTH OF FETAL VENTRO-MESENCEPHALIC GRAFTS FROM NIGRA TO STRIATUM IN THE 6-OHDA LESIONED RATS. Y. Wang*, L. T. Tien and B. J. Hoffer. Dept. of Pharmacology, National Defense Medical Center, Taipei, Taiwan and Dept. Pharmacology, Univ. Colorado Health Sciences Center, Denver, CO. 80262

Previous reports have indicated that grafting of fetal ventro-mesencephalic (VM) tissue to the nigra region of unilaterally 6-OHDA-lesioned animals, in conjunction with kainate injection between nigra and striatum, restores nigrostriatal tyrosine hydroxylase immunoreactivity. Glial cell line derived growth factor (GDNF), a potent trophic factor for dopaminergic (DA) neurons, has been found to be upregulated by kainate. In this experiment, we investigated the bridging effect of GDNF injection on intra-nigral transplants. Adult Sprague-Dawley rats were anesthetized and unilaterally injected with 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. The completeness of lesions was tested by measuring methamphetamine -induced rotations. One to two months after 6-OHDA administration, fetal VM tissues were grafted in the lesioned nigral area followed by injection of GDNF 100 µg, along a tract from nigra to striatum. Animals receiving transplantation and GDNF injection showed a significant decrease in rotation one to three month after grafting. Immunocytochemical studies indicated that tyrosine hydroxylase -positive neurons and fibers were found in the nigra and striatum, respectively, after grafting. No effects of similarly injected BDNF were seen. These results indicate that fetal nigral transplantation and GDNF injection may restore the nigrostriatal DA pathway in the Parkinsonian animals and supports the trophic activity of GDNF on midbrain DA neurons. This research is supported by grants from the National Science Council of R.O.C and USPHS of U.S.A.

592.2

GRAFT-DERIVED DOPAMINERGIC INNERVATION OF STRIATAL INTERNEURONS. B. Rajakumar, A.W. Hrycyshyn*, N. Rajakumar and B.A. Flumerfelt. Departments of Anatomy & Cell Biology and Psychiatry, University of Western Ontario, London, Ontario, Canada, N6A 5C1

Intrastriatal grafting of embryonic dopaminergic (DA) cells is widely employed as an alternative treatment in Parkinson's disease. It is believed that functional restoration is achieved partially through reinnervation of the host by the grafted DA neurons. In this study, it was hypothesized that the graft-derived DA neurons re-establish normal synaptic contacts with somatostatin-, parvalbumin-, calretinin- and acetylcholine-containing interneurons of the host striatum. Unilateral substantia nigra lesions were made by stereotaxic injection of 6-hydroxydopamine in adult rats. The extent of the lesions was assessed using rotational behaviour following amphetamine injection. Suspensions of ventral mesencephalic cells from 14-15 day old embryos were stereotaxically grafted into the DA depleted striatum. Three months postgrafting, animals with rotational recovery were perfusion-fixed with a solution containing 4% paraformaldehyde, 0.08% glutaraldehyde and 15% picric acid. Vibratome sections were double-immunolabeled using cobalt-intensified DAB and DAB as the chromogens for interneurons and DA terminals respectively. Light microscopic observations revealed close apposition between DA fibres and striatal interneurons. Ultrastructural examination revealed numerous appositions between graft-derived DA terminals and dendrites and somata of all four types of interneurons. Moreover, graft-derived DA terminals made synaptic contact with dendrites of cholinergic interneurons similar to that seen in normal animals. [Supported by MRC Canada].

592.3

TIME-COURSE AND SPECIFICITY OF EFFERENT GROWTH FROM NEURAL GRAFTS PLACED INTO THE NEONATAL STRIATUM. K. Wictorin*, M. Olsson, M. A. Gates, K. Bjerregaard, K. Campbell and A. Björklund. Wallenberg Neuroscience Center, Lund, Sweden.

When embryonic striatal tissue is implanted into the lesioned *adult* rat striatum, efferent projections are formed into the adjacent globus pallidus (GP), but not much further into the host. It is well known from other transplantation models that the *neonatal* brain serves as a permissive host environment for fibre growth from implanted cells. In the present study we implanted embryonic (E 13.5-14) tissue derived from the mouse lateral ganglionic eminence (LGE) into the postnatal day (P) 3 rat striatum, and used a mouse specific antiserum (M6) to study the time-course of the efferent growth. At 5 days post-grafting efferent fibres had grown through the GP and into the entopeduncular nucleus. By 7 days the outgrowth had reached into the most rostral aspect of the substantia nigra (SN), and by 11 days this structure was densely innervated. In separate animals this outgrowth was compared to that occurring from grafts of tissue from either the cerebral cortex (CC) or rhombencephalic lip (RL) placed into the P1 striatum. Whereas the LGE implants specifically innervated above all the GP and SN, the CC tissue extended dense projections into certain thalamic nuclei and also grew further into the brainstem. The RL grafts did not provide any significant outgrowth away from the striatal implant site.

This study demonstrates the permissive nature of the neonatal brain for outgrowth from embryonic implants also in the striatal grafting model. The outgrowth follows a distinct time-course which is compared to that of normal striatal development. The efferent growth is highly specific and dependent on the type of tissue that is implanted.

Supported by the Swedish MRC, the Kock and Wiberg Foundations.

592.4

HETEROGENEOUS INTEGRATION OF GLIA FROM PHYLOGENETICALLY DIVERSE REGIONS OF THE MURINE EMBRYO AFTER TRANSPLANTATION TO THE NEONATAL RAT. Monte A. Gates*, Martin Olsson, Kristoffer Bjerregaard and Anders Björklund Department of Medical Cell Research, Univ. of Lund, Sweden

Glial cells support neuronal migration and growth during central nervous system (CNS) development. Recent studies show that this support can be regionally specified, so that glia from particular areas of the developing CNS best promote the migration and/or growth of specific groups of neurons. The idea, then, that glia from various regions of the developing CNS are heterogeneous seems possible.

In the present study, dissociated cells from the embryonic day 14 (E14) mouse cerebral cortex (CC), ventral mesencephalon (VM), or rhomb-encephalic lip (RL) were transplanted to a site between the substantia nigra (SN) and neostriatum of postnatal day 1 (P1) rats, and the integration of donor glia revealed via immunocytochemistry using the murine- specific, glial antibody M2. While the distribution of glia from the donor VM was restricted to the internal capsule, globus pallidus and SN of the host, glia from CC and RL regions were additionally distributed throughout much of the adjacent thalamus. Such heterogeneous integration reflects a variability in the potential for glial cells to integrate across brain regions and suggest a regional diversity in the responses of glia to the milieu(s) of the CNS. Beyond an interesting developmental phenomenon, the potential for glia to integrate across CNS regions seems relevant to current cell replacement paradigms where the integration of donor glia may affect the migration and growth of their neuronal counterparts. Currently, we are grafting similar donor cells into other loci of P1 rats to determine the potential for additional heterogeneous responses by phylogenetically diverse glia. *Supported by the Swedish MRC*

592.5

A RAT MODEL OF L-DOPA-INDUCED DYSKINESIA: NEUROCHEMICAL CHANGES IN THE BASAL GANGLIA AND EFFECTS OF INTRASTRIATAL DOPAMINE-RICH TRANSPLANTS. M.A. Cenci, C.S. Lee and A. Björklund*. Wallenberg Neuroscience Centrum, Sölvegatan 17, 223 62 Lund, Sweden.

The development of dyskinesia is one of the major drawbacks of long-term L-DOPA treatment in Parkinson's disease, and the mechanisms underlying this adverse side-effect are largely unknown. We have developed a rat model of L-DOPA-induced dyskinesia which is suitable for studies aimed at elucidating the pathophysiology of this phenomenon, or at testing possible remedies to it.

Rats sustaining unilateral injections of 6-hydroxydopamine (6-OHDA) in the mesostriatal dopamine pathway received daily injections of L-DOPA (8 mg/kg L-DOPA plus 15 mg/kg benserazide i.p.) for 21 days. During this period, part of the rats gradually developed abnormal involuntary movements, which appeared 20-120 min after each L-DOPA dose, and were classified in four groups (orolingual movements, limb dyskinesia, axial dystonia, hyperlocomotion), as well as quantified daily in a scale from 0 to 4. An in situ hybridization analysis of neurotransmitter-related mRNA expression in the basal ganglia of L-DOPA-treated rats revealed that levels of preproenkephalin (PPE) mRNA, glutamic acid decarboxylase (GAD67) mRNA, and prodynorphin (PDyn) mRNA in the dopamine-denervated caudate-putamen, as well as GAD67 mRNA expression in the globus pallidus ipsilateral to the 6-OHDA lesion, were positively correlated with the rats' cumulative dyskinesia scores. However, among the latter markers, only striatal PDyn mRNA expression was clearly treatment-dependent (3 times higher in the L-DOPA treated group than in non-treated lesion-only controls).

Intrastriatal transplants of fetal ventral mesencephalic tissue dramatically reduced the severity of L-DOPA-induced dyskinesia and abolished the associated neurochemical changes (i.e. hyperexpression of mRNAs encoding for PPE, PDyn and GAD67). In addition, the transplants prevented the development of abnormal involuntary movements upon increasing L-DOPA doses in non dyskinesia-prone rats.

supp. by Tore Nilsson and Åke Wiberg Foundations

592.6

NIGRAL MICROTRANSPLANTATION TO THE CAUDATE -PUTAMEN UNIT OR NUCLEUS ACCUMBENS: CORRELATION OF SENSORIMOTOR BEHAVIORS AND DOPAMINE CONTENT AS MEASURED BY IN VIVO VOLTAMETRY. G.Falkenstein°, C.Rosenthal°, T.Reum^, A.Marburger^, M.Samii°, R.Morgenstern^, G.Nikkhah°* / ° Neurosurgical Clinic, Nordstadt Hospital, Haltenhoffstr. 41, D-30167 Hannover, ^ Institute of Pharmacology and Toxicology, Medical Faculty (Charité), Humboldt-University at Berlin, D-10098 Berlin

The aim of the study was to further elucidate mechanisms of graft induced behavioral recovery. We approached this issue in a comparative study including a) a wide spectrum of tests for complex sensorimotor behavior and b) electrochemical measurements for graft derived reconstitution of striatal dopamine levels.

Three groups of unilaterally 6-OHDA lesioned rats received the same total amount (1,5 µl) of cell-suspension (200,000 cells/µl) derived from E14 ventral mesencephalon. In two groups the grafts were implanted into the caudate-putamen unit (CPU), distributed either over two deposits in one tract or over six deposits in three tracts. The third group received two deposits in one tract into the nucleus accumbens (NAc). The comparative study assessed spontaneous (such as disengage behavior, skilled forelimb use, side-stepping and -falling) and drug induced rotational behavior. Preliminary behavioral results indicate good graft survival, e.g. amphetamine-induced rotation was completely compensated two weeks after the transplantation as seen with intrastriatal grafts. In addition, intrastriatal grafts lead to a partial recovery in stepping behavior as well as in skilled forelimb use, which was not observed in the NAc-grafted group. Studies on long-term behavioral effects and in vivo voltametric measurements of dopamine levels are in progress and will be reported at the meeting.

(Supported by non-commercial grant.)

592.7

ALTHOUGH ADRENAL MEDULLA GRAFTS PLACED INTO THE LATERAL VENTRICLE, GROW BETTER THAN THOSE PLACED INSIDE THE DOPAMINE DENERVATED STRIATUM, THEIR FUNCTIONAL CONSEQUENCES OVER THE MOTOR DISTURBANCES ARE EQUIVALENT. V. Anaya-Martínez, E. Montiel-Flores, J. Espinoza-Villanueva, M. R. Avila-Costa, N. Manzano-León, L. Colín-Barenque and F. García-Hernández*. Neurosciences. ENEP Iztacala. UNAM. Ap. 314.Tlalnepantla, México. 54090.

When grafted into the dopamine denervated striatum, adrenal medullary tissue exhibits poor survival rates, which may limit its capability for reducing motor disturbances. In this study we correlated the reduction of motor deficits with the grafting site and graft survival. Male Wistar rats, exhibiting apomorphine (0.25 mg/Kg) induced rotation (more than 200 turns in 30 min), after the unilateral injection of 6-OHDA into the medial forebrain bundle, were grafted with fetal adrenal medullary tissue as follows: Group 1 (n=9): received 1 µl placed into the ipsilateral lateral ventricle, Group 2 (n=8): received 1 µl placed inside the ipsilateral striatal parenchyma and Group 3 (n=9): received 5 µl also inside the ipsilateral striatal parenchyma. Apomorphine induced rotation was tested again 15, 30, 45 and 60 days after grafting, term after which all rats were sacrificed for histological analysis of tyrosine hydroxylase (TH) immunostained serial sections of the striatum where the transplant was found. With the aid of a computer assisted system, the volume of the prevailing transplanted tissue was established as well as the number and size of the TH immunoreactive cells within the graft. Our results show that although ventricular grafts exhibited a modest loss of the original graft volume and abundant and bigger TH immunoreactive cells, the reduction of apomorphine induced rotation was the same as in intrastriatal grafts, who had only a few and small TH immunoreactive cells, with high loss of the original transplant volume.

SUPPORTED BY PAPIIT IN-203192 and IN-205694, DGAPA. UNAM.

592.8

TRANSPLANTATION OF FRESH CELL SUSPENSIONS VERSUS CULTURED TISSUE OF FETAL RAT MESENCEPHALON TO 6-HYDROXYDOPAMINE LESIONED RATS. M. Meyer, H.R. Widmer, L. Evtouchenko, R. Guzman, R.W. Seiler, J. Zimmer[2] and C. Spenger*. Dept. of Neurosurgery, University of Bern, Bern, Switzerland. [2]Dept. of Anatomy and Cell Biology, University of Odense, Odense, Denmark.

Adult Sprague-Dawley rats received unilateral injections of 6-hydroxydopamine into the nigrostriatal bundle, and the completeness of the lesions was assessed by D-amphetamine-induced rotations. Rotating animals were randomly divided into a control group (sham operation), a group intrastriatally grafted with fresh cell suspension from fetal (E13) ventral mesencephalon and a group intrastriatally grafted with 7 days old free floating roller tube (FFRT) cultures derived from fetal (E13) ventral mesencephalon. Post-operative evolution in rotational behaviour was evaluated at several time points post-transplantation (p.t.), and long-term survival of tyrosine hydroxylase-immunoreactive (TH-ir) neurons was assessed in serial sections of the grafted brains (12 weeks p.t.).

Three, 5 and 9 weeks post-grafting rats grafted with fresh cell suspensions (N=9) showed an average reduction in D-amphetamine-induced rotations of 50%, 101%*** and 121%*** and rats grafted with cultured tissue (N=9) an average reduction of 73%**, 103%*** and 104%***, respectively (**=p<0.01;***=p<0.001). Sham-operated animals displayed no significant changes in rotational behavior in the same period. Histological examination of the grafts revealed similar numbers of TH-ir neurons in suspension grafts (mean ± SEM = 756 ± 99, N=9) compared to tissue culture grafts (mean ± SEM = 728 ± 92, N=9).

In conclusion, the similar extent to which culture grafts survive and are effective as compared to fresh suspension grafts illustrates the feasibility of effective in vitro maintenance of fetal nigral tissue prior to transplantation.

SNF: 31-45558.95; BBW: 93.0349 and the Swiss Parkinson's Association.

592.9

INTRASTRIATAL INJECTION OF ADENOVIRUS EXPRESSING TYROSINE HYDROXYLASE ATTENUATES ROTATIONAL BEHAVIOR IN 6-OHDA RATS BY PREVENTING AGONIST-INDUCED SENSITIZATION. S. Gancher*, L. Muldoon, M. Pagel, and B. Davidson. Department of Neurology, Oregon Health Sciences University, Portland Oregon 97201.

In rats with unilateral 6-OHDA lesions, the intracerebral inoculation of recombinant viruses containing the gene for tyrosine hydroxylase (TH) has been shown to attenuate drug-induced rotational behavior. After 6-OHDA lesioning, rats were treated with six daily injections of apomorphine (0.1 mg/kg), resulting in a significant increase in contralateral rotations which reflected behavioral sensitization. After intrastriatal inoculation of recombinant adenovirus containing the gene for human TH (1µl, 10^9 particles/µl) and a three week recovery, the response to apomorphine fell to levels first observed with initial apomorphine exposure and remained unchanged over six daily apomorphine injections. A group of rats were similarly treated with apomorphine, received intrastriatal injections of vehicle, and allowed to recover for six weeks prior to repeated administration of apomorphine. These rats also exhibited a response which was initially reduced, but which then did increase following subsequent daily apomorphine treatments. These results suggest that a) sensitization to apomorphine in 6-OHDA rats is extinguished over several weeks without treatment, and b) replacement of TH into the striatum results in an attenuated response to apomorphine by interfering with the development of sensitization. These finding suggest that in this model, sensitization to agonist-induced rotational behavior depends on the extent of dopamine depletion. (Supported by NINDS KO81539 and VA Merit Review).

592.10

EFFECTS OF GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR ON DOPAMINERGIC TRANSPLANTS TO THE 6-OHDA DENERVATED STRIATUM J.L. Mott[1], S. Eken[2], K. Bowenkamp[2], G.A. Gerhardt[2,3,5], S. Henry[1], B.J. Hoffer[2,3,5], C. van Horne[4], and A-Ch. Granholm*[1,2,5]. Depts. of Basic Science[1], Pharmacology[2], and Psychiatry[3], Neurosci Program[5], Univ of Colo HSC, Denver, CO 80262; Brigham & Women's Hospital[4], Harvard Med School, Boston, MA, 02115.

In Parkinson's disease, dopaminergic cells of the substantia nigra degenerate, disrupting the nigrostriatal pathway. One approach to replace these lost neurons is to transplant mesencephalic tissue into the striatum. In an attempt to improve survival of transplanted dopaminergic neurons, we have pretreated the graft with the dopaminotrophic factor glial cell line-derived neurotrophic factor (GDNF). Mesencephalic grafts from fetal Fischer 344 rats (E14-E15) were pre-incubated in GDNF (1 µg/µL) or the control protein, cytochrome c (1 µg/µL), and transplanted into the striatum of adult hosts that had been previously lesioned by receiving medial forebrain bundle injections of 6-hydroxydopamine. Host rats received intrastriatal injections of GDNF or cytochrome c (10 µg each) adjacent to the graft at ten days and four weeks postgrafting. The animals were tested for apomorphine-induced rotation behavior, and were perfused for immunohistochemical analysis of transplant morphology at six weeks postgrafting. GDNF and cytochrome c treated groups both showed significant motor improvement. Morphological evaluation revealed that the GDNF-treated grafts were larger than control grafts. Additionally, tyrosine hydroxylase (TH) immunohistochemistry and image analysis showed a significantly higher staining density in the transplanted area, indicating better innervation, and further analysis revealed significantly larger TH-positive cells in the GDNF transplants than in controls. This experiment supports the positive effects of GDNF on dopaminergic cells, and suggests that transplantation in conjunction with neurotrophin application could be a successful treatment strategy. Supported by USPHS grants MH49661 and NS09199.

592.11

CHARACTERIZATION OF IN VIVO SURVIVAL OF EMBRYONIC DOPAMINERGIC CELLS AFTER SELECTIVE SORTING USING A FLUORESCENT NEUROTENSIN DERIVATIVE. A.F. Sadikot*, M. Alonso-Vanegas, M.P. Faure, K. McDonald and A. Beaudet. Montreal Neurol. Inst., Univ. McGill, 3801 University St., Montreal, QC, Canada H3A 2B4.

Current methods for transplantation of embryonic midbrain result in delivery of a heterogeneous population of cells containing dopaminergic neurons, gabaergic neurons, glia, and mesenchymal elements. The fact that dopaminergic neurons of the midbrain are selectivey endowed with high affinity receptors for neurotensin (NT) (Szigethy et al, '89), has allowed us to selectively tag embryonic DA cells with fluo-NT and sort these neurons from other cell types by flow cytometry (Faure et al, '94;'96). Rat mesencephalic cells were harvested at embryonic day 15, mechanically dissociated, cultured for 7 days, and then labelled with fluo-NT. DA cells were then separated from other cell types using a fluoresence activated cell sorter (FACS). 5 µl of a solution containing 10, 000 cells/µl of Hanks balanced salt solution was then stereotactically transplanted into the dopamine-denervated adult rat striatum. In a control group, comparable numbers of unsorted embryonic cultured cells were transplanted into the dopamine-denervated adult rat striatum. At one month after transplantation, animals were perfused with aldehydes and processed for immunohistochemistry for tyrosine hydroxylase as a marker for dopaminergic cells. Sorted cells were noted to survive and grow processes resulting in partial reinnervation of the denervated striatum. By comparison, unsorted DA cells showed more exuberant cell processes and greater density of striatal reinnervation. Our results suggest that cell sorting of embryonic DA cells is feasible, and DA cells survive the process of fluo-NT labeling and FACS separation in a condition that allows for subsequent cell survival *in vivo*. Support: Parkinson Foundation and Medical Research Council of Canada.

592.12

TIRILAZAD MESYLATE INCREASES SURVIVAL OF EMBRYONIC DOPAMINERGIC NEURONS IN VITRO. A. Othberg, M. Keep*, P. Brundin[1] and O. Lindvall.
Section of Restorative Neurology, Department of Clinical Neuroscience,
[1]Section of Neuronal Survival, Department of Physiology and Neuroscience,
Wallenberg Neuroscience Center, S-221 85 Lund, Sweden.

The survival of embryonic dopaminergic neurons of rat or human origin after grafting to the striatum is low (5-20%). Transplantation of tissue from at least 3-4 embryos per side is therefore needed to obtain therapeutic improvement in a patient with Parkinson's disease. For the further development of neural transplantation into a clinically useful treatment for a larger number of patients, the survival of grafted dopamine neurons has to be increased. Lazaroids are compounds which have been shown to improve survival of rat dopamine neurons in cell culture and after grafting to the rat brain (Nakao et al., Proc. Natl. Acad. Sci. USA. 1994, 91:12408-12), probably by inhibiting lipid peroxidation. The only lazaroid approved for human use is tirilazad mesylate (Freedox, Pharmacia-Upjohn). To determine if tirilazad has the utility shown for other lazaroids, it was used here in cultures of rat ventral mesencephalon (VM), and in rat and human VM suspensions,which were treated the same way as in clinical transplantations. After 7 days in culture, we observed a 2.4-fold higher number of surviving tyrosine hydroxylase-positive, presumed dopaminergic, cells with 0.3 µM tirilazad as compared to control. The viability studies showed significantly faster decline of the number of surviving neurons in control compared to tirilazad over time up to 30 hrs. These results show that tirilazad has neuroprotective effects on embryonic VM neurons similar to those of other lazaroids. Tirilazad should therefore be useful to improve graft survival in clinical transplantations, thereby reducing the amount of embryonic tissue required to induce significant symptomatic relief in patients with Parkinson's disease. Supported by the Swedish Medical Research Council.

592.13

THE IMPORTANCE OF DISSECTION MEDIUM AND LAZAROIDS FOR CELL SURVIVAL AT GRAFTING I. Strömberg*, L. Björklund, L. Evtouchenko, and C. Spenger. Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden, Dept. of Neurosurg. Univ. Hosp. of Bern, Switzerland

The trials of grafting catecholamine tissue to patients suffering from Parkinson's disease have reached the point that increased cell survival is needed to enhance graft efficacy. Animal experiments have been focused on trophic factors in this issue, but another approach to yield better survival might be to remove some of the toxic mechanisms which occurs during the dissection and transplantation procedures. In this study we have examined the effect of lazaroid U-74006F, which may inhibit oxidative stress by reducing free radical formation. The intraocular grafting model has been used where fetal ventral mesencephalon has been implanted to the anterior chamber of the eye. During dissection either Hanks' balanced salt solution (HBSS) or Dulbecco's modified Eagle medium (DMEM) has been used and a preincubation in U-74006F at 0.3 μM for 15 min prior to transplantation was performed. Weekly injections of U-74006F was performed for two weeks and cell counts of tyrosine hydroxylase (TH) - positive neurons were performed at 3 weeks. Control grafts were treated with either HBSS or DMEM. TH-immunohistochemistry revealed that grafts dissected and treated with DMEM possessed twice as many neurons as that found in HBSS treated grafts. Injections of 5 μl of 3 μM U-74006F diluted in DMEM revealed insignificant increase in TH-positive neurons. A 10-fold increase in the doses of U-74006F significantly increased the neurons from 333±56 cells in DMEM treated controls to 812±188 TH-positive neurons after lazaroid treatment. In conclusion, both the selection of dissection medium and the treatment with lazaroids seems to be important to enhance the cell survival.

592.14

SIMULTANEOUSLY COGRAFTED STRIATAL TARGET TISSUE ENHANCES NEURITE OUTGROWTH AND NEURON VIABILITY OF GRAFTED MESENCEPHALIC DOPAMINE NEURONS C.E. Sortwell[1]*, T.J. Collier[2], B.C. Blanchard[1] and J.R. Sladek, Jr.[1] [1]Department of Neuroscience, Chicago Medical School, North Chicago, IL 60064 and [2]Department of Neurological Science, Rush Presbyterian Medical Center, Chicago, IL 60612.

Trophic factors derived from target striatum are crucial for nigrostriatal pathway formation and maintenance. Evidence collected in vitro demonstrates that striatal cells may be capable of exerting both a trophic effect on dopamine (DA) neuron survival and a tropic effect on neurite extension. However, previous in vivo studies have demonstrated only a tropic effect of cografted striatum on grafted DA neurons. The aim of the present study was to characterize the tropic and/or trophic effects of striatal cografts on grafted mesencephalic DA neurons. Unilaterally, 6-OHDA-lesioned male, Fischer 344, rats were grafted with either an embryonic (E15) mesencephalic cell suspension alone or with both mesencephalic and striatal cell suspensions (E15, mixed or seperate conditions) to the striatum. Lesioned rats receiving no graft served as controls. Rotational assessment was conducted following amphetamine challenge at 1 week prior, and 4 and 8 weeks following grafting. After behavioral assessment, rats were immunostained using antisera directed against tyrosine hydroxylase (TH) and analyzed histologically. Results confirm previous findings of striatal **tropic** effects where DAergic fiber outgrowth is enhanced when striatum is separately cografted and TH rich patches are observed within mixed striatal and mesencephalic cografts. This study also demonstrates for the first time that simultaneously cografted striatum can exert a **trophic** effect on grafted mesencephalic DA neuron survival. Mesencephalic cell suspension grafts that were mixed with striatal cells or separately cografted with striatal cells contained 2 and 3-fold higher cell counts of DA neurons, respectively, than solo mesencephalic grafts. Separate cografts appeared to induce enhanced behavioral recovery when compared to solo grafts. These results suggest that striatal target tissue, particularly when placed at a distance from mesencephalic grafts, can be used to augment DA neuron viability and neurite outgrowth. This ability might be important for sustained behavioral recovery from the neurodegeneration in Parkinson's disease. Supported by NIH grant F32 NS09646-01 (CES).

592.15

IN VIVO EVALUATION OF ADENOVIRAL VECTOR CONTAINING THE DOPAMINE D_2 RECEPTOR GENE IN RAT AND MOUSE STRIATA. H. Umegaki , H. Ikari , J. Chernak, J. Hengemihle, G. Roth, D. Ingram* Gerontol. Res. Ctr., Natl. Inst. Aging, NIH, Baltimore, MD 21224; Dept. Geriatric Med., Nagoya Sch. Med., Nagoya, JAPAN.

Decline in striatal dopamine D_2 receptors (D_2R) is observed in mammalian aging as well as in neurodegenerative diseases such as Huntington's disease and late-stage Parkinson's disease. To assess the feasibility of gene transfer of D_2R, we (Ikari et al., *Mol. Brain Res.* **34**:315, 1995) constructed a replication-deficient adenoviral vector containing rat D_2R cDNA (AdCMV.DopD_2R). Applying an apomorphine-induced rotational model, we assessed the function of AdCMV.DopD_2R *in vivo*. Starting 4 wk after injection of AdCMV.DopD2R into rat striatum, we observed the predicted contralateral rotational behavior. To determine how this behavior was coupled to receptor expression, we performed autoradiography with the D_2R-specific ligand, [^{125}I]iodosulpride. Densitometric evaluation showed an increase of ligand binding in vector-injected sites that peaked at day 5 and declined to basal levels by 3 weeks after injection. Thus, small, undetectable increases in D_2R may affect rotational responses or it may be delayed or dependent on factors other than D_2R levels, which we are now examining. To assist in this approach, we have injected AdCMV.DopD_2R into mouse striatum, the smaller size of which may allow a relatively greater increase over endogenous D_2R levels than in rats. We have confirmed increased ligand binding and apomorphine-induced rotational behavior in this mouse model.

592.16

CO-GRAFTS OF MUSCLE CELLS AND MESENCEPHALIC TISSUE INTO HEMIPARKINSONIAN RATS: BEHAVIORAL AND HISTOCHEMICAL EFFECTS. F.G.Kaddis*, E.D.Clarkson and C.R.Freed, Depts. of Medicine and Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Conditioned medium from muscle cell cultures (MC-CM) has been shown to increase the expression of tyrosine hydroxylase (TH) in rat mesencephalic cultures (Iacovitti et al. Molec. Brain Res. 16:215, 1992). To see if muscle cells can improve embryonic midbrain transplants in a rat model of Parkinsons disease, we have tested the effects of MC-CM on embryonic mesencephalic and striatal cultures and then co-transplanted newborn rat muscle cells along with embryonic dopamine cells into denervated rat striatum. Results showed that: (A) MC-CM increased the number of TH neurons in ED15 midbrain cultures but did not stimulate the expression of TH in ED15 striatal cultures, (B) co-transplants of muscle cells with ED15 mesencephalic cells improved striatal reinnervation and more rapidly reversed circling behavior to methamphetamine than ED15 mesencephalic tissue grafts alone.

IPSILATERAL CIRCLING TO 5 MG/KG METHAMPHETAMINE, RPM

	Pre-Grafting	*Months Post-Grafting*		
		1	2	3
Control	4.0±0.1	5.0±1.1	4.2±1.8	1.3±0.7
Muscle	5.0±0.5	2.1±0.7	0.9±0.7	0.6±0.5

We conclude that muscle cells improve the behavioral and histochemical effects of embryonic dopamine cell transplants. Supported by NINDS NS18639 and The National Parkinson Foundation

592.17

PREINCUBATION WITH GROWTH FACTORS IMPROVES SURVIVAL OF EARLY MESENCEPHALIC GRAFTS IN HEMIPARKINSONIAN RATS. D.J.Zastrow*, W.M.Zawada, E.D.Clarkson, F.Adams, P.Bell, and C.R.Freed. Div. Clin. Pharmacol., Univ. Colorado School of Medicine, Denver, CO 80262.

Poor survival of transplanted embryonic dopamine (DA) neurons in patients with Parkinson's disease limits the usefulness of this evolving therapy. We have previously shown that apoptosis is in part responsible for the loss of DA neurons *in vitro*. Programmed cell death in DA neurons can be reduced by GDNF or a combination of IGF-1 and bFGF. In an effort to improve the survival of DA neurons in grafts, we have transplanted tissue strands from E15 rat ventral mesencephalon into 6-OHDA-lesioned rats. Each freshly obtained embryonic tissue strand was prepared from 1/2 of a ventral mesencephalon and incubated for 2 hrs on ice in L-15 medium containing GDNF (100 ng/ml), IGF-I (1500 ng/ml), and bFGF (150 ng/ml) and controls in L-15 alone. Tissue strands were grafted immediately following incubation and animals were sacrificed either 1 or 7 days post-transplant. Dopamine neurons were identified by tyrosine hydroxylase immunoreactivity. Growth factor pretreatment improved DA neuron survival by 55% in grafts after 1 day *in vivo* and by 57% after 7 days *in vivo*. To further enhance DA neuron survival *in vivo* by methods aimed at preventing apoptosis, we have studied *in vitro* the effects of inhibitors of cysteine proteases and calpain (putative mediators of apoptosis). Cultures from dispersed mesencephalon were grown for 3 days in serum-enriched medium. Ac-YVAD-chloromethylketone, an inhibitor of interleukin-1β converting enzyme, at 1 μM and 10 μM improved survival of DA neurons by 24% and 31%, respectively. Calpain inhibitor I (1μM) rescued 40% of DA neurons. This inhibitor was toxic to cultures at concentrations of $\geq$10μM. Prevention of apoptotic cell death with growth factors and protease inhibitors may improve the viability of DA neurons grafted for Parkinson's disease. Supported by NS 18639, GM 07063, and the Natl. Parkinson Foundation.

592.18

INCREASED DOPAMINERGIC ACTIVITY CAUSED BY STRIATAL INJURY IS ASSOCIATED WITH AXONAL SPROUTING. G.T. Liberatore[1], D.I. Finkelstein[2], J.Y.F. Wong[1], M.K. Horne[2], G.A. Donnan[1] and D.W. Howells[1]*. [1]Departments of Medicine and Neurology, University of Melbourne, Austin and Repatriation Medical Centre (Austin Campus), Heidelberg, Victoria 3084, Australia and [2]Department of Anatomy, Monash University, Clayton, Victoria 3168, Australia.

We have previously shown that striatal injury causes activation of the nigro-striatal dopamine system. This response is characterised by stimulation of pre-synaptic dopamine activity (increased tyrosine hydroxylase activity, increased concentrations of dopamine and its metabolites and increased density of pre-synaptic dopamine uptake sites) with an accompanying down regulation of the density of the predominantly post-synaptic D_1 and D_2 dopamine receptors. This response could be due to either, increased activity within existing dopamine terminals and/or branching and sprouting of new dopamine terminals within the striatum. To investigate the latter, normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treated male C-57 black mice were anaesthetised and the left striatum lesioned using a Scouten wire knife. Counting silver stained axons and their branches demonstrated that striatal injury causes axonal sprouting one month post damage. Injection of dextran-biotin into the substantia nigra and then monitoring its transport into the striatum, shows that some of this neuronal sprouting is of nigral origin. Tyrosine hydroxylase immunohistochemistry, on alternate sections, demonstrates that a proportion of this sprouting is dopaminergic. A better understanding of these processes and their regulation may play a important role in improving treatment of Parkinsons disease.

Research is funded by the AHMRF and NH&MRC.

592.19

TRANSPLANTS OF FETAL VENTRAL MESENCEPHALIC TISSUE INTO THE MIDBRAIN ATTENUATE D1 AGONIST-INDUCED ROTATIONAL BEHAVIOR, D.M. Yurek* and J.A. Cooper. Depts. of Surgery/Neurosurgery and Anatomy & Neurobiology, Univ. of Kentucky College of Medicine, Lexington, Kentucky 40536.

The function of dopamine release within the substantia nigra pars reticulata (SNR) is not clearly understood despite the long-standing evidence that dopamine is somatodendritically released. Somatodendritic release of dopamine may serve an autoregulatory function for dopaminergic neurons and may also regulate the activity of striatonigral neurons, which are known to have receptors of the D1 subtype on nerve terminals located within the SNR. We recently demonstrated that rotational behavior elicited by SKF 82958 is effectively blocked by intranigral administration of the D1 receptor antagonist, SCH 23390, which suggests that dopamine release within the midbrain contributes to rotational behavior induced by dopaminergic drugs.

In this study young adult rats were given unilateral 6-hydroxydopamine lesions and cannula were implanted to allow for the administration of dopaminergic drugs into the SNR ipsilateral to the lesion. Rotational behavior was tested after administration of the following drugs: SKF 82958, 7.0 µg intranigral or 0.01 mg/kg (s.c.); quinpirole, 10.0 µg intranigral or 0.2 mg/kg (s.c.); and amphetamine 5.0 mg/kg (i.p.). Animals did not show significant rotational behavior to intranigral administration of quinpirole. One week after rotational testing was completed animals received midbrain transplants of fetal mesencephalic tissue; transplants were targeted for the dorsolateral aspect of the SNR. Retests for rotational behavior began 4 weeks posttransplantation. Animals receiving midbrain transplants showed significant reductions in rotational behavior after intranigral or s.c. administration of SKF 82958. Midbrain transplants did not significantly reduce rotational behavior elicited by quinpirole (intranigral or s.c.) or amphetamine. This research was supported by USPHS NS 29994.

592.20

UNILATERAL FETAL MESENCEPHALIC GRAFTS IN NON-LESIONED RATS REDUCE THE RATE OF HIGH AFFINITY DOPAMINE UPTAKE IN THE CONTRALATERAL STRIATUM: AN *IN VIVO* VOLTAMMETRIC STUDY. A. Marburger, R. Morgenstern *, D. Schönfuß, N. Drandarevski and T. Reum, Inst. Pharmacol. and Toxicol., Medical Faculty (Charité), Humboldt University at Berlin, D-10098 Berlin, Germany

Fetal mesencephalic grafts as a potential alternative treatment in Parkinsons' disease have been examined very thoroughly in the last decade. The graft-induced recovery of motor dysfunctions in the 6-OHDA model of Parkinsonism is generally related to the survival of dopaminergic cells, their expression of tyrosine hydroxylase, and the release of dopamine (DA) in the denervated striatum. However, there is growing evidence for further effects of the graft involving the contralateral side in the complex event of functional recovery. Recently we showed that fetal grafts in previously 6-OHDA lesioned rats induce a reduction of DA reuptake mechanisms in the contralateral striatum *in vivo* and *in vitro*.

In order to see whether these effects are independent of a previous lesion and whether they are occuring in the grafted striatum as well, we examined the extracellular DA elimination in non-lesioned but grafted rats with fast cyclic voltammetry. Rats were grafted with solid fetal midbrain intracerebroventricularly (i.c.v.) and with intrastriatal cell suspensions. Extracellular DA clearance was measured after electrical stimulation of the medial forebrain bundle before and after treatment with the high affinity DA uptake inhibitor GBR 12909. Rate constants for the high affinity DA uptake were calculated with the aid of a kinetic model.

In i.c.v. grafted animals, both in the ipsi- and contralateral striatum the rate of high affinity DA uptake was reduced compared to sham-grafted controls, whereas in suspension-grafted rats this reduction was only observed on the contralateral side. This shows that i.c.v. and intrastriatal DAergic grafts without preceeding lesion affect the DA uptake of the host striatum. These results are in good agreement with those observed contralaterally in 6-OHDA lesioned rats. The functional relevance of our findings is not fully understood, but it may be considered that impaired DA reuptake leads to higher concentrations of functionally 'active' DA in the synaptic cleft and that the benefit of DAergic grafts could be at least partially due to a down regulation of DA reuptake.

Supported by grant 01 ZZ 9101 (BMFT Germany)

AGING PROCESSES: TOXICITY, INFLAMMATION, AND NON-NEURONAL CELLS

593.1

EFFECTS OF CHRONIC ALCOHOL CONSUMPTION AND AGING ON PROENKEPHALIN mRNA. M.J. Druse-Manteuffel* and N.F. Tajuddin. Mol. Cell. Biochemistry Dept., Loyola U. Chicago, Stritch School of Medicine, Maywood, IL 60153

Previous studies from this laboratory demonstrated age-related changes in the dopaminergic system in both nigrostriatal and mesocorticolimbic brain areas. As an extension of our prior work, we examined the effects of aging and chronic alcohol consumption on the expression of the gene encoding proenkephalin (PE). *In situ* hybridization histochemistry was used to detect PE mRNA in the striatum, nucleus accumbens and frontal cortex. PE mRNA was detected using an ^{35}S-labeled synthetic oligonucleotide probe, corresponding to the 48 base pairs, complementary to bases 388 to 435 of PE mRNA (Yoshikawa et al., 1984; Hammer et al., 1993). PE mRNA was quantitated in 5 and 24 month male Fischer 344 rats that consumed a control or ethanol-containing liquid diet for 6 weeks.

The results of these studies demonstrated an age-related decline in PE mRNA levels in both the rostral striatum and in the nucleus accumbens. There was also an ethanol-associated decrease in PE mRNA at 5 months. No age- or ethanol-associated changes were detected in the frontal cortex.

This research was supported by a grant from the USPHS - AA08451-04.

593.2

RESPONSE OF BASAL FOREBRAIN NUCLEAR FACTOR KAPPA B TO HYPEROXIA *IN VIVO*. T. Toliver*, L. Tong, D. Rassin and J.R. Perez-Polo. Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77555-0652.

We previously reported that DNA-binding levels of nuclear factor kappa B (NFκB) are altered in the aged rat basal forebrain and hippocampus. We propose that age-associated oxidative stresses contribute to altered NFκB activity with aging. In order to test the hypothesis that NFκB responds to oxidative stress *in vivo*, we exposed rats to 100% oxygen under isobaric conditions for various amount of time. Analysis of nuclear NFκB DNA-binding activity by electrophoretic mobility shift assay and protein levels by western blot analysis demonstrates that basal forebrain levels of NFκB were unaffected following 6 hours of hyperoxia, significantly decreased following 12 and 24 hours of hyperoxia, and restored to almost normal levels following 48 hours of hyperoxia. The data suggest that NFκB in basal forebrain responds to oxidative stress, perhaps to alter gene transcription in an attempt to promote survival under oxidative stress. This is publication 59a from USPHS grant PO1 AG10514 awarded by NIA. Also supported by NINDS NS 18708.

593.3

CHANGES IN AP-1 DNA BINDING ACTIVITY IN THE RAT BRAIN DURING HYPEROXIA-INDUCED OXIDATIVE STRESS. L. Tong*, Tracy Toliver and J.R. Perez-Polo. Dept. of HBC&G, UT Med. Branch, Galveston, TX 77555-0652

Oxidative stress appears to contribute to aging-associated neurodegenerative diseases such as Alzheimer's disease. The AP-1 transcription factor is made up of a family of regulatory proteins that are activated by oxidative stress. The purpose of the present study is to examine the AP-1 DNA binding activity and its component proteins in the rat brain under oxidative stress induced by hyperoxia. Young male Fischer-344 rats were exposed to 100% oxygen under isobaric conditions for various times. After hyperoxic treatments, the brains were dissected, and nuclear protein extracts were prepared. The AP-1 DNA binding activities in different brain regions were analyzed by electrophoretic mobility shift analysis (EMSA). The participating protein species were identified by supershift and Western blotting. There were elevated AP-1 DNA binding activities in hippocampus and basal forebrain induced by hyperoxic treatment, followed by a restoration to near control levels. These results suggest that AP-1 signaling is involved in brain responses to oxidative stress. Supported in Part by NINDS-NS18708. This is publication 60A from USPHS grant P01 AG10514 awarded by NIA.

593.4

EFFECTS OF OXIDATIVE STRESS ON SYNAPTIC MEMBRANE Ca^{2+} TRANSPORTERS: IMPLICATIONS FOR BRAIN AGING. M.L. Michaelis*, J. Huschenbett, and A.. Zaidi. Dept. of Pharmacology/Toxicology & Ctr. Neurobiol./Immunol. Res., Univ. of Kansas, Lawrence, KS 66045.

The plasma membrane Na^+/Ca^{2+} exchanger and Ca^{2+}-ATPase are critical for $[Ca^{2+}]_i$ regulation, and the kinetic properties of these systems change in aged rat brain. Such age-related changes could be due to oxidative modification of Ca^{2+} transporting proteins as a result of chronic oxidative stresses and accumulation of oxidized proteins. These studies were undertaken to determine the sensitivity of these neuronal Ca^{2+} transporters to *in vitro* exposure of synaptic membranes to oxidizing conditions. We initially tested the effects of two azo-initiators, AAPH and ACVA, agents that generate peroxyl free radicals via thermal decomposition, thus permitting one to calculate the amount of free radicals produced under controlled conditions. AAPH, the more hydrophilic agent, produced 15 - 70% inhibition of exchanger activity across AAPH concentrations estimated to generate 6 to 60µM free radicals. When the lipophilic agent ACVA was tested, inhibition ranged from 30 - 95% at 6µM to 60µM concentrations. The Ca^{2+}-ATPase was even more sensitive to exposure to these free radical generating systems, showing nearly complete loss of activity with ~20:M free radicals generated by AAPH and 5:M free radicals by ACVA. These results suggest that generation of free radicals within the membrane disrupts the activity of both Ca^{2+} transporters more strongly. Studies in which a xanthine/xanthine oxidase system was used to generate superoxide revealed that the Ca^{2+}-ATPase is also very sensitive to the presence of this free radical. Ongoing studies are examining the effects of H_2O_2 and peroxynitrite on the activity of these Ca^{2+}-regulating systems. (Supported by Alz. Dis. Assoc., AG12993 , and Higuchi Biosciences Ctr., U. of KS).

593.5

REGIONAL LEVELS OF 8-OHDG, A MARKER OF OXIDATIVE DAMAGE TO DNA, IN RAT BRAIN AND THE LACK OF EFFECT OF CHRONIC L-DOPA TREATMENT. D.M. Togasaki*, M.K. Shigenaga, V. Jackson-Lewis, S. Przedborski. Dept. of Neurology, Columbia University, New York, NY, and Dept. of Molecular and Cell Biology, University of California, Berkeley, CA.

Oxidants may injure nigrostriatal neurons in Parkinson's disease by attacking cell constituents, including nucleic acids. L-Dopa might exacerbate this injury by elevating oxidative stress. 8-Hydroxydeoxyguanosine (8-OHdG) is a product of oxidative damage to deoxyguanosine (dG) residues in DNA; it can be assayed with HPLC methods. We measured 8-OHdG (normalized with the levels of dG) in five brain regions of male Sprague-Dawley rats (n=3-4/group) injected twice daily for 40 days with L-dopa methyl ester/benserazide (50 and 12.5 mg/kg in 0.9% saline, i.p.) or vehicle. Rats were sacrificed 24 hours or seven days after the last injection, and total cellular DNA from each region was isolated, digested, and analyzed. Biogenic amines and their metabolites were also measured. There are differences in the amount of 8-OHdG in various brain regions. The substantia nigra contains the largest amount (14.4±1.8 μmoles 8-OHdG/mole dG, mean±SEM), followed by the striatum (12.1±1.4), the frontal cortex (10.5±0.8), the hippocampus (9.4±0.8), and the cerebellum (8.8±0.6). The nigral level is significantly greater than the levels in cortex, hippocampus, and cerebellum. L-Dopa treatment has no effect, nor does the longer washout time. The level of 8-OHdG shows a positive correlation with the amount of serotonin in these areas, suggesting an association between serotonin levels and oxidative damage. There is no correlation between the levels of 8-OHdG and dopamine or its metabolites. The lack of effect of L-dopa upon 8-OHdG levels may be due to: normal rats might be able to compensate for additional levodopa; the additional 8-OHdG formed may be too low to be measured; prolonged treatment might be necessary; damage might be selective for mitochondrial DNA. Supported by: the Lowenstein Foundation, the NIH, and the Parkinson's Disease Foundation

593.6

OXIDATIVE STRESS PROMOTES MITOCHONDRIAL IRON SEQUESTRATION IN CULTURED ASTROGLIA. L. Bernier*, G. Bernatchez and H.M. Schipper. Lady Davis Institute, Jewish General Hospital, and Department of Neurology and Neurosurgery, McGill University, Montreal, Canada H3T 1E2

Cysteamine (CSH) induces the appearance of iron-rich, peroxidase-positive cytoplasmic granules in cultured rat astroglia, which are identical to those which progressively accumulate in the aging subcortical brain. Both *in situ* and in culture, these glial inclusions derive from degenerate mitochondria in the context of a generalized cellular stress (heat shock) response. In the present study, we demonstrate that CSH up-regulates transcription of heme oxygenase-1 (HO-1) in cultured astroglia followed by the sequestration of non-transferrin bound, non-heme ^{55}Fe by the mitochondrial compartment. CSH-induced HO-1 expression can be blocked by iron chelators (deferroxamine, phenanthroline) and protein synthesis inhibitors, and mitochondrial iron trapping is recapitulated by H_2O_2 or menadione exposure. Our findings indicate that oxidative stress is the mechanism responsible for CSH-induced sequestration of non-heme, non-transferrin bound iron in astroglial mitochondria. CSH-stressed astroglia may serve as a useful model to investigate mechanisms of iron sequestration in the normal senescent brain, in Parkinson's disease, and in other aging-related neurodegenerative disorders.

Supported by the Medical Research Council of Canada

593.7

TAURINE-DOPAMINE (DA) INTERACTIONS IN THE STRIATUM. B. Eppler*, D.R. Wallace, M.A. Pelleymounter, R.M. Booze, S.C. Liu and R. Dawson,Jr. Dept. of Pharmacodynamics, Univ. Florida, Gainesville, FL 32610, Dept. Pharmacology, Univ. Kentucky, Lexington, KY 40536 and Dept. Neurobiology, Amgen, Inc, Thousand Oaks, CA 91320.

Taurine is a sulphur containing amino acid that is involved in both neuromodulatory and antioxidant functions and may interact with the CNS DA system. The aim of the present series of studies was to examine age-related changes in striatal taurine content and investigate some potential interactions between taurine and the DA system.

Aged (26 months) and adult (5 months) male Long-Evans rats were behaviorally tested using the Morris water maze task and then were sacrificed for the determination of brain taurine by HPLC. Additional experiments assessed the ability of taurine and related amino acids to inhibit $FeCl_3$ stimulated DA oxidation using an assay for quinone formation. DA receptor binding (D3 subtype) was determined in the presence and absence of 20 mM taurine using [^{3}H]-7-OH-N,N-di-n-propyl-2-aminotetralin (DPAT) in striatal membrane preparations oxidatively damaged with the combination of 200 μM DA and 100 μM $FeCl_3$. Finally, the *in vivo* effects of depleting brain taurine in adult Sprague-Dawley rats with 1% β-alanine were assessed on striatal [3H]-DPAT binding.

Striatal taurine content was depleted ($p<0.05$) in learning impaired aged rats (n=8) compared to adult rats (n=8). Aged rats that were unimpaired behaviorally (n=7) did not differ significantly in striatal taurine content from the adult controls. Taurine content was not altered in the hippocampus or temporal cortex of aged rats. Taurine was found to significantly inhibit iron-stimulated DA oxidation and protect D3 receptors from oxidative damage. Treatment with β-alanine caused a 25% increase in striatal D3 receptors and D3 receptors have been reported to increase in aging.

Collectively, these findings suggest that taurine may protect vulnerable striatal neurons from age-related oxidative damage. (Funded by grants from Taisho Pharmaceutical Co., Ltd. and NIH AG10747, AG10836 and AG 00242.)

593.8

INDUCTION OF NITRIC OXIDE SYNTHASE IN MOTONEURONS OF THE AGED RAT SPINAL CORD. **K. Kanda*, S. Asaki and E. Nomoto. Dept. of Central Nervous System, Tokyo Metropol. Inst. of Gerontol., Tokyo 173, Japan.**

Loss of motoneurons with advancing age has been shown in both human and experimental animals. However, underlying mechanisms for motoneuronal death in the aged remains unclear. It has been proposed that the survival of motoneurons is maintained by neurotrophic factors. Recently, it has been suggested that nitric oxide is involved in motoneuronal cell death induced by deprivation of trophic factor. We examined in the present experiments if nitric oxide synthase (NOS) was induced in motoneurons in aged rats.

Experiments were performed on male Fischer rats which had been raised under the specific pathogen free condition. The animals (three age groups:15-17, 24 and 29-30 months old) were anesthetized with Nembutal (50 mg/kg, ip), and perfused transcardially with a cooled fixative (1.25% glutalaldehyde and 1% paraformaldehyde in phosphate buffer, pH 7.6). The lumbar spinal cord was removed and immersed in 30% sucrose in phosphate buffer overnight. Serial sections were cut and stained with histochemistry for reduced nicotinamide dinucleotide phosphate diaphorase (NADPH-d). NADPH-d positive cells in the ventral horn of L4 to L6 were counted. In some rats, Fluorogold was injected into the sciatic nerve 8 days before perfusion to identify motoneurons.

Motoneurons in the lumbar spinal cord of young rats were not stained for NADPH-d under the present experimental conditions. On the other hand, NADPH-d positive, large neurons, which were presumably alpha motoneurons, were found in the ventral horn of aged rats. The number of such NADPH-d positive neurons was increased with advancing age from 24 months to 29-30 months. Appearance of NADPH-d positive motoneurons seems to coincide with motoneuronal loss, and it appeared that the both phenomena advanced similarly. The findings in the present study suggest that expression of NOS plays an important role in cell death of motoneurons in the aged rat.

Supported by the TMIG.

593.9

EXTRACELLULAR SPACE DIFFUSION PARAMETERS IN THE RAT BRAIN DURING AGEING. E. Syková*, T. Mazel and T. Roitbak. Dept. Neurophysiol., Inst. Exp. Med. ASCR, Prague 4, Czech Republic.

The changes in extracellular space (ECS) diffusion parameters influence non-synaptic transmission and intercellular communication in the CNS. The morphological changes during ageing, including cellular loss, astrogliosis, demyelination and swollen astrocyte processes, may result in changes in ECS diffusion parameters, such as the ECS volume fraction α (α = ECS volume / total brain volume) and ECS tortuosity $\lambda = (D/ADC)^{1/2}$, where D is the free diffusion coefficient and ADC is the apparent diffusion coefficient in the brain. We studied the diffusion parameters in vivo by the real-time iontophoretic method using TMA^+-selective microelectrodes. Measurements were done in the cortex, corpus callosum (white matter, WM) and hippocampus of ageing rats (aged 26-32 months). The α in senescent rats (n = 23) was significantly lower than in young adults (n = 25) (all values are expressed as mean±S.E.; data from young adults are given in parentheses): in the cortex α=0.18±0.003 (0.21±0.003), in the WM α=0.15±0.004 (0.19±0.004), in the pyramidal cell layer of the CA1 region of hippocampus (CA1pyr) α=0.09±0.005 (0.12±0.004) and in CA1 stratum radiatum (CA1rad) α=0.13±0.006 (0.16±0.008). Tortuosity λ was significantly lower in the cortex, where λ =1.51±0.02 (1.60±0.01), but it was significantly higher in CA3rad, where λ =1.49±0.02 (1.41±0.02). We conclude that significant changes in ECS diffusion parameters which accompany morphological changes during ageing can contribute to impairment of signal transmission, greater susceptibility to anoxia, changes in behavior and memory impairment. Supported by GACR 309/96/0884 and IGA MZ 3423-3.

593.10

NITRIC OXIDE SYNTHASE PROTEIN IMMUNOREACTIVITY (NOS-IR) IS ALTERED IN CRANIAL MOTOR NUCLEI IN RESPONSE TO INJURY AND AGING IN F344 RATS. R.L. Benton*, P. Grammas[1] and J.M. Jacob. Depts. of Anat. Sci. and [1]Pathology, Univ. of Oklahoma HSC, Oklahoma City, OK 73190.

Nitric oxide (NO), a freely diffusible molecule, has been implicated in a variety of neuromodulatory and neurotoxic functions in the nervous system. Both aging and peripheral nerve injury result in a multitude of physiological, morphological and biochemical changes in the perikaryon. The objective of this study is to investigate the effect of age and nerve injury on NOS expression in motor neurons and blood vessels of the facial nucleus (CN VII). Injured motor neurons were labeled with the retrograde tracer FluoroGold; animals were killed 1, 3 or 7 days following transection of the facial nerve. Using polyclonal antibodies directed against the three isoforms of NOS (inducible NOS, endothelial NOS, brain NOS), the distribution of protein immunoreactivity (IR) was determined. Our results indicate that NOS-IR is differentially expressed in motor neurons and blood vessels. iNOS-IR is re-expressed within brain microvessels located in CN VII nucleus of old (24 mo) unoperated rats whereas no iNOS-IR is present in mature (6 mo) rats. There is no basal iNOS-IR or bNOS-IR in motor neurons of either age. eNOS-IR is restricted to microvessels and is unchanged with age. Axotomy results in the induction of iNOS-IR and bNOS-IR in cranial motor neurons. Aging and injury differentially affect the expression of specific isoforms of NOS and may be consistent with a neuroprotective role for NO.

Partially supported by a grant to PG (NS30457) and JMJ (AFAR).

593.11

AGE-RELATED CHANGES IN THE RHESUS MONKEY: ASSOCIATION OF INFLAMMATORY PROTEINS AND AMYLOID WITH COGNITION, J.A. Sloane, M.F. Pietropaolo, D.L. Rosene, M.B. Moss, B.L. Martin-Razzaboni*, T. Kemper and C.R. Abraham. Depts. Pathology, Biochemistry, Neurology, Medicine, and Anatomy and Neurobiology, Boston Univ. Sch. of Med., Boston, MA 02118.

In order to assess whether amyloid plaque accumulation can account for age-related cognitive impairment that begins at about 20 years of age, we measured plaque density in the brains of rhesus monkeys 5 to 31 years old. Amyloid plaques accumulate with age, starting at 25 and escalating at 30 years of age. Although both cognitive dysfunction and plaque number increase with age, amyloid plaques do not appear to be related to the cognitive dysfunction observed. Some of the oldest monkeys with severe cognitive impairment had few amyloid plaques while others with only mild cognitive impairment had abundant plaques. As an alternative potential mechanism for age-related cognitive decline, we examined cerebral white matter, in which we have previously observed dramatic morphological alterations, for the presence of inflammatory changes. Using western blotting and immunohistochemical techniques, we found increased activation of microglia and astrocytes with age. It appears that upregulation of inducible nitric oxide synthase (iNOS), an inflammation-related enzyme produced principally by activated astrocytes and microglia, occurs earlier in age than plaque accumulation. Furthermore, our preliminary results suggest iNOS expression may correlate with age-related cognitive impairment. (Supported by NIH grant PO1-AG00001)

593.12

GLUCOCORTICOID RECEPTOR (GR) MRNA EXPRESSION IN ASTROCYTES AND MICROGLIA IN THE AGED RAT BRAIN. D. Bryan, K. Sugaya, D. Personett, E. Skiba, S.J. Xu, M. Gallager and M. McKinney* Mayo Clinic Jacksonville, Jacksonville, FL 32224 and University of North Carolina, Chapel Hill, NC 27599

Our laboratories are investigating candidate gene expression in an aging rat model in which spatial learning is quantitated using a water maze. We reported global glial activation and βAPP mRNA elevation in learning-impaired Long Evans rats. Adrenal steroids are known to regulate protein synthesis, and age-related effects on glia have been reported. Glucocorticoids are also known to regulate βAPP mRNA expression in hippocampal neurons. Thus, glucocorticoids may be factors in the alterations in gene expression we observed in this aging animal model. In the current study, we examined GR type I and II mRNA expression in astrocytes and microglia, by using *in situ* hybridization histochemistry (ISHH) combined with immunohistochemistry for GFAP and CD2 (OX42) in the same brain sections, respectively. We observed expression for both types of GR mRNA in hippocampal neurons, astrocytes and microglia. These data indicate that ISHH combined with immunohistochemistry for glia can be used to address the involvement of adrenal steroids in the alteration of neuronal or glial gene expression in the aging brain.

593.13

ALTERATIONS OF MN-SUPEROXIDE DISMUTASE (MN-SOD) AND β-AMYLOID PRECURSOR PROTEIN (βAPP) GENE EXPRESSION WITHIN SPECIFIC CELL TYPES IN THE AGED LONG-EVANS RAT. E. Skiba, K. Sugaya, D. Bryan, D. Personett, S.J. Xu*, M. M. Nicolle, M. Gallagher and M. McKinney Mayo Clinic Jacksonville, Jacksonville, FL 32224 and Universityof North Carolina, Chapel Hill, NC 27599

Our laboratories are investigating candidate gene expression in an aging rat model in which spatial learning is quantitated in a water maze. We have previously reported that hippocampal Mn-SOD, βAPP and glial fibrilliary acidic protein (GFAP) mRNA levels are correlated with memory impairment in these animals, suggesting the existence of oxidative stress and glial activation. In the present study, we address which types of cells are involved in these alterations we found in this animal model. Mn-SOD and βAPP gene expression in astrocytes, microglia and cholinergic cells were investigated using *in situ* hybridization (ISHH) combined with immunostaining for GFAP, CD2 (OX-42) and choline acetyltransferase (ChAT), for each of these cell types, respectively. βAPP mRNA in Mn-SOD immunopositive cells was also examined by this technique. Neurons appeared to be the major sites of alteration of Mn-SOD mRNA in the aged hippocampus. Hippocampal neurons, including scattered cells in the hilus, exhibited strong Mn-SOD immunoreactivity and expressed high levels of βAPP mRNA. In GFAP-immunostained material, hilar cells expressing high βAPP mRNA levels appeared to be surrounded by astrocyte processes. Cholinergic cells of the basal forebrain had higher Mn-SOD mRNA expression and (probably) stronger Mn-SOD immunoreactivity than surrounding non-cholinergic cells. OX-42 positive microglia were prominent in the both basal forebrain and hippocampus. These results indicate that in the aging brain glial activation may be a source of neuronal oxidative stress and that neurons may respond to such stress by elevating their levels of Mn-SOD, and/or βAPP.

593.14

ASTROCYTES IN THE AGED RAT SPINAL CORD FAIL TO INCREASE GFAP mRNA FOLLOWING SCIATIC NERVE AXOTOMY. S.A. Gilmore*, T.J. Sims and C.J.M. Kane. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Marked changes in the astrocyte population in the brain occur with aging and are paralleled by increase in the levels of astrocyte glial fibrillary acidic protein (GFAP). Aging also alters the astrocyte response to injury, but the nature of these changes is not clear due to the diversity of models employed. This study was designed to: (1) establish whether astrocytic changes similar to those in the brain occur with aging in the spinal cord, and (2) define the effect of aging on the astrocytic responses following a non-invasive injury to the spinal cord. The levels of GFAP mRNA were quantified in the lumbar spinal cord of young (2 month old) and old (8, 11 and 17 month old) Charles River CD rats by Northern hybridization and densitometry. The level of GFAP mRNA was increased 0.4-fold ($p<0.01$) in rats ≥8 months old compared to rats 2 months old; there was no difference between rats aged 8, 11 or 17 months. Non-invasive injury was performed by axotomy of the sciatic nerve with analysis at 4 days post-injury. The lumbar cord of young rats responded with a 0.5-fold increase in GFAP mRNA levels compared to uninjured controls ($p<0.001$). In contrast, the levels of GFAP mRNA in the aged cord following axotomy remained unchanged compared to age-matched, uninjured controls. Immunohistochemical analysis of GFAP in the lumbar cord paralleled the changes in GFAP mRNA. Thus, astrocytes in the spinal cord exhibited increased expression of GFAP mRNA with aging; and in contrast to young rats, the levels of GFAP mRNA in aged rats did not increase in response to injury. (Supported in part by NIH AG12411).

NEUROGLIA AND MYELIN IV

594.1

Immunohistochemical Localization of Transferrin Binding Protein in the Avian Retina Sa Sun Cho*[1] Arnold G. Hyndman,[2] John J. Lucas. Dept. of Anatomy, Seoul National Univ. College of Med., Seoul 110 Korea; [1]Dept. of Biological Science Rutgers Univ., Piscataway, NJ 08855, USA; [2]Dept. of Biochemistry and Molecular Biology, State Univ. NY Health Science Center, Syracuse, NY 13210, USA

Transferrin binding protein(TfBP) is a glycoprotein purified from the chick oviduct that exhibits transferrin binding activity. Recent work has shown TfBP is a previously described heat shock protein 108(HSP 108). Antiserum to TfBP has been found to selectively label oligodendrocytes in the avian brain(Brain Res 674:15, 1995). The aim of this study was to examine TfBP immunoreactivity in the avian retina using immunohistochemical methods. A strong TfBP-immunostaining was evident in the small cells in the avian retina. The immunoreactive cells were interposed between ganglion cells in the 8th layer and occurred along the nerve fibers in the 9th layer of retina. In the whole mount preparation, TfBP-labeled cells displayed the unique morphological features of brain oligodendrocytes : the fine processes that extended radially from the perikarya could be traced to terminals located around or along optic nerve fibers. These immunoreactive cells were distributed throughout the retina with diminishing toward the periphery of the retina. A diffuse staining at lower level was also visible in the inner segment of the photoreceptor layer. Given the fact that the avian retina has myelin in the 8th and 9th layers, the distribution and morphology of TfBP-immunoreactive cells observed in this study suggests strongly that TfBP-labeled cells are retinal oligodendrocytes responsible for production and maintenance of myelin in the avian retina.

594.2

IRON AND MANGANESE UPTAKE IN GLIAL CELL CULTURES FROM HYPOTRANSFERRINEMIC MICE. A. Takeda*, A. Devenyi, and J. Connor Depts. of Neruoscienc & Anatomy, and Pediatrics Penn State University School of Medicine, Hershey PA 17033

Hypotransferrinemic (Hp) mice are normally occuring mutants with a splicing defect in the transferrin (Tf) gene. The resulting phenotype expresses <1% of the normal plasma circulating levels of Tf. Consequently, this animal offers the opportunity to examine the role of Tf in iron distribution to cells. In this study, we use mixed glial cultures from Hp mice and unaffected wild type controls to determine the contribution of Tf known to be produced and secreted by glial cells in culture to iron uptake by glial cells. The mice expressing the Hp mutation are pale compared to unaffected littermates and can be identified at birth. For the tissue culture analysis, the brains of Hp and unaffected animals were removed within 2-3 days of birth and mixed glial cultures were prepared according to standard procedures. The cultures were established in 10% fetal calf serum for 7 days. Subsequently, the cultures were changed to a defined, serum-free, Tf containing or Tf-free medium for 1 day followed by addition of $^{59}FeCl_3$ to the medium for 3,6 or 24 hrs. In the absence of Tf, iron uptake was elevated in both normal and Hp mice at 3 and 6 hrs but not at 24 hrs. The amount of iron uptake in the Hp mouse mixed glial cultures was 2-10X higher than in the normal mice over a 3 hr incubation period. The absence of Tf in the medium of the Hp mouse cultures did not affect iron uptake by the cells in the culture. However, the absence of Tf in the normal mouse cultures resulted in a nearly 2X increase in iron uptake by microglia. The efflux of iron from the cells in the culture was influenced by Tf in the medium. More iron remained in cells from both Hp and normal mice if Tf was removed from the medium. The efflux of iron in the absence of Tf in the medium was greater in the wild type mouse cultures than in the Hp mouse cultures. These results demonstrate that Tf is not required for iron uptake into mixed glial cell cultures, indeed its absence resulted in an increase in iron uptake. The presence of Tf can influence the cellular distribution of iron and the rate of iron efflux from cells. These results suggest the presence of an non-Tf dependent iron delivery system in mixed glial cells and also suggest that may regulate the intracellular concentration of iron. Supported by NS 22671 Funds from the Children's Miracle Network.

594.3

OLIGODENDROCYTES DERIVED FROM MOUSE BRAIN TRANSIENTLY EXPRESS A FERRITIN RECEPTOR *IN VITRO*. S. Hulet,* S. Powers, and J.Connor. George M. Leader Family Laboratory for Alzheimer's Disease Research. Pennsylvania State University. The Milton S. Hesrshey Medical Center. Hershey, PA.

Traditionally ferritin has been thought of as the intracellular iron storage protein because of it's abitity to store ferric iron in it's hollow core. Iron in ferritin is considered unavailable for toxic radical producing reactions. Recently, we have identified a ferritin receptor in the brains of adult C57 B1/6 mice. This novel protein binds ferritin in a saturable and competitive manner characteristic of receptor- ligand interactions. Autoradiographic analysis reveals the ferritin receptor is predominantly located in white matter tracts in the adult mouse. This distribution is in direct contrast to the distribution of transferrin receptor which is found heavily throughout cortex and shows very low distribution throught white matter tracts. The goal of this study is to identify the specific cell types *in vitro* that bind ferritin.

We used 2-4 day old C57 B1/6 mice to generate mixed glial cell cultures. The mixed glial cell cultures were incubated for 1 hour with fluorescein labeled ferritin. Cultures were labeled concomitantly with antibodies to different cell specific markers. Fluorescent microscopy revealed that two populations of cells were taking up the ferritin. 1) Small, round, non-process bearing cells sitting on the top of the astrocyte bed layer which were O4 and O1 positive. However, process bearing 04+ or 01 + cells were negative for ferritin uptake. 2) Elongated nonprocess bearing cells which were LDL+ which is a microglial marker. The GFAP + bed layer of astrocytes were ferritin uptake negative. This evidence strongly suggests that oligodendrocytes transiently express a ferritin receptor. The significance of this research lies in the ability of each ferritin molecule to carry up to 4,500 iron atoms, while the traditional iron transport protein transferrin can deliver only 2 atoms of iron to the cell. Thus ferritin may be the iron transport protein of choice for oligodendrocytes. Supported by NS22671 (NIH)

594.4

TRANSFER AND EXPRESSION OF GALACTOSYLCERAMIDASE MEDIATED BY ADENO-ASSOCIATED VIRUS. H.Chen[1], D. M. McCarty[2], S. Gowen[2], D. Wenger[5], K. Suzuki[1,4*], K. Suzuki[1,3]. UNC Neuroscience Center[1], Gene Therapy Center[2], Dept. of Pathology & Laboratory Medicine[3], Neurology & Psychiatry[4], University of North Carolina, Chapel Hill, NC 27599, and Division of Medical Genetics, Thomas Jefferson University, Philadelphia, PA 19107[5].

We have explored the possibility and strategies of gene therapy for neurodegenerative diseases using the twitcher mouse as an animal model. Twitcher is an authentic murine model of globoid cell leukodystrophy (GLD, Krabbe disease) in humans caused by a deficiency of the lysosomal enzyme, galactosylceramidase. Lack of this enzyme activity results in degeneration of oligodendrocytes due to an accumulation of the toxic metabolite, psychosine, followed by demyelination, with infiltration of macrophages (globoid cells). Thus, we targeted transfer of the galactosylceramidase gene into oligodendrocytes. A recombinant adeno-associated virus (rAAV) vector containing 2.1 kb of human galactosylceramidase cDNA (rAAV galc) was constructed under the control of the murine myelin basic protein (MBP) promoter/enhancer. Infection of primary normal mouse glia cells and a transformed oligodendocyte cell line with rAAV galc indicated that the trans-galactosylceramidase gene was expressed. *In vivo*, doses of approximately 2 x 10^4 infectious units of rAAV galc in 2 µl of saline were injected into the right cerebral hemisphere of normal mice. At several time points after the injection, the mice were killed, total RNA extracted and mRNA purified. By RT-PCR analysis, expression of the specific trans-galactosylceramidase gene was found at least up to 2 months. Further investigation to evaluate the duration and distribution of galactosylceramidase expression is now underway. (Supported by research grants, NS24453, NS24289, T32-HD07201 and a Mental Retardation Research Center grant, HD03110 from USPHS).

594.5

LOW EXTRACELLULAR Na^+ CONCENTRATION PROMOTES STIMULATION OF GLUCOSE UTILIZATION BY K^+ IN CULTURED ASTROGLIA. J.Gotoh, M.J.Law and L.Sokoloff*. Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, MD 20892.

Previous studies (Takahashi et al., *PNAS*, 92:4616-4620;1995) demonstrated stimulation of glucose metabolism in cultured astroglia by agents, e.g., veratridine or monensin, that promote Na^+ entry into the cells. Membrane depolarization by elevated extracellular K^+ concentrations ($[K^+]_o$) in the 2.7-56 mM range, however, had no consistent effects on glucose utilization under standard incubation conditions with bicarbonate buffer and extracellular Na^+ concentration ($[Na^+]_o$) in the 105-155 mM range. To investigate the possibility of a relationship between $[K^+]_o$ and $[Na^+]_o$ on astroglial energy metabolism, we have compared the effects of increased $[K^+]_o$ on glucose utilization in cultured astroglia incubated in normal and in nominally Na^+-free media. Astroglia were prepared from newborn rat cortex and studied after 20-24 days in culture. Cells were incubated in Dulbecco's bicarbonate-buffered saline (DBS) containing 2 mM glucose, tracer amounts of 2-deoxy-D-[^{14}C]glucose ([^{14}C]DG), and 2.7, 5.4, 28, or 56 mM $[K^+]_o$ for 15 min followed by a 5 min incubation in [^{14}C]DG-free buffer for efflux of unmetabolized [^{14}C]DG. Na^+-free conditions were obtained by substitution of choline for Na^+. In experiments carried out with 10 different cultures no significant changes in [^{14}C]DG phosphorylation were observed with increasing $[K^+]_o$ during incubations in medium containing normal $[Na^+]_o$. Incubation in Na^+-free DBS reduced [^{14}C]DG phosphorylation below rates in medium containing normal $[Na^+]_o$, but unmasked stimulations of [^{14}C]DG phosphorylation by elevated $[K^+]_o$ above 2.7 mM that appeared to be concentration-dependent (4.2%, $p<0.05$, at 5.4 mM; 38%, $p<0.01$, at 28 mM; 57%, $p<0.01$, at 56 mM).

These results suggest that K^+ effects on astroglial energy metabolism are normally obscured by the energy metabolism associated with Na^+-dependent processes but become apparent when these processes are limited by low $[Na^+]_o$.

594.6

POTASSIUM ACCUMULATION IN REACTIVE GLIAL CELLS IN SITU. W. Walz*, I.A. Paterson and W.A. Wuttke. Dept. Physiology, Univ. Saskatchewan, Saskatoon, SK S7N 5E5, Canada.

Adult rats, previously treated with kainic acid, exhibited loss of neurones and gliosis in the CA1 layer of the hippocampus within 3d. We impaled cells of hippocampal slices (400 µm) with two channel microelectrodes and these cells had a membrane potential of -74 mV (3.5 mM $[K^+]_o$). Lucifer yellow injections combined with immunostaining showed that most of the cells were GFAP positive and coupled to up to 29 other cells. Of these coupled cells 75% were GFAP positive. Quite unexpectedly we found GFAP negative cells coupled to these GFAP positive cells. In another set of experiments one electrode channel contained a K^+-sensitive resin. $[K^+]_i$ was 78 mM (E_K = -79 mV). Increase of external $[K^+]$ to 10 mM led to an increase of the internal $[K^+]$ by 24 mM. Bumetanide had no effect on the accumulation, whereas barium doubled the time course to reach a plateau value. Ouabain exposure decreased the internal $[K^+]$, but did not affect the K^+ uptake. We conclude that these glial cells from gliotic tissue retain their K^+ clearance properties and that K^+ accumulation is based on Na^+, K^+-ATPase and Donnan-mediated KCl influx. In addition these cells are well coupled, however their role as spatial buffers is questionable since our data suggest the existence of a significant Cl^- conductance. Supported by the MRC of Canada.

594.7

INTRACELLULAR ACIDIFICATION IN MOUSE CEREBRAL ASTROCYTES CAUSED BY RAPID INFLUX OF AMMONIUM IONS T. N. Nagaraja and N. Brookes*. Dept. of Pharmacol. & Exptl. Therap., Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Exposure of most cell types to 20 mM NH_4Cl produces a rapid intracellular alkalinization as free NH_3 diffuses inward and is protonated in the cytoplasm. Typically, the alkalinization declines slowly, over a time course of several minutes, in the continued presence of NH_4Cl (Boron & De Weer, *J. gen. Physiol.*, 67:91, 1976). We report here an unconventional response to NH_4Cl in primary cultures of mouse cerebral astrocytes, observed using the pH indicator BCECF to measure intracellular pH (pH_i) fluorometrically. After a brief alkalinization, which peaked in 2-4 sec, the astrocytes exhibited a rapid and intense acidification in the continued presence of 20 mM NH_4Cl. Intracellular pH declined with a half-time of 5-7 sec to reach a sustained level 0.44±0.004 pH units (±SE, n=3) below the initial resting pH_i in the nominal absence of HCO_3^-. This sustained acid shift reached 0.36±0.02 pH units (n=6) in response to 1 mM NH_4Cl, and saturated at 5 mM NH_4Cl. However, the initial rate of acidification did not show saturation. The initial rate of acidification produced by 1 mM NH_4Cl (0.61±0.07 pH units/min, n=6) declined by 82% in the presence of 25 µM Ba^{2+}, by 34% in the presence of 0.1 mM bumetanide, and by 24% in the presence of 5 mM ouabain. The combination of Ba^{2+} plus bumetanide prevented net acidification. Our results suggest that both active and passive influx of NH_4^+ acidifies the astrocytes by sustaining an outward diffusion gradient of NH_3 (compare NH_4^+ transport in the thick ascending limb of Henle's loop; Good, *Annu. Rev. Physiol.*, 56:623, 1994). The primary route of entry for NH_4^+ was via inwardly-rectifying K^+ channels. This influx is driven by the inside-negative membrane potential. A second uptake route was via the Na^+-K^+-$2Cl^-$ cotransporter. (Supported by NIEHS grant ES03928).

594.8

H^+-ATPASES AND GLIAL pH-REGULATION. Ch. Volk, T. Albert, O. Kempski*, Inst. Neurosurg. Pathophysiol., Univ. Mainz, 55101 Mainz/Germany

Glial cells play a major role in cerebral ion homeostasis and in the control of cerebral tissue pH. Several pH-regulating mechanisms, such as Na^+/H^+- and Cl^-/HCO_3^--antiport or Na^+/HCO_3^--cotransport are present in astrocytes. Here we studied the contribution of proton-translocating ATPases (H^+-ATPases), so far best described in various epithelial cell types. Intracellular pH (pH_i) of C6 glioma cells and rat astrocytes was measured in vitro using the pH-sensitive dye BCECF under strict control of extracellular parameters. To clarify the role of H^+-ATPases, inhibitors of several subtypes of H^+-ATPases were applied in HEPES-buffered media: 1 mM N-ethylmaleimide (NEM, blocks several types of H^+-ATPases), NBD-Cl (50 µM, more specific for the vacuolar subtype), ortho-vanadate (100 µM, inhibitor of E_1E_2-type ATPases), and oligomycin (10 µM, specific for the F_oF_1 mitochondrial H^+-ATPase). After application of NEM a pH_i-decrease was observed in C6 glioma and astrocytes. The pH_i in C6 cells fell within 15 min from 7.04 ± 0.03 to 6.70 ± 0.03 ($p<0.001$ compared to controls without NEM). After 15 min a slow partial pH_i recovery was seen and after 60 min pH_i was 6.87 ± 0.04. Exposure to NEM in Na^+-free media to prevent Na^+/H^+-exchange caused a more pronounced pH_i reduction: after 20 min pH_i had decreased from 7.07 ± 0.03 to 6.44 ± 0.04, while Na^+-free media alone had only a minor effect. Application of NBD-Cl caused a pH_i-decrease of 0.40 ± 0.07, comparable to that caused by NEM. In rat astrocytes pH_i decreased from 7.04 ± 0.03 to 6.61 ± 0.08 with NEM, and from 7.08 ± 0.11 to 6.67 ± 0.03 with NBD-Cl. Vanadate or oligomycin had no effect. Inhibition of ATP-supply by iodoacetate (2.5mM) also went along with glial acidification. A delayed increase of cell volume was seen with NEM or NBD-Cl, suggesting activation of Na^+/H^+-exchange and swelling below a threshold of approx. pH_i=6.7. The data demonstrate that H^+-ATPases of the vacuolar type contribute significantly to pH_i-control in glial cells at physiological extracellular pH. (Supported by DFG Ke 338/3-2)

594.9

EVIDENCE FOR OUTWARDLY RECTIFYING AND CFTR-LIKE CHLORIDE CHANNEL EXPRESSION IN CULTURED MAMMALIAN ASTROCYTES. C.D. Lascola* and R.P. Kraig. The Committee on Neurobiology and Department of Neurology, University of Chicago, Chicago, IL 60637.

Astroglial anion channels are likely to play important roles in K^+ homeostasis, pH regulation, and volume control. Resting astrocytes in culture normally have a negligeable chloride conductance. Following changes in cell morphology and cytoskeletal actin, however, astroglial whole-cell Cl^- conductance increases (Lascola and Kraig, *J Neurosci*, 1996). We have recently identified two candidate Cl^- channels that may contribute to morphology-dependent whole-cell Cl^- currents. In excised, inside-out patches, we observed an outwardly rectifying Cl^- channel activated by prolonged depolarization (n=17), high ionic strength (600 mM NaCl; n=5), or exposure to 0.025% trypsin (n=3). The slope conductance in symmetrical solutions of 150 mM NaCl (10 TES; 1 $MgCl_2$) was 71pS at positive potentials (60 to 80 mV) and 36 pS at negative potentials (-60 to -80mV). Application of 200 µM DIDS produced flicker block; 1 mM Zn^{2+} produced complete block. In excised patches with symmetrical 150 NMDG-Cl solutions and 180 nM PKA (catalytic subunit), we also observed small, ohmic single Cl^- channels with a conductance of ~10 pS (n=5), suggesting the presence of CFTR-like Cl^- channels. In whole-cell experiments, inclusion of CTP-AMP (n=5) or the PKA subunit (n=3), both with 5 mM Mg-ATP, also induced a Cl^- current ~200 pA at 100 mV. This conductance was blocked by 1 mM DPC, but not 200 µM DIDS. Incubation overnight in serum-free Ringer, followed by application of 5 µM cytochalasin D, appeared to sequentially activate both CFTR-like and outwardly rectifying channels in cell-attached patches. Together, these results suggest astrocytes, which maintain aspects of epithelial organization in brain, also express Cl^- channels previously characterized in airway and gastrointestinal epithelia. These channels appear to be modulated by changes in the actin cytoskeleton, and may therefore contribute to Cl^- conductances accompanying changes in astroglial morphology. (Supported by NS-19108.)

594.10

OSMOLALITY DEPENDENCE OF ANION AND POTASSIUM CONDUCTANCES IN CULTURED ASTROCYTES G. Li* and J.E. Olson Departments of Emergency Medicine and Physiology & Biophysics, Wright State University School of Medicine, Dayton, Ohio 45401.

Astrocytes are thought to modulate neuronal excitability in the CNS by controlling local ion and amino acid concentrations. Volume-sensitive anion channels mediating amino acid efflux have been described in C6 glioma and MDCK cells. We studied the electrophysiology of cultured astrocytes to identify osmolality-dependent channels which might contribute to volume regulation. In the whole cell patch configuration with 140 mM KCl in the electrode, slope conductance was determined with voltage steps between -90 mV and -50 mV from a holding potential of -70 mV. Astrocyte conductance increased by 775±332% (mean±SEM) and cell capacitance was unaltered when the cells were transferred from isoosmotic (290 mOsm) to hypoosmotic (200 mOsm) medium (N=6). Niflumic acid (250 µM) completely reversed the conductance increase while 5 mM $BaCl_2$ had no effect. No conductance change occurred in 200 mOsm medium when the patch solution contained 140 mM K-gluconate rather than KCl. However, a hypoosmotically induced, niflumic acid-sensitive conductance increase was measured when 200 mM taurine (pH=8.2) replaced chloride salts in the patch electrode and bathing solutions. In 140 mOsm medium, astrocyte membrane conductance increased 1326±408% relative to that in isoosmotic medium (N=9). Niflumic acid (250 µM) and $BaCl_2$ (5 mM) inhibited 60.1±11.4% (N=9) and 82.4±15.0% (N=5) of this increase, respectively. We conclude activation of anion channels in 200 mOsm medium can lead to chloride and taurine loss from astrocytes. Potassium channels are activated with greater cellular swelling. Supported by Kettering Medical Center and the Ohio Research Challenge Fund.

594.11

ION AND AMINO ACID CONTENTS OF ASTROCYTES GROWN IN HYPOOSMOTIC MEDIUM. J.E. Olson* Depts of Emergency Medicine and Physiology & Biophysics, Wright State Univ Sch Med, Dayton, Ohio 45401.

Osmolyte contents of cultured astrocytes were measured during 24 hr in hypoosmotic medium to examine mechanisms of brain adaptation to prolonged hypoosmotic hyponatremia. Medium osmolality was reduced from 310 mOsm (isoosmotic) to 220 mOsm by altering the NaCl concentration of serum-supplemented growth medium (Olson et al., Neurochem Res, 6:1337-1343, 1981). After 1, 12, or 24 hr, cells were incubated for 5 min in osmotically matched saline containing [^{3}H]-3-O-methylglucose plus [^{14}C]-sucrose as markers of intracellular and extracellular spaces, respectively. Radioactivity, potassium, amino acid, and protein contents were determined by liquid scintillation counting, atomic absorption spectroscopy, HPLC, and the Folin reaction, respectively. Cell volume in isoosmotic medium was 5.3±0.2 µl/mg protein (mean±SEM, N=15). After 1 hr in hypoosmotic medium the volume was elevated by 30% ($p<0.05$) but returned to normal at 12 and 24 hr. Taurine content fell by 88% in the first hour from an initial value of 137±9 nmol/mg protein and remained at 11-26 nmol/mg protein for the remainder of the exposure period ($p<0.05$). Aspartate decreased by 54% from its initial value of 37±5 nmol/mg protein after 1 hr in hypoosmotic medium and was unchanged thereafter ($p<0.05$). Cell contents of potassium, glutamate, glutamine, asparagine, tyrosine, and alanine were not altered at any time. The data support the importance of taurine as an osmolyte for astrocyte volume regulation and suggest potassium lost from brains of hypoosmotic hyponatremic animals does not come from astroglial cells. Supported by BRSG funds of Wright State University and the Department of Emergency Medicine Research Fund.

594.12

Characterization of volume regulated chloride currents in human astrocytoma cells.

N. Ullrich* and H. Sontheimer. Neurobiology Research Center and Dept. of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham AL, 35294 and Interdepartmental Neuroscience Program, Yale Univ. School of Medicine, New Haven CT 06510.

Unlike neurons, glial cells can divide postnatally and, under neoplastic conditions, can divide rapidly to form astrocytomas. We have recently described a chloride current that characterizes both primary cultures and established cell lines of human astrocytomas and glioblastomas. These currents are outwardly rectifying, are mediated by a ~100pS channel, and are sensitive to chlorotoxin, a 36aa scorpion venom toxin that also blocks epithelial chloride channels. Because rapidly proliferating astrocytomas in humans are clinically associated with peritumoral edema, we were interested to learn whether these chloride currents can be modulated by changes in osmolality typically associated with edema. Using the whole-cell and cell attached patch clamp technique we recorded from astrocytoma cells and observed chloride currents in all cells. Astrocytoma cells responded to a hypotonic challenge (66% osmolarity) with cell swelling as well as with an increase in Cl^- current amplitude by ~50% of control. Chronic exposure to osmotic challenges altered proliferation as determined using ^{3}H-thymidine incorporation. Control and sister cultures were grown for 24 hours in media with the osmolality adjusted to (in mOsm): 145, 205, 265, 325, 375, or 410. Proliferation was maximal ~325 mOsm and decreased in either hypo- or hyperosmolar solutions. Addition of chloride channel blocker chlorotoxin (590nM) to the hyposmotic solutions partially overcame the osmotically-induced changes in proliferation. These results suggest that astrocytoma chloride currents associated with the swelling response may be involved in regulation of cell volume. We are presently investigating the adaptive role of chloride channels for cell survival during osmotic challenges.

594.13

Electrophysiological characterization of astrocytes and astrocytoma cells in slices from human biopsies and experimental intracranial brain tumors.

A. Bordey, N. Ullrich and H. Sontheimer*. Neurobiology Research Center and Dept. of Physiology & Biophysics, Univ. of Alabama at Birmingham.

We studied astrocytoma cells in acute slices from patient biopsies and in slices from tumors experimentally induced in SCID mice by injection of D54MG cells and compared their properties to normal astrocytes in rat hippocampal slices. Recordings were obtained by the whole-cell patch-clamp technique with Lucifer Yellow in the pipette to mark cells for subsequent identification with antibodies to GFAP and for visualization of cell-cell coupling by means of dye spread. Astrocytoma cells were extensively dye coupled. On average, dye spread to more than 20 neighboring cells. Normal astrocytes rarely coupled to more than 3 neighboring cells. Resting membrane potential of astrocytoma cells was -47mV and -20mV in slices from human biopsies and in SCID mice as compared to -65mV in astrocytes from adult rats (P30-P34). Like cultured astrocytoma cells, astrocytoma cells in tumor slices expressed large outwardly rectifying chloride currents in response to depolarizing voltage steps. Currents activated at potentials >45mV, did not inactivate during voltage steps and showed outward transients upon termination of voltage steps. Tail current analysis showed current reversal close to the predicted equilibrium potential for Cl^- ions (+3mV). Cl^- currents were not observed in normal astrocytes which, however, consistently expressed voltage-activated Na^+ and K^+ currents. Indeed, TTX-sensitive Na^+ channels were observed in most astrocytes (90%) and, unlike previously suggested, were still present in adulthood. While in cultured astrocytoma cells and in experimental tumors Cl^- currents were the predominant current type expressed, 92% of astrocytoma cells in slices from acute biopsies additionally expressed voltage-activated TTX-sensitive Na^+ currents (mean peak amplitude of -210pA) like those observed in normal adult astrocytes (-490pA). As such, astrocytomas from patient biopsies express mixed features typical of normal astrocytes and those of astrocytoma cell lines. (R01-NS-31234)

594.14

DOWN-REGULATION OF MOUSE ALDOSE REDUCTASE mRNA IN THE SCHWANN CELLS BUT NOT IN THE ENDOTHELIAL CELLS OF SCIATIC NERVE BY HYPERGLYCEMIA. D. T. W. Fu, A. Y. W. Lee, C. X. F Lin, S. S. M. Chung and S. K. Chung*. Institute of Molecular Biology. The University of Hong Kong.

Previously, it has been reported that reduction of blood flow and conduction velocity deficit in the nerve occurs within 1 wk of hyperglycemic induction. It has been suggested that the activation of the first enzyme, aldose reductase, of the polyol pathway, in the endothelial cells causing the hypoxic-ischemic related damage in the nerve might contribute to these functional deficit. However, our previous finding showed that AR mRNA is not expressed by the endothelial cells but is strongly expressed by the Schwann cells. Here, we report the regulation of AR and its related proteins, mouse vas deferens proteins (MVDP) and fibroblast growth factor-regulated factor-1 (FR-1) and sorbitol dehydrogenase (SD) mRNA in the Schwann cells by short-term hyperglycemia. Groups of 6-8wk old female and male mice were treated with streptozotocin (STZ, 200mg/kg body wt), 50% galactose or vehicle only. After 1, 2, and 3 wks of treatment, the sciatic nerves from the matching group were dissected after cervical dislocation. *In-situ* hybridization was performed on 7 micron frozen sections using 35S-labelled gene-specific probes. To our surprise, the AR mRNA in the Schwann cells of female mice was dramatically reduced after 1 and 2 wks of hyperglycemia. However, the male mice show less dramatic but gradual decline in the level of AR mRNA in the Schwann cells. Only small changes in AR mRNA expression was observed in galactose-fed mice. No significant induction of AR, MVDP, FR-1 and SD mRNA expression in the endothelial cells with hyperglycemia. These findings should help us to further understand the function of these proteins and their potential role in diabetes-related neuropathy. (HKU 359/94M)

594.15

MODULATION OF RAT MICROGLIA O_2^- GENERATION BY THE MARINE 14KDA PHOSPHOLIPASE A_2 INHIBITOR MANOALIDE. A.M.S. Mayer, E. Presto and W.C. Prozialeck* Pharmacology Dept., Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, IL 60515.

As of October 1, 1995 we have initiated a project whose long-term objective is to characterize marine natural products with potential for the treatment of neuroinflammatory disorders. For this purpose we have begun by establishing an *in vitro* paradigm , i.e. harvested rat primary microglial cells, since these cells have been shown to release neurotoxic superoxide anion (O_2^-) upon immune stimulation. The specific aims of these experiments were the following: a) to establish a well-defined protocol for the isolation and histochemical characterization of rat brain microglia; b) to characterize agonists that would stimulate rat microglia O_2^- generation and c) to determine the effect of marine 14kDa phospholipase A_2 inhibitors on O_2^- generation. O_2^- release was assessed by the SOD-inhibitable reduction of ferricytochrome C. Our results were the following: a) a well-defined *ex vivo* protocol for the isolation of rat brain microglia was successfully established; b) positive identification of *in vitro* rat brain microglia cultures was performed by established histochemical techniques (OX-42 and LDL); c) phorbol 12-myristate 13-acetate (PMA) was determined to be a convenient agonist to stimulate rat microglia O_2^- generation in a dose- and time-dependent manner and d) the potent 14kDa phospholipase A_2 inhibitor, the marine natural product manoalide inhibited O_2^- generation in a dose- and time-dependent manner. Studies are presently underway to a) characterize other agonists that will stimulate and/or prime microglial O_2^- generation, b) determine additional marine natural products that might modulate microglial O_2^- generation and c) initiate mechanism of action studies on active compounds.

Supported by California Sea Grant College (R/MP-73) and Midwestern Univ.

594.16

NF1*-deficient mouse Schwann cells are angiogenic, invasive and can be induced to hyperproliferate: reversion of some phenotypes by an inhibitor of farnesyl protein transferase.** **Haesun A. Kim and Nancy Ratner, Department of Cell biology, Neurobiology and Anatomy, College of Medicine University of Cincinnati, Cincinnati, OH, 45267

Neurofibromatosis type 1 (NF1) is one of the most common inherited human autosomal diseases. Formation of neurofibromas is one of the major defining features of NF1. Schwann cells may be the primary pathogenic cells in neurofibromas since majority of cells in the tumors are Schwann cells and Schwann cells, but not fibroblasts, from neurofibromas show angiogenic and invasive properties and are found without association to neurons within tumors.

The *NF1* gene product, neurofibromin, functions as a Ras-GAP, suggesting that loss of NF1 might lead to tumor formation due to inadequate regulation of Ras Increased Ras-GTP has been demonstrated in Schwann cells isolated from *NF1*-deficient mice. However, elevated Ras activity in these cells is correlated with decreased cell proliferation suggesting that additional genetic and/or epigenetic alterations might be required for Schwann cell hyperplaisia.

Here we show that Schwann cell derived from *NF1*-deficient mice are invasive and induce angiogenic responses *in vitro*. Furthermore, colonies of hyperproliferating *NF1* null mutant Schwann cells develop in the absence of serum that show growth factor independent proliferation, lose contact with axons *in vitro*, and decrease expression of the Po but fail to grow in soft agar; hyperproliferating cells develop after a week lag in Schwann cells heterozygous at *NF1*. An anti-Ras drug, the farnesyl protein transferase inhibitor, L-739,749, is able to inhibit mutant Schwann cell hyperplasia, but not invasion, suggesting a requirement for Ras activation in *NF1* deficient Schwann cells for acquisition of some tumor phenotypes. Supported by NIH NS-28840 DAMD J3030

594.17

MICROGLIAL AND IMMUNE CELL RESPONSE TO INTRACRANIAL GLIOMA: MODIFICATION BY IL-10 AND IL-2. M. A. Wilson*, P. Johnston, A. M. Fulton, and J. Laterra. Kennedy Krieger Research Institute; Depts. of Neurology, Oncology, & Neuroscience, The Johns Hopkins Univ. School of Medicine; Cancer Center and Dept. of Pathology, Univ. of Maryland, Baltimore, MD 21205.

Genetically modified cells that secrete cytokine gene products offer a promising approach to treatment of primary brain neoplasms, which are often fatal. Immortalized rat brain endothelial cells (RBEZ-Hygro) were engineered to express interleukin-2 (RBEZ-IL2); 9L rat gliosarcoma cells (9L-Neo), syngeneic to Fisher 344 rats, were engineered to express interleukin-10 (9L-IL10). Adult Fisher 344 rats (5/group) received intrastriatal injections of 2 x 10^6 RBEZ cells (Hygro or IL2) and 10^5 9L cells (Neo or IL10) as follows: 1) RBEZ-Hygro + 9L-Neo; 2) RBEZ-IL2 + 9L-Neo; 3) RBEZ-Hygro + 9L-IL10; 4) RBEZ-IL2 + 9L-IL10. Rats were sacrificed 15 days later. Tumor growth was assessed in H & E-stained sections. Microglial and immune cell responses were assessed using immunocytochemical detection of the microglia/macrophage antigens CR3 (OX-42), MHC-I (OX-18), MHC-II (OX-6), the T-cell antigens CD4 (W3/25, also on microglia), CD8 (OX-8, also expressed on NK cells in rats), a pan-T-cell antigen (OX-52), and the NK-cell antigen NKR-P1 (3.2.3). Tumor cross sectional areas were reduced to 66% of control (group 1) in the RBEZ-Hygro/9L-IL10 group ($p<0.01$). At the cellular ratios used, IL-2 alone (group 2) had no effect on tumor size. However, IL2 combined with IL-10 (group 4) dramatically reduced tumor size to 18% of control ($p<0.001$). A number of differences were noted between groups in the morphology, density and immunoreactivity of microglia. For example, in the absence of IL-10, CD8 was expressed in small round cells (presumed cytotoxic T-cells/NK cells). However, in both groups expressing IL-10, CD8 was remarkably elevated in a second population of cells with the morphologic features of microglia/macrophages. This suggests a role for CD8+ microglia/macrophages in the observed antitumor effect of IL-10, which is enhanced in the presence of IL-2. Support: NIH NS-01329, Robert Leet & Clara Guthrie Patterson Trust.

594.18

A COMMON MORPHOLOGICAL RESPONSE OF ASTROCYTES TO DIFFERENT BRAIN INJURIES: THE "DARK" ASTROCYTES. Z. Toth[1,3], L. Seress*[2], C.E. Ribak[3] and F. Gallyas[1]

Depts. of [1]Neurosurg. & [2]Physiol., Univ. Med. Sch., Pecs, Hungary and [3]Dept. of Anat. & Neurobiol., Univ. of Calif., Irvine, CA 92717, U.S.A.

Several cell types of various mammalian tissues can be transformed into a special morphological state, the so-called "dark" cell. The goal of this study was to determine whether glial cells have a dark form. Using a special silver method, a considerable number of astrocytes became stainable in rats subjected to compressive or concussive head injuries and following seizures caused by intraperitoneal administration of pentylenetetrazole or kainic acid. Several brain areas displayed argyrophilic "dark" astrocytes in light microscopic preparations. Semi-thin 2 µm plastic sections stained with toluidine blue showed that these astrocytes were hyperbasophilic. Thin sections for electron microscopy displayed hyperelectron-dense somata and processes of these astrocytes. These processes were identified by their typical shape, inclusion of glial filaments, and their apposition to capillary walls. "Dark" astrocytes with similar features were also observed in postmortem conditions of rat brains that were treated with head injuries. Based on these observations it is concluded that: 1) the nature of the intracellular pathological event in these astrocytes is the same as that of "dark" neurons or "dark" cells of non-neural tissues, and 2) the formation of these astrocytes is governed by biophysical rather than metabolic processes.

[Supported by OTKA T16570 to FG and NIH Grant NS 15669 to CER]

594.19

AUTOLOGOUS ASTROCYTE CULTURES FROM ADULTS: LONG TERM CHARACTERIZATION AND THERAPEUTIC POTENTIAL. S.A. Withiam-Leitch*, R.C. Chou, R.J. Plunkett and T.J. Langan. Depts. of Neurol. and Neurosurg., School of Medicine, SUNY, Buffalo, N.Y., 14222.

Our laboratories described previously the recovery of adult astrocytes from gelatin implanted into injured rat striata and demonstrated their secretion of neurotrophic substances. Eventual therapeutic use of these astrocytes requires their expansion and long-term characterization.

Adult astrocytes survived in culture >10 mos. and were dramatically expanded through repeated passage. The type 1 astrocytic phenotype persisted after ≥6 passages as demonstrated by staining for the astrocyte markers GFAP, 7B11, and RAN2. The proliferative kinetics, however, differed markedly between early and late passage cultures. Primary cultures become confluent in 10-14 d, while passage ≥ 5 times caused confluence and proliferation arrest in only 1-2 d. Both early and late passage cultures secreted neurotrophic agents, as demonstrated by addition of astrocyte-conditioned media to a dorsal root ganglion bioassay. Neurite outgrowth occurred after 8 passages. Immunocytochemistry identified further the components of this neurotrophic potential. Late and early passage cells expressed the extracellular matrix molecule laminin, which facilitates neurite extension, and tenascin, which is known to guide neuronal processes. Both early and late passage astrocytes contained Glial Derived Neurotrophic Factor (GDNF), which promotes recovery in animal Parkinsonism.

In summary, astrocytes from injured adult rat brain survive in culture for many months, can be expanded dramatically, and even after repeated passage produce molecules with potential roles in brain regeneration.

(Supported by N.I.H. grant NS-30718 to T.J.L.)

594.20

CHRONIC IMPLANTATION OF SILICON SUBSTRATE ELECTRODES IN THE GUINEA PIG CORTEX: TISSUE TOLERANCE. P. A. Finger, D. J. Anderson*, J. A. Wiler, J. F. Hetke & R. A. Altschuler. Kresge Hearing Research Inst.; Dept. Electrical Engineering & Computer Sci., Univ. Michigan, Ann Arbor, MI 48019.

Multichannel electrode arrays fabricated using integrated circuit processing techniques have been developed for stimulating and/or recording in the central nervous system. These devices can be used in their planar form or can be assembled into 3-D structures for volume interaction with the tissue. We have examined the tissue compatibility of these devices after a three month placement in the guinea pig neocortex. Animals were fixed with paraformaldehyde for LM assessment or paraformaldehyde and glutaraldehyde for EM assessment. Brains were carefully removed, blocked, electrodes removed and blocks embedded in plastic and sectioned perpendicular to the electrode tracts. Six micron sections were cut and stained with Paragon stain for LM evaluation and thin sections cut for TEM evaluation. Oval electrode tracts were visible in the sections. They were surrounded by several lamina of glial processes. Neurons between the tracts and in the general region of the tracts had normal numbers and appearance, except for occasional flattening of the most proximal neurons. In several animals, the region at the base of the electrode tracts had increased gliosis and reactive cells, often asymmetric, particularly in animals where the probe platform became embedded in the cranium, rather than remaining free-floating on cortical surface. In conclusion, there is excellent tissue tolerance to a 3 month placement of various configurations of silicon substrate electrodes into guinea pig cortex, with minimal tissue reaction.

Supported by NIH grants P41 RR 09754 and NO1 NS 42303

594.21

SPECIES DIFFERENCES IN THE EFFECTIVENESS OF THE GLIAL-SPECIFIC METABOLIC BLOCKER FLUOROACETATE (FAC). T. C. Pellmar* and D. O. Keyser. Radiation Pathophysiology and Toxicology Department, AFRRI, Bethesda, MD 20889-5603, USA.

We previously reported that the metabolic integrity of glial cells in field CA1 of the guinea pig hippocampal slice is critical to maintenance of synaptic transmission (Keyser & Pellmar *Glia* 10:237, 1994). We have now tested the sensitivity of two other species, rats and monkeys, to the glial-specific metabolic inhibitor fluoroacetate (FAC). There is a dose-dependant reduction of ATP levels in hippocampal slices in all three species. Guinea pigs are most sensitive (IC_{50}= 31 μM) followed by monkeys (IC_{50}= 413 μM) and then rats (IC_{50}= 7.4 mM). Field potentials were recorded in field CA1 of rat and guinea pig hippocampal slices. In the guinea pig, micromolar concentrations of FAC caused a significant decrease in synaptic potentials within 30 min (IC_{50}= 65 μM), while in the rat concentrations of 10-20 mM were required to impair synaptic transmission (IC_{50}= 40 mM). Synaptic potentials in rat slices often showed transient decreases, with recovery occurring during the FAC exposure. These results suggest that species differences exist in glial responses to FAC and that phylogenetic order does not predict the degree of sensitivity. The differences may be due to disparate sensitivities of glial cells to FAC or to dissimilarities in glial-neuronal coupling. These results highlight the need to recognize species differences and to focus more research on the complexities of glial-neuronal communications.

Supported by AFRRI under work unit 00105.

PRESYNAPTIC MECHANISMS: RELEASE AND RECYCLING

595.1

SNAP-25 IS DIFFERENTLY REGULATED COMPARING WITH GAP-43 AND SYNAPTOPHYSIN IN DECENTRALIZED SCGs X.-E Hou*, K. Lundmark and A. Dahlström Dept. of Anatomy and Cell Biol., Göteborg University, S-413 90, Sweden

During development SNAP25, a 25-kD synaptosome associated protein, is strongly induced during late stages of axonal growth and at the onset of synapse formation. In contrast, GAP43, a growth associated protein, is highly expressed during the stage of axonal outgrowth and is decreased upon synaptogenesis. In our previous work it was observed that GAP-43 was up-regulated in the decentralized SCG together with some synaptic vesicle proteins, for instance synaptophysin. In the present work, we have studied changes in SNAP25 in the decentralized SCG and one of the nerve terminal areas (iris) innervated by the adrenergic SCG neurons, using immunofluorescence and immunoblotting methods. The content of immunoreactive SNAP25 showed no obvious changes in the nerve terminal network of irides after decentralization of SCG, by removing 15-20 mm of the cervical preganglionic trunk. On the other hand, in nerve terminals (probably preganglionic) in the SCG, SNAP25 was clearly decreased one day after decentralization, as was GAP-43 and synaptophysin. However, at later post-decentralization intervals (3, 8, 14 and 28 days) many GAP43 and synaptophysin positive varicose nerve terminals and nerve fibers were again observed in the SCG, apparently devoid of SNAP25. These newly appearing terminals are in all likelyhood not regenerated preganglionic terminals, but emanate from other sources, possibly from the principal neurons. Immunoblot of ganglia after decentralization showed an increase in GAP43 and synaptophysin but not in SNAP25. Together with our previous results, the results may indicate that the newly synthesized synaptic vesicles in decentralized principal neurons have a reduced content of SNAP25 in comparison to GAP43 and synaptophysin. Possibly, no increase in SNAP25 synthesis is necessary since a large fraction of this protein is located in the axolemma of the axons and nerve terminals, and it would therefore be possible for newly formed vesicles to recruit SNAP25 from the axolemma in sufficient amounts to match their need in order to function properly. Further experiments to explore the mechanism on the differential regulation of synaptic vesicle associated proteins in the SCG are in progress.

Supported by the Swedish MRC (2207) and by the Swedish Soc. for Med. Res.

595.2

INHIBITION OF CLATHRIN ASSEMBLY BY THE HIGH AFFINITY BINDING OF PHOSPHATIDYLINOSITOL(3,4,5)P_3 TO THE SYNAPSE-SPECIFIC CLATHRIN ASSEMBLY PROTEIN AP-3. W. Hao~, Z. Tan^, K.K. Reddy*, Q. Du~, J. R. Falck*, S. B. Shears^, and E. M. Lafer*~. ~ Institute of Biotechnology, University of Texas Hlth. Sci. Ctr., San Antonio, TX, 78245; ^Inositol Lipid Section, Laboratory of Cellular and Molecular Pharmacology, NIEHS, Research Triangle Park, NC, 27709; *Dept. of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX 75235.

It is now evident that in various cell types many of the proteins involved in vesicle traffic either participate in the metabolism of inositol lipids and inositol phosphates, or they tightly bind these compounds; however, the general significance of these observations remains to be determined. Within the nervous system, it is very important to elucidate these regulatory pathways in order to understand basic mechanisms of synaptic vesicle biogenesis and recycling. It is likely that regulation of these steps of the synaptic vesicle life cycle contribute to the changes in synaptic strength which underlie learning and memory. We have analyzed the interactions of the synapse-specific clathrin assembly protein AP-3 with inositol derivatives. In previously published studies (JBC 270:1564-8,1995) we showed that the amino terminal domain of AP-3 binds with high affinity to highly phosphorylated inositol polyphosphates, and that this binding inhibits AP-3 mediated clathrin assembly. We now report that AP-3 also binds with high affinity to a water soluble analog of PtdIns(3,4,5)P_3 (DiC_8PtdIns(3,4,5)P_3), the consequence of which is potent inhibition of AP-3 mediated clathrin assembly. We have also found that AP-3 is a good substrate for protein kinase A and casein kinase II, and we are characterizing the effects of AP-3 phosphorylation on the binding of AP-3 to inositol lipids and inositol phosphates.

Supported by NINDS NS29051 (EML) and NIGMS GM31278 (JRF).

595.3

HETEROGENEOUS PHENOTYPES OF DROSOPHILA MUTANTS IN SYNAPTIC VESICLE RECYCLING. D. Stimson*[1], P. S. Estes[2], K. S. Krishnan[3], and M. Ramaswami[1,2]. [1]ARL Division of Neurobiology, and [2]Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ, 85721. [3]Molecular Biology Unit, TIFR., Homi Bhabha Rd, Bombay, India, 400005.

In *Drosophila*, synaptic vesicle recycling requires the activity of the *shibire* (*shi*) gene product, a small GTPase homologous to mammalian dynamin. At restrictive temperature, shi^{ts} mutants undergo a complete block of synaptic vesicle endocytosis and eventually become paralyzed, following a depletion of synaptic vesicles at the neuromuscular junction (NMJ). Using a *Drosophila* larval NMJ preparation, we are investigating other mutations which potentially disrupt synaptic vesicle recycling, including $stoned^{ts}$ (*stn*), which enhances the paralytic phenotype of shi^{ts}. In addition to conducting electrophysiological analyses, we are developing optical methods to assay rates of synaptic vesicle recycling and endocytosis at mutant synapses. Our analyses of wildtype, *shi*, and *stn* synapses suggest that mutations which compromise the efficiency of synaptic vesicle recycling need not manifest themselves exclusively as defects in synaptic vesicle endocytosis. Indeed, under certain conditions, such mutations can alter levels of synaptic vesicle exocytosis.

NIH training grant (T32NS07363), grants from HFSB, McKnight endowment fund, NSF (IBN9511062)

595.4

ALTERNATIVE SPLICING GENERATES TWO SYNAPTOJANIN ISOFORMS WITH DISTINCT MEMBRANE BINDING PROPERTIES. A.R. Ramjaun and P. S. McPherson*. Montreal Neurological Inst., Dept. Neurology & Neurosurgery, McGill Univ., Montreal, QC, Canada H3A 2B4

Synaptojanin is a nerve terminal enriched inositol 5-phosphatase of 145 kDa which is thought to function in synaptic vesicle endocytosis. cDNA clones encoding the protein contain two open reading frames (ORFs) separated by an in-frame stop codon. ORF1 encodes the 145 kDa form of the protein which contains a proline-rich domain at its C-terminus with consensus sites for SH3 domain-mediated interactions. A 170 kDa isoform of synaptojanin, with additional SH3 domain-binding consensus sequences appears to be produced by the expression of ORF1 and ORF2. Here, we demonstrate that the two synaptojanin isoforms are generated by the alternative use of a 27 nucleotide exon which encodes the stop codon. Whereas the 145 kDa isoform is highly enriched in adult brain, the 170 kDa isoform is excluded from this tissue and has a widespread distribution in non-neuronal cells. Unlike the 145 kDa isoform, which can be removed from membranes by a low salt wash, the 170 kDa isoform remains membrane associated, even in the presence of 1 M salt. Consistent with this finding, the 170 kDa form, but not the 145 kDa form, can be isolated from membranes as part of a large molecular weight complex suggesting that the additional SH3 domain-binding sites present in the 170 kDa protein mediate a stable interaction with membranes, likely through an SH3 domain-containing protein(s). These properties may allow the 170 kDa isoform of synaptojanin to play a unique, and perhaps more general role in endocytosis, as compared to the 145 kDa isoform. (Supported by MRC-Canada and the Alfred P. Sloan Research Foundation).

595.5

GENERATION OF MICE LACKING SYNAPTOGYRIN (P29).
R. Janz*, R.E. Hammer and T.C. Südhof. Howard Hughes Medical Institute, UT Southwestern Medical Center, Dallas, TX, 75235-9050.

Synaptogyrin (p29) is an integral synaptic vesicle protein with four transmembrane domains (Stenius et al., 1995, JCB). It has a weak but significant homology to synaptophysin (p38), which has a similar overall structure and membrane topology. We have now cloned the mouse gene of synaptogyrin. The exon-intron structure showed conservation between the mouse and a homologous *C. elegans* gene, indicating a high degree of evolutionary conservation. In order to elucidate the biological function of the protein, we constructed a knockout vector that deletes the second coding exon of the gene and replaces it with the neomycin resistance gene. The vector was used for homologous recombination in embryonic stem cells and several homologous recombined clones where isolated. Heterozygous mice were derived from injection of the cells into mouse blastocysts and breeding of chimaeric offspring. Interbreeding of heterozygous mice then led to the generation of homozygous knockout mice which showed a total lack of the protein, as shown by western blot analysis, whereas the levels of other synaptic vesicle proteins like synaptophysin and synaptobrevin II showed no obvious change. The null mutant is viable, and a homozygous line could be established. These mice will be used for clarifying the biological role of synaptogyrin in an in vivo system and to study genetic interaction with other synaptic proteins by generating double knockout mice using already existing knockout mouse lines, such as synaptophysin (McMahon et al., 1996, PNAS).

Supported by the Howard Hughes Medical Institute, the W. Keck Foundation and a fellowship of the Deutsche Forschungsgemeinschaft.

595.6

CLEAVAGE OF SNAP-25 WITH BOTULINUM TOXIN A REDUCES THE CALCIUM SENSITIVITY OF THE RELEASE MACHINERY IN HIPPOCAMPUS. M. Capogna *, R. A. McKinney, B. H. Gähwiler and S. M. Thompson. Brain Research Institute, University of Zurich, CH-8029 Zurich Switzerland

The role of SNAP-25 in neurotransmitter release was examined by incubating rat hippocampal slice cultures with botulinum toxin A (BoNT/A, 30-60 ng/ml for 48 hrs). No immunoreactivity for the presynaptic protein SNAP-25 could be detected in treated cultures. The frequency of both spontaneous and mEPSCs, recorded in CA3 cells, was strongly reduced (30- and 12-fold) compared to control cultures. Furthermore, application of phorbol ester or α-latrotoxin did not produce Ca^{2+}-independent increases in mEPSC frequency after BoNT/A treatment. In contrast, spontaneous EPSCs were elicited in BoNT/A treated cultures when $[Ca^{2+}]_0$ was raised from 2.8 to 5 or 10mM (0.09 ± 0.02, 0.8 ± 0.2, 4.5 ± 0.9Hz, respectively). In addition, ionomycin increased the mEPSC frequency in the presence of 10mM Ca^{2+} (from 0.09 ± 0.02 to 2.6 ± 0.7Hz). Log-log plots of unitary EPSC amplitude vs $[Ca^{2+}]_0$, obtained with pair recording from CA3 cells, were linear, with a slope ~4 in control cultures. In BoNT/A treated cultures this relation also had a slope ~4 but was shifted to the right. Vesicular exocytosis is thus possible from synapses in which SNAP-25 has been cleaved by BoNT/A, although the sensitivity of the release machinery for Ca^{2+} is decreased, without changes in Ca^{2+} cooperativity.

Supported by the Swiss National Science Foundation (31-42174.94)

595.7

UNC-13 EXPRESSION IN WILD-TYPE AND MUTANT *C. ELEGANS*, R. J. Eustance, J. S. Duerr*, A. L. Duke, and J. B. Rand. Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

We are using a model organism, the nematode *Caenorhabditis elegans*, to study the processes underlying synaptic neurotransmission. The *unc-13* gene is important for normal neuronal function and may be part of a mechanism regulating neurotransmitter release. Mutations in *unc-13* result in resistance to aldicarb, an anti-cholinesterase. UNC-13 contains a region homologous to the regulatory region of PKC (Maruyama and Brenner, 1991) with phorbol ester and phospholipid binding properties, but its function in neurons is currently unknown.

We have generated antibodies against UNC-13 using fusion proteins representing amino acids 25-578 (A) or 903-1286 (B) of the 1734 amino acid UNC-13 protein (both fragments exclude the PKC homology region). Anti-UNC-13 sera appear to stain most or all neurons. Antibodies generated against A (anti-A) label synaptic regions; antibodies against B (anti-B) label neuronal processes uniformly. Mutations in *unc-104*, which cause mislocalization of synaptic vesicles, do not profoundly alter either staining pattern. We have examined twenty *unc-13* alleles; most show a severe decrease in staining with anti-A, but none show any change with anti-B. Our current hypothesis is that UNC-13 is located in synaptic regions (but probably not in synaptic vesicles) and is recognized by anti-A. We hypothesize that anti-B recognizes UNC-13 and another possibly related neuronal protein We are interested in identifying this protein and characterizing genes and proteins that interact with *unc-13*.

Supported by grants from NIH, NSF, OCMM, and OCAST.

595.8

A *DROSOPHILA* NEUREXIN IS REQUIRED FOR SEPTATE JUNCTION FUNCTION IN EMBRYONIC DEVELOPMENT, BLOOD BRAIN BARRIER FORMATION, AND IMAGINAL PATTERNING. J.T. Littleton*, M.A. Bhat, S. Baumgartner, K. Broadie and H.J. Bellen. Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030.

Intercellular junctions such as adherens and tight/septate junctions mediate cellular adhesion and serve as centers to organize proteins for specific intercellular signaling pathways. Neurexins are a family of transmembrane adhesion like molecules that were first identified as extracellular neuronal receptors for latrotoxin. Based on their structure and protein-protein interactions, it has been proposed that neurexins may function in synapse formation, synaptic vesicle docking or synapse modification. Interestingly, the role of neurexins may not be confined to the nervous system. Recently, the CASK protein has been identified as an intracellular ligand of neurexins. CASK is expressed in both neuronal and non-neuronal tissues and shares homology with a family of septate junction and synapse associated proteins. We have isolated and characterized a novel member of the neurexin family, Neurexin IV, that is specifically expressed in septate junctions of epithelial and glial cells of *Drosophila*. *nrx IV* encodes a transmembrane protein with EGF repeats and laminin G domains with an overall structure similar to that of rat and human Neurexins. In non-neuronal tissues, absence of NRX IV results in mislocalization of the septate junction specific protein CORACLE, a protein 4.1 homolog. Mutant embryos that lack NRX IV activity are paralyzed and have impaired ability to propagate action potentials, indicating that absence of NRX IV in glia causes a breakdown of the blood nerve barrier. The phenotypes of viable mutants reveal that NRX IV is required for ommatidial polarity in the compound eye and pattern formation in wing development. We propose that NRX IV is a transmembrane protein component of septate junctions that is essential for their function in embryonic development, blood brain barrier formation, and imaginal patterning.

This work was supported by an NIH training grant to J.T.L. K.B. is supported by the Wellcome Trust and H.J.B. is an Associate Investigator of HHMI.

595.9

SYNAPSIN ISOFORMS ISOLATED FROM LAMPREY REVEAL NOVEL CONSERVED DOMAIN STRUCTURE G. Stefani, H. T. Kao*, O. Shupliakov, L. Brodin, P. Greengard and V.A. Pieribone Lab. of Mol. and Cell. Neurosci., The Rockefeller University, NY, NY, 10021; Dept. Neurosci. Karolinska Inst., Stockholm, Sweden.

The synapsins are a family of neuronal phosphoproteins that have been implicated in the regulation of vesicle clustering and neurotransmission. Four mammalian proteins, designated synapsins Ia, Ib, IIa, and IIb, are formed by alternative splicing of two genes (synapsin I and II genes). Comparison of the sequence for each synapsin isoform suggests a domain model for the synapsins: all the synapsin isoforms share homology with each other at the amino part of each protein, while the carboxy ends show divergence.

The lamprey is an ancient vertebrate that possesses large pre-synaptic elements which are ideal for studying the physiology of an isolated synapse (*Nature* 375 (1995) 493-7). We have used antibodies directed against mammalian synapsins to screen a lamprey cDNA expression library, and have isolated cDNA clones encoding 3 different lamprey synapsins. Preliminary analysis of these clones indicates that they are similar to mammalian synapsin Ia, IIa and IIb. In contrast to the synapsins from mammals (human, bovine and rat) which show high protein sequence homology throughout, lamprey synapsins show high homology to mammalian sequences only over certain regions of the sequence while other domains are highly divergent. These are the first non-mammalian vertebrate synapsins to be isolated and the identification of highly conserved subdomains has revealed new structural and functional information about the synapsins. Supported by PHS grant # MH39327, MH19908, NARSAD and the Scottish Rite Schizophrenia Research Program.

595.10

SNAP-25 BINDING DOMAINS OF SYNTAXIN: AN ANALYSIS OF PRIMARY AND SECONDARY STRUCTURE. P. Zhong[1] *, Y. Chen[2], D. Tam[1], D. Chung[1], R.H. Scheller[2], G.P. Miljanich[1]. [1]Neurex Corporation, Menlo Park, CA 94025 and [2]Department. Molecular & Cellular Physiology, HHMI, Stanford University Medical Center, Stanford, CA 94305

Interaction between syntaxin 1A and SNAP-25 is a critical step in synaptic vesicle docking and fusion. In the present study, a simple microtiter plate binding assay was developed to study this protein-protein interaction quantitatively. A panel of peptides was prepared to encompass different sequences of syntaxin's H3 domain, the region important for SNAP-25 binding to syntaxin. Affinities of the peptides for SNAP-25 were then determined to evaluate the roles of regions of the H3 domain in this protein-protein interaction. A region of 32 amino acids (amino acids 189-220 of syntaxin) was found to be the minimal binding domain. Peptides spanning the C-terminal region (amino acid 221-266) of the H3 domain failed to bind to SNAP-25; however, supporting domains that reside within this region are required for optimal binding activity. Thus, extension of the C-terminus of the minimal binding domain greatly enhanced the affinity for SNAP-25. Selected mutations in evolutionarily conserved residues of a putative amphiphilic α-helix within the minimal binding domain greatly reduced affinity for SNAP-25. Circular dichroism (CD) confirmed the presence of amphiphilic α-helicity in the syntaxin H3 domain and the 32-mer minimal binding domain. The CD analysis further suggests that integrity of the 32-mer domain is crucial for maintaining the helical conformation of the entire H3 domain and the C-terminal region is responsible for further stabilizing this conformation. Thermal stability analysis demonstrated that this α-helicity is required for SNAP-25 binding activity and for inhibition by syntaxin peptides of [^{3}H]-norepinephrine release from PC12 cells. Funded by Neurex Corp.

595.11

LONG TERM POTENTIATION AND BIOCHEMICAL ALTERATIONS IN THE EXOCYTOTIC APPARATUS. J. E. A. Braun and D. V. Madison* (Department of Molecular and Cellular Physiology, Stanford University Medical Center, Stanford CA 94305)

Upon the pharmacological induction of persistant synaptic potentiation with 4-aminopyridine in rat hippocampal slices, we observed a two-fold increase in an SDS resistant protein complex, composed of the proteins SNAP-25, syntaxin and VAMP. Potentiation was recorded in field EPSP's resulting from Schaffer collateral stimulation in area CA1 of rat hippocampal slices. Hippocampal slices were subsequently solubilized in Triton X-100, isolated by SDS-PAGE and quantitated by densitometry. In addition, larger 7S and 20S complexes, composed of synaptotagmin, α-SNAP and NSF as well as SNAP-25, syntaxin and VAMP were isolated by immunoprecipation or glycerol gradient fractionation and quantitated by densitometry. These protein complexes, or "SNARE complexes", have been proposed to account for synaptic vesicle "docking" to the presynaptic plasma membrane. Each step of the exocytotic pathway is thought to be mediated by a specific protein complex-such that a sequence of protein complex assemblies and dissassemblies underlie the exocytotic cycle. The correlation of an increase in synaptic efficacy with an increase in "docking complexes" implicates more docked vesicles in processes expressing LTP. Supported by Conte Center MH48108.

595.12

ISOLATING AND IDENTIFYING SYNAPTIC RIBBON ASSOCIATED PROTEINS Tri-Hung Nguyen* and Grant W. Balkema. Biology Department, Boston College, Chestnut Hill, MA 02167.

We have now identified four proteins that cosediment with synaptic vesicles and are labeled by the monoclonal antibody (B16). The B16 mAb has been found to label synaptic ribbons in the retina and recognizes multiple protein bands in a retinal homogenate. One epitope was found on the 88-kDa protein, aconitase, which is found in the synaptosomal fraction. The epitope was mapped to a ten amino acid region (DTYQHPPKDS) containing a double proline "kink". Here, we report the purification and identification of these proteins.

Cow retinas were isolated and washed in iso-osmotic sucrose solution. Retinas were gently homogenized and centrifuged at different speeds (150, 800 and 25,000 x g) to isolate photoreceptor synaptosomes. Relatively pure populations of synaptosomes were hypo-osmotically lysed to release synaptic ribbons and synaptic vesicles. Retinal synaptic vesicles were sedimented by centrifugation after a high speed spin (100,000 x g). Samples from each centrifugation step were analyzed on SDS-polyacrylamide gels and Western blots.

The B16 mAb recognizes four proteins: 66-, 70-, 100- and 110-kDa that cosediment with synaptic vesicles as indicated by anti-synaptophysin antibody. We have isolated, sequenced and identified three peptides resulting from protease digestion of the 100-kDa protein to be the PTB-associating splicing factor, that is rich in proline. These results are consistence with the idea that the B16 mAb recognizes proteins with a shared conformational epitope and bind to synaptic vesicles.

(Supported by NSF IBN-9320039 and Boston College Research Fund.)

595.13

NT75, THE S-7B8 ANTIGEN, IS A COMPLEX COMPOSED OF SNAP-25 AND SYNAPTOBREVIN/VAMP. T.C. Ritchie*, D. Kluge, S. Zielske and J.D. Coulter. Dept. of Anatomy & Neuroscience Program, University of Iowa, Iowa City, IA 52242.

The S-7B8 antibody recognizes a nerve terminal antigen that is neuron-specific and is both developmentally regulated and differentially regulated in distinct populations of mature neurons. Initially, the S-7B8 antigen was identified as a 75 kDa protein, NT75, on Western Blots of non-reduced proteins. However, purified NT75, when reduced and boiled, resolves into two bands of 29 (p29) and 17 kDa. Tryptic fragments of the major protein, p29, were subjected to microsequencing, and a total of 53 residues of high confidence sequence were obtained from three peptides. A search of protein databases showed the p29 sequence to have 100% identity with the published sequence of SNAP-25. A comparison of Western blot staining with commercial antibodies and antibodies to p29 and NT75 confirmed the identity of p29 as SNAP-25 and showed that the 17 kDa subunit of NT75 is synaptobrevin/VAMP. Immunocytochemical staining patterns of S-7B8 and anti-SNAP-25 are largely similar, as both antibodies yield selective dense labeling in subpopulations of nerve terminals in adult spinal cord and brain. The experiments show that the S-7B8 antigen is SNAP-25. Our previous studies on S-7B8 are consistent with the idea that the function of SNAP-25 is not restricted to synaptic vesicle cycling, but that SNAP-25 is also likely to have roles in the formation, maintenance and plasticity of distinct populations of nerve terminals. Supported by NIH grant NS23783.

595.14

CHOLINE ACETYLTRANSFERASE UNDERGOES COVALENT MODIFICATION BY THE ADDITION OF LONG CHAIN FATTY ACIDS. M. C. Resendes, R. Jane Rylett*, Department of Physiology, University of Western Ontario, London Ont. Canada, N6A 5C3.

The neurotransmitter acetylcholine is synthesized by the enzyme choline acetyltransferase (ChAT) which exists in both hydrophilic (cytosolic) and amphiphilic (membrane-bound) form. Distinct physiological roles have not been ascribed to the different subcellular fractions of ChAT, nor is it clear how the different forms of enzyme are generated or how distribution within the different pools is regulated. The covalent attachment of primarily myristic or palmitic fatty acid to a wide range of cellular proteins is known to dramatically alter both their subcellular distribution and function. Analysis of amino acid sequences deduced from published cDNAs reveals that human, rat, pig and mouse ChAT contains elements of myristoylation and/ or palmitoylation consensus sequences. To examine if ChAT undergoes modification by acylation, cholinergic human neuroblastoma LA-N-2 cells were incubated in the presence of ^{3}H-myristic or ^{3}H-palmitic acids (0.5 and 1 mCi/mL media, 24 h, respectively). Following the labelling period, ChAT was immunoprecipitated and separated by SDS-PAGE. Gels were either immunoblotted for ChAT or lanes were cut at 2mm intervals and digested in 30% H_2O_2 for quantitation of ^{3}H. In both ^{3}H-myristic or ^{3}H-palmitic labelled cells, a 20-30 fold increase in ^{3}H activity was observed in gel slices corresponding to the location of ChAT immunoreactivity from immunoblots. Based upon this data, it would appear that ChAT can exist as an acylprotein in cholinergic cells. Whether acylated ChAT regulates the function or the subcellular localization of the enzyme is currently being investigated.

Supported by the Medical Research Council of Canada

595.15

TRANSLOCATION OF TETANUS TOXIN H_N-L THROUGH MEMBRANES. T. Lücke, H. Göschel, T. Binscheck, U. Weller[†], and H. Bigalke*. Institute of Toxicology, Hannover Medical School, 30623 Hannover, Germany. [†]Institute of Microbiology, Johannes Gutenberg University, 55101 Mainz, Germany.

Tetanus toxin (TeTx) is composed of two chains. The light chain (L) proteolyses synaptobrevin II and the heavy chain (H) navigates the toxin from the extracellular space into the cytosol via the endosomal compartment. The passage through the endosomal membrane occurs only at low pH. Under conditions corresponding to those prevailing in endosomes, the light chain linked with the N-terminal half of the heavy chain (H_N-L) inhibited transmitter release from chromaffin cells and motonerve-endings. This inhibition was not prevented by the ATPase inhibitor, bafilomycin A1, indicating that H_N-L bypassed the endosome on its route into the cytosol. At physiological pH H_N-L was ineffective. At low pH, but not at neutral pH, H_N-L formed pores in inside-out patches derived from the plasma membrane of chromaffin cells. Whereas the pore formation was also caused by both TeTx and H_N, only TeTx, and not H_N, blocked exocytosis. The C-terminal half of the heavy chain (H_C) and L neither formed pores nor did they inhibit noradrenaline release. We conclude that H_N, at low pH, mediates the passage of TeTx through the endosomal membrane. The passage may be accompanied by pore formation, which is recorded as an increase in membrane conductance. (Supported by the German Research Council, Bi 274/8-1.)

595.16

BASSOON, A NOVEL PRESYNAPTIC PROTEIN FROM RAT BRAIN. E.D. Gundelfinger*, K. Langnäse, L. Sanmarti-Vila, H. Wex, K. Richter, J.T. Fränzer, C.C. Garner[#]; BL-Institute for Neurobiology, P.O.Box 1860, D-39008 Magdeburg, Germany and [#]Neurobiol. Res. Center, Univ. of Alabama, S-Birmingham, AL 35213-0021, USA

The knowledge of structure and function of the molecular components is prerequisite to the functional comprehension of synapses at a molecular level. To get access to novel synaptic proteins we have screened a rat brain expression library with polyclonal antisera against a rat brain synaptic protein preparation. Bassoon is one of the gene products newly identified by this approach. The partial nucleotide sequence of the Bassoon cDNA does not have any correlate in public databases. Northern analysis of various rat tissues identified a brain-specific transcript of 12kb. A wide distribution of Bassoon transcripts in the rat brain has been revealed by *in situ* hybridization. Antibodies against bacterially expressed Bassoon fusion protein recognize multiple bands in the molecular weight range of 150 to >300 kd on Western blots of rat brain protein preparations. The signal is highly enriched in the synaptic junctional protein fraction. Immunohistochemically Bassoon has been detected in synaptic neuropil regions of all parts of the brain. At the ultrastructural level, an ecxclusive presynaptic localization has been observed, e. g. in excitatory mossy fiber terminals in the hippocampus as well as in parallel fiber terminals in the molecular layer and mossy fiber terminals in the granular layer of the cerebellum. Immunoreactivity appears to be restricted to the active zone. Therefore, we hypothesize that Bassoon could play a role in the presynaptic machinery organizing the presynaptic cytomatrix and/or orchestrating events of the synaptic vesicle cycle. Supported by the German Federal Government (BMBF) and the Land Saxony-Anhalt.

595.17

SELECTIVE DEPLETION OF CLEAR SYNAPTIC VESICLES AND ENHANCED QUANTAL TRANSMITTER RELEASE AT FROG MOTOR NERVE ENDINGS PRODUCED BY TRACHYNILYSIN, A TOXIN ISOLATED FROM THE STONEFISH (*Synanceia trachynis*) VENOM. C. Colasante[1,2], F.A. Meunier[1], A.S. Kreger[3], J. Molgó[1] and J. Bruner[1]*. [1]Lab. Neurobiologie Cellulaire et Moléculaire, C.N.R.S., 91198 Gif sur Yvette Cedex, France; [2] Centro de Microscopía Electrónica, Univ. Los Andes, Mérida, Venezuela; [3]Medical Sciences Research Institute, Herdon, Virginia 22070, USA.

The protein toxin trachynilysin (TLY) significantly increased spontaneous quantal acetylcholine (ACh) release from motor endings, as detected by recording miniature end plate potentials (MEPPs) in isolated frog cutaneous pectoris neuromuscular preparations. Ultrastructural analysis of nerve endings in which quantal ACh release was stimulated to exhaustion by 3 hrs exposure to TLY revealed swelling of nerve terminals and a marked depletion of small clear synaptic vesicles (SVs). The number of large dense core vesicles (LDCVs) per terminal cross-section remained unaffected. LDCVs contained calcitonin gene-related peptide (CGRP), as revealed by colloidal gold immunostaining, and TLY-treated nerve endings exhibited similar CGRP-like immunofluorescence as untreated endings. TLY-induced ACh release caused permanent incorporation of SV membrane into the axolemma as revealed by synaptophysin immunolabeling. We conclude, that the ability of stonefish (*S. trachynis*) venom to elicit spontaneous quantal ACh release from motor nerve endings is a function of TLY, which selectively stimulates the release of Svs and impairs their recycling but, does not affect the release of LDCVs. Supported by Direction des recherches Etudes et Techniques (grant 94/067).

LONG-TERM POTENTIATION: PHYSIOLOGY IV

596.1

ODQ, A SELECTIVE INHIBITOR OF NO-ACTIVATED GUANYLYL CYCLASE, BLOCKS THE INDUCTION OF LONG-TERM DEPRESSION IN RAT HIPPOCAMPUS.

A.T. Gage* and P.K. Stanton. Departments of Neuroscience & Neurology, Albert Einstein College of Medicine, Bronx, NY 10461-1602

The putative retrograde messenger nitric oxide (NO) is controversially suggested to play a role in inducing both long-term potentiation (LTP) and long-term depression (LTD) of synaptic strength. NO activates two separate enzymes,an NO-stimulated guanylyl cyclase (NOGC) and an ADP ribosylase. The putative guanylyl cyclase inhibitor LY83583 has been reported to block induction of LTP. However, this compound is a more potent inhibitor of nitric oxide synthase, leaving the question of the role of cyclic GMP unanswered for either LTP or LTD. We utilized a recently developed selective NOGC inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), to determine whether NOGC activation is necessary for the induction of hippocampal LTD.

We recorded in *in vitro* hippocampal slices from 13-28 day old male Sprague-Dawley rats in an interface chamber at 34° C. Schaffer collateral axons synapsing on CA1 pyramidal neurons were stimulated each 60 sec, and evoked excitatory postsynaptic potentials (epsps) recorded in stratum radiatum. After a 30 min baseline, 2 μM ODQ was bath applied for 1 hour, after which low-frequency stimuli (LFS; 1 Hz/15 min) were given to induce LTD. ODQ alone had no significant effect on Schaffer collateral-evoked epsp slopes. However, it did block the induction of LTD as assessed 60 min post-LFS. Control LTD in untreated slices = -22.9 ± 3.2% ($p<0.05$, paired t-test, n=22); ODQ-treated slices = -5.7 ± 4.7% (n=16, no significant LTD). In some slices, after an initial LFS in the presence of ODQ, the drug was washed out for 60 min, after which a second identical LFS now elicited stable LTD. At previously potentiated synapses, ODQ showed less effective blockade of LTD. Thus, we conclude that NOGC activation is a necessary step in the induction of *de novo* LTD, but, perhaps, only a partial contributor to depotentiation. (Supported by NIMH Grant #45752)

596.2

INDUCTION OF HIPPOCAMPAL LTD REQUIRES RELEASE OF CA^{2+} FROM SEPARATE PRE- AND POSTSYNAPTIC STORES AND ACTIVATION OF PRESYNAPTIC CAM KINASE II. Magali Reyes, Allyson T Gage and Patric K. Stanton*. Dept. of Neuroscience, Albert Einstein Coll. Med., Bronx, NY 10461.

Postsynaptic increases in $[Ca^{2+}]$ and activation of Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) are both necessary to induce long-term potentiation (LTP) of synaptic strength. A smaller rise in postsynaptic $[Ca^{2+}]$ also appears to be required to induce long-term depression (LTD). It is not known whether Ca^{2+} for LTD comes from influx and/or intracellular stores, if pre- and/or postsynaptic stores are necessary, or if activation of CAMKII is required to induce LTD.

Bath application of thapsigargin or cyclopiazonic acid, which deplete all intracellular Ca^{2+} pools, blocked induction, but not maintenance, of LTD by low frequency stimuli (1Hz/15min) at Schaffer collateral-CA1 synapses in hippocampal slices. Injecting thapsigargin into single CA1 pyramidal cells also prevented LTD. Selectively depleting Ca^{2+}-gated stores with bath applied ryanodine also blocked LTD, but postsynaptic injection did not. Thus, LTD requires Ca^{2+} release from *presynaptic* ryanodine stores and *postsynaptic* IP_3-gated stores.

Extracellular application of the CAMKII inhibitor KN-62, at a concentration which blocks LTP, also prevented induction of LTD. In contrast, impaling CA1 pyramidal neurons with electrodes containing KN-62 did not impair LTD, although it did block LTP in the same cells. Thus, *presynaptic* CAMKII is necessary to induce LTD, while *postsynaptic* CAMKII plays a role in LTP. (NIMH Grant #45752)

596.3

DE-POTENTIATION OF LTP OF SYNAPSES BETWEEN PRIMARY AFFERENTS AND DORSAL HORN NEURONS. J.C. Marvizon* and E.A. Mayer. CURE: Dig. Dis. Res. Cent., Dept. of Medicine, UCLA, Los Angeles, CA 90073.

Transversal spinal cord slices with attached dorsal roots and ganglions were prepared from 14-30 days rats as described by Randic et al., J. Neurosci. 13, 5228-5241 (1993). Intracellular recordings from neurons in the substantia gelatinosa were used to study their synaptic responses to electrical stimulation of the dorsal root. The average resting membrane potential and membrane input resistance of 22 neurons was -68 ± 2 mV and 170 ± 16 MΩ, respectively. Synaptic plasticity was induced by delivering a tetanus to the dorsal root consisting of three trains of 1 s at 10 s intervals of high frequency (100 Hz) pulses (6-20 V, 0.4 ms). This tetanus produced LTD in two neurons, evidenced by a long lasting (40 min) decrease in EPSP amplitude. LTP was induced in three other neurons by the same tetanus accompanied by a depolarization of the neuron to about -60 mV. The membrane potential was kept constant at -85 mV except during the tetanus. After the presence of LTP was established for 30 min., a second, identical tetanus was delivered while hyperpolarizing the neuron to -100 mV. Except for a short initial increase (post-tetanic potentiation), the evoked EPSPs were significantly lower than after the first tetanus but higher than the baseline, remaining unchanged for the rest of the recording (40 min). A similar de-potentiation of LTP was observed in three neurons. These results indicate that the membrane potential of substantia gelatinosa neurons determines the direction of the synaptic plasticity induced by tetanic stimulation of the dorsal root. Moreover, since changes in the membrane potential restricted to the duration of the tetanus are sufficient to produce this effect, they seem to be involved in induction but not the expression of synaptic plasticity. Further studies are necessary to determine whether LTD and de-potentiation of LTP are the same or different phenomena in these neurons. (Supported by a Pilot and Feasibility Award, NIH 5P30 DK41301)

596.4

LOW FREQUENCY STIMULATION OF AFFERENT Aδ-FIBERS INDUCES LONG-TERM DEPRESSION OF PRIMARY AFFERENT NEUROTRANSMISSION. J. Sandkühler* and M. Randić. Department of Veterinary Physiology and Pharmacology, Iowa State University, Ames, IA, 50011.

Fine primary afferent nerve fibers including nociceptors terminate in laminae I and II of the spinal dorsal horn. It is believed that this region is important for plastic changes in spinal nociception. Here we have identified a robust long-term depression (LTD) of primary afferent neurotransmission in Aδ-fibers which can be induced by repetitive low frequency stimulation (LFS) of dorsal roots (DRs). Intracellular recordings were made from lamina II neurons in transverse slices of young (18-27d) rat spinal cord. Electrical stimulation of L4 or L5 DRs evoked stable mono- or polysynaptic EPSPs in all neurons studied. Conditioning stimulation of DRs consisted of 900 pulses (10V, 0.1ms) given at 1 Hz. As a quantitative parameter of synaptic strength the peak amplitude of Aδ-fiber-evoked EPSPs was measured. LFS of afferent Aδ-fibers caused a stable, long-lasting decrease in synaptic efficacy (EPSP amplitude decreased to 40.8 ± 10.3% of control; n=8). When A- and C-fibers were recruited conditioning stimulation was as effective as A-fiber stimulation alone. This form of LTD required activation of postsynaptic NMDA receptor, since D-APV (50μM), the NMDA receptor antagonist, reversibly reduced or abolished the generation of LTD (86.1 ± 13.5%, n=7). LTD is independent of $GABA_A$ or glycine receptor activation. In the presence of calyculin A (100nM), an inhibitor of protein phosphatases 1 and 2A, LTD was somewhat more expressed (to 15.0 ± 6%, n=4). Following LTD synaptic strength could be increased to its original maximal level, indicating that LTD is reversible and not due to deterioration of individual synapses. This LTD of primary afferent neurotransmission may be involved in long-lasting segmental antinociception following afferent stimulation. *Supported by the NINDS, the National Science Foundation and Deutsche Forschungsgemeinschaft.*

596.5

A ROLE FOR *MU* OPIOID RECEPTORS IN LONG-TERM DEPRESSION IN SUBSTANTIA GELATINOSA OF THE SPINAL CORD, J. Zhong and M. Randić*, Dept. of Veterinary Physiology and Pharmacology, Iowa State University, Ames, IA 50011.

Evidence indicates that *mu* opioid receptor activation attenuates primary afferent neurotransmission, and is also involved in the mediation, or modulation, of activity-dependent synaptic plasticity in the brain. We have reported the existence of long-lasting decrease in synaptic strength in the spinal substantia gelatinosa (SG) neurons resulting from the low frequency stimulation (LFS) of primary afferent Aδ-fibers (Sandkühler and Randić, *this meeting*). The underlying mechanism of this phenomenon is not known. To assess the role of opioid receptors in the induction of long-term depression (LTD) of primary afferent neurotransmission we examined whether naloxone, a non-selective opioid receptor antagonist, and CTAP, a selective *mu* opioid receptor antagonist, could affect the LFS-induced LTD using conventional intracellular recording from the SG neurons in transverse slices of young rat (17-22-d-old) spinal cord. The strength of primary afferent neurotransmission was assayed by measuring the peak amplitude of EPSPs that result from stimulation of primary afferent fibers in L4 or L5 dorsal roots (DRs) with electrical shocks of 3-8V (0.1ms duration) intensity. Conditioning stimulation of DRs, which consisted of 900 pulses given at 1 Hz, produced a long-term depression of EPSP amplitudes (to 40.8 ± 10.3% of control, n=8) in SG neurons when Aδ-fibers were recruited. In the presence of extracellular naloxone (1μM), or CTAP (100nM), the same LFS did not cause LTD (CTAP: 136.1 ± 30.8%, n=6). This finding suggests that the activation of *mu* opioid receptors is required for LTD of EPSPs at primary afferent synapses with neurons in the SG. *Supported by the NINDS and the NSF.*

596.6

INDUCTION OF NEOCORTICAL DEPOTENTIATION AND LONG-TERM DEPRESSION IN THE ADULT BEHAVING RAT. David J. Froc*, Christopher Trepel, and Ronald J. Racine. Department of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

We have shown that long-term potentiation (LTP) in the chronically prepared rat requires multiple, spaced stimulation sessions. Long-term *depression* (LTD), in which baseline responses are depressed, and *depotentiation*, in which previously potentiated responses are depotentiated towards baseline levels, have both been demonstrated in slice preparations. However, there is little evidence of these phenomena in the awake, freely moving rat. Here we report that both neocortical depotentiation and LTD can be readily induced in the adult behaving rat. Animals were implanted with stimulating and recording electrodes located in the corpus callosum and anterior neocortex (parietal area 1), respectively. The depotentiation group received sixty 8-pulse (0.1 msec biphasic ; 1259 μA), 24 msec, 300 Hz (train frequency=0.1 Hz) trains per day to induce asymptotic levels of potentiation prior to LF stimulation. I/O measures were also taken on a daily basis, to monitor the effects of this stimulation protocol, and immediately prior to and after LF stimulation. LF stimulation consisted of 900, 1259 μA pulses delivered at 1 Hz. Control animals were similarly potentiated to asymptote but did not receive LF stimulation. Following LF stimulation, all experimental animals showed a significant decrease in the late component of the evoked field potential which was still evident 24 hours later. Depotentiation was less pronounced and more transient in contralateral than in ipsilateral recording sites. The LTD animals received the same LF stimulation used to induce depotentiation. Relative to controls a significant reduction in the amplitude of the evoked field potential was observed. We have demonstrated that neocortical synapses are bidirectionally modifiable and that LTD may be induced in naive synapses following one LF stimulation session in the adult behaving rat.

596.7

BRAIN-DERIVED NEUROTROPHIC FACTOR BLOCKS LONG-TERM DEPRESSION IN RAT VISUAL CORTEX, Y. Akaneya, T. Tsumoto* and H. Hatanaka†. Dept. of Neurophysiology, Biomed. Res. Ctr., Osaka Univ. Med. Sch., Suita, 565 Japan and †Div. of Prot. Biosynth., Inst. for Prot. Res., Osaka Univ., Suita, 565 Japan

Brain-derived neurotrophic factor (BDNF) is suggested to play a role in formation of ocular dominance columns and input-dependent modification of neuronal function in developing visual cortex. Since long-term depression (LTD) is supposed to be a synaptic basis for such a use-dependent alteration in structure and function of visual cortex, we have studied effects of BDNF on the induction of LTD in visual cortical slices prepared from young rats. Layer II/III field responses evoked by test stimulation of layer IV at 0.1 Hz were not significantly changed by BDNF at the concentration of 20 ng/ml. On the other hand, BDNF at this concentration blocked the induction of LTD of field responses by low-frequency stimulation (1 Hz for 15 min) of layer IV. This action of BDNF was antagonized by K252a, an inhibitor of receptor tyrosine kinases, at the concentration of 200 nM. When K252a alone was applied to slices, LTD of the stronger magnitude than in control slices was induced while responses to test stimulation were not significantly changed, suggesting that endogeneous BDNF may prevent synapses from being depressed to some degree during low-frequency inputs. These results suggest that BDNF may play a role in use-dependent changes in synaptic connectivity in the developing visual cortex. Supported by a Grant-in-aid from the Japanese Ministry of Education, Science, and Culture.

596.8

LTD INDUCTION IS NMDAR-INDEPENDENT, AND DEPENDENT ON T-TYPE Ca CHANNELS ACTIVATION AND Ca RELEASE FROM INTRACELLULAR STORES Y. Wang, M.J. Rowan and R. Anwyl*, *Physiology, Trinity College, Dublin 2, Ireland.*

Long-term depression (LTD) can be induced by a period of low frequency stimulation (LFS). In the present studies, we have carried out an investigation into the type of Ca channel responsible for LTD induction.

LTD of epsps or whole-cell patch-clamped epscs in rat dentate gyrus was induced by prolonged LFS of 900 stimuli at 1Hz, or by an intracellular pairing procedure under voltage clamp conditions. LTD induced by these two procedures measured 40±2%, n=8 and 37±1%, n=7, respectively, at 30 min post-stimulation. The induction of LTD was independent of activation of NMDA receptors. Thus LTD of epscs induced by the pairing procedure in the presence of the NMDA receptor antagonist D-AP5 (100μM) measured 35±1%, n=5, a value not significantly different from control (P<0.001). The induction of LTD of field epsps by 1Hz LFS was inhibited by Ni (50μM), but not by nifedipine (10μM), the LTD measuring 1±1%, n=5, and 36±2%, n=5, in Ni and nifedipine respectively. These experiments suggest that the induction of LTD may be mediated by Ca influx via T-type, but not L-type, voltage-dependent Ca channels. Some evidence was also found that the induction of LTD requires the release of Ca from intracellular stores. Thus the induction of LTD of epscs was significantly inhibited by intracellular application of ruthenium red (20 μM), an agent known to bind to, and block the release of Ca from, the intracellular enoplasmic reticulum ryanodine receptor (LTD =1±2%, n=5). LTD of the field epsp was also inhibited by thapsigargin, an agent known to deplete intracellular Ca stores by inhibiting endoplasmic reticulum Ca-ATPase. Thus in 2μM thapsigargin, LFS of 1Hz induced LTD of 2±1%, n=5, a significant inhibition of LTD.

596.9

CESIUM PREVENTS LTD MAINTENANCE AND CAUSES EPILEPTIFORM ACTIVITY BY AN EFFECT ON BOTH NEURONAL AND GLIAL CELLS. S. Gasparini+, R. D'Ambrosio, D. DiFrancesco and D. Janigro*, Univ. of Milan+ and Dept. of Neurosurgery, Univ. of Washington, Seattle, WA

We have investigated the effects of brief and prolonged applications of extracellular cesium (Cs^+) on the electrical properties of CA1 pyramidal cells, interneurons and astrocytes by using the *in vitro* hippocampal slice. Field potential recordings revealed that Cs causes synchronous, interictal-like bursting and prevents maintenance of long-term depression (LTD). Whole cell recordings showed that brief (<2 min.) bath exposures to Cs^+ caused pyramidal cell hyperpolarization associated with decreased membrane conductance due to blockade of an inward h-type current. After prolonged exposures a late depolarizing response was observed; this effect was not associated with changes in membrane conductance and vanished when the rate of slice perfusion was increased. Recordings from interneurons revealed that I_h is expressed in a subpopulation of interneurons and that Cs effects on interneurons expressing I_h are comparable to those observed in pyramidal cells. Consistent with this effect, the early component of the IPSP recorded in pyramidal cells was decreased by Cs^+. However, evoked population-spikes were depressed by Cs^+. The specific neuronal I_h blocker Zeneca ZD-7288 failed to affect LTD and did not cause synchronous bursting. We concluded that Cs^+ actions on LTD and Cs-induced epileptiform activity were not due exclusively to its effects on neurons. Recordings from astrocytes revealed that, in contrast to pyramidal cells, glial cells were depolarized by Cs^+. Cs^+ depressed glial depolarizations induced by elevation of $[K]_o$. Thus, the effects of Cs^+ on CA1 synchronization and synaptic plasticity appear to be mediated by both glial and neuronal cells. Supported by NIH 51614.

596.10

LONG-TERM DEPRESSION IN THE CA1 REGION OF THE ADULT RAT HIPPOCAMPUS *IN VITRO*. N. Kemp and Z. I. Bashir*. Department of Anatomy, Medical School, University of Bristol, Bristol, BS8 1TD, UK.

Long-term depression (LTD) can readily be induced in the CA1 region of young (up to 3 weeks old) but not adult rat hippocampal slices by low frequency stimulation (LFS) of the Schaffer collateral-commissural pathway (SCCP). We demonstrate that under certain conditions LFS can induce LTD in slices from adult rats.

Hippocampal slices were prepared from female adult rats (170-220g; ~ 8 weeks old). The SCCP was stimulated at 0.033 Hz and standard techniques were used to record field excitatory post-synaptic potentials (EPSPs) from the stratum radiatum. Results are expressed as a change in the slope of the field epsp 30 minutes after the end of LFS (900 pulses, 1 Hz) relative to the pre-LFS baseline.

Two periods of LFS separated by at least 30 minutes resulted in only a small depression of synaptic transmission (5 ± 1 % and 3 ± 6 %, respectively; mean ± sem; n=6). In the presence of the adenosine A1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; 100 nM) two periods of LFS resulted in a depression of 8 ± 3 and 17 ± 2 %, respectively (n=6). When the experiments were repeated in the additional presence of the NMDA receptor antagonist, D-2-amino-5 phosphonopentanoate (D-AP5; 50 μM) a depression of 4 ± 3 (n=7) and 13 ± 3 % (n=5) resulted. In a separate series of experiments, LFS in control conditions followed by LFS in the presence of the $GABA_A$ receptor antagonist, picrotoxin (50 μM) resulted in depression of 2 ± 7 and 18 ± 9 %, respectively (n=5).

These results show that LTD can be induced in adult rat hippocampal slices if LFS is given either during adenosine A_1 receptor blockade or during $GABA_A$ antagonism. The LTD obtained in the presence of DPCPX appears to be NMDA receptor independent since it is not blocked by D-AP5.

Supported by the Wellcome Trust.

596.11

TWO FORMS OF HOMOSYNAPTIC LONG-TERM DEPRESSION COEXIST AT HIPPOCAMPAL CA3-CA1 SYNAPSES

Stéphane H.R. Oliet*, Robert C. Malenka and Roger A. Nicoll.
Depts. of Cellular & Molecular Pharmacology, Physiology and Psychiatry; University of California, San Francisco CA 94143-0450.

In hippocampal CA1 pyramidal neurons of neonatal rats (3-7 days old), activation of L-type calcium channels paired with the activation of metabotropic glutamate receptors induces a long term depression (LTD) of synaptic efficacy at CA3-CA1 synapses [Science 264: 1148-1152, 1994]. This form of LTD appeared to be independent of NMDA receptor (NMDAR) activation and is not associated with changes in quantal size. Interestingly, hippocampal neurons in culture express a form of LTD with similar properties [Neuron 16:103-111, 1996]. This is different from the homosynaptic LTD typically described in older animals (14-30 days) which requires activation of NMDARs [Neuron 9:967-975, 1992] and is accompanied by a decrease in quantal size [Science 271: 1294-1297, 1996]. It was postulated that the differences between these two forms of LTD is related to the ages of the animals. In order to test this hypothesis, we carried out experiments with rats of different ages (6 to 38 days), using standard electrophysiological techniques. Preliminary results indicate that both forms of LTD coexist in neonatal (6-7 days) and older animals (19-38 days). These two forms could be studied in isolation using either APV or MCPG to respectively block the NMDA-dependent and -independent LTD. We are presently carrying out experiments to determine the interaction between both forms of LTD. *Supported by the International Human Frontier Science Program and the NIH.*

596.12

POSTSYNAPTIC COMPONENT IN THE MAINTENANCE PHASE OF LONG-TERM DEPRESSION OF THE RAT HIPPOCAMPUS.

K. Kato[(1)], T. Inoue[(2)] * and K. Mikoshiba[(1)(2)], [(1)] Lab. of Physiology, CalcioSignal Net Project, ERATO, JRDC, Meguro-ku, Tokyo 153, JAPAN, [(2)] Dept. of Molecular Neurobiology, Institute of Medical Science, Univ. of Tokyo, Tokyo 108, JAPAN

We have reported that adenosine is involved in the induction of long-term depression (LTD) in the CA1 region of the rat hippocampus after the application of low frequency stimulation (2 Hz, 15 min; LFS) to the Schaffer Collateral. We have investigated components that composed the depression of field EPSPs. One component which recovered rapidly after LFS could be explained as the depletion of transmitter at presynaptic terminals by the sequential stimulations. Another component which recovered slowly after LFS was eliminated by the application of 1 μ M 8-cyclopentyl-1,3-dimethylxanthine (8-CPT), suggesting that an increase of adenosine activity occurs. The other component which lasted more than one hour was the maintenance phase established by the unknown mechanism. Quantum analysis using a whole cell clamp configuration in a slice was introduced to test whether the expression of LTD was maintained at pre or postsynaptic site. This result demonstrates that LTD could be expressed mainly at presynaptic site for 10 to 20 min after LFS. However, this presynaptic expression of LTD was gradually reduced in a while and the post synaptic expression became gradually dominant. we concluded that at least partially LTD is expressed at postsynaptic site and the presynatpic expression could be explained partially by the enhancement of adenosine activity.

596.13

CALCIUM SIGNALS IN SPINES AND DENDRITES UNDERLYING THE INDUCTION OF HIPPOCAMPAL LONG-TERM DEPRESSION.

Y. Kovalchuk, J. Eilers, J. Lisman* and A. Konnerth. Univ. des Saarlandes, 66421 Homburg, Germany.

We combined whole-cell recordings and ratiometric (nonconfocal) Ca^{2+}-imaging to determine the postsynaptic Ca^{2+} signals underlying the induction of homosynaptic long-term depression (LTD) in hippocampal CA1 pyramidal cells. We found that subthreshold conditioning trains (5 Hz, 3 min) were sufficient to reliably induce LTD. With this conditioning protocol, the induction of LTD was associated with an elevation in $[Ca^{2+}]_i$ that was restricted to the active spiny dendrite. Interestingly, during the initial period of stimulation, $[Ca^{2+}]_i$ levels built up gradually and then partially decayed as stimulation continued. Suprathreshold stimulation produced an LTD of a similar extent, even though the associated Ca^{2+} transient had a larger amplitude and occurred within the entire dendritic tree and in the cell body. Applying confocal imaging, we found that subthreshold excitatory postsynaptic potentials were associated with localized Ca^{2+} transients that could be greatly reduced with AP5. These Ca^{2+} transients were large and fast in active spines, and smaller and slower in the adjacent dendrites indicating that they originated in the spines. During the LTD induction, the fluorescence signals reached about the same peak levels (less than 2 µM) in both the active spines and the adjacent dendritic segments. These results provide strong evidence that synaptic NMDA receptor-mediated Ca^{2+} transients, that are restricted to spines and their adjacent dendrites, are sufficient to induce hippocampal LTD.

Supported by DFG - SFB 246 and INTAS-93-871.

596.14

INDUCTION AND EXPRESSION MECHANISMS FOR STRIATAL LONG-TERM DEPRESSION

S. Choi* and D. M. Lovinger Dept. Mol. Physiol. Biophys., Vanderbilt Univ. Sch. Med., Nashville, TN, 37232.

Repetitive activation of cortical afferent inputs to striatal neurons produces long-term depression (LTD) in striatum. We have studied both the induction and expression mechanisms for striatal LTD using a whole-cell voltage-clamp method in acute striatal slices from 10 - 25-day-old rats. LTD was induced by pairing high frequency stimulation (HFS) at 100 Hz, 1 sec duration along with 1 sec depolarization of the postsynaptic neuron to -10 - 0mV, repeated 4 times at 10 sec intervals. EPSC amplitude was reduced to 54.3±5.3% (n=11) of control by the pairing protocol. LTD was blocked by voltage-clamping the postsynaptic neuron at -80 - -90 mV during HFS (EPSC amplitude=99.96±12.6% of control {n=5}), by dialyzing 10 mM EGTA into the postsynaptic neuron (112±5.4% of control {n=5}) or by bath-applying 10 µM nifedipine (93.5±6.7% of control {n=6}), consistent with the previous finding in adult rats that the induction of striatal LTD depends on postsynaptic calcium influx through L-type calcium channels (Calabresi et al., 1994; J. Neurosci. 14,4871). In order to investigate the expression mechanism of striatal LTD, paired pulse facilitation (PPF) and coefficient of variation (CV) were measured before and after LTD induction. LTD expression was accompanied by increases in PPF and CV (PPF=137.7±7.4% of control, CV=186.9±35.2% {n=11}), suggesting a decrease in release probability during LTD expression. Furthermore, the increases in PPF and CV were inhibited by voltage-clamping the postsynaptic neuron at -80 - -90 mV (PPF=108.8±4.5% of control, CV=108.9±19.3% {n=5}), by dialyzing with EGTA (PPF=107.1±6.4% of control, CV=108.2±8.8% {n=5}) or by applying nifedipine (PPF=101.1±3.0% of control, CV=94.0±9.9% {n=6}), suggesting that the increases in PPF and CV were specific for LTD expression. These observations suggest that, although the induction of striatal LTD is dependent on postsynaptic calcium entry, striatal LTD is expressed, at least in part, by a decrease in the probability of transmitter release. (supported by NS30470)

596.15

PRESYNAPTIC LONG-TERM DEPRESSION AT THE HIPPOCAMPAL MOSSY FIBER-CA3 SYNAPSE.

K. Kobayashi, T. Manabe* and T. Takahashi. Department of Neurophysiology, Institute for Brain Research, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan.

Long-term potentiation (LTP) and depression (LTD) of synaptic efficacy may underlie learning and memory in the brain. The induction of LTP is postsynaptic in the hippocampal CA1 region, whereas presynaptic in the CA3 region. LTD is also well characterized in the CA1 region, but not in the CA3 region. We found that low-frequency stimulation (1 Hz, 15 min) causes LTD of excitatory synaptic transmission between mossy fibers and CA3 pyramidal cells in mouse hippocampal slices. This LTD was input specific and saturable, and could be reversed by high-frequency stimulation. The LTD induction was blocked by an antagonist of metabotropic glutamate receptors (mGluRs). However, activation of mGluRs alone was not sufficient for the LTD induction, indicating the requirement of additional factor(s). The LTD induction was not suppressed by blockade of postsynaptic depolarization, *N*-methyl-D-aspartate receptors or L-type Ca^{2+} channels. These results suggest that the mossy fiber LTD is induced presynaptically by pairing of presynaptic activity and activation of presynaptic mGluRs. The long-term bidirectional regulation of synaptic efficacy at the mossy fiber-CA3 synapse may be important for hippocampal information processing.

Supported by the Ministry of Education, Science and Culture (Japan) Grant-in-aid, the Uehara Memorial Foundation and the Brain Science Foundation.

596.16

ACTIVITY- AND NMDA-DEPENDENT INDUCTION OF LTD OF ENTORHINAL CORTICAL INPUT TO GRANULE CELLS OF THE DENTATE GYRUS, *in vivo*.

M.F. Yeckel*[1] and T.W. Berger.[2] [1]*Div. of Neurosci., Baylor College of Medicine, Houston, TX. 77030, and* [2]*Dept. of Biomed. Engineering, and Program in Neurosci., U. of Southern California. Los Angeles, CA 90089.*

The ability to induce activity-dependent long-term depression (LTD) in hippocampus of adult animals *in vivo* has proven to be problematic. Only recently has this been demonstrated for synaptic input to CA1 pyramidal neurons (Thiels et al., 1994), and has never been observed for perforant path input to the dentate gyrus. In the experiments described here we adapt stimulation parameters designed to provide coincident activation of NMDA receptors and postsynaptic $GABA_A$ inhibition, and shown previously to induce LTD of CA1, to demonstrate for the first time the ability to induce activity-dependent LTD of entorhinal cortical input to granule cells of the dentate gyrus.

Experiments were performed on adult, male New Zealand white rabbits anesthetized continously with halothane. Stimulating electrodes were placed into the ipsilateral angular bundle and optimized for activation of medial perforant path fibers. Using stimulation parameters identical to those for inducing LTD of CA1, paired pulse stimulation of perforant path fibers (100-400 pairs given at a frequency of 0.5-1.0 Hz; 15-30 ms ISI) *did not* induce LTD of evoked granule cell responses (pop. spike or pop. EPSP; n=10). In an effort to enhance NMDA receptor-activation, pairs of two-pulse bursts (ISIs of 2.5-5 ms; interpair intervals of 15-25 ms) were given at a low frequency (0.5-2 Hz; 100-400 repetitions) to the perforant path. Results showed that this pattern of *burst-pair* stimulation is capable of inducing LTD of perforant path-evoked granule cell responses (pop. spike or pop. EPSP; n=12). Induction of dentate LTD was blocked in the presence of the NMDA receptor antagonist d-APV (n=5). Burst-pair stimulation of commissural afferents did not depress CA1 responses. Low-frequency stimulation trains (0.5-2.0 Hz; 900 impulses) did not induce LTD for either perforant path input or for commissural input (n=10). Supported by NIH, Human Fron. Org., and NIMH.

596.17

DIFFERENTIAL INFLUENCE OF INCUBATION DURATION ON THE EXPRESSION OF HOMOSYNAPTIC LTD AND LTP IN MATURE HIPPOCAMPUS IN VITRO. X. Xie* and T.W. Berger *Dept. of Biomedical Engineering and Program in Neuroscience, Univ. of Southern California, Los Angeles, CA 90089.*

The capability of expression of long-term depression (LTD) and long-term potentiation (LTP) at perforant path-dentate granule cell synapse was investigated in hippocampal slices of the adult rabbit. LTP could be reliably induced using high frequency stimulation (100 Hz/40 pulses) in all naive slices incubated between 2 to 12 hours with the similar magnitude of increase. In contrast, the probability of inducing LTD with low frequency stimulation (1Hz/900 pulses) was highly dependent on incubation time: LTD could be induced only in slices incubated for more than 4.5 to 5 hours, but not less. The properties of this LTD are much the same as characterized in young animal by others. First, the LTD is input specific. Second, LTD could be reversed by HFS. Third, induction of LTD was NMDA receptor-dependent. We also observed that LTD could only be induced at zero or low concentration of picrotoxin (15 μM), but not at higher concentration (50 μM). The depressed synapse could still support the expression of LTP. In contrast to the LTD, a similar magnitude of depotentiation of LTP could be induced using LFS throughout the 2 to 12 hour time span of our experiments. Our results suggest that although the synaptic weight seems to remain stable during incubation, the capability to increase or decrease it does undergo unparalleled changes. Supported by ONR, NCRR, Human Frontiers Org., and NIMH.

596.18

CEREBELLAR LONG-TERM DEPRESSION (LTD) IN TWO ULTRA-REDUCED POSTSYNAPTIC PREPARATIONS THAT LACK DENDRITIC SPINE COMPARTMENTS. K. Narasimhan* and D.J. Linden. Department of Neuroscience, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Cerebellar long-term depression (LTD) is a model system of neuronal information storage in which parallel fiber and climbing fiber inputs to a Purkinje neuron (PN), results in a selective and persistent depression of the parallel fiber-PN synapse. A similar phenomenon may be seen in culture when glutamate pulses and PN depolarization, which replace parallel and climbing fiber stimulation respectively, are co-applied. Here we report that LTD may be induced in two ultra-reduced preparations of the PN, an acutely-disassociated preparation which consists of the soma together with a stump of primary dendrite, and a perforated outside-out macropatch of dendritic (but not somatic) membrane. Using an imaging system of sufficient resolution and dynamic range to detect dendritic spines in cultured PNs, no spines could be seen in either acutely dissociated PNs or PN dendritic macropatches. LTD induced by glutamate/depolarization conjunction in these preparations was similar to that seen in the intact PN, in that it was blocked by postsynaptic BAPTA (20 mM), removal of external Ca, application of the PKC inhibitor chelerythrine (10 uM) or the metabotropic glutamate receptor antagonist (+)-MCPG (250 uM). These preparations also retain the property of associativity, as neither glutamate pulses alone nor depolarization alone induced LTD. These findings suggest that the associative computational property of LTD induction cannot depend upon the presence of separate dendritic spine compartments. Furthermore, LTD induction in the dendritic macropatch suggests that somatic/nuclear processes are not required for this process or the initial stages of LTD expression. Supported by PHS MH51106, NARSAD, and the Klingenstein, McKnight and Develbiss Funds.

LONG-TERM POTENTIATION: PHYSIOLOGY V

597.1

Depotentiation of a Facilitated Synaptic Response in the *In Vitro* Amygdaloid Slice. H. Li*, S.R. Weiss, D.-M. Chuang, R.M. Post and M.A. Rogawski. Biological Psychiatry Branch, NIMH and Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

Low frequency stimulation (LFS) of the amygdala has been shown to block the development of amygdaloid kindling produced by repeated application of a high frequency stimulus (Weiss *et al.*, 1995). In an attempt to elucidate the underlying mechanism, we studied synaptic plasticity in rat amygdala slices *in vitro* using similar stimulation protocols. Synaptic responses were evoked by stimulation of the external capsule (EC) or stria terminalis (ST) and intracellular recordings were performed in the basal lateral nucleus of the amygdala. High frequency stimulation (HFS, 100 Hz for 1 sec) of EC or ST induced posttetanic potentiation (PTP) lasting 1.4 ± 0.4 min (n = 3) and short-term potentiation (STP) lasting 8.3 ± 2.6 min(n = 11). STP but not PTP was blocked by 100 μM APV, an NMDA receptor antagonist. LFS (1 Hz) of ST or EC alone induced an enhancement (154 ± 7 %, n = 6) in the amplitude of synaptic responses that were maintained for > 30 min following termination of the stimulation. In contrast, when LFS was applied following recovery from the transient potentiating effect of HFS, there was a transient enhancement (126 ± 4%, n = 8) of the synaptic response followed by persistent synaptic depression (76 ± 7% of baseline response, n = 8). This depressed synaptic response could be repotentiated by HFS. These studies demonstrate that LFS in the *in vitro* amygdaloid slice can depotentiate a facilitated synaptic response. This system may allow analysis of the mechanism by which LFS reverses kindling-induced synaptic plasticity *in vivo*. (Supported by NIMH & NINDS/NIH)

597.2

$GABA_B$ RECEPTOR MEDIATED SUPPRESSION OF INDUCTION OF LONG-TERM POTENTIATION (LTP) IN RAT LATERAL SEPTAL NUCLEUS NEURONS. H. Hasuo*, and T. Akasu. Dept. Physiol., Kurume Univ. Sch. Med., 67 Asahi-machi, Kurume 830 Japan.

Intracellular recordings were made from neurons of rat lateral septal nucleus(LSN) in para-sagittal slice preparations which contain both septum and hippocampus. High frequency stimulations (HFS)(100 Hz, n = 40, 4 trains at 10 s intervals) did not produce a long-term potentiation (LTP) of an EPSP but resulted in a LTP of late hyperpolarizing potential (LHP) in the presence of picrotoxin. The induction of LTP of LHP was blocked by AP5 (100 μM). The monosynaptic LHP evoked in the presence of CNQX (20 μM) did not show LTP. A $GABA_B$ receptor antagonist (CGP35348 or CGP55845A) blocked an LHP but did not affect the EPSP after induction of LTP of LHP. While, the EPSP recorded with Cs-containing electrode or the EPSP recorded in the presence of CGP55845A showed clear LTP after HFS. These data suggest a functional role of $GABA_B$ receptors on the LTP of glutamate receptors in principal neurons of rat LSN. (Supported by Grant-in-Aid (C) 07680904, Japan)

597.3

NITRIC OXIDE SYNTHASE INHIBITORS BLOCK LTP IN SYMPATHETIC GANGLIA. G. F. Altememi, Reem S. Alhijailan and K. A. Alkadhi*, Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX 77204-5515.

The possible role of nitric oxide (NO) in the induction and maintenance of long term potentiation (LTP) in sympathetic ganglia was studied. Transmission in the isolated superior cervical ganglion of rat was evaluated by measuring the postganglionic compound action potential made submaximal by partial block with hexamethonium (0.4 mM). LTP of the nicotinic pathway was induced by a brief train of supramaximal pulses (tetanus, 20Hz for 20 sec) to the preganglionic nerve. The nitric oxide synthase (NOS) inhibitor L-nitro arginine methyl ester (L-NAME, 10 μM) completely prevented the induction of LTP when superfused 30 min before tetanus. Similar treatment with the inactive isomer D-NAME had no effect on the induction of LTP. However, LTP was fully expressed when L-NAME was superfused in the presence of the NO donor sodium nitroprusside (100 μM). The effect of L-NAME on the maintenance phase of LTP was tested by superfusing the drug 60 min after tetanus. The NOS inhibitor completely and reversibly blocked the established LTP. Similar results were obtained using another NOS inhibitor N^G-nitro-L-arginine (L-NOARG, 0.5 μM). Neither L-NAME nor sodium nitroprusside had any significant effect on the basal ganglionic (control level) transmission. These results indicate that both the induction and maintenance of LTP in sympathetic ganglia are dependent on the presence of NO (Supported by an LGIA grant from the University of Houston)

597.4

REVERSAL OF LONG-TERM POTENTIATION IN DEEP LAYERS OF LATERAL ENTORHINAL CORTEX FOLLOWING 1Hz OR 5Hz STIMULATION OF CA1/SUBICULAR BORDER. G.A. Cousens* and T. Otto. Dept. of Psychology, Rutgers, the State University of New Jersey, New Brunswick, NJ 08903.

Recent investigation has revealed that deep layers (IV-VI) of lateral entorhinal cortex (LEC) support homosynaptic long-term potentiation induced by theta burst stimulation (TBS) of the ipsilateral CA1/subicular border in urethane anesthetized rats. The present study was conducted to determine whether LTP in this pathway could be reversed by 1Hz or 5Hz stimulation.

Stable monosynaptic field EPSPs (mean maximum amplitude, .37mV; mean peak latency, 6.5ms) were recorded in deep layers of LEC from urethane-anesthetized (3mg/kg) male Sprague-Dawley rats (300-400g) following test pulses delivered to the CA1/subicular border. Potentiation of EPSP amplitude following two bouts of TBS (11 bursts [four pulses at 100Hz] separated by 140 ms) was significantly reduced by either 1Hz (300 pulses; n=3) or 5Hz (300 pulses; n=3) stimulation. Preliminary data indicate that 5Hz stimulation tended to reduce potentiated EPSP amplitude slightly more effectively than 1 Hz stimulation. Histological verification of electrode placement is currently underway.

These data are consistent with the idea that LEC supports synaptic plasticity induced by biologically-relevant stimulation and that this plasticity can be modulated by 1Hz or 5Hz single-pulse stimulation. It is unclear at present whether reversal of LTP in deep layers of LEC reflects the same cellular mechanisms involved in long-term depression. (Supported by Busch Biomedical Research Support Grant #649383)

597.5

CHARACTERISTICS OF LONG-TERM POTENTIATION OF THE PERIRHINAL CORTEX FOLLOWING STIMULATION OF THE OLFACTORY BULB IN THE CHRONIC PREPARATION. Robert A. Hodgson* and Ronald J. Racine. Department of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S-4K1.

Olfactory bulb (OB) stimulation is known to produce a long-term potentiation (LTP) effect in the OB and piriform cortex in rats in which the monosynaptic component of the response remains unchanged but the late component is potentiated (Stripling and Patneau, 1988). This late-component change is manifested as an increase in field potential amplitude and is thought to reflect selective potentiation of excitatory synapses terminating on inhibitory interneurons. Here we demonstrate a similar effect in the perirhinal cortex (PRh) following OB stimulation. Stimulating and recording electrodes were chronically implanted in the OB and PRh respectively. Thirty 8-pulse trains (pulse frequency: 300 Hz; pulse intensity: 794 μA; train frequency: 0.1 Hz) were delivered to the OB every 24 h. This stimulation produced an amplitude increase in the late-component of the evoked potential but had no effect on the monosynaptic component. Experimental animals deviated significantly from controls who received identical implants but no high-frequency stimulation. Only one set of trains was required to produce a significant potentiation effect, and asymptotic levels were reached around the sixth day of stimulation. The response showed no significant decay two weeks following the termination of trains.

597.6

THE EFFECT OF LTP-INDUCTION ON THE RESPONSE TO OLFACTORY CUES IN THE OLFACTORY SYSTEM IN THE RAT. Y. Xu[1,2], R.J. Racine[2], S. Haykin[1], C.A. Chapman[2] and M. Fahnestock*[3]. Communication Research Lab.[1], Dept. Of Psychology[2], Dept. Of Biomedical Science[3], McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

To test the effects of enhanced synaptic connections in the olfactory system on the processing of olfactory signals, we induced long-term potentiation (LTP) at several points in a polysynaptic circuit running from the olfactory bulb to the dentate gyrus of the hippocampus. EEGs were recorded from indwelling electrodes in the olfactory bulb, piriform cortex, entorhinal cortex and dentate gyrus, and analysed using multichannel spectral analysis before and after the induction of LTP. Toluene, a volatile solvent, was used as the olfactory stimulus. After extended baseline recording, LTP was induced by tetanizing, in succession, the entorhinal cortex, piriform cortex and olfactory bulb. Test field potentials were reliably enhanced in the dentate gyrus, entorhinal cortex and piriform cortex, respectively. Prior to LTP induction, exposure to toluene triggered an oscillatory response in all recording sites which lasted about 0.5-2.0 sec. Power spectra revealed a dominant frequency of about 20 Hz. Coherence between all pairs of recording sites was also maximal at 20 Hz during the toluene presentation. Following the induction of LTP, there was little or no change in power or coherence of spontaneous EEG. However, there was a significant reduction in power and coherence in the toluene-induced signal following LTP induction. The dominant frequency of the oscillation also shifted from 20 Hz to about 30 Hz. We are currently investigating the possibility that the decreased power and coherence reflect a context-dependency for the activation patterns that express response potentiation.

597.7

LONG TERM SYNAPTIC PLASTICITY MODULATES THE MODE OF SIGNALING BETWEEN PYRAMIDAL NEURONS BY REGULATING THE RATE OF ACTIVITY DEPENDENT SYNAPTIC DEPRESSION. M. Tsodyks* and H. Markram of the Dept. Neurobiology, The Weizmann Institute for Science, Rehovot, 76100, Israel.

Synaptic transmission between individual neocortical layer 5 pyramidal neurons was examined using whole-cell patch clamp techniques. We discovered that above a certain frequency of synaptic activation (limiting frequency), the synaptic response is inversely proportional to the frequency. An integrated experimental and computational analysis revealed that synapses which depress rapidly reach this limit at low frequencies and are therefore ineffective in transmitting information about the action potential (AP) rate in presynaptic neurons but are very effective at contributing a phasic signal that emerges when a population of presynaptic neurons changes its activity synchronously. On the other hand, synapses that depress slowly reach this limit only at high frequencies and are therefore more effective at conveying rate information and less effective at conveying temporal information. An examination of how the activity dependent properties of these synapses change following simultaneous activation of pre and postsynaptic neurons (Hebbian pairing protocol) revealed that the form of plasticity observed at these synapses is not an unconditional amplification of the gain of the synapse but an activity dependent temporal redistribution of synaptic efficacy (RSE) during a train of presynaptic APs. RSE modulates the rate of synaptic depression and hence the limiting frequency. RSE thus serves to slide the mode of synaptic transmission along a continuum between rate and temporal signaling. The study was supported by research grants from The Henry S. and Ann S. Reich Research Fund for Mental Health and an ONR grant N00014-96-1-0450.

597.8

A NOVEL ACTION FOR ACETYLCHOLINE (ACh) - MODULATING THE MODE OF SIGNALING BETWEEN PYRAMIDAL NEURONS BY CHANGING THE RATE OF ACTIVITY DEPENDENT SYNAPTIC DEPRESSION. H. Markram* and M. Tsodyks of the Dept. Neurobiology, The Weizmann Institute for Science, Rehovot, 76100, Israel.

We discovered that in synapses between two neocortical pyramidal neurons, the amplitude of the postsynaptic response decreases inversely proportional to the frequency at which the synapse is activated once a certain frequency threshold is crossed (the limiting frequency). We examined the effect of ACh on this unique synaptic property using whole-cell patch clamp techniques. We found that ACh did not merely inhibit synaptic transmission while present, as usually assumed, but rather decreased the rate at which the synaptic response depressed during a train of presynaptic action potentials (APs). The effect of ACh therefore is an activity dependent temporal redistribution of synaptic efficacy. An integrated experimental and computational analysis revealed that synapses which depress rapidly are ineffective in transmitting information about presynaptic AP rates but are very effective at contributing a phasic signal that emerges at the moments when a population of presynaptic neurons changes its activity. On the other hand, synapses that depress slowly are more effective at conveying rate information and less effective at conveying temporal information. ACh therefore serves to slide the mode of synaptic transmission along a continuum away from temporal signaling towards rate signaling. The effect of ACh is the inverse of the synaptic plasticity observed when the pre and postsynaptic neurons are activated simultaneously. The study was supported by research grants from The Henry S. and Ann S. Reich Research Fund for Mental Health and an ONR grant N00014-96-1-0450.

597.9

INDUCTION OF LONG-TERM POTENTIATION IN THE INSULAR CORTEX BY AMYGDALA STIMULATION *IN VIVO*. M.L. Escobar*, V. Chao and F. Bermúdez-Rattoni. Depto. de Neurociencias, Instituto de Fisiología Celular, UNAM, México, D.F. 04510. México.

Long-term potentiation (LTP) is a form of activity-dependent synaptic plasticity that has been investigated mainly in the hippocampus. It is considered likely that similar mechanisms may also account for aspects of naturally occurring plasticity in the neocortex. The agranular insular cortex (IC), a region of the temporal cortex in the rat, corresponding to Krieg's areas 13 and 14, has been implicated as a neural substrate of Conditioned Taste Aversion and recently, it has been also involved in the acquisition and consolidation of spatial and inhibitory avoidance learning tasks. The IC receives direct projections from the basolateral (Bla) amygdaloid nucleus and it is established that both the amygdaloid complex and the IC contribute to the formation and retention of taste-illness memories. The aim of the present study was to examine whether LTP can be induced *in vivo* in the adult rat IC stimulating the amygdalocortical projections from basolateral amigdaloid nucleus. Seven adult male Wistar rats weighing 350-400g were used. IC responses were recorded by using a monopolar stainless steel recording electrode placed in the IC stereotaxic coordinates (AP 1.2, ML 5.5, DV 7). Responses were evoked via direct stimulation of the Bla, using a stainless steel monopolar stimulating electrode at coordinates (AP 2.8, ML 5.0, DV 8.5). Low frequency responses were evoked once every 20 sec throughout a 20 min baseline period. The current intensity that elicited a 50% maximal response was determined and used for all subsequent stimulation, including induction of LTP. The IC field EPSP consisted of small (approximately 0.4 mV) potentials elicited with (50-60μA) current intensities. Tetanic stimulation (1 sec trains of 100 Hz) to the Bla induced LTP in the IC, increasing significantly (50%) synaptic responses to low frequency stimulation during a period of at least 1 hr after conditioning training. These results demonstrate that IC LTP can be induced *in vivo* in adult rats by tetanic stimulation of afferent projections from amygdaloid complex. DGAPA IN 206095.

597.10

NMDA-RECEPTOR DEPENDENT LONG-TERM POTENTIATION (LTP) IN THE ADULT RAT VISUAL CORTEX *IN VIVO* . A.J. Heynen, L.N Cooper*, and M.F. Bear. HHMI/Department of Neuroscience, Brown University, Providence, RI 02912.

Previous studies have demonstrated that LTP of synaptic responses can be induced in the visual cortex *in vitro* . This synaptic enhancement has been shown to be long-lasting, input-specific and dependent on *N*-methyl-D-aspartate (NMDA) receptor activation. In order to determine whether a similar form of synaptic enhancement is present in the intact animal, LTP induction was studied in the adult rat *in vivo*. Male Long-Evans rats (250-400 g) under pentobarbital anesthesia (65 mg/kg i.p.) had a recording electrode positioned in the superficial layers (II/III) of visual cortex and a concentric bipolar stimulating electrode positioned in the ipsilateral lateral geniculate nucleus (LGN). Negative field potentials (FPs) with a latency-to-peak of ~13 ms were recorded in response to 0.2 ms stimulus pulses (50-150 μA) delivered to the LGN. FPs were monitored for 60 min. prior to application of theta-burst stimulation (TBS), which previous work has shown to yield robust synaptic potentiation in rat visual cortex *in vitro*. A significant potentiation in the amplitude of synaptic responses was consistently observed following TBS (FP amplitude 60 min. post-TBS = 141.6 ± 8.3 % of baseline; n = 11). TBS-induced LTP was prevented by prior application of the competitive NMDA-receptor antagonist CPP ((±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, 10 mg/kg i.p.; n = 4). In order to determine the duration of LTP, responses were monitored for up to 11 hrs following application of TBS (n = 4). In all cases LTP remained stable for as long as the preparation was viable. Together these results demonstrate that the characteristic properties of LTP observed in slices of visual cortex are also present in the adult visual cortex *in vivo*.

Supported by HHMI.

597.11

NMDA-INDUCED SYNAPTIC DEPRESSION IN THE CA1 REGION OF THE HIPPOCAMPUS. H.-K. Lee* and M.F. Bear. Dept. of Neuroscience and Howard Hughes Medical Institute, Brown University, Providence, RI 02912.

Induction of homosynaptic LTD in CA1 requires activation of NMDA receptors. However, whether NMDA receptor activation is sufficient to induce LTD is not known. To examine this issue, we studied Schaffer collateral –> CA1 synaptic transmission in hippocampal slices prepared from young adult rats. Bath application of NMDA (20 μM, 3 min) produced a reliable long lasting decrease in field EPSPs (81 ± 2 % of baseline slope, 1 hr post-NMDA application, n = 32 slices). NMDA-induced depression was not stimulation dependent (n = 5), did not require CA3 (n = 6), and occurred in slices maintained both in interface (n = 3) and submersion (n = 29) chambers. NMDA application after LTP resulted in depotentiation (n = 6). Occlusion experiments were performed to test if NMDA-induced depression shares similar mechanisms with homosynaptic LTD. Saturation of homosynaptic LTD with several episodes of 1 Hz (900 pulses) stimulation significantly ($P < 0.01$) reduced the magnitude of NMDA-induced depression (90 ± 1%, n = 5) compared to a second control path in the same slice (77 ± 2%, n = 5). However, NMDA-induced depression did not occlude homosynaptic LTD (n = 3). In addition, bath application of 1 μM okadaic acid (OKA) did not block NMDA-induced depression (NMDA + OKA: 86 ± 2%, n = 8; interleaved NMDA controls: 81 ± 4%, n = 7), while it was effective at blocking homosynaptic LTD (n = 4). Intracellular recordings demonstrated that neurons can tolerate 20 μM NMDA, showing that the depression of the EPSP was not caused solely by excitotoxic cell loss. Interestingly, however, the decrease in the intracellular EPSP was accompanied by a persistent increase in input resistance. To reduce the possible complication of NMDA toxicity, we tried a lower concentration of NMDA (10 μM, 10 min). Depression was not reliably induced by a single application of NMDA (99 ± 3%, n = 11 slices), but multiple applications did lead to a measurable depression (83 ± 3%, measured 20 min post-third NMDA application, n = 4 slices), which showed saturation. Currently, we are testing if 10 μM NMDA-induced depression is blocked by phosphatase inhibitors.

(supported by HHMI)

597.12

ACTIVITY DEPENDENT SYNAPTIC PLASTICITY - A PROPERTY COMMON TO ALL LAYERS OF ADULT MOTOR CORTEX? M.-S. Rioult-Pedotti* and J.P. Donoghue. Dept. of Neuroscience, Brown University, Providence, RI 02912.

We have previously shown that LTP and LTD can be induced in motor cortex layer II/III horizontal connections (Hess & Donoghue, 1994). Is the capacity to produce long term synaptic changes common to all layers of motor cortex or is it restricted to certain motor cortical neurons? Here we have investigated plasticity of horizontal pathways that synapse within layer I upon the apical dendrites of layer III and V pyramidal neurons.

Extracellular fieldpotentials to layer I stimulation were recorded in layer I at distances of .5 mm and 1 mm in parasagittal slices of adult rat motor cortex. Simultaneously, EPSPs were recorded intracellularly from layer III and V pyramidal cells vertically below the field potential recording electrode.

High frequency stimulation (HFS) to one or two horizontally displaced sites, routinely did not induce LTP in layer I (N=5). However, if the $GABA_A$ antagonist bicuculline was applied focally (1 min., 3.5 mM) at the recording site immediately before HFS, the tetanized pathway was potentiated for at least one hour while an unstimulated control pathway remained unchanged (N=5). LTP could be saturated (N=5) and reversed (N=5) by application of low frequency stimulation (LFS). Layer III (N=10) as well as layer V (N=8) neurons produce EPSPs to layer I stimulation, indicating that intra- as well as extracortical neurons have the potential to produce long term synaptic plasticity in layer I. LFS applied to naive connections in layer I produced horizontal LTD of layer I fields (N=5) and additionally of layer III and V pyramidal cell EPSPs, indicating that plasticity of connections in layer I can influence neurons in superficial and deep layers.

These data indicate that the potential for synaptic plasticity in the rat motor cortex is spread across multiple connection pathways and includes both intracortically projecting and output neurons. Supported by NS22517.

597.13

LONG-TERM POTENTIATION IN THE NEOCORTEX: EVIDENCE SUGGESTING THAT PERIRHINAL CORTEX MAY PLAY A ROLE IN MEMORY AND PRECONSOLIDATION MEMORY STORAGE, T.L. Ivanco*, M. Michelin, and R.J. Racine. Department Of Psychology, McMaster University, Hamilton, ON, Canada, L8S-4K1.

The hippocampal region, and the entorhinal, perirhinal, and parahippocampal cortices, have been widely cited for their involvement in information storage. There are extensive reciprocal connections between the structures in this system, and between this system and the neocortex. Although LTP has been extensively studied in the *intrinsic* connections of both the hippocampus and neocortex, LTP in the pathways conveying information between these systems have received little attention. Otto and Eichenbaum (1994) previously reported a potentiation effect in the perirhinal cortex following hippocampal stimulation. The current study was designed to investigate LTP in projections from the perirhinal cortex to the frontal cortex in chronically implanted male Long-Evans rats. Multi-session procedures were used to induce LTP as described by Racine et al. (1995). The main finding was a significant long-term potentiation of the polysynaptic components of the response evoked in the neocortex by perirhinal cortex stimulation. Several sessions were required to achieve asymptotic levels of potentiation. We suggest that perirhinal cortex activation may promote enhancement in cortico-cortical pathways secondarily activated by perirhinal inputs.

This research supported by an NSERC grant awarded to RJR

597.14

NEOCORTICAL LONG-TERM POTENTIATION IN THE ADULT, BEHAVING RAT: INDUCTION AND COOPERATIVITY EFFECTS FOLLOWING MINIMAL WHITE MATTER STIMULATION.

Ronald J. Racine*, David J. Froc and Christopher Trepel. Department of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S-4K1.

While the neocortex is believed to be a primary site for information storage in behaving animals, it has proven resistant to the induction of long-term potentiation (LTP). We have recently shown that LTP can be reliably induced in the neocortex of awake, freely moving animals if multiple sessions of high-frequency stimulation are applied, and we are currently using these procedures to further investigate the properties of neocortical LTP. Here we report that LTP can be induced in the neocortex following the spaced activation of white matter afferents with tetanizing trains of minimal intensity. We also report that LTP induction requires a threshold level of afferent coactivity (cooperativity). Stimulating and recording electrodes were implanted into the corpus callosum and parietal area 1, respectively. Daily LTP-inducing stimulation consisted of thirty 8-pulse, 24 ms trains (pulse frequency: 300 Hz; train frequency: 0.1 Hz). Four groups of animals received trains of varying pulse intensities: 16 μA (single pulses at this intensity evoke early component responses that are 5-10% of maximum amplitude), 32 μA (single pulses trigger responses 8-14% of maximum), 159 μA and 501 μA. A fifth, control group was implanted but received no trains. Relative to controls, all groups showed potentiation of the early (~10.0 ms to peak) component, although the changes were small in the 16 μA and 32 μA groups. Population spike enhancement was also clear in the 159 μA and 501 μA groups within 1 week of train delivery. All but the 16 μA group showed a reliable enhancement of the late (~20.0 ms to peak) component. Even in the 16 μA group, 25% of the animals showed a potentiated late component. Therefore, a threshold for the induction of potentiation appears to exist around the 16 μA mark, indicating a cooperative LTP effect. Moreover, 32 μA trains reliably induced potentiation effects in both components in the adult, freely moving animal, indicating that white matter stimulation is an effective, low threshold means of inducing LTP in this preparation, as long as the stimulation sessions are spaced and repeated.

597.15

CONSOLIDATION INTERVAL PLAYS A CRITICAL ROLE IN THE INDUCTION OF NEOCORTICAL LONG-TERM POTENTIATION IN THE ADULT, BEHAVING ANIMAL. Christopher Trepel*, David J. Froc, Robert A. Hodgson and Ronald J. Racine. Department of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S-4K1.

The neocortex is widely held to be a primary site for the storage of long-term memory and is believed to store information through many repetitions of similar acts of information processing, producing gradual, incremental changes in the synaptic connections between neurons. Here we demonstrate that the induction of neocortical LTP in the chronically prepared animal follows rules similar to those for the consolidation of memory. Stimulating and recording electrodes were implanted into the corpus callosum and anterior neocortex (parietal area 1), respectively. LTP-inducing stimulation sessions consisted of sixty 8-pulse, 24 ms trains (pulse frequency: 300 Hz; pulse intensity: 1259 μA; train frequency: 0.1 Hz). Three groups of animals received stimulation sessions at varying intervals for 10 days: Once every 6 hours (6h, n=5), once every 12 hours (12h, n=5) and once every 24 hours (24h, n=6). A fourth, control group was implanted but received no trains (n=4). Relative to controls, all stimulated groups showed potentiation of both the early (~10.0 ms to peak) and late (~20.0 ms to peak) components of the evoked response. Population spike enhancement was also clear. After 4 stimulation sessions (or a total of 240 trains), none of the 6h animals showed increases in late component amplitude, while the 12h and 24h groups both showed clear increases. Following 8 stimulation sessions, 3/5 of the 6h animals showed an increase in late component amplitude. Over the 10 day induction period, the 24h group received the fewest number of LTP-inducing trains but showed the largest late component potentiation, followed by the 12h and 6h groups, respectively. There were no differences in early component potentiation between the groups over days. These data demonstrate that the stimulus interval is critical for the induction of neocortical long-term potentiation and offer physiological support for the hypothesis that an incremental consolidation process is essential for information storage in the neocortex.

597.16

MONOAMINE AND POSTSYNAPTIC INOSITOL TRISPHOSPHATE RECEPTORS ARE INVOLVED IN LONG-TERM POTENTIATION AT VISUAL CORTICAL INHIBITORY SYNAPSES, Y. Komatsu*. Dep. of Physiology, Kyoto Prefectural Univ. of Med., Kamigyoku, Kyoto 602, Japan.

The induction of long-term potentiation (LTP) at visual cortical inhibitory synapses requires the activation of G protein-coupled receptors in postsynaptic cells. In the present study, the induction mechanism was further examined in developing rat visual cortical slices. Inhibitory postsynaptic currents evoked by layer IV stimulation were recorded from layer V cells using the whole-cell recording method under a blockade of ionotropic glutamate receptors. High-frequency stimulation of layer IV failed to generate LTP when the patch pipette solution contained an inositol trisphosphate (IP_3) receptor antagonist heparin (2 mg/ml) or a Ca^{2+} chelator BAPTA (10 mM). To identify receptors which mediate IP_3 formation and thereby contribute to the LTP induction, involvement of metabotropic glutamate receptors and amine receptors including α_1 adrenergic, histamine H_1, muscarinic and serotonin 5-HT_2 receptors, which are all known to be coupled to IP_3 formation, was tested by intracellular recording. Only bath application of an α_1 adrenoceptor antagonist prazosin or a 5-HT_2 receptor antagonist ketanserin prevented LTP induction in most, but not all, of cells. These results suggest that the LTP induction requires IP_3-induced Ca^{2+} release from internal stores in postsynaptic cells and that the IP_3 formation is at least partly mediated by the activation of monoamine receptors. Supported by Grant-in-Aid for Scientific Reserach Project 07680898 from the Japanese Ministry of Education, Science and Culture.

597.17

POTENTIATION OF SPONTANEOUS EPSCs IN CULTURED CEREBELLAR PURKINJE NEURONS BY A cAMP ANALOG. A.R. Parent* and D.J. Linden. Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

It has recently been shown that cerebellar parallel fiber synapses express a form of LTP which is both induced and expressed presynaptically via activation of a calcium-sensitive adenylyl cyclase and consequent production of cAMP (Salin et al., Neuron 16:797, 1996), in a manner identical to that proposed for hippocampal mossy fiber synapses (Weisskopf and Nicoll, Nature 376:256, 1995). To further characterize this form of synaptic plasticity, whole-cell patch-clamp recordings were made from Purkinje neurons in dispersed cultures of embryonic mouse cerebellum. Isolation of sEPSCs was accomplished using Cs/QX-314-containing internal saline, and external saline supplemented with picrotoxin. With Vh=-70 mV, two forms of inward current were seen; large, broad paroxysmal currents (500-1000 pA peak amplitude, 0.5-3 sec duration) and small brief currents (15-50 pA, 5-15 msec). Both reversed at 0 mV. The large currents were strongly attenuated by a high divalent cation containing external saline, an NMDA-R antagonist or TTX, and the remaining currents were blocked by an AMPA-R antagonist, suggesting that the large currents result from NMDA-R dependent multisynaptic network activity, while the small currents are predominantly monosynaptic and AMPA-R mediated. Application of a cAMP analog (Sp-8-CPT-cAMPS, 10-50 uM, 10 min) produced a >5-fold increase in the frequency of both sEPSCs (recorded in APV) and mEPSCs (recorded in TTX) which persisted >30 min after drug washout. These results are consistent with a presynaptic cAMP-mediated form of LTP at the parallel fiber-Purkinje neuron synapse. Supported by FRSQ Québec, PHS MH51106, NARSAD, and the Klingenstein, McKnight and Develbiss Funds.

597.18

LONG-TERM POTENTIATION AND DEPRESSION (LTP & LTD) IN TURTLE CEREBELLAR PURKINJE CELLS IN ACSF WITH OR WITHOUT PICROTOXIN. C.Y.Chan*, J.Li, L.Cross. Dept. of Physiology, CUNY Med. School, 138th & Convent AV., NY 10031.

Intradendritic recording of 30 Purkinje cells (PC) and simultaneous field potential recording were used to demonstrate and characterize co-elicited LTP and LTD by relatively low-speed repetitive dorsal-cerebellar surface stimulation (referred to here as "training"). Amplitude of the parallel fiber volley was basically unaffected by the training. Picrotoxin (80 μM) seemed to slightly enhance LTD but reduced LTP generation. In cases when LTD was elicited alone, it usually had a 2-phase time course with a fast-recovering phase lasting 20-30 min and a slower tail phase lasting up to 70 min from training. More often, LTD was co-elicited with LTP and appeared as an early transitory (duration 24 +/- 10 min) suppression of the LTP, which lasted 53 +/- 16 min. The disparate durations suggest modulation or other involvements of distinct sets of intracellular processes.

In either recording medium, the intensity of training strongly affected the outcome: E.g. trainings with a 1 min train of 1 Hz pulse triplets, or with weaker current strengths elicited mostly LTP; while training with 0.5 Hz or stronger currents elicited mostly LTD. Evocation of Ca^{2+} spikes during training was correlated to higher incidence and magnitudes of LTD, but activation of climbing fiber responses were not necessary for LTD. Its main effect was suppression of LTP.

LONG-TERM POTENTIATION: PHYSIOLOGY VI

598.1

MECHANISMS OF LONG-TERM POTENTIATION INDUCED BY ELEVATED EXTRACELLULAR CALCIUM

David B. Wheeler* and Richard W. Tsien, Dept. Molecular and Cellular Physiology, Beckman Center B103, Stanford School of Medicine, Stanford, CA 94305-5426

Long-term potentiation (LTP) of synaptic transmission induced by tetanic stimulation has been studied extensively in the hippocampal slice. However, since tetanus can only stimulate a small subset of synapses within the slice, this system is often difficult to use for cellular and molecular studies of the mechanisms underlying LTP. To potentiate a larger proportion of the synapses than can be achieved with tetanic stimulation, we have used the standard hippocampal slice preparation to study LTP induced by elevated $[Ca^{2+}]_o$ (Turner *et al.*, 1982, *Neuroscience*, **237**:1411-16). Field recordings showed that the synaptic strength was increased for several hours following a 10 min period during which $[Ca^{2+}]_o$ was raised from 2 to 10 mM and $[Mg^{2+}]_o$ was reduced from 1.3 mM to 0. The slope of the EPSP at 60 min was 145±4% of control levels (n=10). When $[Mg^{2+}]_o$ was held at 1.3 mM during the exposure to high $[Ca^{2+}]_o$, LTP was not observed (n=6), raising the possibility of a role for NMDA receptors. The importance of NMDA receptors (Bliss *et al.*, 1987, In: *Excitatory Amino Acid Transmission*, pp. 337-40) was confirmed by showing that 25 µM D-AP5 halved the amount of potentiation seen after exposure to 10 mM $[Ca^{2+}]_o$ (n=8). To test the possibility that other Ca^{2+} entry pathways could be important for the induction of LTP, we looked for effects of Ca^{2+} channel blockers. We found that 10 µM nimodipine reduced the potentiation caused by elevated $[Ca^{2+}]_o$, to roughly the same degree as D-AP5 (n=5). Our findings indicate that multiple Ca^{2+} entry pathways underlie the induction of LTP by elevated $[Ca^{2+}]_o$. Further studies are needed to determine the loci of induction and maintenance of Ca-LTP, and to establish the extent to which the mechanisms of Ca-LTP and tetanic LTP overlap.

DBW is a HHMI Predoctoral Fellow. This work was supported by an NIMH Silvio Conte Center for Neuroscience Research award and the Mathers Foundation.

598.2

MODELS OF CALMODULIN TRAPPING BY CAMKINASE IN A DENDRITIC SPINE. W.R. Holmes* and K-W. Tsao. Neurobiology Program, Dept. of Biological Sciences, Ohio University, Athens, OH 45701.

CaMkinase activation by calmodulin following calcium entry into the cell is thought to be important for learning and memory. CaMkinase activation is much prolonged if calmodulin becomes trapped on CaMkinase. Models of a dendritic spine including calcium influx and subsequent calmodulin trapping by CaMkinase were developed to estimate levels of calmodulin trapping in experimental conditions thought to induce LTP or LTD.

Calcium influx through NMDA receptors on a dendritic spine was computed following tetanic input in a dentate granule cell model. Calcium ions could bind to calmodulin, bind to another unspecified buffer, be pumped out of the cell or diffuse to neighboring compartments. Calmodulin that was fully bound with calcium could bind to CaMkinase or to another calmodulin binding protein that had binding kinetics similar to calcineurin. CaMkinase subunits could be in either the free, bound with calmodulin, calmodulin trapped, automonous or capped states as in the model of Michelson and Schulman (J. Theor. Biol. 171:281-290, 1994).

With non-tetanic input, weak tetanic input, or strong low frequency input, almost no calmodulin was trapped by CaMkinase. One reason is that the small volume of the spine required calcium concentration to exceed 1.25 µM before a single calmodulin molecule would be fully bound with calcium. With strong tetanic inputs, large numbers of CaMkinase subunits entered the trapped state. The nonlinearity was particularly steep as the frequency of tetanic input was raised from 20 to 100 Hz.

The results suggest that significant calmodulin trapping by CaMkinase is highly correlated with experimental conditions thought to induce LTP. (Supported by NIMH grant MH-51081).

598.3

DIFFERENTIAL ACTIVATION OF CaMKII AND CALCINEURIN TO LARGE AND MODERATE RISE IN $[Ca^{2+}]_i$: AN ANALYSIS BY MODELING AND SIMULATION

K.Ichikawa* , H.Okamoto and I.Yamaguchi. Foundation Research Lab. Fuji Xerox Co., Ltd., 430 Sakai Nakaimachi Ashigarakamigun Kanagawa, 259-01 Japan

It is suggested that the large and moderate rise in intracellular calcium concentration($[Ca^{2+}]_i$) result in long-term potentiation(LTP) and depression(LTD), respectively. A model for the differentiation of LTP and LTD was proposed in which the net phosphorylation or dephosphorylation of CaMKII was resulted depending on the rise in $[Ca^{2+}]_i$ (Lisman, Proc.Natl.Acad.Sci.USA86, 9574-9578, 1989). In the present study, we analyzed the activity of CaMKII and calcineurin(CaN) assuming a different scheme in which the rivalry of the activity of kinase and phosphatase depending on the rise in $[Ca^{2+}]_i$ resulted in the differentiation of LTP and LTD.

The present scheme was composed of the rise in $[Ca^{2+}]_i$ and the activation of calmodulin(CaM), CaMKII and CaN. A model for the rise in $[Ca^{2+}]_i$ was the same as before(Ichikawa, *et al.*, Soc.Neurosci.Abstr.21, 711.10, 1995). CaM was activated by the binding of four Ca^{2+}. CaMKII was activated by the binding of active CaM(CaM^*). CaN was assumed to be activated by the binding of CaM^* and four Ca^{2+}. The 4-Ca^{2+} bound but no CaM^* bound CaN was assumed to have 10% activity as was reported(Stweart, *et al.*, Eur.J.Biochem.132, 289-295, 1983). Simulations of the present scheme showed that the activity of CaMKII was lower than CaN for the moderate rise in $[Ca^{2+}]_i$, while it was higher than CaN for larger rise in $[Ca^{2+}]_i$. The steady state analysis of a simplified scheme showed the same differential activation of CaMKII and CaN. The difference in the affinity of Ca^{2+} in reactions of CaM-CaMKII complex and CaN was found to play a critical role for this differential activation.

598.4

CA1 LONG TERM POTENTIATION (LTP) IS DIMINISHED BUT PRESENT IN HIPPOCAMPAL SLICES FROM YOUNG α-CAMKII MUTANT MICE.

H.L. Hinds°^, S. Tonegawa^ and R. Malinow°* °Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 and ^HHMI, Center for Learning and Memory and Center for Cancer Research, MIT, Cambridge, MA 02138.

We are interested in using recombinant vaccinia virus as a vector for delivering the wild-type and Thr286Ala point mutant form of the α-CamKII gene back to the CA1 region of hippocampal slices from α-CamKII mutant mice, to determine whether such complementation is sufficient for the rescue of some or all of the physiological defecits observed in these mice. Such analysis would help to determine if the defecits observed are due to an immediate absence of the α-CamKII molecule or from developmental abnormalities in these mice, and could further define the role of CamKII in the post- or pre-synaptic cell in hippocampal CA1 LTP. In pursuit of this aim, we have further analyzed the physiological deficits previously reported in these mutant mice. Mice of a mixed B6/129/Balbc inbred background derived from brother/sister mating of the original mutant line through multiple generations (> 10) were produced using in vitro fertilization. Young mice (10-56 days) were analyzed, with the majority of animals being between the ages of 10-21 days, as these ages are optimal for vaccinia virus infection of acute hippocampal slices. Field recordings in the CA1 region were monitored for extensive baselines and LTP was induced using a 100Hz tetanus (5 trains, 20 pulses per train, 10 seconds between trains) in the presence of picrotoxin. While LTP at 60 minutes post-tetanus was clearly deficient in (-/-) mice (142±9%, n=14) compared with (+/+) control animals (202±24%, n=7), young α-CamKII mutant mice did show a significant level of LTP. This LTP appears to be APV dependent (n=3). This implies that a form of α-CamKII independent LTP is present in young mice that could be dependent upon another kinase, such as the β isoform of CamKII.

Supported by NIH grants to R.M. and S.T.

598.5

EFFECT OF TETANUS ON THE DISTRIBUTION OF AUTOPHOSPHORYLATED CaM KINASE II IN THE CA1 REGION OF RAT HIPPOCAMPUS, Y. Ouyang*, E.M. Schuman, K.M. Harris#, and M.B.Kennedy. Div. of Biology 216-76, Caltech, Pasadena, CA 91125; #Dep. Of Neurol., Children's Hosp., 300 Longwood Ave., Boston, MA 02115.

CaM kinase II (CaMKII) plays a critical role in synaptic plasticity in the hippocampal CA1 region. CaMKII is autophosphorylated after activation, and remains in an active state that is independent of calcium until it is dephosphorylated by protein phosphatases. We raised antibodies specific for the autophosphorylated (PO_4-CaMKII) or nonphosphorylated form of CaMKII. The Abs were used to visualize the distribution of PO_4-CaMKII in hippocampal slices by double-label confocal fluorescence immunohistochemistry. The effect of tetanic stimulation of the Schaffer collateral pathway on levels of PO_4-CaMKII was examined by a "two-pathway" paradigm in which control slices received no or only test stimulation at 2 electrodes and exp. slices received a tetanus at 1 of the electrodes. Slices were fixed and stained 30 min after tetanus. Tetani that caused LTP consistently increased PO_4-CaMKII in somas of CA1 pyramidal neurons. In 80% of experiments, the tetani also produced an increase in PO_4-CaMKII in neuropil (s.radiatum). In 80% of these, the amount of non-PO_4-CaMKII also increased in s. radiatum. At high mag., the staining for PO_4-CaMKII appeared to be concentrated in spines and along short segments of dendrites. The changes in PO_4-CaMKII associated with tetanus were blocked by APV. In summary, tetanic stimulation of the Schaffer collateral pathway increases the level of PO_4-CaMKII in neuronal somas. The tetani usually produce an increase in PO_4-CaMKII in synapses along dendrites. This increase is confined to a subset of synapses, suggesting at least some synapse specificity. Finally, under some circumstances, it appears that the tetanus may increase the amount of CaM KII in dendrites.The data support the idea that autophosphorylation of CaMKII could play a role in the induction or maintenance of LTP. NIH NS17660; MH49176; NSF GER-9023446

598.6

CALCINEURIN INHIBITORS SUPPRESS THE NMDA RECEPTOR-MEDIATED POTENTIALS AND LONG-TERM POTENTIATION IN CA1 AREA OF RAT HIPPOCAMPUS. Y.-F. Lu, A. Moriwaki, K. Tomizawa, H. Onuma, and H. Matsui*. The First Dept. of Physiol., Okayama Univ. Med. Sch., Okayama 700, Japan.

To reveal the involvement of dephosphorylation in the induction of long-term potentiation (LTP), we investigated the effects of Ca^{2+}/calmodulin-dependent phosphatase 2B (calcineurin) inhibitors, FK506 and cyclosporin A (CysA), on the LTP and the depotentiation in CA1 area of rat hippocampal slices. Extracellular recordings were made from the stratum radiatum under stimulation on the Schaffer commissural pathway and the chemicals were applied through a perfusate. FK506 (1-50 μM) and CysA (20-100 μM) decreased the excitatory postsynaptic potentials and prevented the induction of LTP. These effects were dose-dependent and reversible. Analyses of the fiber volley and paired-pulse facilitation revealed that FK506 and CysA influenced postsynaptic mechanisms. FK506 and CysA had no effects on the depotentiation. We further investigated the effects of FK506 and CysA on the NMDA receptor-mediated potentials. The NMDA receptor-mediated potentials were recorded under perfusion of low Mg^{2+} (50 μM) medium in the presence of DNQX (10 μM). FK506 and CysA showed a reversible inhibition on the NMDA receptor-mediated potentials. These results showed that FK506 and CysA prevented the induction of LTP through the inhibitory effects on the NMDA receptors. The results also suggest that calcineurin is involved in the processes of LTP. The differential effects of FK506 on the LTP and the depotentiation may attribute to the partial inhibition on the activity of NMDA receptors and the subsequent attenuation of intracellular Ca^{2+} increase.

598.7

EXAMINATION OF ALTERED HIPPOCAMPAL SYNAPTIC PLASTICITY DURING AGING. C.M. Norris*[1], T.C. Foster[1,2], and S. Halpain[1,3]. [1]Neuroscience Graduate Program, [2]Dept. of Psychology, [3]Dept. of Neuroscience and The Center for Cell Signaling, Univ. Virginia, Charlottesville, VA 22903

Hippocampal slices from adult (4-7 mos) and aged (20-24 mos) Fischer 344 rats were prepared for electrophysiological and biochemical assays. Electrophysiological data demonstrated that low-frequency stimulation (LFS, 900 pulses/1 Hz) of Schaffer collaterals resulted in robust AP5-sensitive long-term depression (LTD) in area CA1 of slices from aged but not adult rats. LTD in aged slices was blocked by raising the bath [Mg^{2+}] to 4 mM and AP5-sensitive LTD of adult slices was revealed only when LFS was delivered in high Ca^{2+} (4 mM) medium. The results suggest that differences in Ca^{2+} signaling underlie age differences in susceptibility to LTD.

Because Ser/Thr protein phosphatases have been implicated in LTD, we have begun to examine the possibility that expression or activity of protein phosphatases is altered during aging. Preliminary data were gathered from immunoblot assays of the level of catalytic subunits for various protein phosphatases. No age differences were observed for PP2A or the Ca^{2+}/calmodulin-dependent phosphatase, PP2B. Previous reports indicate that the cytoskeletal protein MAP2 is a substrate of several Ser/Thr protein phosphatases *in vitro* and is a major target of NMDA receptor-stimulated, PP2B-mediated dephosphorylation in slices. However, preliminary experiments have revealed no age differences in the coupling of NMDA receptors to MAP2 dephosphorylation. Aging-related differences in LTD induction may therefore be due to phosphatase properties not examined in our assays, or to differences in other Ca^{2+}-dependent signaling events at synapses. Work supported by NS31830 to T.C.F and an AFAR grant to S.H.

598.8

The role of protein kinase C on different forms of long-term potentiation (LTP) in the rat hippocampus. G. Y.-P. Ko*, and T. J. Teyler. Dept. Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272-0095, U.S.A.

Protein kinase C (PKC) is a protein phosphotransferase involved in the phosphorylation of receptors and ion channels (Vaccarino, 1988). It may strengthen the efficacy of synaptic transmission, thus providing a basis for learning and memory (Tanaka and Nishizuka, 1994). In hippocampal CA1 area, there are at least two forms of LTP: one is NMDA receptor-dependent LTP (NMDA LTP), and the other is NMDA receptor-independent LTP (non-NMDA LTP) (Grover and Teyler, 1990). NMDA LTP can be selectively induced with a 25 Hz tetanus and blocked by 50 μM APV. Non-NMDA LTP can be induced selectively by 200 Hz tetanic stimulation in the presence of APV and can be blocked by nifedipine, a voltage-dependent Ca^{++} channel (VDCC) blocker (Grover and Teyler, 1990), thus non-NMDA LTP is termed VDCC LTP (Teyler et al., 1994). The expression of NMDA LTP but not VDCC LTP, can be prevented by the NMDA receptor antagonist APV or the non-selective PKC inhibitor H-7 (Grover and Teyler, 1995). In this study, we used the selective PKC inhibitor chelerythrine to investigate the role of PKC on NMDA LTP and VDCC LTP. Chelerythrine (10 μM) abolished the expression of NMDA LTP in a manner similar to the effect of H-7 and decreased the expression of VDCC LTP unlike H-7 which has no effect on VDCC LTP. Chelerythrine had no effect on the induction of both NMDA LTP or VDCC LTP. This result suggests that the expression phases of NMDA LTP and VDCC LTP may share some common enzyme systems.
This work is supported by grant NS-28698 from NIH to Teyler.

598.9

STRAIN-DEPENDENT DIFFERENCES IN HIPPOCAMPAL LTP AND SPATIAL MEMORY. T. Abel*, P.V. Nguyen, R. Bourtchouladze, M.E. Bach, I. Gaprindashvili, P. Jain, and E.R. Kandel. HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

Long-term potentiation (LTP), an activity-dependent form of synaptic plasticity, is a leading cellular model for certain types of memory in the mammalian brain. Many studies have used "reverse" genetics, through the generation of knock-out and transgenic mice, to explore the role of various molecules in LTP and spatial memory. The existence of a variety of inbred strains of mice provides an additional way of exploring the genetic bases of learning and memory. We have used a number of protocols to examine LTP induction and expression in the Schaffer collateral pathway of four different strains of mice: C57BL/6J, CBA/J, DBA/2J, and 129/SvEms/J mice. We found that long-lasting potentiation (> 3 hr), induced by four 100 Hz trains or brief (3 sec) theta-burst stimulation, was robust in C57BL/6J and DBA/2J mice, but was deficient in CBA/J and 129/SvEms/J mice. LTP induced by two 100 Hz trains separated by 20 sec was reduced in 129/SvEms/J and CBA/J mice. By contrast, only the 129/SvEms/J mice showed a deficiency for shorter-lasting potentiation induced by one 100 Hz train. We have begun to assess the performance of these strains on spatial memory tasks, and we have observed strain-related deficits. These findings provide strong support for a genetic basis for some forms of synaptic plasticity that are linked to spatial memory. Furthermore, this work lays the foundation for the genetic identification of the molecular components critical for LTP, learning and memory. [This work has been supported by the Runyon-Winchell Foundation, the MRC of Canada, NIH, and HHMI.]

598.10

BRIEF THETA-BURST STIMULATION INDUCES A cAMP- AND TRANSCRIPTION-DEPENDENT LATE PHASE OF HIPPOCAMPAL LTP IN C57BL/6J MICE. P.V. Nguyen*. Ctr. Neurobiol. & Behav., Columbia Univ., College of Physicians & Surgeons, New York, New York 10032.

Long-term potentiation (LTP) is a form of activity-dependent synaptic plasticity that is thought to be a cellular mechanism for some types of memory. An effective protocol for inducing LTP consists of repeated, brief bursts of stimulus pulses [4 pulses per 40-ms burst, 5 bursts/s (known as "*theta-burst*")]. This pattern mimics the natural activity profiles of hippocampal neurons recorded *in vivo* during exploratory behavior. In the present study, I have found that 3 s of theta-burst induced a late phase of LTP (L-LTP) in the Schaffer collateral pathway of hippocampal slices from C57BL/6J mice (a strain often used for generating transgenic mice). This L-LTP was blocked by APV and by inhibitors of transcription and cAMP-dependent protein kinase (PKA). Sp-cAMPS, a PKA analog, induced L-LTP that was occluded by prior induction of theta-LTP. By contrast, applying an equal number of stimulus pulses in a compressed pattern (60 Hz, 1 s) induced short-lasting potentiation that was insensitive to inhibitors of PKA and transcription. I also found that theta-induced L-LTP was not equally robust across various inbred mouse strains: CBA/J and 129/SvEms/J mice showed deficient L-LTP when compared with C57BL/6J and DBA/2J mice [*see also T. Abel et al., this meeting*]. These results show that gene transcription, mediated by cAMP and PKA, is critical for expression of L-LTP induced by physiological patterns of impulse activity. My findings also suggest a strong genetic basis for the expression of L-LTP induced by theta bursts. [Supported by NIH and HHMI, and by a Fellowship from the MRC of Canada. I thank Eric Kandel for his encouragement throughout this study].

598.11

FYN AND FAK NON-RECEPTOR TYROSINE KINASE REGULATION OF NMDA RECEPTOR SIGNALLING. S. G. N. Grant and S. Nada, Centre for Genome Research and Centre for Neuroscience, University of Edinburgh, Edinburgh , United Kingdom EH9 3JQ.

The induction of long-term potentiation (LTP) in the CA1 region of the hippocampus requires signal transduction cascades involving tyrosine and serine-threonine phosphorylation in the post-synaptic neuron. Mice lacking Fyn, but not Src, Yes, or Abl tyrosine kinase reveal a specific deficit in the induction step of LTP. Synaptic transmission, post-tetanic potentiation and paired pulse facilitation were normal. The *fyn* mutants also showed abnormal hippocampal architecture and impairments in spatial learning.

To identify components of a Fyn-dependent pathway that may be involved with hippocampus function we have studied tyrosine phosphorylated proteins in the kinase mutant mice. Focal Adhesion tyrosine Kinase (FAK) is hypophosphorylated in *fyn* mutants and shows reduced activity in immunecomplex kinase assays. Fyn can bind directly to FAK and modulate the kinase activity and signalling to downstream effector molecules.

We have investigated the role of Fyn and FAK in the phosphorylation of the NMDA receptor. The NMDA receptor 2A and 2B subunits are tyrosine phosphorylated and the mutants reveal that Fyn is required for the phosphorylation of the NMDA receptor. The role of Fyn and FAK may be to regulate the assembly of the NMDA receptor with a post-synaptic signal transduction cascade.

Research supported by BBSRC, Wellcome Trust and Japan Society for the Promotion of Science

598.12

NMDA RECEPTOR-INDEPENDENT LTP AND DIFFERENTIAL EXPRESSION OF trkB RECEPTOR IN BASAL VS APICAL DENDRITES. I. Çavuş* & T. Teyler, Dept. Neurobiology, NE Ohio College of Medicine, Rootstown, OH 44272 USA

Apical dendrites (str. radiatum) of rat hippocampal area CA1 display both NMDA receptor dependent and independent forms of LTP. The NMDA receptor-independent LTP results in increased expression of trkB receptor protein in the apical dendrites and is blocked by tyrosine kinase inhibitors (Çavuş et al. Soc. Neurosci. Abstr. 1993, 376.11; 1995, 245.3). Little is known about the nature of LTP in the basal dendrites (str. oriens) of CA1 pyramidal cells. Here we report that the tetanus parameters used in str. radiatum (200 Hz/200 msec x 10 every 5 sec in 50 μM D,L-APV) also elicit NMDA receptor-independent LTP in str. oriens. Although initially str. oriens LTP was much larger than str.radiatum LTP (63.9 ± 6.0 vs 35.1 ± 7.2, n=6, 20 min post-tetanus), later their magnitudes were comparable (39.8 ± 6.5 vs 34.9 ± 7.6, 80 min post-tetanus). Str.oriens NMDA receptor-independent LTP was also completely inhibited by the tyrosine kinase inhibitor genistein (20 μM, 4.4 ± 10.0, 80min post-tet, n=6). However immunohistochemical analysis with trkB antibody ($gp145^{trkB}$, Santa Cruz) revealed that while this receptor tyrosine kinase is abundant in the somata and apical dendrites of the CA1 pyramidal cells, it is completely absent in the basal dendrites. We conclude that other tyrosine kinases than the trkB receptors are probably involved in the basal dendrite NMDA receptor-independent LTP.

Supported by NS# 28698

598.13

NEUROTROPHINS REGULATE DEVELOPMENT OF TETANUS-INDUCED LONG-TERM POTENTIATION (LTP) AND SYNAPTIC RESPONSES TO HIGH FREQUENCY STIMULATION IN THE HIPPOCAMPUS. A. Figurov*, L. D. Pozzo-Miller, P. Olafsson, T. Wang, and B. Lu. Roche Inst. of Mol. Biol., Nutley, NJ 07110 and Lab. of Devel. Neurobiol., NICHD, NIH, MD 20892-4480

Neurotrophins have traditionally been viewed as trophic factors for neuronal survival and differentiation; the fact that their expression is modified by neuronal activity, however, suggests that they may also play a role in regulating synapse development and plasticity. In developing hippocampus, the expression of BDNF and its receptor TrkB increases in parallel with the development of LTP. Here we report a mechanism by which BDNF regulate tetanus-induced LTP (LTP_T). Exogenous BDNF (2 nM, minimal incubation 2.5 hr) promoted the induction of LTP_T in young (postnatal day 12-13) hippocampal slices, which in the absence of BDNF show only short-term potentiation (STP). NT-4/5 but not NT-3 or NGF had similar effect. The BDNF-facilitated LTP_T was NMDA-dependent, and was not due to a decreased GABAergic inhibition. BDNF did not affect synaptic responses to low frequency stimulation (1 per min) at any ages examined. In contrast, the ability of hippocampal synapses to follow high frequency stimulation was significantly enhanced by BDNF in p12-13 slices. In adult hippocampus, weak tetanus that normally induce only STP produced long lasting enhancement of synaptic efficacy after BDNF treatment. Moreover, TrkB-IgG fusion protein (2 ug/ml), which scavenges endogenous BDNF, reduced the synaptic responses to tetanus as well as the magnitude of LTP_T. Neither BDNF nor TrkB-IgG altered LTP induced by pairing low frequency presynaptic stimulation with postsynaptic depolarization, suggesting that LTP triggering mechanism was not affected. Our results suggest that BDNF may regulate LTP in developing and adult hippocampus by enhancing an ability of synapses to follow high frequency stimulation rather than direct modulation of LTP-triggering mechanisms.

Neurotrophins were provided by Genentech, Inc., CA. TrkB-IgG was a generous gift from Dr. D. L. Shelton (Genentech). P. O. is supported by postdoctoral fellowships from Swiss NSF.

598.14

AN ECTO-PROTEIN KINASE REQUIRED FOR THE MAINTENANCE OF LTP IS LOCALIZED ON THE SURFACE OF THE SYNAPTIC JUNCTION IN THE CLEFT. W. Chen, R.S.Lasher, A. Wieraszko and Y. H. Ehrlich*, CSI/IBR Center for Developmental Neuroscience, City Univ. of NY, Coll. Staten Island, NY 10314 and Dept. C.&S. Biol., Univ. Colorado Med. Sch., Denver, CO 80262.

During the induction of long-term potentiation (LTP) in hippocampal slices ATP is secreted into the synaptic cleft, and a 48K/50K protein duplex becomes phosphorylated by extracellular ATP. All the criteria required as evidence that these two proteins serve as principal substrates of ecto-protein kinase activity on the surface of hippocampal pyramidal neurons have been fulfilled. Addition to the extracellular medium of a monoclonal antibody termed M.Ab.1.9, directed to the catalytic domain of protein kinase C, inhibited selectively this surface protein phosphorylation activity and blocked the stabilization of LTP induced by high frequency stimulation (HFS) in hippocampal slices. This antibody did not interfere with routine synaptic transmission nor prevent the initial enhancement of synaptic responses observed during the 1-5 min period immediately after the application of HFS (the induction-phase of LTP). However, the initial increase in the slope of EPSP as well as the elevated amplitude of the population spike induced by HFS, both declined gradually and returned to pre-stimulus values within 40 mins after HFS was applied in the presence of M.Ab.1.9. Immunoperoxidase localization of the ecto-PK was carried-out with intact, unfixed synaptosomes. The externally-oriented epitope that is recognized by M.Ab.1.9 in this ecto-PK was localized on the surface of the synaptic junctional plasma membrane, within the synaptic cleft. These results provide the first direct evidence that a synaptic, surface ecto-PK localized at the site where ATP is secreted during tetanic stimulation, plays a causal role in the maintenance of stable LTP, an event implicated in the formation of memory in the brain.

Supported by NIH grant HD28788 to YHE.

598.15

Ca^{++}-dependent enhancement of gap junctional conductance.

A. Pereda[1,*], T. Bell[1], A.J. Czernik[2], A.C. Nairn[2], and D.S. Faber[1].
[1]*Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA, and* [2]*Rockefeller University, New York, NY.*

Discontinuous tetanic stimulation of the posterior eighth nerve produces short- and long-term homosynaptic potentiations (STP, LTP) of both the electrotonic and chemical components of the mixed excitatory postsynaptic potential (EPSP) recorded from the Mauthner(M-) cell lateral dendrite. The potentiations are dependent upon activation of postsynaptic NMDA receptors, and presumably, an increased postsynaptic Ca^{++} level, since they are blocked by intradendritic BAPTA injections. In contrast, stimulating paradigms that increase presynaptic Ca^{++} (e.g. paired pulses) enhance chemical transmission but not electrotonic coupling. Thus, we postulated that gap junctional conductance can be potentiated as a consequence of postsynaptic, but not presynaptic, elevations in calcium. We directly tested this hypothesis by injecting Ca^{++} pre-and postsynaptically. For this purpose, $CaCl_2$ (2-4 mM) was added to the electrode recording solution (2.5M KCl, 10mM HEPES, pH 7.2). Presynaptic iontophoretic injections of Ca^{++}, performed while recording either from a single afferent in the eighth nerve and the M-cell dendrite or from afferent terminals in the medulla, did not induce any changes in the amplitudes of orthodromic or antidromic coupling. Presynaptic membrane hyperpolarizations of 5 to 10 mV provided indirect evidence of successful Ca^{++} injections. In contrast, intradendritic injections of Ca^{++} triggered long-term enhancements of both components of the mixed synaptic response, and Ca^{++}-induced potentiation occluded the stimulus induced LTP. The responsible Ca^{++}-dependent pathway most likely involves the activation of the enzyme Ca^{++}-calmodulin kinase II (CamKII) since: i) the induction of LTP could be prevented by prior intradendritic injections of KN-93, an inhibitor of the Ca^{++}-calmodulin-dependent kinases, and ii) immunohistochemical studies with antibodies (G-301) selective for the alpha subunit of CamKII revealed the presence of this enzyme in the M-cell lateral dendrite. This asymmetric effect of Ca^{++} may reflect different properties among gap junction channels (heterologous gap junctions) or the existence of different regulatory mechanisms on either side of the junction. (Supported by NIH grant NS#15335-17)

598.16

EXPRESSION OF MUTANT ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) BLOCKS LONG-TERM POTENTIATION (LTP). D.B. Kantor, S.J. Stary, W.B. Smith, B.M. Sullivan, N. Davidson, E. M. Schuman* Caltech, Division of Biology 216-76 ,Pasadena CA, 91125

Several studies have demonstrated the importance of nitric oxide (NO) as an intercellular signal during some forms of LTP. Evidence from wildtype and neuronal NOS (nNOS) knockout mice suggests that eNOS may catalyze the production of NO following LTP induction. The most notable difference between eNOS and nNOS is the co-translational N-myristoylation, and consequent membrane localization of eNOS. Using antibodies selective for the eNOS isoform, we have shown that eNOS is enriched in hippocampal synaptosomes. We have previously demonstrated that hippocampal slices incubated in the N-myristoyltransferase inhibitor hydroxy-myristic acid (HMA) failed to exhibit LTP. In order to specifically address the role of eNOS in LTP, we have created an adenovirus vector containing a truncated eNOS (TeNOS) which lacks catalytic activity, yet retains the N-terminal sequence required for myristoylation. Control experiments with adenovirus containing the β-galactosidase (β-gal) gene show that infected slices express marker protein in 4-6 hrs, remain electrophysiologically viable for at least 34 hrs, and display normal synaptic transmission and plasticity. In blind experiments, slices infected with TeNOS virus and allowed to incubate 23-34 hrs (mean = 27.3 +/- 2.2 hrs) failed to exhibit LTP, while control slices infected with β-gal virus display normal LTP [mean % baseline 1 hr after tetanus: TeNOS, 99.2 +/- 3.0% (n=9); control, 216.2 +/- 8.5% (n=8)]. TeNOS infected slices display normal synaptic transmission, paired-pulse facilitation, post-tetanic potentiation, and input/output curves. Recent work has shown that the heterodimerization of wildtype eNOS with truncation mutants similar to our TeNOS construct inhibits wildtype enzyme activity, raising the possibility that our construct is acting as a dominant negative. Alternatively, it is possible that TeNOS competitively inhibits myristoylation of wildtype eNOS (or another myristoyl-protein), thus depriving the cell membrane of catalytically active eNOS. Currently, we are creating a chimeric protein to assess directly whether eNOS is the critical myristoyl-protein effected by inhibition of myristoylation. The chimera fuses the transmembrane domain of CD8 to eNOS thus providing an alternate means of membrane association. Following infection, slices will be incubated with the myristoylation inhibitor HMA. We will then determine whether the CD8-eNOS construct rescues the ability of HMA-treated slices to exhibit LTP. *Supported by the National Institutes of Health and the Arnold and Mabel Beckman Foundation.*

598.17

Augmented LTP in mice lacking GluR2 subunit of AMPA receptor. N. Agopyan*, Z.P. Jia, J. Roder. Dept. of Molecular Neurobiology, SLRI, Mount Sinai Hospital, Toronto, Ontario, Canada.

GluR2 subunit of AMPA receptors is highly expressed in the mammalian central nervous system, and neurons expressing this subunit permate less calcium. In order to examine the extent of its involvement in synaptic plasticity, we created mutant mice lacking the GluR2 subunit (Taverna *et al*, this meeting).

Dendritic field responses were elicited by focal stimulation of Schaeffer collaterals and recorded at 32 °C from the CA1 subfield of hippocampal slices. Input-output curves, constructed by plotting the slope of field EPSP versus presynaptic fiber volley amplitude revealed that the efficacy of excitatory synaptic transmission was indistinguishable between the control (+/+) and GluR2 mutant (-/-) mice. The magnitude of paired-pulse facilitation was also not changed over an interpulse interval range of 20 ms to 1 sec between slices obtained from control and -/- mice. However tetanic (100Hz for 200 ms, 5x at 0.1 Hz) stimulation-induced LTP was 2-fold higher in the -/- mice; the normalized EPSP slope at 60 min after tetanus was 167 ± 15 % and 252 ± 17.8 % in +/+ and -/- respectively (n = 20). When the tetanic stimulation was delivered in the presence of 50-100 μM D-AP5, slices from control mice did not exhibit LTP while those from -/- mice displayed a residual LTP, which persisted even in the presence of nifedipine.

In conclusion, our data indicate that Ca^{2+} permeable AMPA receptors can both induce LTP and enhance it by modulating NMDA-dependent component. *(Supported by MRC. N. Agopyan is a MRC Centennial Fellow)*

598.18

AMPA RECEPTOR CHANNEL CONDUCTANCE IS INCREASED IN LTP IN CA1 REGION OF RAT HIPPOCAMPUS. T. A. Benke, W. W. Anderson* and G.L. Collingridge. Dept. of Anatomy, University of Bristol, Bristol, UK.

In order to study the properties of glutamatergic ion channels responsible for synaptic transmission in the hippocampus, dendrites of CA1 pyramidal neurons of ~ 14 day-old rats were voltage-clamped with patch-electrodes (6-8 MΩ when filled with $CsMeSO_4$ based solution) in the whole-cell configuration (30-40 MΩ access resistance) under visual control. AMPA receptor mediated synaptic currents, elicited by focal stimulation of nearby presynaptic fibers with an insulated and sharpened tungsten monopolar electrode (tip ~ 5 μm), were recorded in the presence of picrotoxin (50 μM) at -70 mV in the presence of 1mM Mg^{2+} and were completely abolished by CNQX (10 μM). Typical currents showed relatively fast rise times on the order of 1-2 msec and decay times of 5-9 msec. Pairing of minimal stimulation (0.5-1 Hz) with depolarization (+10-20 mV for 30 shocks, test intensity) resulted in long-lasting (30 minutes) potentiation of these currents with no apparent change in kinetics and an apparent reduction in synaptic failures. Non-stationary analysis of the variance of these currents revealed a basal single channel AMPA receptor conductance of 7-9 pS before pairing and 13-16 pS after pairing. This provides evidence for a post-synaptic mechanism of expression of long-term potentiation in the rat hippocampus.

Supported by the U.S. NIH (NS09894 to T.A.B) and the Medical Research Council (UK).

598.19

HYPOXIC INHIBITION OF LTP IN CA1 OF RAT HIPPOCAMPAL SLICES IS MEDIATED BY THE ACTIVATION OF NMDA RECEPTORS AND NO RELEASE Y. Izumi*, A.M. Benz & C.F. Zorumski, Dept. of Psychiatry, Washington Univ., School of Med., St.Louis, MO 63110.

It is known that the cognitive impairment and learning disability that follow brain hypoxia or asphyxia may not be manifest for some time after the insult. To understand these delayed sequelae, we examined the effects of hypoxic conditions on hippocampal long-term potentiation (LTP), a leading candidate to be a mechanism underlying memory.

Although 20 min oxygen deprivation (replacement of oxygen with nitrogen) completely suppressed excitatory postsynaptic potentials (EPSPs) in CA1 region of rat hippocampal slices, EPSPs were fully restored following reoxygenation.

The maintenance of LTP was not blocked when the oxygen deprivation was introduced 30 min after tetanus (EPSP slope 60 min after reoxygenation; 136 ± 2 %, N=4). However, LTP could not be induced when a tetanus was delivered 100 min after reoxygenation (EPSP slope 60 min after tetanus; 105 ± 4%). Administration of 100 μM D,L-amino-phosphonovaleric acid (APV), a competitive antagonist of N-methyl-D-aspartate (NMDA) receptors, during the oxygen deprivation, overcame the hypoxic inhibition of LTP (142 ± 7 %, N=5). Similarly, 100 μM L-monomethyl arginine (LMNA), a competitive inhibitor of nitric oxide synthase (NOS), administered during oxygen deprivation, but not during reoxygenation, allowed LTP induction by a tetanus delivered 100 min after reoxygenation (127 ± 6%, N=5). The effects of LMNA were blocked by co-administration of 1 mM L-arginine, a substrate of NOS.

These results suggest that the activation of NMDA receptors during hypoxic conditions results in the failure of LTP induction through NO release.

Supported by the Diabetes Research and Training Center at Washington University and Alzheimer's Disease and Related Disorders Program of University at Missouri.

598.20

POSSIBLE INVOLVEMENT OF PLASMIN AND PLASMINOGEN IN LONG-TERM POTENTIATION OF RAT HIPPOCAMPUS. N. Matsuki*, A. Mizutani and H. Saito. Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, JAPAN.

Effects of proteases and protease inhibitors on generation of long-term potentiation (LTP) were investigated in the CA1 and dentate regions of rat hippocampus *in vitro*. Plasmin, a serine protease, and its precursor plasminogen significantly enhanced short-term potentiation (STP) induced by a weak tetanic stimulation, without affecting basal responses. The STP-enhancing effect of plasmin disappeared by concomitant perfusion of α_2-antiplasmin, an endogenous plasmin inhibitor. Other proteases, such as thrombin, trypsin and cathepsin B, did not affect STP. On the other hand, α_2-antiplasmin and leupeptin significantly attenuated LTP induced by a strong tetanus though plasminogen or plasmin itself did not influence LTP. Furthermore, plasminogen and plasmin did not affect NMDA receptor-mediated synaptic responses in the absence of extracellular Mg^{2+}. These results suggest that endogenous plasmin is involved in the mechanism of LTP in CA1 and dentate regions of rat hippocampus and that the STP-enhancing effect of plasmin is independent of NMDA receptors. (Supported partly by Grant-in-Aid 05558095 from the Ministry of Education, Culture and Science of Japan)

LONG-TERM POTENTIATION: PHYSIOLOGY VII

599.1

LONG-TERM POTENTIATION OF DENTATE GYRUS MOSSY CELLS, S. Chattarji*[1,2] and H.E. Scharfman[2,3]. [1]NNC, Yale University, New Haven, CT 06511, [2]Neurol. Res. Ctr., Helen Hayes Hosp., NY State Dept. of Health, W. Haverstraw, NY 10993, and [3]Depts. Pharmacology & Neurology, Columbia Univ., NY, NY 10032

Granule cells (GCs) and pyramidal cells are thought to play a role in learning and memory functions of the hippocampus. Since mossy cells (MCs) of the dentate hilus are synaptically connected with these cells, MCs may also have a role. In support of this hypothesis, loss of hilar cells has been noted in Alzheimer's disease, and animals with hilar lesions display memory deficits. We studied the potential role of MCs in hippocampal plasticity by determining whether MCs exhibit long-term potentiation (LTP), an increase in synaptic efficacy following high frequency stimulation, and a potential cellular substrate of learning and memory.

Intracellular sharp microelectrode recordings were made from adult rat hippocampal slices (400 μm). Buffer contained (in mM) 126 NaCl, 5 KCl, 26 $NaHCO_3$, 2 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, and 10 d-glucose. Electrodes were filled with 4% Neurobiotin in 1M KCH_3COOH so that MCs could be identified morphologically. The perforant path was stimulated tetanically (100 Hz 1 sec trains 1-4 times) in the molecular layer. Extracellular recordings monitored LTP of the GC population spike or population EPSP simultaneously.

Under these conditions, LTP of MCs was rare (2 of 10 cells), as was LTP of GCs (4 of 10 slices), although short-term potentiation occurred. However, in the presence of the $GABA_A$ receptor antagonist bicuculline (5-10 μM), 3 mM $CaCl_2$, and 3 mM $MgSO_4$, LTP was robust (4 of 4 cells). MCs demonstrated LTP in several ways, such as a decrease in stimulus strength required to reach threshold, increased EPSP amplitude, or increased number of action potentials per suprathreshold stimulus. Spontaneous EPSPs increased after LTP. These data suggest that afferent inputs to mossy cells can exhibit LTP and that local inhibitory circuits may regulate this plasticity. Supported by NS 30831 to HES.

599.2

OXYTOCIN INHIBITS HIPPOCAMPAL DENTATE GRANULE CELL LTP. B.G. Smith, M.J. Wayner and D.L. Armstrong*. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78249-0662, USA.

Oxytocin is a hypothalamic peptide produced in the paraventricular nucleus. It is synthesized by single large clusters of neurons that give off multibranched axons to several distant targets. Oxytocin decreases spontaneous motor activity and retention of a learned response. The purpose of this study was to determine the dose relationship effects of oxytocin on hippocampal granule cell long-term potentiation (LTP). Oxytocin in doses of 1, 5, 10, 30, 50, 100, and 200μg/kg was given to male Harlen Sprague-Dawley rats. To determine these effects of oxytocin, we examined LTP synaptic changes *in vivo*. We stimulated the medial perforant path and recorded from dentate granule cells. Urethane anesthetized rats were injected with the appropriate dose of oxytocin 2 hours and 15 minutes prior to tetanization. The results show that oxytocin can effectively block LTP in doses of 50 and 100μg. Oxytocin did not significantly decrease or inhibit LTP when given in the lower concentrations. Oxytocin has an inhibitory effect on hippocampal dentate granule cell LTP that is probably related to the previously reported impairment of spontaneous motor activity and retention of learned responses.

Supported by The Council for Tobacco Research, USA, Inc., Grant #4038.

599.3

THE 2B SUBUNIT OF THE NMDA RECEPTOR IS ON DENTATE GYRUS INTERNEURONS AND ITS TYROSINE PHOSPHORYLATION MAY CONTRIBUTE TO LOCAL CIRCUIT PLASTICITY. G. Richter-Levin[1]*, K. Rosenblum[2], S. Hazvi[2] and Y. Dudai[2]. Haifa University[1] and the Weizmann Institute of Science[2], Israel .

We have recently reported that gustatory experience enhances tyrosine phosphorylation of a set of synaptic proteins in the rat insular cortex (*PNAS* 92: 1157), including the 2B subunit of the NMDA receptor (NR2B). A similar effect was found following the induction of LTP in the rat dentate gyrus (*Soc. Neurosci. Abst.* 21: 20.1 and *submitted*). Immunocytochemistry with antibodies to NR2B revealed that this subunit is preferentially located in dentate interneurons, at least part of which are GABAergic. The cellular localization of NR2B suggests a role in modulation of local circuit properties. To further investigate this hypothesis, we compared frequency-dependent inhibition (FDI) in the dentate gyrus before and after LTP induction. FDI was assessed by increasing stimulus frequency from 0.1 to 1 Hz. Before induction of LTP the population spike (PS) was reduced to 33+/-13% of its size during stimulation at 0.1 Hz (n=7). At one hr after LTP induction FDI was reduced (PS size inhibited to only 52+/-13% of its size during stimulation at 0.1 Hz, $p<0.01$, n=8). The NMDA channel blocker MK801 (2 mg/kg i.p.) applied 10 min after LTP induction further reduced FDI (PS became 82+/-7% of its original size, n=7). It is suggested that in the dentate gyrus, NMDA receptors on inhibitory interneurons play a role in regulating circuit connectivity, and that tyrosine phosphorylation of NR2B, which in neuronal culture regulates NMDA receptor mediated currents (Wang & Salter, *Nature* 369: 233), may contribute to this mechanism of plasticity. (Supported by the US-Israel Binational Science Foundation, Jerusalem.)

599.4

ARACHIDONIC ACID STIMULATES PROTEIN TYROSINE KINASE IN HIPPOCAMPUS: IMPLICATIONS FOR LTP IN DENTATE GYRUS

M.A. Lynch* and B. McGahon Dept. Physiology, Trinity College, Dublin, Ireland.

The mechanism by which arachidonic acid (AA) and the metabotropic glutamate receptor agonist, ACPD, interact to increase glutamate release in long-term potentiation (LTP) involves activation of phospholipase C (PLC)[1]. The finding that AA and ACPD alone increase PLC activation, while coincident application of both agents is required to increase glutamate release, indicates that a threshold must be reached before activation of PLC is translated into increased glutamate release. We report here that this threshold relies on tyrosine phosphorylation, and subsequent activation, of PLCγ by AA and activation of PLCβ by ACPD.

Synaptosomes were prepared from rat hippocampus and incubated in Krebs solution containing [^{3}H]-inositol to assess PLC activity or [^{32}P]-γ-ATP to assess phosphorylation of the substrate poly[Glu^{4}Tyr^1]. AA-induced PLC activity was inhibited by the tyrosine kinase inhibitor genistein, while it did not affect ACPD-induced PLC activity. AA also increased phosphorylation of poly[Glu^{4}Tyr^1]. Immunoblotting of SDS gel separated proteins from synaptosomes incubated in the presence of AA or ACPD probed with anti-phosphotyrosine and anti-PLCγ antibodies revealed that AA, but not ACPD, increased tyrosine phosphorylation of PLCγ. LTP was induced in dentate gyrus in urethane-anaesthetized rats. Synaptosomes prepared from potentiated tissue showed an increase in tyrosine phosphorylation of PLCγ compared to controls.

These data provide evidence that AA increases tyrosine kinase activity in hippocampus. The data also suggest that one role for AA in LTP is phosphorylation of PLCγ, which together with stimulation of PLCβ by ACPD, allows a threshold activation of PLC to be reached which translates into increased glutamate release.

[1]McGahon B and Lynch MA (1996) Neuroscience 72, 847-855.

We acknowledge the financial support of the Health Research Board of Ireland.

599.5

RYANODINE GENERATES A LOW FREQUENCY INDUCED LTP WHICH IS NMDAR INDEPENDENT BUT mGLUR AND L-TYPE VOLTAGE-GATED Ca CHANNEL DEPENDENT R. Anwyl, Y. Wang, M.J. Rowan, and J. Gigg*. Dept Physiology, Trinity College, Dublin 2, Ireland.

The induction of LTP was investigated in slices of the rat dentate gyrus in the presence of ryanodine, an agent which is known to selectively bind to the intracellular ryanodine receptor (RyR-receptor) Ca channels which regulate Ca release from intracellular Ca stores.

In control media, high frequency stimulation (HFS) induced LTP, and prolonged low frequency stimulation (LFS), 5Hz, 900 pulses, induced long-term depression of field epsps and patch clamped epscs. In the presence of ryanodine, at a threshold concentration of 1μM, HFS-induced LTP was inhibited, whereas LFS induced LTP. The N-methyl-D-aspartate receptor (NMDAR)-antagonist D-AP5, (200μM), did not prevent the induction of LTP by LFS in the presence of ryanodine. This demonstrates the NMDAR-independence of LTP induction in ryanodine. Furthermore, AP5 reversed the block of HFS-induced LTP by ryanodine, showing that activation of NMDAR inhibiits LTP induction in ryanodine. The induction of LTP by LFS in the presence of ryanodine was blocked by the L-type voltage-gated Ca channel blocker nifedipine, and also by Ni, a commonly-used T-type voltage-gated Ca channel blocker. The LFS-induced LTP in ryanodine was inhbited by the metabotropic glutamate receptor antagonist MCPG. The LFS-induced LTP in ryanodine was also blocked by ruthenium red, an agent known to block RyR channel opening.The results of the present studies emphasize the importance of intracellular Ca stores in the induction of LTP.

599.6

A SELECTIVE nNOS INHIBITOR, TRIM, BLOCKS BOTH LTP AND LTD IN RAT DENTATE GYRUS IN VITRO

J Wu, Y Wang,[1] R Anwyl[1] and MJ Rowan* Dept of Pharmacology and Therapeutics, [1]Dept of Physiology, Trinity College, Dublin 2, Ireland

The possible role in the induction of synaptic plasticity of NO generated by the neuronal isoform of NO synthase (nNOS) was investigated in the rat hippocampus *in vitro*. Field population EPSPs were recorded in the medial perforant pathway in the dentate gyrus of submerged slices maintained at 32^{O}C in standard media containing picrotoxin (100μM). Brief bursts of high-frequency stimulation (HFS, 200 Hz) induced a short-term potentiation (STP; 197±43% baseline, $p<0.05$) and long-term potentiation (LTP; 167±20%, $p<0.05$) in control slices. The selective nNOS inhibitor 1-(2-trifluoromethylphenyl) imidazole (TRIM, Handy *et al*, Brit J Pharm 116, 2349), at a concentration (100μM) which had no effect on baseline transmission, significantly reduced STP (127±18%, $p<0.05$) and completely blocked LTP (90±8%). This effect was prevented by co-administration of the substrate amino acid of NOS, L-arginine. Low-frequency stimulation (LFS, 900 pulses at 1 Hz) induced stable long-term depression (LTD; to 69±8%, $p<0.05$) in control slices. Pretreatment with TRIM (100μM) abolished LTD (92±13%). L-Arginine also opposed this effect.

NO generated by nNOS appears to play a critical role in the induction of both LTP and LTD in the dentate gyrus *in vitro*. This is consistent with our previous findings in the CA1 region *in vivo* (Doyle *et al*, J Neurosci 16, 418).

Supported by the Health Research Board of Ireland and the Wellcome Trust

599.7

INVOLVEMENT OF PROTEIN TYROSINE PHOSPHORYLATION IN SYNAPTIC TRANSMISSION AND LTP IN DENTATE GYRUS. H. Xiong*, J. M. Wojtowicz and M. Salter[$] Dept. of Physiology, University of Toronto, Toronto, Ont., Canada M5S 1A8, and Div. of Neuroscience, Hospital for Sick Children[$], Toronto, Ont., Canada M5G 1X8.

Several lines of evidence indicate that pre- and post-synaptic kinases are involved in molecular mechanisms underlying long-term potentiation (LTP). Tyrosine kinases are abundant in the central nervous system and may possibly be involved in LTP. In this study we investigated the effects of tyrosine kinase inhibitors on synaptic transmission and synaptic plasticity in the dentate gyrus of rat hippocampal slices (400μm in thickness) taken from 2-4 week old Wistar rats. Applied by bath at a concentration of 50 μM, Genistein, a tyrosine kinase inhibitor, was found to block the induction of LTP evoked by tetanic stimulation of the medial perforant path. The amplitude of LTP was reduced from 162.2 ± 22.8% of control to 82.8 ± 35.2% of control by Genistein. Pre-incubation of slices with Lavendustin A, another tyrosine kinase inhibitor, had similar depressant effects on LTP. In addition to its effect on LTP, Genistein was also found to inhibit basal synaptic transmission and post-tetanic potentiation in dentate gyrus, possibly through a presynaptic mechanism. These results suggest that protein tyrosine phosphorylation is involved in regulation of synaptic transmission and synaptic plasticity in dentate gyrus.

Supported by EBEWE, Austria and MRC of Canada.

599.8

PROLONGED MEMBRANE DEPOLARIZATION AND INCREASED NUMBER OF ACTION POTENTIALS DURING INDUCTION OF LONG-TERM POTENTIATION IN RAT DENTATE GYRUS. **S. Wang* and J.M. Wojtowicz, Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.**

In dentate gyrus from immature rats (16-30 days), tetanic stimulation fails to produce long-term potentiation (LTP) in a large proportion (65%) of granule neurons. We have addressed possible mechanisms which can enhance the depolarization required for induction of LTP. Under the whole-cell current-clamp configuration, four 0.5 s, 100 Hz pulse trains, separated by 10 s intervals, were used to induce LTP in the medial perforant path-dentate granule cell synapses in the hippocampal slice preparation. It was observed that during the four trains, the number of action potentials increased in parallel with the duration of membrane depolarization in successive trains. This phenomenon was only seen in those cells in which LTP was successfully induced after the tetanic stimulation. Using extracellular field potential recordings to examine the changes during the tetanic pulse trains, it was noticed that in each train the evoked synaptic responses were initially increased then progressively depressed towards the end of the train. However, the first response of each train was larger in the successive trains. Activation of muscarinic receptors by carbachol is known to block the M current and the slow after-hyperpolarization potential in CA1. When we tested the carbachol effects in dentate gyrus, carbachol (10 µM) enhanced the synaptic responses during high frequency trains. Average LTP induced in the presence of carbachol was 132% larger than control. We conclude that cholinergic input may contribute to the induction of LTP by prolonging postsynaptic depolarizations during tetani. Supported by MRC of Canada.

599.9

HIPPOCAMPAL LONG-TERM POTENTIATION AND KINDLING-INDUCED POTENTIATION IN THE FREELY MOVING GUINEA-PIG. T. H. Gilbert*, L. P. Spongberg and G. C. Teskey. Behav. Neurosci. Res. Grp., Dept. of Psychology, Univ. of Calgary, Calgary, AB, Canada T2N 1N4.

Compared to rats, guinea-pigs show a number of differences in kindling behaviour, seizure progression and the induction and decay parameters of piriform evoked responses following either high-frequency stimulation (HFS) or kindling. Understanding the nature of these differences may help to inform us about the underlying neuroplastic mechanisms. Thus, this study was undertaken to examine long-term potentiation (LTP) and kindling-induced potentiation (KIP) of the perforant path - dentate gyrus synapse, in awake behaving guinea-pigs.

Mixed-strain guinea-pigs were chronically implanted with bipolar stimulating electrodes in the perforant path (PP) and bipolar recording electrodes the dentate gyrus (DG). Following a two week recovery period, baseline input/output (I/O) responses were generated. These were based on the application of rectangular 200 μsec pulses at 11 intensities through the PP electrodes. In the LTP protocol, I/O responses were taken for 3 days before HFS and immediately following HFS. Follow-up I/Os were then taken daily for 3 days and then once weekly for at least 3 weeks. In the KIP protocol, I/O responses were taken 3 days before kindling stimulation, immediately following the first afterdischarge (AD), and 24 hrs after the first AD. The animals were then stimulated once per day for 4 consecutive days. Follow-up I/O responses were then taken for 3 days following the last AD and once weekly for at least 3 weeks.

Preliminary results of the LTP data showed no change in the evoked response with the application of only one session of high frequency stimulation. Preliminary results of the KIP data showed an increase in the amplitude of the evoked response 24 hrs after the first AD and a further increase following the subsequent ADs. However, there appears to be a rapid decay of the evoked response within 2-3 days. Supported by NSERC.

599.10

A NOVEL IMMEDIATE-EARLY GENE, Ti3, IS INDUCED DURING SEIZURE AND LTP. K. Inokuchi*, A. Kato, K. Hirai and F. Ozawa. Mitsubishi Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan.

The prolonged maintenance of hippocampal LTP depends on *de novo* protein and RNA synthesis, indicating an involvement of altered gene expression in long-lasting changes in synaptic efficacy. We have employed PCR-based mRNA differential display to identify a set of genes that are induced by neural activity in the rat hippocampus. One of the cDNAs isolated, denoted Ti3, was rapidly induced in the hippocampus following the intraperitoneal injection of pentylenetetrazole (PTZ). The increase in Ti3 mRNA was mainly observed in granule cells of the dentate gyrus, while no or very weak induction in the CA1 and CA3 region. Ti3 represents one of the immediate-early genes since the transcripts were induced by PTZ even in the presence of protein synthesis inhibitor cycloheximide. Ti3 mRNA was approximately 7 kb in size. DNA sequence analysis of the cDNA clone and search of the EMBL and GenBank databases showed that Ti3 seems to be novel and not related to any known genes. Under the urethane-anesthesia, repeated high frequency stimulation given to the perforant pathway, which elicited a long-lasting (>10 h) LTP measured from the dentate gyrus, caused a drastic increase in Ti3 mRNA at granule cell layer of the hippocampus ipsilateral to the stimulating electrode 45 min after the delivery. The increase persisted at least for 3 h. Preinjection of a specific NMDA receptor antagonist, MK801, completely blocked the increase in the level of Ti3 mRNA as well as LTP induction, suggesting a correlation between the induction of Ti3 transcription and of LTP. (Supported in part by Grant-in-Aid #07279106, the Ministry of Education, Science, sports and Culture of Japan.)

599.11

LEVELS OF THE mRNA ENCODING SYNTAXIN 1B ARE DIFFERENTIALLY INCREASED IN THE CORTEX, ACCUMBENS AND HIPPOCAMPUS FOLLOWING ACQUISITION OF A SPATIAL REFERENCE OR WORKING MEMORY TASK. S. Davis*, J. Rodger, A. Hicks, J. Mallet and S. Laroche. NAM, CNRS URA1491, Université Paris-Sud, 91405 Orsay, France; LGN, CNRS UMR9923, Hôpital de la Pitié Salpêtrière, 75013 Paris, France.

It has been suggested that the presynaptic vesicle protein, syntaxin 1B is implicated in mediating a form of transsynaptic plasticity, as induction of LTP on the perforant path leads to an increase in the mRNA in the dentate granule cells and a subsequent increase in the protein at the mossy fibre terminal zone. We hypothesised that this may be a mechanism for the propagation of plasticity through interconnected neural networks. To test this hypothesis, the mRNA levels of syntaxin 1B were measured in several brain structures of rats which had been trained to criterion on either a spatial working or reference memory task on the radial arm maze (5-7 days). The data show that rats learning a spatial working memory task showed a significant increase (approx 30-40%) in syntaxin 1B mRNA in the dentate gyrus, CA3 and CA1 of the hippocampus compared with controls, whereas rats learning a spatial reference memory task showed an increase in the prelimbic region of the prefrontal cortex (80%) and the shell of the accumbens (80%). Naive and motor control groups showed no structure specific change and no group showed specific change in either the motor or parietal cortices. As syntaxin has been shown to be a marker of transsynaptic plasticity and as the hippocampus has monosynaptic connections to both the prelimbic region and the accumbens that support LTP induction, we believe the differential increase in syntaxin 1B mRNA may reflect one possible mechanism underlying the propagation of plasticity through hippocampal connections with other structures, such that continuous manipulation of information (as in a working memory task) occurs in the hippocampus but as information becomes consolidated across time (as in a reference memory task) it is represented in efferent connections of the hippocampus. Further studies are under investigation to test changes in the mRNA expressing syntaxin 1B at different stages of learning. Supported by a grant from the HFSP.

599.12

SYNAPSIN I AND SYNTAXIN 1B: KEY ELEMENTS IN THE CONTROL OF NEUROTRANSMITTER RELEASE ARE REGULATED BY NEURONAL ACTIVATION AND LONG-TERM POTENTIATION *IN VIVO*. A. Hicks, S. Davis, J. Rodger, A. Helme-Guizon, S. Laroche* and J. Mallet. LGN, CNRS UMR9923, Hôpital de la Pitié Salpêtrière, 75013 Paris, France; NAM, CNRS URA1491, Université Paris-Sud, 91405 Orsay, France.

Little is known about the regulatory effects of synaptic plasticity on the proteins of the exocytotic machinery *in vivo*. At different times following the induction of long-term potentiation (LTP) or increasing neuronal activity in neurones within the dentate gyrus *in vivo*, *in situ* hybridisation revealed that from the many mRNAs that encode proteins involved in regulated exocytosis, only those encoding synapsin I and syntaxin 1B were specifically increased. Increased neuronal activity by high-intensity stimulation to evoke a response matching that seen in LTP, produced short-lasting unilateral changes visible two hours after tetanus (94±26% for syntaxin 1B and 20±6% for synapsin I), whereas induction of LTP resulted in long-lasting postsynaptic changes visible at least five hours after tetanus (98±13% for synapsin I and 120±34% for syntaxin 1B). Furthermore, LTP induction produced contralateral changes visible also at five hours (52±15% for synapsin I and 95±41% for syntaxin 1B). These changes suggest an overall increase in neuronal excitation within a neuronal network can be differentiated from a change in synaptic strength at a specific subset of synapses, where only plastic changes lead to enduring modification. Thus we demonstrate that synaptic plasticity can lead to relatively long-term changes in selective members of the exocytotic machinery. Altered concentrations of key vesicle proteins may thus provide the means for modulation of neurotransmitter release over long time periods. The persistent postsynaptic increase, induced by LTP, in mRNAs encoding these presynaptic proteins has important implications for the propagation of signals downstream from the site of LTP induction in hippocampal neural networks, and highlights a candidate cellular mechanism for mediating the propagation of plastic changes in such networks, which may be important in the laying down of memories. Supported by HFSP.

599.13

SYNAPTIC STRUCTURAL BASIS OF LTP: SPECIFIC MORPHOLOGICAL CHANGES 24 HOURS POST-LTP INDUCTION IN THE RAT DENTATE GYRUS. A. Weeks, T. Ivanco, J. LeBoutillier, R. J. Racine and T. L. Petit*. Dept. of Psychology and Prog. in Neuroscience, University of Toronto, 1265 Military Trail, Scarborough, Ontario, CANADA, M1C 1A4.

Long-term potentiation (LTP) involves a long-lasting enhancement of electrophysiological response following tetanic stimulation which is thought to arise from synaptic plasticity. The present study examined synapses in the stimulated middle molecular layer of the rat dentate gyrus following high frequency stimulation of the medial perforant path. Implanted non-stimulated animals and the non-stimulated inner molecular layer served as controls. The animals were sacrificed 24 hours after the final stimulation of the LTP group. The number of synapses in each of the molecular layers was estimated using an unbiased dissector technique. The shape of each synapse and the presence of perforations in the post synaptic density were recorded. The maximal lengths of the pre- and postsynaptic elements were also measured. The results did not indicate changes in all of the structural features that have been observed at shorter post-induction intervals. However, there was a significant increase in the number of concave and irregularly shaped synapses in the tissue stimulated during LTP. These synaptic types were also found to be significantly smaller in the LTP tissue. It is possible that these synaptic shapes underlie increases in synaptic efficacy. Their increase in number may, therefore, help support the potentiated electrophysiological response observed 24 hours after the induction of LTP. **Research Supported** by grants from the Natural Sciences and Engineering Research Council of Canada to T.L.P.

599.14

THE Ca^{2+} CHANNEL BLOCKER Ni^{2+} REDUCES THE INDUCTION OF THE HEBBIAN FORM OF LTP AT HIPPOCAMPAL MOSSY FIBER SYNAPSES A. Kapur* and D. Johnston, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Long-term potentiation (LTP) at mossy fiber synapses does not require the activation of NMDA receptors, but may have two forms, called Hebbian and non-Hebbian, based on factors required for induction (Urban and Barrionuevo, *J. Neurosci.*, 1996). The Hebbian form depends on postsynaptic membrane potential and intracellular Ca^{2+}. In this study we tested the hypothesis that Ni^{2+}-sensitive Ca^{2+} channels are one source for the rise in postsynaptic $[Ca^{2+}]_i$ that permits the induction of the Hebbian form of LTP. Perforated-patch recordings of mossy fiber EPSPs were obtained from CA3 neurons in hippocampal slices by stimulating with a glass pipette in stratum lucidum. Test pulses were delivered at 0.1 Hz, and LTP was induced with high frequency stimulation (HFS) of 15 bursts delivered at 5 sec intervals, with each burst consisting of 7 pulses at 100 Hz. Depolarizing pulses were injected into the recorded cell, in conjunction with the stimulus bursts, to evoke spikes during the bursts. In the presence of NMDA-receptor blockers (50 μM D,L-APV, or APV + 10-20 μM MK-801) and $GABA_A$ blockers (10 μM bicuculline + 10 μM picrotoxin), LTP was induced in 6/7 cells. The EPSP slope 15 min after HFS was potentiated by 72±26% (mean± SEM). When $NiCl_2$ (25–100 μM) was added to the bathing medium, the same HFS induced LTP in only 7/14 cells. The EPSP slope 15 min after HFS was potentiated by only 29±7%. In 4 of the 7 cells that did not show LTP in the presence of $NiCl_2$, LTP could be induced after washing out the Ni^{2+}. These results suggest that Ca^{2+} entry through Ni^{2+}-sensitive channels plays a role in the induction of the Hebbian form of LTP at mossy fiber synapses. Further studies will be needed to determine whether the effects of Ni^{2+} are being mediated through a block of the low- or high-voltage activated Ca^{2+} channels that are known to be sensitive to Ni^{2+}. (MH44754, MH48432, NS11535).

599.15

PROPERTIES OF LTP INDUCTION IN THE CA3 REGION OF THE PRIMATE HIPPOCAMPUS. G. Barrionuevo*, N.N. Urban, D.A. Henze, D. A. Lewis *Departments of Neuroscience and Psychiatry and Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, PA 15260.*

We performed experiments to compare LTP induction in primate CA3 to previous work in the rat. Transverse hippocampal slices (400 μm) were prepared from young cynomologous macaque monkeys. Field EPSPs were recorded in the CA3 region following stimulation of mossy fiber (MF) or collateral/associational (C/A) axons.

Application of HFS to C/A fibers resulted in LTP of the field EPSP recorded in *s. radiatum* (132 ± 7% of control, N=5). LTP induction at this synapse was reversibly blocked by the addition of 25 μM D-APV to the bathing medium (N=4).

Presumed MF field EPSPs were recorded in *s. lucidum* in response to granule cell stimulation. Unlike in guinea pig, but like in rat, MF responses were insensitive to L-AP4 (N=7). Thus, this drug could not be used to screen for pure MF responses. D-APV (25 μM) and in some cases MK-801 (10 μM) were added to the bathing medium to block NMDA-dependent LTP. MF LTP was induced using two patterns of HFS (Urban and Barrionuevo, *J. Neurosci.* In press). Long trains of HFS (L-HFS: 100 pulses at 100 Hz, repeated 3 times) induced LTP that included a large early component. L-HFS applied during blockade of synaptic transmission by 10 mM kynurenic acid (KYN) also induced LTP following the washout of the KYN (137 ± 6% LTP at 35 minutes post HFS, N=6), suggesting that L-HFS can induce a presynaptic non-Hebbian form of LTP. L-HFS also induced LTP in 10-30 μM naloxone (131 ± 8% of control, N=12). Brief trains of HFS (B-HFS: 8 pulses at 100 Hz repeated 10 times) induced LTP of the MF field EPSP under control conditions, but not when administered in the presence of KYN (N=110 ± 12% of control, N=4), suggesting that LTP induction by B-HFS requires postsynaptic depolarization. Together, these data suggest that the induction of synaptic plasticity in the primate CA3 region is similar to what we have observed in the rat. Supported by NS24288, MH51234, a HHMI predoc. fellowship (to NNU) and an NIMH predoc. Fellowship (to DAH).

599.16

MONOSYNAPTIC PERFORANT PATH-CA3 RESPONSES DISPLAY ASSOCIATIVE LTP *In Vivo.* *Brian E. Derrick* and Joe L. Martinez, Jr.* Division of Life Sciences, The University of Texas, San Antonio, TX 78249.

Direct medial and lateral perforant path projections to the CA3 region of the hippocampus display LTP (Breindl et al., 1993). However, studies have not yet addressed associative interactions within medial and lateral perforant path-CA3 projections, or between the perforant path projections and other afferents to area CA3. Adult (>90 day) male Sprague Dawley rats were anesthetized with pentobarbital and maintained at 37° C. Using stereotaxic coordinates, perforant path-CA3 responses were evoked by stimulation of the extreme dorsomedial or lateroventral aspects of the angular bundle as described previously (Breindl, *ibid*). In addition, commissural-CA3 responses were evoked by stimulation of the contralateral CA3 region. In each experiment, responses from two of the pathways were evoked alternately at a rate of 0.33 Hz. In one pathway, responses were evoked at intensities that elicited responses 20-25% of the maximal field EPSP amplitude. The alternate pathway was stimulated at intensities producing a response that was 50% of the maximal EPSP amplitude. After a 20 min period of stable baseline responses, the 50% intensity pathway was stimulated (five 2 sec trains of 100 Hz, 50 msec bursts at intervals of 200 msec) to produce LTP in this pathway, and to verify independence of the two afferent systems by the lack of LTP in the 25% intensity pathway. Thirty min following this stimulation, the 25% pathway was stimulated alone to verify that this intensity was ineffective in inducing LTP. Thirty min later, both the 25% and 50% pathways were stimulated simultaneously. Using this paradigm, we observed that medial and lateral perforant path-CA3 afferents display associative LTP with each other or with commissural-CA3 afferents. Thus associative LTP can occur between medial perforant path-, lateral perforant path-, and commissural-CA3 synapses.

Supported by DA04195 & the Ewing Halsell Endowment (J.L.M.) and a UTSA Faculty Research Award (B.E.D.).

599.17

INTENSITY-DEPENDENT BIDIRECTIONAL PLASTICITY AT THE MOSSY FIBER-CA3 SYNAPSE. *J.A. Reyes, B.E. Derrick, and J.L. Martinez, Jr.* Div. of Life Sciences, Univ. of Texas, San Antonio, TX 78249

Data supporting theories of bidirectional modification (LTD and LTP) of synaptic strength are observed in a number of neocortical and archicortical synapses, suggesting that NMDA receptor-dependent LTP and LTD display different induction thresholds dependent on the level of postsynaptic activity, and perhaps the degree of postsynaptic depolarization. The mossy fiber synapse also displays bidirectional modifications of synaptic strength. However, both LTP and LTD at this synapse appear independent of NMDA receptor activation. We reported previously that both homosynaptic mossy fiber LTD and LTP are dependent on mossy fiber activity and the activation of opioid receptors (Derrick and Martinez, Nature, in press). However, the dependence of stimulation intensity (cooperativity) on the induction of homosynaptic LTD and LTP has not been investigated in this pathway. In anesthetized adult rats, we used a stimulation paradigm (two, 1 sec, 100 Hz trains) that reliably elicits mossy fiber LTP, and varied the intensity of mossy fiber stimulation. Current intensities eliciting mossy fiber field EPSPs 25% and 33% of the maximum response amplitude produced a homosynaptic LTD of the mossy fiber field EPSP slope. Stimulation at current intensities eliciting responses 40% and 50% of maximum response amplitude elicited LTP of mossy fiber responses. Importantly, in agreement with bidirectional rules suggesting a null effect at the cross-over point between LTD and LTP, an intermediate current intensity (37%) did not produce significant changes in mossy fiber responses. These data suggest that homosynaptic mossy fiber LTD and LTP display mechanisms of induction that display different thresholds of intensity. This data is consistent with the BCM model, for this cooperative effect likely is related to the level of postsynaptic activity, and possibly postsynaptic depolarization. Supported by DA04196, NSF6567 (J.L.M.); a UTSA Faculty Research Award (B.E.D.) and an APA- Minority Postdoctoral Fellowship 2T32MH18882-08 (J.A.R.)

599.18

PROPERTIES OF LONG TERM POTENTIATION BETWEEN PAIRS OF HIPPOCAMPAL NEURONS IN ORGANOTYPIC CULTURE

P. Pavlidis* and D.V. Madison. Department of Molecular and Cellular Physiology, Stanford University School of Medicine. Stanford CA, 94305.

We are studying associative LTP between monosynaptically coupled pairs of pyramidal cells. We use 7-14 day old organotypic interface cultures of hippocampal slices taken from 7-10 day old rats. We first showed that LTP can be induced reliably by pairing postsynaptic depolarization with extracellular stimulation of afferent axons at 1 Hz for 1 minute. To determine whether LTP could be observed between pairs of neurons, we used dual whole-cell recordings from nearby pyramidal cells within area CA3 or between CA3 and CA1. Monosynaptic EPSCs could be reliably observed following the stimulation of an action potential in the presynaptic cell with a depolarizing current pulse. We found that LTP of the EPSC could be induced between pairs of cells by pairing depolarization of the postsynaptic cell with presynaptic spike delivery at 1 Hz for 1 min. The LTP is associative, as 1 Hz stimulation or depolarization alone do not result in LTP. The LTP is pathway specific, as potentiation of an extracellularly stimulated pathway did not result in potentiation of the pair synapse; likewise, inducing LTP in the pair did not result in LTP of the extracellularly evoked response. Thus this LTP has many of the properties of associative LTP in acute slices. These results set the stage for an exploration of the role of the presynaptic neuron in the potentiation. We plan studies in which the presynaptic cell will be pharmacologically manipulated specifically via the presynaptic recording electrode. Supported by the Conte Center for Neuroscience Research (MH48108).

599.19

GAP-43 IS EXPRESSED WITH THE INDUCTION OF MOSSY FIBER LTP. E.J. Barea-Rodríguez*, S. Peña de Ortiz, M. Escobar, J. A. Reyes and , J.L. Martinez, Jr. Division of Life Sciences, University of Texas, San Antonio, TX 78249.

Growth Associated Protein (GAP-43) is associated with axonal growth and synaptic plasticity. In the present study we investigated the expression of GAP-43 after the induction of mossy fiber long-term potentiation, a form of plasticity which depends on the activation of opioid receptors. Animals were handled for 7 consecutive days to minimize stress. Rats were then anaesthetized and were implanted with stimulating electrodes in the mossy fibers and recording electrodes in the CA3 pyramidal cell region. Baseline evoked responses were collected for 20 min and this was followed by the delivery of high-frequency stimulation (2, 100Hz, 1 sec) which induced LTP. Animals were sacrificed at 18 and 24 hrs after LTP induction and the brains were sectioned and prepared for *in situ* hybridization. In sham animals, low frequency stimulation resulted in a moderate bilateral increase in GAP-43 mRNA in all hippocampal subfields when compare to intact controls. LTP resulted in a pronounced decrease in GAP-43 mRNA levels in area CA3 at 18 hrs when compared to sham. In contrast, GAP-43 mRNA was robustly increased in the dentate gyrus 24 hrs after LTP induction. This increase was blocked by prior administration of the opioid receptor antagonist naloxone (10 mg/kg, i.p.). These results suggest that GAP-43 may be involved in the long-term changes in synaptic plasticity associated with mossy fiber long-term potentiation and its expression, under the conditions used in this study, is regulated by opioid receptors. *This work was supported by NSF (J.L.M., grant IBN 9411-564) and a NSF postdoctoral fellowship (S.P.O.)*

599.20

HZF-3 IS AN IMMEDIATE-EARLY GENE IN MOSSY FIBER LTP AND IS INDUCED BY TRAINING IN THE MORRIS WATER MAZE. J. Onton, S. Peña de Ortiz*, J. Reyes, C. Vallejos, E.J. Barea-Rodriguez, and , J.L. Martinez, Jr. Division of Life Sciences, University of Texas, San Antonio, TX 78249.

Induction of mossy fiber LTP in anesthetized rats results in enhanced expression of HZF-3, an immediate-early orphan receptor related to NGFI-B. HZF-3 mRNA levels increase ipsilaterally in the dentate gyrus one hour after mossy fiber LTP induction and remain elevated after 5 hours. The importance of this gene activation to the establishment of LTP is strongly suggested by the fact that the opioid receptor antagonist naloxone blocks both the induction of LTP and the upregulation of HZF-3 mRNA levels. To determine whether HZF-3 expression is also regulated during learning we studied the changes in HZF-3 mRNA which occur after training in the Morris water maze. Rats were subjected to two training sessions of 10 trials each in which they were allowed to search for the submerged platform for 60 seconds. Animals were sacrificed 5 hours after initial exposure to the maze and HZF-3 mRNA levels were studied using *in situ* hybridization. Training resulted in a pronounced increase in HZF-3 mRNA in the hippocampal formation, entorhinal cortex, subiculum, and several white matter areas, including the fimbria, and internal capsule. Naloxone (10 mg/kg), injected 15 minutes prior to the first training session resulted in a complete blockade of HZF-3 mRNA upregulation in all brain areas but the internal capsule. Together, these studies support the notion that HZF-3 is an important immediate-early gene associated with changes in synaptic plasticity which occur during spatial learning and provide evidence that opioidergic neuromodulation is involved in leaning as well as in LTP. *This work was supported by NSF (J.L.M., grant IBN 9411-564) and a NSF postdoctoral fellowship (S.P.O.)*

600.1

COMPARISON OF TWO INDEPENDENT FORMS OF LTP IN AN ANIMAL MODEL OF IN UTERO COCAINE EXPOSURE
J.S. Martinez, J.Z. Little, W.L.R. Cruce* and T.J. Teyler Dept. Of Neurobiology, Northeastern Ohio Univ. Coll. Of Med., Rootstown, Ohio 44272

The effects of in utero cocaine exposure on two independent forms of LTP were studied in hippocampal area CA1. Using 400 μm hippocampal slices from a rabbit model of gestational cocaine exposure we compared NMDA-receptor dependent and independent forms of LTP. Slices were maintained in an interface chamber at 33° C and extracellular electrodes were used to record field EPSPs. NMDA-receptor dependent LTP was induced by afferent fiber activation at 25Hz for 1 second. NMDA-receptor independent LTP was in the presence of 50 μM APV by four tetanic bursts of 200 Hz each lasting .5 seconds. Differences in LTP kinetics and magnitude were analyzed. Possible mechanisms underlying observed differences will be discussed.
This project was supported by NIDA Grant #DA06871

600.2

CHARACTERIZATION OF LIPOSOME MEDIATED TRANSFECTION OF MOUSE ORGANOTYPIC HIPPOCAMPAL CULTURE SYSTEM.
Christine L. Olsson#, Loan B. Nguyen^, Alfred T. Malouf*, and Bruce L Tempel#. VM Bloedel Hearing Res. Ctr., Dept. of Otolaryngology# and Neurosurgery^, U.W., Seattle, WA 98195, Dept. of Pediatrics* 6003, Case Western Reserve Univ., Cleveland, OH 44106.

In order to study focal alterations in the expression of mouse K channel genes we have developed a mouse organotypic hippocampal slice culture system. Our culture conditions allow hippocampi from 3-5 postnatal day C3HeB/FeJ mice to be maintained in vitro for up to 2 months. To characterize the system, cultures were fixed at day 11 in vitro, a time corresponding to 14 days postnatal, and processed for Timms staining, cresyl violet staining, or immunocytochemistry using antibodies against mKv1.1 or mKv1.2 protein. Staining patterns were similar to control 14 day postnatal hippocampus. Adult hippocampus showed different staining patterns, most obviously with Timm's staining and ICC. Since the culture system appears to model hippocampal development in vivo, we attempted to develop a focal transfection system using plasmid-liposome complexes delivered by pressure injection. Plasmid constructs contained the CMV early gene promoter and myc epitope tagged K channel genes. At 11 days in vitro, cultures were injected near the pyramidal cell layer with plasmid-liposome complex, liposome alone or plasmid DNA alone. The cultures were fixed and stained with myc antibody from 1 to 7 days after injection to assess introduced gene expression. Cultures injected with DNA alone showed no transfection. Approximately 30% of the cultures injected with plasmid-liposome complexes expressed myc tagged K channel protein. Cresyl violet staining of injected slices indicated no obvious cell mortality. These results suggest that the mouse organotypic hippocampal culture models normal hippocampal development and that pyramidal cells in these cultures can be focally transfected with plasmid-liposome complexes. Supported by NIH grant NS27206.

600.3

EFFECTS OF HYPERCAPNIA ON HIPPOCAMPAL LONG-TERM POTENTIATION IN ANESTHETIZED RATS.
M. Yoshioka*, Y. Itoh, M. Matsumoto, H. Togashi, K. Mori and H. Saito Dept. of Pharmacol. Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

The effects of hypercapnia, which has been reported to impair consciousness, on the long-term potentiation of the population spike in the hippocampus were studied in vivo. Experimental conditions of hypercapnia were obtained by loading of 13 % CO_2 (F_{ICO2} = 0.13) with normal O_2 concentration (F_{IO2} = 0.21) for 80 min in halothan-anesthetized rats. Arterial CO_2 and O_2 tension was 104.09 ± 12.86 and 90.71 ± 18.89 mmHg (mean ± S.D., n = 18), respectively. A stainless steel bipolar electrode with 0.5 mm tip separation (0.1 mm in each tip diameter) was lowered into Schaffer's collaterals to stimulate. A monopolar glass-coated recording electrode (approximately 20 mm in tip diameter) was placed in the CA1 region. Under these conditions the amplitude of the population spike was reduced. After delivery of tetanic stimulation (400 Hz, 10 burst of 8 pulses, inter-burst interval 1 s) population spike height was not enhance relative to the baselines. Unloading of inspired CO_2 unmasked population spike amplitude enhancement to 150% of the basal levels. Following the second delivery of the same high frequency trains, population spike height was still enhanced to the levels of control rats. These results suggest that hypercapnia may suppress both hippocampal synaptic transmission and its long-term potentiation.

600.4

SYNAPSES IN MATURE HIPPOCAMPAL SLICES (CA1) WITH AND WITHOUT LONG-TERM POTENTIATION (LTP)
K.E. Sorra* and K.M. Harris, Division & Program in Neuroscience, Children's Hospital and Harvard Medical School, Boston, MA 02115.

Hippocampal slices are a model system in which to study the cellular mechanisms of synaptic transmission and plasticity (LTP). To establish whether changes in synapse number underlie the enduring expression of LTP in area CA1, hippocampal slices were evaluated electrophysiologically and the number and structure of synapses were analyzed. Experiments involved: (1) an across slice comparison, and (2) a within-slice experimental design. The unbiased series sample analysis, and 3-D reconstructions of dendritic segments were used to evaluate synapse number in s. radiatum at 2 hours posttetanus. In the across slice comparisons, slices were tetanized either in the presence or absence of 50 µM APV, and untetanized slices also served as controls. Results from 7 young adult rats revealed synapse number was not significantly different between the LTP, untetanized control and APV conditions. For the within slice experiments, a "coulombic" control (5 Hz) and LTP pathway (100 Hz) were monitored independently. Quantitative anatomical analysis of these slices also revealed no significant difference in synapse number between the LTP and control sites. Together, these results suggest that the increased synaptic efficacy characteristic of LTP in area CA1 does not involve synaptogenesis at 2 hours posttetanus. To address whether enduring LTP involves structural modification of existing synaptic elements, quantitative analysis of synapse dimensions is ongoing.
Supported by NSERC (KES), NIH-NINDS #NS21184 and the MR center grant P30-HD18655 from NICHD.

600.5

LTP AND LTD ARE INDUCED BY ACTIVATION OF DISTINCT NMDA RECEPTOR SUBTYPES.
S. Hrabetova*, T. C. Sacktor, Depts. of Pharmacology and Neurology, State University of New York at Brooklyn, Brooklyn, NY 11203.

In the hippocampal CA1 region, a common trigger for the induction of homosynaptic LTD and LTP is the increase in postsynaptic calcium through the activation of NMDA receptors. The mechanism by which the activation of the NMDA receptor leads to these opposing forms of synaptic plasticity is not known. Here we report evidence indicating that LTP and LTD are induced through the activation of pharmacologically distinct subpopulations of NMDA receptors.

Hippocampal slices were prepared from Sprague-Dawley rats (16-21 days). Field excitatory postsynaptic potentials (EPSPs) were recorded from stratum radiatum using standard extracellular technique. For LTP induction, 100Hz tetanization of Schaffer collaterals was given for 1 sec, producing enhancement of synaptic transmission (149.3 ± 2.4% of the baseline 30 min after tetanization, $p < 0.001$, n = 8). LTD was induced by 3Hz stimulation for 5 min (73.0 ± 3.2%, $p < 0.005$, n = 8). We examined the effect of two different competitive antagonists of the NMDA receptor, D-2-amino-5-phosphonovaleric acid (D-AP5) and 3-((RS)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP). CPP, in contrast to D-AP5, binds with high affinity to conventional NMDA-receptor subtypes, but not to atypical subtypes that are relatively independent of Mg^{2+}-mediated voltage-dependent blockade. As previously reported (Dudek and Bear, PNAS 89: 4363, 1992; Mulkey and Malenka, Neuron 9: 967, 1992), applications of D-AP5 (50 µM) blocked both LTP and LTD (102.1 ± 0.9%, 99.5 ± 1.7%, respectively, n = 8). In contrast, CPP (10 µM) prevented LTP (101.7 ± 1.7%, n = 8) but not LTD (76.5 ± 4.8, $p < 0.005$, n = 8).

We conclude that the induction of LTD and LTP requires the activation of different NMDA-receptor subpopulations. Since the only atypical NMDA subunit expressed in the hippocampus is NR2D, we propose that LTD is specifically induced through activation of NMDA receptors containing the NR2D subunit.
Sponsored by grants from the NIH and the Epilepsy Foundation of America.

600.6

HFS-induced LTP in area CA1 of the rat hippocampus is not a model for spatial learning
C Hölscher[1]*, L McGlinchey[1], R Anwyl[2], M J. Rowan[1]
[1]Dept of Pharmacology and Therapeutics, [2]Dept of Physiology, Trinity College, Dublin 2, Ireland

Long-term potentiation (LTP) in the hippocampus has been suggested to model memory formation. Recently it was shown that LTP in the dentate gyrus of the hippocampus can be blocked without affecting spatial learning, and that mossy-fiber LTP in area CA3 is not required for spatial learning. Here we show that the presynaptic metabotropic glutamate receptor (mGluR) agonist (1S,3S)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3S-ACPD; 100 nmol *icv*) depressed baseline (55% of control) and totally blocked LTP induced by high-frequency stimulation (HFS; 600 pulses at 200 Hz; 180±20% LTP in control, 91±21% in ACPD group; $p<0.0001$) in the CA1 region in urethane anaesthetised rats. 50 nmol 1S,3S-ACPD also blocked LTP but did not affect baseline (101±9% of baseline after drug injection). The block of LTP was prevented by the presynaptic mGluR antagonist MCCG (151±9% LTP; MCCG 500nmol +ACPD 50nmol group).

In contrast, 1S,3S-ACPD (100 nmol *icv*) only marginally impaired learning a spatial task in a water maze or radial arm maze. No effect was seen in the acquisition phase of the water maze or the radial arm maze while a small effect was detected in the transfer tasks (distance swum in target quadrant 37±3% for control; 29±3% for drug group; $p<0.05$). Swim speed or behavior in the open field was not affected.

The results contradict the assumption that LTP in CA1 is a valid model for synaptic plastic events that might occur during learning.

Supported by the Health Research Board of Ireland.

600.7

APICAL DENDRITIC LOCATION OF SLOW AHP CHANNELS IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS

P. Sah * and J. M. Bekkers The Neuroscience Group, Discipline of Human Physiology, Faculty of Medicine, University of Newcastle, Newcastle, NSW 2308 & Division of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia.

In CA1 pyramidal neurons trains of action potentials are followed by a slow afterhyperpolarization (AHP) which is due to activation of a calcium-activated potassium current (sI_{AHP}). The AHP is responsible for spike frequency adaptation and is modulated by a range of neurotransmitters including noradrenaline, acetylcholine and glutamate. In this study we have examined the cellular location of active sI_{AHP} channels. Hippocampal slices were prepared from 18-21 day-old rats and whole-cell recordings made using the "blind" approach. First we measured the "switchoff" of the AHP current and compared it with that of two GABAergic synaptic currents (IPSCs) with known somatic and dendritic locations. For the sI_{AHP}, the switchoff had a time constant of 7.4 ± 0.4 ms , compared with 3.5 ± 0.5 ms and 8.8 ± 0.3 ms for the somatic and dendritic $GABA_A$ IPSCs respectively. Analysis of the data using a passive cable model of the CA1 pyramidal cells indicated that active AHP channels are concentrated on the proximal dendrites within about 200 µm of the soma. Excitatory postsynaptic potentials (EPSPs) evoked in *Str. Radiatum* had their amplitudes shunted more during the AHP (0.81 ± 0.04) than did EPSPs evoked in *Str. Oriens* (0.98 ± 0.07) indicating that sI_{AHP} channels are restricted to the apical dendritic tree. We then examined one possible consequence of this AHP-induced apical shunt for synaptic integration. A brief tetanic stimulus in *Str. Radiatum*, which in control conditions produced short-term potentiation (STP), produced long-term potentiation (LTP) when the AHP was blocked. These results suggest that, in addition to its role in spike frequency adaptation, the AHP works as an adjustable gain control, variably shunting synaptic potentials arising in the apical dendritic tree. (Supported by The Sylvia and Charles Viertel Foundation and the Australian Research Council)

600.8

LTP EXPRESSED IN THE HIPPOCAMPAL CA1 REGION FOLLOWING 100 HZ OR THETA-PATTERNED STIMULATION IS ENHANCED BY $GABA_A$RECEPTOR ANTAGONISM. C.A. Chapman*, Y. Perez, and J.-C. Lacaille, Centre de Recherche en Sciences Neurologiques et Département de Physiologie, Université de Montréal, Montréal, Canada, H3C 3J7.

Long-term potentiation of excitatory synaptic responses in hippocampal CA1 pyramidal neurons is also accompanied by increases in GABA inhibition generated by local interneurons. The impact of potentiated inhibitory inputs on the enhancement of postsynaptic responses in CA1 pyramidal cells was assessed here by monitoring the effects of tetanization on stratum radiatum field potentials evoked by stimulation of Schaffer collaterals in the presence and absence of inhibition. Conventional rat hippocampal slices (400µm, 32°C, n=8/group) were tested either in normal ACSF, or with the $GABA_A$ receptor antagonist bicuculline (20 µM; BIC) to block inhibition. LTP was induced using either a 1 sec 100 Hz train (HFS), or 3 episodes of theta-burst stimulation (TBS; 4 pulses at 100 Hz, repeated at 5 Hz for 1 sec). In both control and BIC-containing media, the initial decay of responses during the first 10 min post-tetanus was larger after HFS than TBS. In normal ACSF, both stimulation patterns resulted in a similar mean potentiation of the population EPSP slope and peak amplitude (≈115%) 30 min after tetanization. In BIC, both TBS and HFS produced a larger LTP of the field EPSP slope (149±14.1 and 127±9.5%, respectively) and amplitude (136±9.9 and 113±8.0%, respectively). Bicuculline alone (10 µM, n=10) enhanced the field EPSP slope (121±8.5%) and amplitude (128±6.2%). These results indicate that removal of inhibition results in an enhancement of excitatory responses of CA1 pyramidal cells and also facilitates LTP of these responses. The increased LTP of excitatory responses observed in BIC could be due to a block of long-lasting enhancement of inhibition in CA1 pyramidal neurons.

(Supported by MRC, NSERC, FRSQ and FCAR)

600.9

ACTIVITY-DEPENDENT β-ADRENERGIC MODULATION OF CA1 LTP

M.J. Thomas*, T. Moody, M. Makhinson, T.J. O'Dell, Dept. of Physiology and the Interdepartmental Ph.D. Program for Neuroscience, UCLA School of Medicine, Los Angeles, CA 90024

β-adrenergic receptor (βAR) activation has a central role in the memory enhancement that occurs during heightened states of emotional arousal. Although βAR activation may enhance memory formation by modulating long-term potentiation (LTP), the cellular basis of this modulation is not fully understood. We thus investigated the effects of the βAR agonist isoproterenol (ISO) on LTP in the CA1 region of mouse hippocampal slices. ISO alone (1.0 µM for 10 mins) had no persistent effect on synaptic transmission (n = 8) but enabled the induction of LTP by 1 min. of 5 Hz synaptic stimulation (field EPSPs were 109.8 ± 7.6% of baseline 45 mins. after 5 Hz stimulation alone, mean ± SEM, n = 6, and were 169 ± 15.6% of baseline following 5 Hz stimulation in ISO, n = 5). In contrast, 15 seconds of 5 Hz stimulation induced an ISO-insensitive potentiation (EPSPs were 150.1 ± 15.8% of baseline, n = 10 after 5 Hz/15 sec. stimulation in the absence of ISO and were 154.3 ± 20.2% of baseline following 5 Hz/15 sec. stimulation in ISO, n = 5). Like ISO, the protein phosphatase inhibitor calyculin A (CA, 0.5 to 1.0 µM) enabled the induction of LTP during 5 Hz/1 min. stimulation (EPSPs were 101.5 ± 5.1% of baseline in controls, n = 7 and were 171.8 ± 15.2% of baseline in CA treated slices, n = 11) but had no effect on LTP induced by 5 Hz/15 sec. stimulation (EPSPs were 154.6 ± 8.7 in controls, n = 7 and were 145.5 ± 10.9% of baseline in CA treated slices, n = 7). This suggests that 1 min., but not 15 sec., of 5 Hz stimulation activates protein phosphatases that oppose the induction of LTP. Thus, βAR activation selectively enables the induction of LTP by patterns of synaptic activity that activate protein phosphatases. Supported by grants from NIMH (MH52876) and the Esther A. and Joseph Klingenstein Fund to T.J.O, and an ARCS award to T.M.

600.10

BRIEF TRAINS OF 5 HZ STIMULATION INDUCE NMDA RECEPTOR AND PROTEIN KINASE A-DEPENDENT LTP IN THE CA1 REGION OF THE HIPPOCAMPUS. T. Moody, M.J.Thomas, M. Makhinson, T.J. O'Dell*, Dept. of Physiology and the Interdepartmental Ph.D. Program for Neuroscience, UCLA School of Medicine, Los Angeles, CA 90024

We have observed that brief trains of 5 Hz synaptic stimulation (duration = 30 sec., intensity = 50% of max.) induces NMDA receptor-dependent LTP in the CA1 region of mouse hippocampal slices (EPSPs were 144.1 ± 7.8% of baseline 45 min. after 5 Hz stimulation in control experiments, mean ± SEM, n = 18 and were 117.3 ± 4.8% of baseline when 5 Hz stimulation was delivered in 100 µM APV, n = 7). 5 Hz stimulation did not induce LTP at commissural/associational fiber synapses in the CA3 region of the hippocampus (EPSPs were 101.3 ± 9.4% of baseline, n = 9), suggesting that 5 Hz stimulation cannot induce LTP at all synapses capable of NMDA receptor-dependent, high-frequency stimulation-induced LTP. Thus, different mechanisms may be involved in high vs. low-frequency stimulation-induced LTP. Consistent with this notion the protein kinase A (PKA) inhibitor H89 blocked the induction of LTP by 5 Hz stimulation in the CA1 region (EPSPs were 162.1 ± 8.7% of baseline in vehicle (DMSO) controls, n = 8 and were 108.4 ± 4.7% of baseline in slices exposed to 10 µM H89, n = 8) but had no effect on the induction of LTP by 2 trains of 100 Hz stimulation (control: 183.0 ± 11.2% of baseline, n = 6, H89: 183.0 ± 7.5, n = 8). The phosphatase inhibitor calyculin A (CA, 0.5 to 1.0 µM) had no effect on LTP induced by 5 Hz stimulation (EPSPs were 158.2 ± 10.9% of baseline, n = 8), but restored the induction of LTP in H89 treated slices (EPSPs were 165.6 ± 8.4% of baseline, n = 6). These results suggest that PKA is particularly important for the induction of LTP during short trains of 5 Hz stimulation in the CA1 region of the hippocampus, perhaps by inhibiting protein phosphatases. Supported by grants from NIMH (MH52876) and the Esther A. and Joseph Klingenstein Fund to T.J.O. and an ARCS award to T.M.

600.11

THE LONG-TERM POTENTIATION IN THE HIPPOCAMPAL CA1 AREA DEPENDED ON THE SPATIO-TEMPORAL INFORMATION

T. Aihara[1] * , M. Mizuno[1], M. Tsukada[1], H.Saito[1], and H. Kato[2]
[1]Dept. of Information-Communication Engineering, Tamagawa Univ., Machida,Tokyo, 194, and [2]Dept. of Physiology, Yamagata Univ., School of Medichine, Yamagata, 999-23, Japan.

We investigated the amount of LTP induced by "spatio-temporal pattern" stimuli. The stimuli consisted of weak ("w") and strong ("s") electric pulses which stimulated different areas of Schaffer region. Intensity of the stimulus "s" and that of "w" were adjusted to be a half and one tenth of that producing the maximum amplitude of the population spike. Spatio-temporal pattern stimuli consisted of the same number of component "s" (100 pulses) and "w" (100 pulses) and are identical in the spatial coincidence, but are different in the time correlations; for the stimulus with strong-positive correlation long trains of "s" or "w" are prevalent, while for the stimulus with strong-negative correlation "s" and "w" tend to alternate.

The results suggest that the LTP is sensitive both to the cooperation of input stimuli (the spatial coincidence) from ensembles of neurons impinging upon the target cell and to temporal correlation of stimuli.

By using the optical-imaging method, we also observed that the spatial distribution of LTP was different according to the difference in the spatio-temporal pattern of stimuli.

600.12

PRIMING ACTIVATION OF GROUP I METABOTROPIC GLUTAMATE RECEPTORS FACILITATES LTP INDUCTION IN CA1. C.R. Raymond, A.S. Cohen, and W.C. Abraham*. Department of Psychology, University of Otago, Dunedin, New Zealand.

Previously we have shown that LTP induced in area CA1 of rat hippocampus can be facilitated by prior "priming" activation of metabotropic glutamate receptors (mGluRs; Cohen & Abraham, in press). We have now investigated the receptor specificity of this effect and whether it can be observed throughout the LTP induction function. Several durations of theta-burst stimulation (TBS) were used to induce varying LTP in CA1 slices from young adult male rats. In control slices, mild TBS (5 bursts) just above threshold for LTP induction generated a 15 ± 1% (n=5) increase in fEPSP slope measured 1 hour post-tetanus. A 10 min application of the specific Group I mGluR agonist 3,5-dihydroxyphenylglycine (DHPG, 20 µM), 30 min prior to the mild TBS, transiently suppressed synaptic transmission but significantly enhanced subsequent LTP (43 ± 10%, n=7, $p<0.05$). Maximal LTP induced by repeated TBS, however, was not facilitated by DHPG pre-treatment. Furthermore, LTP produced by a very weak tetanus (2 bursts) was the same in control (6 ± 4%, n=7) and DHPG-treated slices (5 ± 3%, n=5). Facilitation of LTP was not mediated by Group II mGluRs since slices primed with (2S,3S,4S)-α-(carboxycyclopropyl)glycine (L-CCG-I, 10 µM) yielded LTP (13 ± 8%, n=5) that was not significantly different from control. Similarly, priming with the ionotropic receptor agonist NMDA (20 µM) did not enhance LTP. Finally, activation of muscarinic ACh receptors by carbachol (1-50 µM) did not prime LTP induced by a mild TBS. These findings indicate that prior selective activation of Group I mGluRs facilitates the induction of submaximal LTP, probably via biochemical sequelae to phospholipase C activation and subsequent hydrolysis of phosphoinositide.

(Supported by the New Zealand Health Research Council.)

600.13

EXCESSIVE HIGH-FREQUENCY STIMULATION REVERSES LTP BY NMDA- AND ADENOSINE-MEDIATED MECHANISMS. W.C. Abraham, C. Coussens* and A. Huggett. Department of Psychology, University of Otago, Dunedin, New Zealand.

Recent data from our lab has indicated that an inverted U-shaped function describes the relation between the number of high-frequency trains and the induction of LTP, in the dentate gyrus in vivo. We investigated this phenomenon further in CA1 slices from young adult male rats. The inverted U-shaped induction function was confirmed in CA1. Thus, peak LTP (56% increase in EPSP slope) occurred after 8 trains of 'theta-burst stimulation' (TBS), but little or no LTP resulted after 24 (10%) or 32 (8%) trains of TBS (i.e., "over-stimulation"). Over-stimulation also inhibited the subsequent induction of LTP by additional TBS for 60-90 min. The lack of LTP after over-stimulation suggests a reversal of LTP as induced by the initial trains of TBS. Over-stimulation could not produce this reversal, however, if LTP were given 10 min to stabilize first. Both D-AP5 (100 μM) and DPCPX (50 nM, an A_1 antagonist), applied to the perfusate after the initial few trains of TBS, prevented the reversal of LTP by the remaining trains. On the other hand, little LTP occurred when over-stimulation was given in the presence of 20 μM nimodipine (19%), or when extracellular Ca^{2+} was lowered from 2.5 mM to 2.0 mM (17%). These data indicate that over-stimulation causes a reversal of recently established LTP by NMDA- and adenosine-mediated mechanisms. (Supported by the New Zealand Health Research Council.)

600.14

Facilitation of NMDA receptor-dependent homosynaptic LTD by the type II corticosteroid receptor agonist RU-28362. C. Coussens, D.S. Kerr*, and W.C. Abraham. Departments of Psychology and Pharmacology*, University of Otago, Dunedin, New Zealand.

Activation of the low-affinity type II corticosteroid receptor (CSR) has been associated with a decrease in the magnitude of LTP both *in vivo* and *in vitro*. However, the role of type II CSR activation in the induction of homosynaptic LTD has not been firmly established. As previously shown, two episodes of low-frequency stimulation (LFS; 600 pulses at 1 Hz, each episode separated by 10 min), delivered to the Schaffer collaterals of area CA1 at 0.5 mV field EPSP strengths, did not significantly induce LTD of field EPSPs (-3 $\pm$ 4%, n=6). The addition of the type II CSR agonist RU-28362 to the perfusate for 2 hours prior to recording facilitated the induction of LTD in a dose-dendent manner. RU-28362 (1 μM) resulted in a non-significant depression (-10 $\pm$ 3%, n=4), while perfusion with 10 μM RU-28362 resulted in a significant synaptic depression following LFS (-27 $\pm$ 4%, n=9, $p<0.01$). The facilitation of LTD by RU-28362 was blocked by co-application of the type II CSR antagonist RU-38486 (30 μM, n=3). The LTD observed in the presence of RU-28362 is dependent on NMDA receptor activation, since LTD was blocked by co-application of D-APV (50 μM; -9 $\pm$ 3%, n=6) and was readily induced following APV washout (-22 $\pm$ 3%, n=4, $p<0.01$). One known effect of RU-28362 is to enhance voltage-sensitive calcium currents (Kerr et al., 1992, PNAS 89:8527-8531). However, bath application of nimodipine (an L-type calcium channel antagonist, 20 μM) did not prevent the facilitation of LTD by RU-28362 (-20 $\pm$ 3%, n=7). The role of N-type calcium channel activity in this phenomenon is currently under investigation. (Supported by the New Zealand Health Research Council.)

600.15

ACTIVITY-INDUCED ENHANCEMENT OF HB-GAM EXPRESSION IN RAT HIPPOCAMPAL SLICES: IMPLICATIONS FOR LONG-TERM POTENTIATION ? S.E. LAURI*,T. TAIRA, K. KAILA AND H. RAUVALA Department of Biosciences, Division of Animal Physiology and Laboratory of Molecular Neurobiology, Institute of Biotechnology, P.O.Box 17, 00014 University of Helsinki, Finland. Email: slauri@helsinki.Fi

Heparin-binding growth-associated molecule (HB-GAM) is a developmentally regulated secretory protein with neurite outgrowth-promoting activity. The putative role of HB-GAM in synaptic plasticity was studied with conventional electrophysiological methods and *in situ*-hybridization in rat hippocampal slices. High-frequency stimulation (HFS, 100 Hz/1s) leading to induction of LTP increased the expression of HB-GAM mRNA in the CA1 pyramidal neurons. A combination of antagonists of NMDA receptors (80 μM AP5 + 50 μM ketamine) and postsynaptic voltage-gated calcium channels (50 μM Ni^{2+} + 10 μM nimodipine) inhibited the HFS-induced enhancement in HB-GAM expression, while a less complete inhibition was seen with an NMDA receptor block only. The effects of HB-GAM on synaptic transmission and plasticity were studied by injecting recombinant HB-GAM into the CA1 dendritic area. The presence of excess HB-GAM inhibited LTP specifically and dose-dependently, but had no effect on the initial induction of potentiation or on the slope or amplitude of fEPSPs evoked by low-frequency baseline stimulation. The results indicate that high-frequency stimulation inducing LTP leads to a calcium-dependent enhancement in HB-GAM expression, and imply a role for this extracellular protein in the regulation of synaptic function in hippocampus. *Supported by the Academy of Finland.*

600.16

HIPPOCAMPAL LTP IN SEGMENTAL TRISOMIC TS65DN MICE F.E.N. LeBeau, N.Varma, J. Wagner, B.K. Krueger*, P.J. Yarowsky[1] and B. E. Alger. Departments of Physiology and Pharmacology[1], University of Maryland School of Medicine, Baltimore, MD, 21201.

Spatial learning and memory have been shown to be impaired in the segmental trisomic 16 mouse (Ts65Dn), a possible model for Down syndrome. We wished to determine whether abnormalities in hippocampal LTP could account for these deficits. Experiments were performed blind and data from 50 slices (26 from 12 Ts65Dn mice, 24 from 11 control mice of either sex) were analyzed. Synaptic field EPSPs were recorded extracellularly in CA1 *stratum radiatum* of hippocampal slices during stimulation (every 10 or 30 seconds) of the Schaffer collateral-commissural pathway. LTP was induced using 3 trains of stimuli (100 Hz for 1 sec) at 10 minute intervals. Changes in synaptic efficacy were determined as increases in EPSP slope measured 25-30 minutes (early-LTP) or 50-60 minutes (late-LTP), following the last tetanic stimulus. Increases of >20% above control were considered significant. Early-LTP was seen in 19/24 Ts65Dn slices and 21/26 control slices, with a mean increase in slope of 72% ±8 (mean ± s.e.m.) and 80% ±11 respectively. There was no significant difference between these two groups ($p = 0.45$). Late-LTP was observed in 8/12 Ts65Dn slices and 7/8 control slices with mean increases of 71% ±12 and 64% ±11 respectively. These differences were also not significant ($p = 0.69$).

These preliminary results suggest that LTP in the CA1 region of Ts65Dn mice is normal, and cannot account for the observed impairment in spatial learning.

NIH NS22010, A610686 and SRIS University of Maryland.

600.17

SHORT-TERM POTENTIATION LOWERS THE INDUCTION THRESHOLD FOR LONG-TERM POTENTIATION J.C. Fitzgibbons* & P.E. Schulz, Dept. Neurol. & Div. Neurosci., Baylor College of Medicine, Houston, Tx 77030.

High frequency stimulation (HFS) induces potentiation of synaptic efficacy with at least three phases: a brief, APV-insensitive post-tetanic potentiation, a decremental short-term potentiation (STP), and a sustained long-term potentiation (LTP). It has been unclear whether LTP and STP are expressed via the same or different mechanisms. We have obtained two lines of evidence from extracellular recordings in rat hippocampal area CA1 suggesting that STP may in fact be a separate form of potentiation. First, saturation of LTP does not decrease the magnitude of STP, and second, LTP expression is associated with a change in paired-pulse facilitation, while STP expression is not. While these data suggest differing mechanisms of expression, there is an interesting relationship between STP saturation and LTP induction. With progressive increases in the duration of HFS, the amount of STP increases. We find that as STP is saturated, LTP is first induced, which suggests that the two forms of potentiation may share similar induction mechanisms. If STP is a separate form of potentiation from LTP, what is its function? We have found that, while a subthreshold stimulation will induce only STP, two subthreshold stimuli separated by 1 minute can produce LTP, suggesting that STP may lower the LTP induction threshold for several minutes. This data supports the hypothesis that, while LTP and STP may have similar mechanisms for induction, they may utilize different expression mechanisms. In addition, the data suggests that one function of STP may be to lower the LTP induction threshold, so that two subthreshold stimuli may induce LTP (supported by NIH).

600.18

FIRING OF POSTSYNAPTIC ACTION POTENTIALS DURING TETANIZATION IS REQUIRED FOR INDUCTION OF NMDA-RECEPTOR INDEPENDENT LTP IN AREA CA1 OF RAT HIPPOCAMPUS. L.M. Grover*. Dept. Physiology, Marshall Univ. Sch. Med., Huntington, WV 25755.

High frequency (200 Hz) tetanization can induce an NMDA-receptor independent LTP in hippocampal area CA1. This LTP is blocked by nifedipine, suggesting involvement of high threshold, voltage-dependent Ca^{2+} channels (VDCCs). Because postsynaptic action potentials activate high threshold dendritic VDCCs (Spruston et al., *Science*, 1995; Magee & Johnston, *Science*, 1995), postsynaptic firing may be critical during induction of NMDA-receptor independent LTP. To test this possibility, whole cell, current clamp recordings were made from CA1 pyramidal cell somata. Stimulating electrodes were placed in stratum radiatum to activate two independent afferent inputs. Under control conditions (50-100 μM APV, n=10 cells), 200 Hz tetanization of one input increased EPSP slopes by 38±10%, with no change in the nontetanized input (-5±7%). Loading cells (n=6) with QX-314 (5 mM) blocked postsynaptic action potentials and abolished NMDA-receptor independent LTP (change in EPSP slope for tetanized input = -3±14%, for nontetanized input = 9±9%). Hyperpolarizing neurons (n=6) to -100 to -120 mV by constant current injection greatly reduced the number of action potentials during tetanization and also abolished NMDA-receptor independent LTP (EPSP change for tetanized input = -16±17%, for nontetanized input = -34±13%). These results indicate that firing of action potentials in postsynaptic neurons is necessary for induction of NMDA-receptor independent LTP, mostly likely as a stimulus for activating high threshold VDCCs. Postsynaptic spiking is probably not sufficient for induction of this LTP, however, since metabotropic glutamate receptors are also required (Little et al., *Neurosci. Lett.*, 1995). (Supported in part by the NSF and Marshall Univ.)

600.19

OPTICAL RECORDING OF TRISYNAPTIC PATHWAY IN RAT HIPPOCAMPAL SLICES WITH A VOLTAGE-SENSITIVE DYE. Y. Nakagami*, H. Saito and N. Matsuki. Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, JAPAN.

In the present study, voltage-sensitive dye and an optical recording system with a 128 × 128 photodiode array were utilized to investigate the spatial and temporal propagation of rat hippocampal trisynaptic pathway. Sequential propagation in the hippocampus was clearly detected in DG, CA3 and CA1 when the perforant pathway was stimulated. Duration of excitation in the stratum radiatum of CA3 was significantly longer than that in other hippocampal area. The long-lasting dendritic excitation is probably important to integrate synaptic transmission and may be related with epileptogenesis. When long-term potentiation (LTP) was induced by a tetanic stimulation (100 Hz for 1 sec), the excitatory region dramatically broadened out and the onset latency in the stratum radiatum of CA1 was reduced by as much as 60 %, indicating an increase of conduction velocity. The optical recording of excitation in the hippocampus is a powerful technique to analyze neural networks related to learning and memory process including LTP. (Supported by Grant-in-Aid from the Ministry of Education, Culture and Science of Japan)

LONG-TERM POTENTIATION: PHYSIOLOGY IX

601.1

INCREASED NEURONAL EXCITABILITY DURING DEPOLARIZATION-INDUCED SUPPRESSION OF INHIBITION. J.J. Wagner[1]* and B.E. Alger[2]. 1. Dept. Pharm. Sci., North Dakota State Univ., Fargo, ND 58105 and 2. Dept. Physiol., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Brief (1sec) depolarization of CA1 pyramidal cells results in the Ca^{2+}-dependent induction of a retrograde signal which acts presynaptically to inhibit spontaneous and evoked GABAergic synaptic responses for approximately 1 min (see Pitler and Alger, 1995, TINS 18 (8): 333-340 for review). Inhibition plays a critical role in regulating the extent of excitatory transmission, and this depolarization-induced suppression of inhibition (DSI) is potentially an important mechanism by which the degree of GABAergic influence may be transiently modified.

Because of the temporal overlap of EPSPs and IPSPs following s. rad. stimulation in the rat hippocampal slice preparation, we predicted that the excitatory component would be enhanced during DSI due to the decrease of the inhibitory component. We tested the effects of DSI on synaptic transmission using whole-cell voltage-clamp recordings to monitor EPSC/IPSC responses from CA1 pyramidal cells. Following a 20/30 mV depolarization from a holding potential of -50/-60 mV, the peak amplitude (23±5%), initial slope (13±6%), and inward charge (42±9%) of the EPSC were all significantly enhanced during the DSI period (n=10). In current-clamp recordings, the probability of synaptically evoked action potential firing was increased from 13±4% to 46±9%, n=4. Thus the induction of DSI can indirectly lead to an increase in the efficacy of excitatory synaptic transmission in the hippocampus. Supported by NS30219 & NS22010 to B.E.A and DA09603 to J.J.W.

601.2

AN INFLUENCE OF GABAergic FEEDBACK INHIBITION ON LTP AND LTD IN THE CA1 REGION OF THE HIPPOCAMPUS. P.M. Steele* & M.D. Mauk, Dept. of Neurobiology and Anatomy, Univ of Texas Medical School at Houston; Houston, Tx. 77225.

Previous studies of the CA1 region in hippocampus have shown that prolonged low frequency stimulation induces long-term depression (LTD) and high frequency stimulation induces long-term potentiation (LTP). Between the stimulation frequencies that induce LTD and LTP is a threshold frequency, where on average no change in synaptic strength is observed. Here we demonstrate that the threshold frequency is influenced by inhibitory synaptic transmission. Standard techniques were used to prepare hippocampal slices from Spraque-Dawley rats (5-9 weeks old). The γ-aminobutyric acid type A receptors $GABA_AR$ agonist muscimol (3nM) increased the threshold frequency, so LTD was induced at higher stimulation frequencies (> 10 Hz). The $GABA_A$ antagonist picrotoxin (50μM) decreased the threshold frequency, so LTD was induced only at very low stimulation frequencies (.25-.5 Hz). These findings suggest that modulation of inhibitory interneuron activity may control the ability to induce LTD or LTP by sliding the threshold frequency (i.e. increased inhibitory interneuron activity will favor LTD induction and decreased activity will favor LTP induction). To investigate the effects of feedback inhibition on the threshold frequency, we paired antidromic stimulation of the alveus (4 pulses at 100 Hz given every 500 msec.) with afferent stimulation at 8 Hz. The induction frequency of 8 Hz is close to the threshold frequency where no net change in synaptic strength is induced (105 ± 13.3%, n=5). We find that 8 Hz stimulation presented during antidromic activation of pyramidal cell spikes induced LTD (73 ± 4%, n=4, $p<.05$). Since this effect was blocked by picrotoxin (120 ± 9%, n=4), we propose that pyramidal cell driven recurrent inhibition via $GABA_AR$ modulates the threshold frequency between LTD and LTP. Such inhibitory feedback modulation may play an important role in maintaining pyramidal cell activity in its effective dynamic range.
Support: R29 MH46904 (MDM)

601.3

BICARBONATE-MEDIATED GABA DEPOLARIZING COMPONENT AND SYNAPTIC PLASTICITY. C. Debray, D. Diabira, I. Chaudieu*, Y. Ben-Ari & H. Gozlan. INSERM U-29, 123, Bld de Port-Royal, 75014 -Paris (France).

In the adult CNS, GABA exerts an inhibitory control over the activity of NMDA receptors but also generates a bicuculine-sensitive depolarizing response, following intense electrical stimulation. Several mechanisms are involved in this depolarizing response including a high permeability of bicarbonate ions through $GABA_A$ receptors (Staley et al., Science 1995). Since depolarizing responses have been observed in the hippocampus and particularly in dendrites where NMDA receptors are localized, we have examined the role of the bicarbonate-mediated $GABA_A$ depolarization in the induction of NMDA receptor-dependent long-term potentiation (LTP) in CA1. Experiments were conducted in slices of adult rats in a bicarbonate buffer (CTRL), in the presence of acetazolamide or in a HEPES buffer to suppress the bicarbonate-mediated $GABA_A$ depolarization. In CTRL conditions LTP of AMPA receptors was similarly induced in the presence or in the absence of bicuculine and the inhibition of the bicarbonate-mediated $GABA_A$ depolarization has no significant effect on the induction of LTP. As the depolarization induced by $GABA_A$ receptors may be less efficient than that provided by AMPA receptors, these were transiently blocked during the train. Again, no modification of the induction of LTP was observed in either conditions. In contrast, when AMPA receptors were permanently blocked and when NMDA receptors were boosted by a low concentration of magnesium ions, the LTP of NMDA receptors is affected by the inhibition of bicarbonate-mediated $GABA_A$ depolarization. This suggests that in physiological conditions the contribution of this depolarizing component is not sufficient to relieve the high magnesium block of NMDA receptors but in conditions that enhance the level of excitability of NMDA receptors such as in certain pathological conditions, this component may play a role.
Supported by INSERM and the Medical Research Foundation

601.4

ARE AMPA RECEPTORS ESSENTIAL FOR THE INDUCTION OF LTP IN PHYSIOLOGICAL CONDITIONS? N. Chevassus au Louis*, D. Diabira, C. Debray, Y. Ben-Ari & H. Gozlan. INSERM U29, 123, Bld de Port-Royal, 75014 -Paris (France).

There is a general argument that in the CA1 region of the hippocampus the induction of long term potentiation (LTP) is controlled by NMDA receptors, blockade of AMPA receptors during tetanization does not prevent the induction of LTP. However, this has been shown using non-physiological conditions i.e. high Ca2+ or low Mg2+ concentrations (see Kauer et al., Neuron 1988; Muller et al., Science 1988). Indeed, in keeping with these earlier studies, tetanic stimulation of Schaffer collaterals (2 x 100 Hz, 1s) induces a LTP in CA1, i) in presence of 4 mM Ca^{2+} and 4 mM Mg^{2+} (+ 87%, n= 3/4), or ii) in presence of a low concentration(0.02-0.1 mM) of Mg^{2+} (+ 54% n= 2/3).

We repeated the above experiments in physiological conditions, in a bicarbonate buffer containing 2 mM Ca2+ and 1.3 mM Mg2+, and with no $GABA_A$ antagonists, expecting also a LTP of AMPA receptors upon washing out of CNQX. Surprizingly, no synaptic modification of these receptors was observed 30 min after tetanization (+ 3% n= 0/6). The plasticity of the slice was carrefully checked using a different stimulation pathway in the same slice before the application of CNQX (+47% n=6/6). Similar results were obtained in a HEPES buffer (n=6/6). Thus, in physiological conditions LTP cannot be induced when AMPA receptors are blocked during the train. These results suggest that AMPA receptors are essential for the induction of LTP in physiological conditions.
Supported by INSERM and the Medical Research Foundation

601.5

Activation of metabotropic glutamate receptors induces EPSP-spike potentiation in area CA1 of the submerged rat hippocampal slice. **N.A. BREAKWELL* M.J. ROWAN and R.ANWYL. Depts of Physiology and Pharmacology and Therapeutics, Trinity College Dublin.**

EPSP-spike (E-S) potentiation refers to a potentiation of the population spike (PS) which cannot be accounted for by a commensurate potentiation of the EPSP. E-S potentiation induced by high frequency stimulation (hfs) has been well documented as a shift to the left of the input/output (I/O) curve describing PS as a function of EPSP, but mechanisms involved in E-S potentiation remain unclear. We examined the role of mGluRs in E-S coupling. (1S,3R)-ACPD induced a long lasting potentiation of the population spike (PS) amplitude which was consistently larger than that of the EPSP measured in the cell body area. E-S potentiation was confirmed by a shift in the I/O curve following (1S,3R)-ACPD washout and by analysis of data interpolated from regression lines. This E-S potentiation was not observed in the presence of the gamma-aminobutyric acid A ($GABA_A$) receptor antagonist, picrotoxin (50μM), suggesting an mGluR mediation of GABAergic inhibitory transmission. E-S potentiation induced by hfs, which was of the same magnitude as that induced by (1S,3R)-ACPD, was blocked by the mGluR selective antagonist (+)-a-Methyl-4-carboxyphenylglycine (+MCPG; 250μM). These results suggest that uncoupling of the E-S relationship is mediated by mGluRs and provide further evidence that both synaptic and E-S potentiation induced by (1S,3R)-ACPD share common mechanisms of expression with hfs induced long-term potentiation .

N.Breakwell, supported by Wellcome

601.6

IMAGING OF PROPAGATION OF PRE- AND POST-SYNAPTIC NEURAL ACTIVITIES IN CA1 REGION OF RAT HIPPOCAMPAL SLICES. T. Akaike*[1], Wang, J.[1], M. Sokabe[1], N. Kuno[1], A. Itoh[2] and T. Nabeshima[2]. [1]Dept. Physiol., [2]Dept. Neuro-psychopharmacol. and Hosp.Parm. Nagoya Univ. Sch. of Med., Nagoya 466 Japan.

Our aim is to see how neural activities evoked in CA1 region of rat hippocampal slices spread to neighboring regions. We used an optical imaging method (Fujifilm Deltaron 1700: 0.6 ms/frame, 128x128 pixel) and a voltage sensitive dye (RH482). Neural activities were evoked by an electrical stimulation of either Schaeffer collaterals and/or commissural fibers in the radiatum and lacunosum moleculare (RLM) layers or those in oriens (Ors) layer. We used a technique of paired pulse facilitation to confirm correspondence between neural responses and optical signals. By applying CNQX in perfusing solution we could record optical signals of a primary component of neural activities, i.e., action potentials evoked in the passing fibers. By subtracting them from optical records obtained in normal solution we could calculate their secondary component which seem postsynaptically to be generated by primary activities. The fact that secondary neural activities propagate further than primary ones suggests that some feedford activities originating either from pyramidal cells or from interneurons propel the neural propagation. To see whether recurrent collaterals of pyramidal cells are necessary for the propagation we made incisions either across RLM layers leaving Ors layer intact or across Ors layer with intact RLM layers. A cut of RLM layers completely blocked the propagation, whereas neural activities could propagate after a cut of Ors layer. It suggests that interneurons may play more important role for propelling the propagation than recurrent collaterals of pyramidal cells.

601.7

ECTOPIC PACEMAKER GENERATOR IN AXON TERMINALS ASSOCIATED WITH EPILEPTIFORM ACTIVITY AND LTP. V.L.Ezrokhi, A.M.Kasyanov, Yu.I.Alexandrov*. Institute of Higher Nervous Activity and Neurophysiology and Institute of Psychology*, The Russian Academy of Sciences, 5a Butlerov St., Moscow, 117865, Russia.

Intracellular and extracellular recordings were used to study ectopic (i.e. nonsomatic) action potential (AP) generation occurring in three experimental environments. First, in callosal neurons during electographic seizures induced by various means (penicillin, strychnine, electroconvulsant) in rabbit cortex. Second, in CA3 pyramidal cells during LTP in the CA1 induced by theta-rhythm stimulation of Schaffer collaterals in rat hippocampal slices. Finally, we studied strychnine-treated inhibitory neurons situated on the slow- and fast-adapting mechanosensory neurons of the crayfish during molting. Antidromic AP bursts were recorded in the soma of callosal neurons simultaneously with as well as between the paroxysmal EEG waves, for up to 2 hrs after the cessation of the paroxysmal activity in the opposite hemisphere, and also as a consequence of kindling-like electrical stimulation. Occurrence of ectopic APs in CA3 pyramidal cells during LTP in CA1 was found to be dependent on the extracellular calcium concentration, and varied from single ectopic APs (2.2 mM) to ectopic AP bursts (1.3 mM). In the isolated crayfish stretch receptor, ectopic APs were observed in the axon terminals of the inhibitory neuron. AP bursts in an inhibitory terminal evoked IPSPs recorded from the postsynaptic membrane of one neuron and inhibited another neuron via the axon reflex. The AP bursts in the axon terminal arised either spontaneously or following orthodromic AP, as well as after a depolarization shift of the postsynaptic membrane or extracellular polarization. Our data reveal a long-term increase of axon terminal excitability, resulting in the generation of ectopic AP ranging from epileptiform activity to LTP. *The work was support by grant RFBR 96-04-48842.*

601.8

LONG- AND SHORT-TERM PLASTICITY OF THE TEMPOROAMMONIC PATHWAY IN RAT HIPPOCAMPAL SLICE. H. Dvorak* and E. M. Schuman. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

We are interested in activity-dependent modulation of the temporoammonic pathway, the direct projection from entorhinal cortex to area CA1 of the hippocampus. To isolate the temporoammonic pathway in a hippocampal slice, the dentate gyrus is removed and a cut is made through stratum radiatum in distal CA1. The temporoammonic pathway is stimulated in stratum lacunosum-moleculare distal to the cut through radiatum, and a field response is recorded in the same layer on the other side of the cut. Low frequency (1 Hz) stimulation applied to the temporoammonic pathway for 10 min resulted in a modest depression of the field response 20-30 min after cessation of low-frequency stimulation (mean±s.d. % of baseline of initial EPSP slope: 82.6±11.5) in nine out of ten slices. This depression is dependent upon NMDA receptor activation; in the presence of the NMDA receptor antagonist AP5 (50 μM), no significant depression was observed (mean % of baseline: 104±22, n=6). In five slices, the response recovered to baseline values (mean % of baseline: 104±9.2) after tetanic stimulation (4x100 Hz for 1 sec) of the temporoammonic pathway. We are also examining short-term plasticity by applying paired-pulse stimulation to the temporoammonic pathway. Unlike the Schaffer collateral response, in which facilitation is seen only at interstimulus intervals (ISIs) of less than 400 ms (at 22-25°C), the temporoammonic pathway shows a significant facilitation at ISIs as long as 800 ms. We are currently using intracellular recording to investigate the basis for this facilitation. Preliminary results suggest a differential effect of paired-pulse stimulation on excitatory and inhibitory postsynaptic potentials.

This work was supported by an HHMI predoctoral fellowship to HD and by the NIH, John Merck Fund, and the Pew Charitable Trusts.

601.9

LONG-TERM POTENTIATION AT SINGLE FIBER INPUTS TO HIPPOCAMPAL CA1 PYRAMIDAL CELLS. J.T.R.Isaac*, G.O.Hjelmstad, R.A.Nicoll and R.C.Malenka, Depts.of Psychiatry, Physiology and Cellular and Molecular Pharmacology, University of California, San Francisco, CA 94143.

Despite extensive investigation, it remains unclear whether presynaptic and/or postsynaptic modifications are responsible for the expression of LTP. We attempted to address this using minimal stimulation and analysis of failures. In a first group of experiments we used whole-cell recordings and minimal stimulation at an intensity which elicited EPSCs on approximately 50% of trials. Following pairing LTP was associated with an increase in potency (194±22%), defined as the average size of the successes, and a shift in the amplitude histogram from small events to large in 8/8 cells. There was also a significant reduction in failure rate in 4/8 cells. In a second set of experiments (n=8) perforated patch-clamp recordings were used. The likelihood of reliable activation of a single axon and a presumptive single site was maximized by using stimulus intensity ramps in which EPSCs exhibited an abrupt threshold, and a plateau in amplitude and failure rate for at least 2 further increases in intensity. In 4 of these cells, paired pulses were also applied during the baseline which induced paired pulse facilitation, but a potency ratio (potency of 2nd response/potency of 1st) of ≤1. This indicated a single release site was likely being activated. In these experiments LTP caused an increase in potency (414±94%) and a shift from smaller to larger amplitude events in all 8 cells. There was a decrease in failure rate in 4/8 cells. In 2 of these cells paired pulse data was collected during LTP and an increase in the paired pulse potency ratio was observed consistent with an increase in n. In a final set of single axon experiments we attempted to induce LTP in the presence of 5 mM Ca^{2+} which raised baseline Pr to 0.84±0.05 (n=5). LTP was reliably induced in 5/5 cells (247±58%), and was associated with an increase in potency (238±57%). These data suggest that LTP is accompanied by an increase in q and is very difficult to explain by an increase in Pr alone. *Supported by the Wellcome Trust, NSF and NIH.*

601.10

LONG-TERM POTENTIATION IN CA1 OF THE NEONATAL RAT. J.A. Cummings*[∞], J.T.R. Isaac[§], R.A. Nicoll[†‡], and R.C. Malenka[§‡], Neuroscience Graduate Program[∞], Depts. of Cellular and Molecular Pharmacology[†], Physiology[‡], Psychiatry[§], and Center for Neurobiology and Psychiatry[§], University of California @ San Francisco, San Francisco, CA 94143.

It has often been reported that long-term potentiation of synaptic strength is difficult or impossible to obtain at the Schaffer collateral - CA1 hippocampal synapse of neonatal rats (J. Physiol. 346, 27 [1984]; J. Neurosci. 13, 2910 [1993]). Most recently, it has been reported that LTP results from an increase in the probability of neurotransmitter release (p), and that p of neonatal CA1 synapses is very high, thus precluding LTP (Science 269, 1730 [1995]). In contrast to these results, we have found that it is possible to induce LTP in hippocampal synapses of neonatal (P4-P8) rats. Tetanic stimulation of CA1 fields (4X100 Hz, 1sec) resulted in a potentiation to 132% ± 9% (S.E.M.) of the original baseline fEPSP slope (n=7), and pairing of neonatal CA1 cells (60 pulses at 2 Hz with cell held at 0 mV) resulted in an average 187% ± 32% potentiation (n=10). The discrepancy between our own and previous results has many possible explanations, among them the possibility that p is not as high in our own preparations as it was in the previous studies. To gain insight into the differences between our own and previous results, we are currently examining LTP at presumptive single synaptic sites (Eur. J. Neurosci. 7, 1882 [1995]; Neuron 14, 795 [1995]) using perforated patch recordings.

Supported by grants from the National Institutes of Health (R.A.N., R.C.M.), the Wellcome Trust (J.T.R.I.), and the Howard Hughes Medical Institute (J.A.C.).

601.11

LONG-TERM POTENTIATION AND FUNCTIONAL SYNAPSE INDUCTION DURING EARLY HIPPOCAMPAL DEVELOPMENT. G. M. Durand*, Y. Kovalchuk and A. Konnerth. I. Physiologisches Institut, Universität des Saarlandes, 66421 Homburg/Saar, Germany.

In the adult mammalian central nervous system, excitatory synaptic transmission is mediated mostly by co-localized AMPA and NMDA receptors. Our results indicate that, at their earliest stages of development, hippocampal glutamatergic synapses involve almost exclusively NMDA receptors, forming a "silent" neuronal network. By combining confocal calcium imaging with whole cell recordings in hippocampal slices, we found that these immature synaptic contacts are localized on dendritic shafts and not on spines as in the case of mature synapses. During the first week of development, the hippocampal glutamatergic network becomes gradually functional due to the transformation of preformed NMDAergic synaptic contacts into conducting synapses, a process that we termed functional synapse induction. The early form of glutamatergic synaptic transmission was sensitive to CNQX and cyclothiazide indicating that it is mediated by AMPA receptors. Interestingly, synapse induction shared several properties with long-term potentiation (LTP). Thus, it was long-lasting, input-specific and easily triggered experimentally by pairing presynaptic stimulation with postsynaptic depolarization. We conclude that the induction of functional synapses is governed by Hebb's rule of association. Moreover, our data indicate that LTP or a closely related process may be involved in the activity-dependent formation of the glutamatergic network in early development.

Supported by the Deutsche Forschungsgemeinschaft (SFB 246). G.M.D. is supported by the Alexander von Humboldt-Stiftung and the Human Frontier Science Program.

ACETYLCHOLINE RECEPTORS: NICOTINIC—PHARMACOLOGY II

602.1

α_7/5HT$_3$ CHIMERIC HOMOMERS: SIMILARITY TO PC12 α-BUNGAROTOXIN RECEPTORS. S.V. Rakhilin, P. Atluri, R.C. Drisdel*, E. Ko, F. Rangwala, S. Salman, W.N. Green. Department of Pharmacological & Physiological Sciences, University of Chicago, Chicago, IL 60637.

The nicotinic acetylcholine receptor (AChR) α_7 subunit forms homomeric α-bungarotoxin receptors (BuTxRs) in *Xenopus* oocytes. However, attempts to express α_7 homomers in heterologous mammalian systems have met with little success. In contrast, a subunit chimera, which contains the N-terminal toxin binding domain of the α_7 subunit and the C-terminal domain of the 5-hydroxytryptamine (5HT$_3$) subunit, is expressed as a BuTxR homomer in mammalian cells (Corringer et al. 1995. JBC. **270**). In the present study, α_7/5HT$_3$ homomers were stably expressed (~500,000 surface sites/cell) in tsA201 cells and characterized in parallel with BuTxRs from PC12 cells (Drisdel et al., this meeting). α_7/5HT$_3$ BuTxRs bind BuTx with high affinity (K_D~2x10^{-10} M, 4°C) and sediment as a single 10S peak on sucrose gradients. The cross-linked receptor-^{125}I-BuTx complex also migrates as a 10S peak and at ~250 kDa based on SDS-PAGE. Inhibition of cell surface ^{125}I-BuTx binding by agonists and antagonists is similar to that observed for PC12 cells. Hill coefficients for ACh, carbachol, cytisine, and nicotine were between 2 and 3. Based on the inhibition of cell surface ^{125}I-BuTx binding by bromoacetylcholine alkylation of cysteines at the ligand binding site, the chimeric receptor possesses high (~70%) and low (~30%) affinity sites, analogous to the PC12 receptor. Our data indicate that the α_7/5HT$_3$ BuTxRs, like the PC12 BuTxRs have at least three nonequivalent toxin binding sites per receptor and thus possess structural and pharmacological properties similar to BuTxRs described in PC12 cells. Supported by NSF IBN-9319656, Council for Tobacco Res. Scholar Award (W.N.G.), and Am. Heart Assoc. Sr. Res. Fellowship. (S.V.R.).

602.2

THE PC12 α-BUNGAROTOXIN RECEPTOR HAS MORE THAN TWO TOXIN BINDING SITES. R.C. Drisdel, P. Atluri, A.P. Fox, A.B. Harkins*, E. Ko, E. Potter, S.V. Rakhilin, F. Rangwala, W.N. Green. Department of Pharmacological & Physiological Sciences, University of Chicago, Chicago, IL 60637.

We have characterized an α-bungarotoxin receptor (BuTxR) in PC12 cells (~40,000 surface sites per cell) that sediments as a single 10S peak on sucrose gradients. Cross-linked cell surface receptor-^{125}I-BuTx complexes also migrate as a 10S peak on sucrose gradients and as a single band of 290-300 kDa based on SDS-PAGE. PC12 receptors bind BuTx with high affinity (K_D~1x10^{-10} M, 4°C) and are precipitated by α_7-specific antibodies. The nicotine-induced whole cell current in these cells is almost completely blocked (~90% inhibition) by BuTx (0.5 μM) or methyllycaconitine (1 μM) and is similar to BuTxRs in other preparations. Inhibition of cell surface ^{125}I-BuTx binding with a panel of nicotinic acetylcholine receptor (AChR) agonists and antagonists reveal a distinct nicotinic pharmacological profile. Hill coefficients for acetylcholine, carbachol, cytisine, and nicotine were between 2 and 3. Inhibition of cell surface BuTx binding by bromoacetylcholine (BrACh) alkylation of cysteine residues at the ligand binding site indicates two different classes of BrACh binding sites, high affinity sites (~70%, IC_{50}=3 μM) and low affinity sites (~30%, IC_{50}=2 mM). Our results are consistent with the presence of at least three nonequivalent toxin binding sites per receptor and indicate that the neuronal BuTxR is structurally different from other AChRs. Supported by NSF IBN-9319656, Council for Tobacco Research Scholar Award (W.N.G.), and Am. Heart Assoc. Sr. Research Fellowship (S.V.R.).

602.3

Differential discrimination between human and rat α7 nAChR by GTS-21 and its primary metabolite, 4-OH,2 Methoxybenzylidene anabaseine R.L. Papke*, E. M. Meyer, and C.M. de Fiebre University of Florida, Department of Pharmacology.

GTS-21 (3-(2,4)-dimethoxybenzylidene anabaseine, DMXB) is a neuronal nicotinic partial agonist, highly selective for rat α7 receptors. The activation of α7 receptors has been proposed to provide a mechanism for cytoprotection of PC12 cells and cognitive enhancement in a rat model of cholinergic hypofunction. The ability of GTS-21 and other anabaseine derivatives to elicit maximal responses from α7 receptors expressed in *Xenopus* oocytes appears to be limited both by variable efficacy for receptor activation and by a secondary noncompetitive inhibitory activity. Therefore, we define the alpha7 activity factors (A7AF) as the product of the response at 20 μM (relative to 500 μM ACh) and the residual control response remaining after a 20 μM application. DMAC (3-(4)-dimethylamino–cinnamylidine anabaseine), a compound previously reported to have high α7 efficacy (0.93, relative to 500 μM ACh), has an A7AF of only 0.32, due to high residual inhibition. In contrast, while at 20 μM, GTS-21 stimulates responses of rat α7 receptors that are only 0.20 the 500 μM ACh control responses, GTS-21 has an A7AF of 0.20, because the application of a 20 μM solution produces no significant inhibition of subsequent ACh responses. 4-OH,2Methoxy-benzylidene anabaseine (4-OH BA) is the primary metabolite of GTS-21 (B. Kem, personal communication), and 4-OH BA has a A7AF of 0.35 for rat α7 receptors.

When DMAC was applied to *Xenopus* oocytes expressing human α7 receptors, the calculated A7AF was 0.11, and the GTS-21 A7AF for human receptors was only 0.034. In contrast, the A7AF of 4-OH BA for human receptors was 0.31, greater than that of GTS-21 for the rat α7 receptors. These results suggest that for effects depending on α7 receptor activation, GTS-21 may have limited effectiveness at eliciting *in vitro* effects with human cell lines. However in humans, and potentially other primates, *in vivo* administration of GTS 21 may produce significant levels of α7 receptor activation based on the conversion of GTS-21 to an active metabolite.

These experiments were supported by Taiho Pharmaceuticals. Anabaseine compounds were generously provided by Dr. B. Kem and Taiho Pharmaceuticals. We thank Jon Lindstrom for the human α7 clone.

602.4

The disposition of use-dependent binding sites in nAChR. M. M. Francis[1,2]*, Kyung Choi[3], Ben Horenstein[3] and R.L. Papke[1,2] Univ. of Florida, Departments of [1]Pharmacology, [2]Neuroscience and [3]Chemistry, Gainesville, Florida.

Neuronal nicotinic acetylcholine receptors (nAChRs; e.g. $\alpha3\beta4$) are more sensitive to a class of noncompetitive inhibitors known as ganglionic blockers than muscle-type nAChRs (e.g. $\alpha1\beta1\gamma\delta$). Bis-TMP-n compounds are potent use-dependent bi-functional inhibitors of nAChRs. These compounds are symmetrical conjugates of methylated piperidine rings connected by a flexible aliphatic linker chain consisting of "n" number of carbons. Bis-TMP-10 inhibits neuronal ($\alpha3\beta4$) and chimeric $\alpha1\beta1(\beta4TM2)\gamma\delta$ nAChRs with prolonged kinetics of recovery as compared to inhibition of muscle-type nAChRs, localizing the site responsible for conferring prolonged kinetics of inhibition to eight amino acids in the pore-lining second transmembrane domain of the neuronal beta subunit ($\beta4$).

Since the slow reversibility of inhibition of neuronal nAChRs by bis-TMP-10 seems to arise from an ability of the compound to bind to multiple sites within the receptor complex, we have been able to estimate the separation distance between sites by examining the kinetics of inhibition after application of bis-TMP-n compounds. $\alpha1\beta1(\beta4TM2)\gamma\delta$ receptors showed complete recovery from inhibition five minutes after co-application of ACh with bis-TMP-8 while responses were inhibited by 93±01% five minutes after co-application of ACh with bis-TMP-10. This result demonstrated the maximal separation distance between use-dependent sites to be between 20Å and 23Å in chimeric $\alpha1\beta1(\beta4TM2)\gamma\delta$ receptors. However, additional studies of compounds composed of identical piperidine rings connected by rigid linker groups showed the separation distance to be between 15.2Å and 18.3Å. While $\alpha1\beta1(\beta4TM2)\gamma\delta$ and $\alpha3\beta4$ nAChRs showed comparable sensitivities to inhibition by bis-TMP-n compounds at the time of co-application, they differed in their kinetics of recovery suggesting that the potential for binding to adjacent beta subunits in the neuronal receptor complex alters the requirements for bi-functional inhibition. This result opens the possibility of using this class of compounds as probes for subunit arrangement and stoichiometry in nAChRs. This work was supported by NIH R01 NS3288-0A2 to RLP and NIMH NRSA 1 F31 MH11258-01 to MMF.

602.5

PACAP RECEPTOR ACTIVATION INHIBITS α7-CONTAINING NEURONAL ACETYLCHOLINE RECEPTORS. D. Pardi*, J. F. Margiotta. Dept of Physiology & Biophysics, Mount Sinai School of Med, NY, NY 10029, and Dept of Anatomy & Neurobiology, Med. College of Ohio, Toledo, OH, 43699.

Two major classes of nicotinic AChRs are present on chick ciliary ganglion neurons. One contains the α7 AChR subunit, displays rapid desensitization, and is blocked by α-bungarotoxin (αBgt). The second class lacks α7 subunits, desensitizes much more slowly, and is unaffected by αBgt. We previously showed that peak ACh-evoked responses are enhanced ~50% after increasing cAMP levels in the neurons, either by injecting cAMP or by activating pituitary adenylate cyclase activating peptide (PACAP) type I receptors. Interestingly, after blocking adenylate cyclase with 2',-5'-dideoxyadenosine (ddA), PACAP treatment resulted in ~50% reduction in the ACh response (ddA alone was without effect) suggesting that a parallel, cAMP-independent, PACAP-activated signaling pathway inhibits some AChRs. In order to identify the AChRs targeted by such signals, ciliary ganglion neurons were pretreated with 200uM ddA and 10nM PACAP, and then challenged with 500uM ACh or 20uM Nicotine. In ddA/PACAP treated neurons, the rapidly desensitizing, initial component of the agonist induced response was reduced 36.21±12.3% ($p<0.001$, n=16 cell pairs), while the slowly desensitizing components were unaffected ($p>0.25$). In parallel experiments, αBgt abolished the rapidly desensitizing initial component, indicating that α7 containing AChRs are inhibited by PACAP-activated cell signals. In other systems, PACAP has been shown to increase both cAMP and IP3/Ca++. We find that PACAP elevates inositol phosphate (IP) levels in ciliary ganglion neurons with a 10nM dose causing an 85.06%±7.47 increase ($p<0.05$, n=16 separate platings). A possible link between IP production and selective inhibition of αBgt-sensitive AChRs is the expected increase in [Ca++]i previously shown to be necessary for arachidonic acid-mediated inhibition of α7-containing AChRs on ciliary ganglion neurons (Vijayaraghavan et al., 1995). Supported by NIH NS24417.

602.6

DIFFERENTIAL BLOCK BY MECAMYLAMINE OF NICOTINIC SYNAPSES ON SYMPATHETIC B AND C NEURONS W-X Shen* and JP Horn Department of Neurobiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

We have previously shown that the blocking effect of mecamylamine differs between sympathetic B and C neurons. The IC_{50} for block of nicotinic transmission is 25 μM in the B system and 5 μM in the C system (Shen & Horn, Soc. Neurosci. Abstr. 19, 1532, 1993). We have now analyzed the underlying mechanism for this differential effect by recording nicotinic synaptic currents under voltage-clamp.

In 6 B cells, mecamylamine (1-8 μM) reduced the amplitude and speeded the decay of EPSCs in a voltage- and concentration-dependent manner. The mechanism was further analyzed using a sequential reaction model. The blocking rate constant (k_{+B}^*) for mecamylamine was 6.4 x 10^7 M^{-1} s^{-1} at -100 mV. A potential change of -91 mV gave an e-fold shift in k_{+B}^*. This corresponds to a binding site for mecamylamine that senses 28% of the membrane field. In 3 C cells mecamylamine showed similar effects. In one cell, we succeeded in recording EPSCs over a range of holding potentials and drug doses. The k_{+B}^* for mecamylamine block was 1.2 x 10^7 M^{-1} s^{-1} at -100 mV. A potential change of -144 mV gave an e-fold shift in k_{+B}^*, which corresponds to a binding site 18% into the membrane field.

These results indicate that mecamylamine acts in a similar manner on B and C neurons. Its ability to distinguish between receptors on the 2 cell types probably arises from the difference in their mean open times. (Supported by a Grant-in-Aid from the AHA, PA Affiliate).

602.7

METHYLLYCACONITINE A COMPETITIVE INHIBITOR OF THE α7 DESENSITIZED OPEN MUTANT L247T . S. Bertrand*, E. Palma, P.J. Corringer, S. Edelstein, J.P. Changeux and D. Bertrand University of Geneva, 1211 Geneva, Switzerland, Institut Pasteur, 75734 Paris Cedex France.

The natural insecticide methyllycaconitine (MLA) competitively inhibits the wild type homomeric α7 neuronal nicotinic acetylcholine receptor in the picomolar range. We have now examined the action of this compound on the desensitized open mutant L247T. We find that in contrast to other competitive inhibitors such as dihydro-β-erythroidine (DHβE) MLA applications induce no detectable current but reversibly block successive ACh-evoked currents. MLA IC_{50} of the L247T mutant (~30 pM) is comparable to that exhibited by the wild type receptor. Furthermore, recovery from MLA blockade follows a sigmoidal time course suggesting the existence of multiple binding sites for this compound. L247T inhibition by MLA resembles the effect of the snake toxin α-bungarotoxin which suggests that the binding sites for these two compounds might partly overlap.

Work supported by HFSP, the Swiss National Foundation, OFES and the EEC.

602.8

ATROPINE INHIBITS NEURONAL NICOTINIC RECEPTORS

R. Zwart* and H.P.M. Vijverberg. Research Institute of Toxicology, Utrecht University, P.O. Box 80.176, NL-3508 TD Utrecht, The Netherlands.

The neurotransmitter acetylcholine (ACh) acts on two distinct types of receptors: G-protein coupled muscarinic receptors (mAChRs) and ionotropic nicotinic receptors (nAChRs). Atropine is a selective antagonist of mAChRs as compared to endplate nAChRs. Little is known, however, about effects of atropine on neuronal nAChRs. The finding that the recently cloned α9 nAChR is sensitive to atropine (Cell 79,705-715) prompted us to investigate the effects of atropine on various nAChR subunit combinations expressed in *Xenopus* oocytes.

1 μM atropine inhibited 1 mM ACh-induced ion currents of α4β2 nAChR to 75% and of α4β4 to 45% of control values. Possible contributions of endogenous mAChRs in oocytes to the effects of atropine have been excluded. The concentration dependence of the effect of atropine on 100 μM ACh-induced ion current revealed an IC50 of 660 nM for α4β4 nAChR. At high ACh concentrations, 1 μM atropine inhibited α4β4 nAChR-mediated ion currents by an apparent non-competitive mechanism. However, at very low ACh concentrations atropine potentiated α4β4 nAChR-mediated ion currents. The EC50 of the concentration-effect curve for ACh was not significantly affected, but the Emax was reduced to 49% in the presence of 1 μM atropine. The slope factor of the curve was slightly reduced in the presence of atropine, which is likely caused by the atropine-induced potentiation at low ACh concentrations. Atropine-induced inhibition of the α4β4 nAChR was voltage-dependent, suggesting open nAChR channel block as the mode of action. Supported by the Netherlands Organization for Scientific Research (N.W.O. #903.42.011).

602.9

INHIBITION OF THE HUMAN α7 NICOTINIC RECEPTOR BY PARTIAL AGONISTS. C.A. Briggs* and D.G. McKenna. Neuroscience Research, D-47W, Bldg. AP10, Abbott Labs, 100 Abbott Park Rd., Abbott Park, IL 60064.

Previous studies using human α7 nicotinic acetylcholine receptor (nAChR) expressed in *Xenopus* oocytes identified substances, such ABT-089 (see also Sullivan *et al.*, this volume) that acted as inhibitors at low micromolar concentrations and as weak partial agonists (≤15%) at higher concentrations (0.1-1 mM). The mechanism of inhibition could include channel block, competitive antagonism or receptor desensitization. In evaluating the last possibility, we determined the relative potencies and efficacies of a variety of standard compounds to act as inhibitors as well as activators of the human α7 nAChR expressed in *Xenopus* oocytes. Responses were measured using two-electrode voltage clamp in the presence of 10 mM Ba^{2+} to prevent secondary activation of Ca^{2+}-dependent Cl^- currents, and 2 μM atropine to prevent activation of endogenous muscarinic receptors. Acetylcholine (ACh), (-)-nicotine, (+)-nicotine and dimethylphenylpiperazinium (DMPP) each inhibited the response to ACh with IC50 values 60- to 130-fold more potent than their EC50 values to activate the α7 nAChR. The stereoselectivity of nicotine as activator was maintained when it was applied as an inhibitor. At the IC50 concentrations, these agonists activated slow responses that were only 0.6% to 1.2% of the maximal response. (-)-Cotinine elicited a 1% response at 1 mM, and inhibited the response to ACh with an IC50 of 175 μM. L-Lobeline, surprisingly, acted only as an antagonist, eliciting no agonist-like response up to a concentration of 1 mM while inhibiting the response to ACh with an IC50 of 8.5 μM. These results are consistent with the hypothesis that weak partial agonists can appear to be more potent and effective as inhibitors than as activators of the rapid nAChR response due to receptor desensitization.

[supported by Abbott Laboratories]

602.10

THE ENDOGENOUS CANNABINOID, ANANDAMIDE, POTENTLY INHIBITS THE FUNCTION OF NICOTINIC α_7 RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES. M. Oz*, E. Podrasky, A. Ravindran, S. Singhal, L. Zhang and F. F. Weight Laboratory of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205..

Anandamide is a recently isolated endogenous ligand for cannabinoid receptors that mimics effects of cannabinoids in the CNS. Currently, little is known about cellular mechanism of anandamide actions. Recently, several studies have suggested that the modulation of brain type nicotinic acethylcholine receptors (nAChR) may be involved in some of the behavioral effects of several drugs of abuse. In this study, the effect of anandamide on the function of recombinant, homomerically expressed α_7 receptors was investigated in *Xenopus* oocytes. In the concentration range 1 nM to 5 μM, anandamide reversibly inhibited the current activated by 10 μM nicotine. The inhibition was concentration and time dependent. The IC_{50} value for the steady-state inhibition was 29 nM, and the apparent Hill coefficient was 1.2. The effect of anandamide was not dependent upon membrane potential. Maximum responses to nicotine were also inhibited, and increasing the concentration of nicotine did not affect the inhibitory effect of anandamide. By contrast, maximal current amplitudes mediated by mouse muscle nAChR were not altered by anandamide up to the concentration of 100 nM. The results provide evidence that the endogenous cannabinoid, anandamide, can selectively and potently inhibit the function of brain type nicotinic ACh receptors. This work was supported by the intramural research program of NIAAA/NIH.

602.11

CHARACTERIZATION OF SIB-1508Y, THE ACTIVE ENANTIOMER OF A NOVEL NICOTINIC ACETYLCHOLINE RECEPTOR (NAChR) AGONIST, SIB-1765F. F. Menzaghi*, A.J. Sacaan, R.T. Reid, E. Santori, L.D. Correa, P.B. Adams, K.T. Whelan, V.B. Risbrough, T.S. Rao, J.S. Schneider[1] and G. K. Lloyd. SIBIA Neurosciences Inc., La Jolla, CA 92037, and Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19102[1].

SIB-1765F [(±)-5-ethynylnicotine fumarate] is a novel NAChR agonist (Soc. Neurosci. Abs. 25, #11.10, 1995). In this investigation, we characterized SIB-1508Y and SIB-1680WD, the two isomers of SIB-1765F. SIB-1508Y and SIB-1680WD displaced the binding of [^{3}H]-nicotine to rat cortical membranes with IC_{50} values of 3 and >100 nM, respectively. The rank order of potency at releasing striatal dopamine (DA) *in vitro* was SIB-1508Y>SIB-1765F>>SIB-1680WD. In freely moving rats, both nicotine and SIB-1508Y increased hippocampal ACh release. Dopamine D1 receptor activation subsequent to NAChR-induced DA release appears to be important in SIB-1508Y-evoked hippocampal ACh release as evidenced by its sensitivity to SCH-23390. Nicotine and SIB-1508Y appear to release hippocampal ACh through distinct mechanisms as the response to the former is both Mec and DHβE sensitive while the response to the latter is sensitive only to DHβE. The locomotor stimulating actions of SIB-1765F appear to be due to its active isomer SIB-1508Y as SIB-1680WD did not act as a partial agonist. Similar to SIB-1765F, SIB-1508Y potentiated the action of L-DOPA in reserpine-induced hypolocomotion. Nicotine was inactive in this model. An inactive dose of SIB-1508Y potentiated the effects of an inactive dose of L-DOPA in improving the latency for object retrieval in MPTP-treated cynomolgus monkeys (J. Schneider et al., this meeting). In addition, SIB-1508Y showed antidepressant activity in the learned helplessness model in rat while nicotine was inactive (Studies performed with ITEM-Labo, Paris, France). These results taken together suggest potential utility of SIB-1508Y in treating motor, cognitive and affective deficits in Parkinson's disease.

602.12

LOBELINE: AN ATYPICAL NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR (NACHR) AGONIST. A. i. Sacaan*, F. M. Menzaghi, R. T. Reid, E. M. Santori, L. D. Correa, K. T. Whelan, G. K. Lloyd, and T. S. Rao. SIBIA Neurosciences Inc., La Jolla, CA 92037.

Lobeline (LOB) and nicotine (NIC) are known to show differential pharmacological effects. These include a lack of effect of LOB on ^{86}Rb-efflux from mouse fibroblasts stably transfected with cDNAs encoding the chick α4β2 subunits and on prepulse inhibition in an acoustic startle assay in rats. In this study, effects of LOB were compared to NIC and SIB-1765F [(±)-5-ethynylnicotine fumarate] in several assays. Unlike NIC and SIB-1765F, LOB-induced increases in striatal and olfactory tubercle DA release were relatively mecamylamine (Mec) and dihydro-β-erythroidine (DHβE)-insensitive and Ca^{+2}-independent. NIC-, but not LOB-induced hippocampal NE release was Mec-sensitive and Ca^{+2}-dependent. LOB attenuated basal striatal ACh release in a sulpiride-independent mechanism. In contrast to NIC, LOB attenuated N-methyl-D-aspartate (NMDA)-evoked striatal ACh release in a Mec or DHβE-insensitive manner with an IC_{50} of 35 μM. LOB however, did not affect NMDA-evoked hippocampal NE release suggesting that LOB is not a general NMDA antagonist. Both NIC and LOB increased hippocampal ACh release *in vivo*. Unlike NIC and SIB-1765F which increased striatal and n. accumbens DA release *in vivo* in naive rats and induced turning behavior in 6-OHDA-lesioned rats, LOB decreased striatal DA in a Mec-sensitive manner, did not significantly alter n. accumbens DA release and failed to induce turning behavior. Consistent with this profile, NIC and SIB-1765F, but not LOB increased locomotor activity in rats that were acclimated to activity cages. In addition, LOB produced pronounced transient locomotor depressant effects in naive rats as compared to NIC and SIB-1765F. In contrast to NIC, the locomotor depressant effects of LOB were insensitive to blockade by NAChR antagonists Mec, hexamethonium (Hex) and DHβE. Repeated administration of LOB (2 mg/kg/sc twice a day for 4 days) resulted in a tolerance to locomotor depressant effects and did not alter locomotor stimulant effects of SIB-1765F (20 mg/kg, sc) suggesting a lack of partial agonist properties for LOB in this response. These contrasting *in vitro* and *in vivo* profiles of LOB as compared to NIC confirm the atypical profile of LOB which is possibly due to activation of distinct NAChRs and/or non-nicotinic pathways.

602.13

PHARMACOLOGICAL INVESTIGATION OF LOBELINE, A NICOTINIC RECEPTOR LIGAND. M.I. Damaj * and B.R. Martin. Department of Pharmacology and Toxicology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298.

Lobeline, a naturally-occurring alkaloid with a binding affinity comparable to that of nicotine, has a complex pharmacological profile. In this study we evaluated the pharmacological responses of lobeline in different behavioral assays and investigated the acute and chronic tolerance to this drug in mice. Male ICR mice were used to test the effects of s.c. lobeline on locomotor activity, motor coordination, body temperature and antinociception (tail-flick test). Lobeline-induced hypomotility, hypothermia and motor impairment was dose-dependent but lobeline was less potent than nicotine. However, lobeline's effects were not blocked by neither mecamylamine nor dihydro-β-erythroidine, two known nicotinic antagonists. On the other hand, lobeline elicited little antinociception by itself but significantly enhanced nicotine's antinociceptive effects. In another group of mice, lobeline injected intrathecally (i.t.) induced a dose-dependent antinociceptive effect. Acute tolerance to lobeline-induced hypothermia, motor impairment and antinociception (after i.t. injection) did not develop after one-dose or repeated injections. In contrast, tolerance developed to lobeline's effects after chronic treatment with lobeline (15 mg/kg twice a day for 10 days). Furthermore, cross-tolerance to nicotine-induced hypomotility and hypothermia was observed in mice rendered tolerant to lobeline. Further studies are needed to explain the differential effects of lobeline and its interaction with nicotine. (Supported by NIDA grant #DA-05274).

602.14

PRESYNAPTIC NICOTINIC RECEPTORS ENHANCE GLUTAMATE RELEASE IN THE HIPPOCAMPAL SLICE AND RAISE Ca IN SINGLE MOSSY FIBER TERMINALS. R. Gray* and J.A. Dani. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Nicotine applied locally onto the dendrites of a CA3 neuron increased the frequency of glutamatergic miniature excitatory postsynaptic currents (mEPSCs). Furthermore, when mossy fiber presynaptic terminals where loaded with fura-2, local application of nicotine induced a Ca influx that could be measured from single mossy fiber terminals.

Hippocampal slices (200-400 μm) were prepared from Sprague-Dawley rats. Na and Ca channels were inhibited by 1 μM TTX and 200 μM Cd. CA3 neuron were whole-cell voltage clamped and mEPSCs were collected before, during, and after a pressure puff of 20 μM nicotine directed toward the dendrites. The frequency but not the amplitudes of the mEPSCs increased, consistent with a presynaptic effect. We then loaded presynaptic mossy fiber terminals in the CA3 region with fura-2AM. Pressure puffs of 20 μM Nic produced changes in the fura-2 signals from individual mossy fiber terminals consistent with a presynaptic Ca influx. The results indicate that cholinergic innervation or applied nicotine is capable of altering glutamatergic synaptic transmission in the hippocampus. The results are consistent with presynaptic nicotinic receptors mediating a Ca influx that then enhances glutamate release. Supported by NIH, NINDS; Smokeless Tobacco Research Council.

602.15

EVIDENCE THAT PHOSPHORYLATION EVENTS AND PROTEIN SYNTHESIS ARE IMPORTANT IN NICOTINIC RECEPTOR TURNOVER IN BOVINE ADRENAL CHROMAFFIN CELLS. B.W. Wenger, R.T. Boyd*, and D.B. McKay. Division of Pharmacology, The Ohio State University College of Pharmacy, Columbus, OH 43210

In these studies, the ability of bromoacetylcholine (brACh) to induce nicotinic acetylcholine receptor (nAChR) turnover in cultured bovine adrenal chromaffin cells was investigated. Several protein kinase inhibitors and a protein synthesis inhibitor were also tested for their ability to affect turnover. Under reducing conditions, brACh treatment causes a rapid loss of nAChR-stimulated adrenal catecholamine release, but is without effect on catecholamine release stimulated with depolarizing concentrations of KCl (56mM). The effects of brACh are concentration-dependent (IC_{50}, ~300 nM) and at concentrations >10 μM, brACh eliminates >90% of nAChR-stimulated release. Recovery of nAChR-stimulated secretory response following brACh treatment occurs slowly. After 100 μM brACh treatment, a functional recovery rate of ~2-3% per hr is observed, with full recovery achieved within 48 hrs. The protein synthesis inhibitor, cycloheximide (5 μg/ml), prevents recovery of nAChR-stimulated secretion. When present during the recovery period (24 hr), the protein kinase inhibitors, chelerythrine chloride (10 μM), K252a (1μM) and H-7 (50 μM), prevent full recovery of the functional response, while having no effects on control, non-brACh treated cells. H-8 (50 μM), another protein kinase inhibitor, has little or no effect on recovery. These studies support the ability of brACh to induce nAChR turnover. These studies also provide evidence that nAChR functional recovery involves protein synthesis and that phosphorylation events contribute to the recovery process.

602.16

ROLE OF NITRIC OXIDE IN NICOTINIC RECEPTOR INDUCED MYOPATHY. M.D. El-Dada* and M. Quik. Dept. Pharmacology, McGill Univ., Montreal, Quebec, Canada.

Previous studies have shown that nicotinic cholinergic agonists mediate a calcium-dependent muscle cell degeneration. Recently nitric oxide (NO) synthase was identified in skeletal muscle. The present experiments test whether this intracellular messenger is involved in agonist induced myopathy. Both the irreversible NO synthase inhibitor L-N-iminoethylornithine (NIO) and L-nitroarginine methyl ester, a reversible inhibitor, protected the muscle cells from the myopathic effects of nicotine. These results suggest that nicotinic receptor stimulation produces an increase in NO to result in muscle cell degeneration. In line with this interpretation, exposure of the muscle cultures to the NO donor sodium nitroprusside (SNP) resulted in a dose dependent decline in myotube branch points. Neither NIO nor SNP altered the binding of ^{125}I-α-bungarotoxin to muscle cells in culture, indicating that the effect of these agents was not mediated through an interaction at the nicotinic receptor. In many systems, NO mediates its effects through cGMP. Exposure of the muscle cultures to cGMP analogs produced a muscle cell degeneration which was not additive with that of nicotine, while the guanylate cyclase inhibitor LY 83583 protected against the myopathic effects of nicotine. The present results suggest that nicotinic receptor activation results in skeletal muscle degeneration through the production of NO and a subsequent activation of guanylate cyclase. Supported by the MRC and Verum Foundation.

602.17

NICOTINIC RECEPTOR MEDIATED CALCIUM MOBILIZATION AND ENHANCED GROWTH IN CELLS STABLY EXPRESSING RAT α7 RECEPTORS. M. Quik* and B. Weinberger. Dept. Pharmacology, McGill Univ., Montreal, Quebec, Canada.

Our previous work had demonstrated stable expression of rat α7 α-bungarotoxin (α-BGT) binding sites by GH_4C_1 rat pituitary cells, a clonal line which does not endogenously express nicotinic receptors. The stably expressed α7 sites were similar to rat brain α-BGT receptors in terms of binding affinities, pharmacological profiles, kinetic properties and molecular size, suggesting that they represent a good model system for studying the α7 receptor. The present data show that nicotinic receptor agonists increase intracellular calcium mobilization (fura 2-AM) in α7/GH_4C_1 cells in a dose dependent manner with EC_{50}'s of 0.2, 2, 5 and 10 μM for epibatidine, nicotine, dimethylphenylpiperazinium and acetylcholine, respectively. The agonist evoked responses were completely inhibited by α-BGT, methyllycaconitine and d-tubocurarine. Interestingly, the α7/GH_4C_1 cells exhibited differential growth related properties as compared to control cells. Nicotine at 10^{-5} M resulted in a long term increase in cell number, which was not observed in untransfected cells or transfected cells not expressing α-BGT receptors. In addition, the cells expressing receptors appeared more adherent than control cells. The present results suggest that activation of stably expressed α7 α-BGT receptors, which results in a mobilization of intracellular calcium, may lead to long term effects on cellular growth. Supported by the MRC and Verum Foundation.

602.18

NICOTINE STIMULATED RELEASE OF [^{3}H]NOREPINEPHRINE FROM CULTURED LOCUS COERULEUS CELLS. K.A. Gallardo*, F.M. Leslie. Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

The structure and function of neuronal nicotinic acetylcholine receptors (nAChR) are of primary importance in elucidating the effect of nicotine on the developing central nervous system (CNS). Morphological and molecular studies of these pentameric ion channels suggest receptor subtype heterogeneity exhibited by differential subunit expression in rat brain. Upon stimulation of central nAChRs diverse cellular responses can be measured depending on the age and type of tissue being examined. We have chosen to assess the functional properties of a subpopulation of nAChRs by examining the release of norepinephrine (NE) from primary dissociated locus coeruleus cells in culture. Cells were harvested from rostral rhombencephalon of E14 Sprague-Dawley rat embryos, cultured for 4 days, then pre-loaded with [^{3}H]NE. Following a washout period, the effect of various nicotinic agonists and antagonists on [^{3}H]NE release was examined. Nicotine was a full agonist with an EC_{50} of 0.63 $\pm$ 0.07 uM, DMPP was as a weak partial agonist, whereas cytisine, lobeline, and DHβE were without agonist effect but antagonized nicotine's actions. Preliminary data indicate the presence of a functional $\alpha_4\beta_2$ receptor subtype when compared to existing pharmacological profiles of known receptor subunit combinations. An anatomical survey was also performed using *in situ* hybridization techniques to confirm the presence of α_4 and β_2 mRNA, and to establish the presence of any other nAChR subunit mRNA. Embryonic, neonatal and adult time points were analyzed. These data suggest that the nicotinic receptors on LC cells during early embryogenesis are of the $\alpha_4\beta_2$ subtype, but does not exclude the presence of other combinations. Work in progress involves a more complete pharmacological characterization of the nAChRs in this system. *PHS grant* #19319 and 30109.

602.19

INDUCTION OF C-FOS PROTEIN IN THE RAT BRAIN AFTER ACUTE AND REPEATED INJECTIONS OF NICOTINE. A.M. Mathieu, C. Pages, B. Zalc*, M.J. Besson. Lab. Neurochimie-Anatomie, IDN, UPMC, URA 1488, 9 Quai St Bernard, 75005 & Lab. Neurobiology Cell. Mol. Clin., INSERM U134, 75013 Paris

The detection of the immediate early gene c-fos acting as a third messenger, coupling cell surface events to gene expression, has been widely used as a functional marker. In order to identify brain regions potentially involved in psychostimulant properties of nicotine, we have analyzed the induction of c-Fos protein 90 min after a single (0.35 mg/kg, sc.) and a repeated (0.35 mg/kg, 3x day, 15 days, sc.) injection of nicotine. A high number of c-Fos positive nuclei was observed in the anterior part of the cerebral cortex (medial prefrontal, cingulate and orbital cortices) and in the posterior part whereas a moderate number of positive nuclei was observed in the sensorimotor cortex. The pyriform cortex exhibited a particular strong immunoreactivity. In subcortical structures, c-Fos positive nuclei were visualized in the dorsal striatum (only in the medial part) and in the nucleus accumbens (rostral pole and cone of the shell). More caudally, immunostained nuclei were localized in the ventral pallidum and the substantia innominata. An induction was also obvious in the superficial layers of the superior colliculus and in the pontine nucleus. A prior mecamylamine injection (1mg/kg, ip) antagonized the nicotine effects. Following repeated injections of nicotine, c-Fos was still expressed in these various structures but the number of c-Fos positive nuclei was less abundant, suggesting a desensibilisation of c-Fos expression. In all these responsive regions, a high to moderate density of nicotine binding sites and of mRNA encoding subunits of the nicotinic receptor has been described. Several regions (thalamus, cerebellum, mesencephalic DA neurons) with high levels of nicotinic receptors failed, however, to express c-Fos. The region specific effects suggests that the nicotine-induced c-Fos expression may involved a specific nicotinic receptor subunit combination.
Supported by MESR (contract 94 VO253)

ACETYLCHOLINE RECEPTORS: NICOTINIC—RECOMBINANT

603.1

Isolation and Localization of the Human Neuronal Nicotinic Acetylcholine Receptor Alpha 2 Subunit. L. Monteggia*, A. Weverst, J-M Roch, E. Touma, K. Idler, H. Schroder†, and J.P. Sullivan Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064-3500 and †Department of Anatomy, University of Koln, Koln, Germany.

Neuronal nicotinc acetylcholine receptors (nAChRs) are members of the ligand gated ion channel gene superfamily. nAChRs are composed of two types of subunits, ligand-binding α subunits (α2-α9) and structural β subunits (β2-β4). The structural, biophysical, and pharmacological properties of a number of human nAChRs have been reported however, no information regarding the human α2 subunit currently exists. In rat the α2 subunit is characterized by a very restricted localization and discrete pharmacological properties. In this present report, we describe the initial characteristics of the human α2 subunit. Human α2 was cloned from whole human brain and shown to contain an open reading frame of 1587bp. The proposed mature α2 protein is composed of 472 amino acids preceeded by a leader sequence of 57 residues. The α2 protein has three potential N-linked glycosylation sites at residues 79, 129 and 235. Additionally, potential protein kinase C phosphorylation sites exist at residues 132, 242 and 488 while a potential tyrosine kinase phosphorylation site exists at residue 468. The human α2 subunit has 84% amino acid identity with rat α2. In situ hybridization studies to assess the distribution of the α2 transcript in human brain are ongoing. (Supported by Abbott Laboratories)

603.2

The General Anesthetic Propofol inhibits ACh response in heterologously expressed a3b4 nAChRs and in Sympathetic neurons in culture.
P. Flood, R. Girod*, J. Ramirez-Latorre and L. Role. Departments of Anesthesiology and Anatomy and Cell Biology in theCenter for Neurobiology & Behavior, Columbia Univ., P&S, 722 W. 168th St. NY, NY 10032.

General anesthetic agents are a diverse variety of particularly toxic compounds used in clinical medicine today. Many of these agents have been shown to act potently and specifically at ligand gated ion channels in the CNS. We have recently reported potent inhibition of the α4β2 type nAChR. Here we report inhibition of the α3β4 nAChR and inhibition of ACh mediated current in sympathetic ganglia cells in culture by the general anesthetic, propofol.

RNA for the chick α3 and β4 subunits were expressed in *Xenopus laevis* oocytes, and studied with standard two electrode voltage clamp methodology. The oocytes expressing α3β4 nAChRs were exposed to a pulse of 1 μM ACh in a continuous perfusion chamber with or without an escalating concentration of Propofol from 12nM to 2μM (Clinical EC_{50} x 1/32-5). ACh mediated current from heterologously expressed α3β4 receptors was reduced by propofol application to a maximum inhibition of 60% in the presence of 2 μM propofol. IC_{50} for these receptors was 0.22μM propofol.

Sympathetic ganglia were dissected from E11 chick and cultured for 3-5 days. Whole cell currents were recorded from these sympathetic neurons. 5 second pulses of 5 μM ACh were pressure applied to the neurons and evoked whole cell currents were measured using standard patch clamp methodology. 400 nM Propofol was preapplied for ten seconds prior to ACh application as above. Ach mediated current in sympathetic neurons was reduced 90% by 0.4 μM propofol.

Propofol inhibits the ACh mediated currents in heterologously expressed α3β4 nAChRs and in sympathetic neurons in culture. Inhibition in the cultured neurons was more profound, perhaps because of the participation of other subunit combinations of nAChRs which are more potently inhibited by propofol, such as α4β2 (IC_{50} = 0.1 μM).
(Supported in part by NIDA DA09366 to LWR.)

603.3

NICOTINIC RECEPTOR SUBUNIT mRNAs IN DEVELOPING RAT HIPPOCAMPUS VIA RT-PCR. T. Moreira, E. F. R. Pereira, D. R. Burt*, Dept. of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201.

Nicotinic responses in hippocampal neurons increase during development. We studied the level of mRNAs for several neuronal type nicotinic subunits (α_3, α_4, α_5, α_7, β_2, β_3, β_4) in postnatally developing neurons in the hippocampus (days P1, P7, P16 and P21). We extracted total RNA from rat hippocampi dissected at the different ages and reverse transcribed (RT) it into cDNA with random priming. We made a pair of primers spanning at least one intron for each subunit and performed polymerase chain reactions (PCR). The β_4 band was stronger at P7 and P16 than at other ages (P1 and P21). Similar results were seen with α_3. The composition $\alpha_3\beta_4$ is proposed to be one of the possible subunit combinations of native nicotinic receptors expressed in hippocampal neurons. The mRNA signals for both subunits appear to rise together. The α_5 band was present at P1, P7 and P16 but hardly detected at P21. The α_4 band was detected strongly in all ages but peaked in intensity at P16. The β_2 band was present throughout development, with a gradual increase with age. Thus, although $\alpha_4\beta_2$ is another proposed receptor combination in native hippocampal nicotinic receptors, the correlation of developmental patterns of the two subunits is not as clear as for $\alpha_3\beta_4$. The α_7 mRNA appeared to be by far the most abundant, with a slight increase during development and a possible peak at P16. The α_7 homopentamer is thought to give to rise to the most widespread type of response, type IA, in hippocampal cultures. These qualitative changes in mRNA levels *in vivo* appear to be consistent with existing functional data for changes in nicotinic response patterns in hippocampal neurons developing *in vivo* and *in vitro*. (Supported in part by NIH grant HD16596 to DRB and FAPEMA scholarship to TM.)

603.4

THE ROLE OF THE CYSTINE LOOP IN ACETYLCHOLINE RECEPTOR ASSEMBLY. W. N. Green, S. V. Rakhilin* and C. P. Wanamaker. Department of Pharmacological & Physiological Sciences, University of Chicago, Chicago, IL 60637.

Nicotinic acetylcholine receptors (AChRs) contain α, β, γ and δ subunits assembled into $\alpha_2\beta\gamma\delta$ pentamers. A highly conserved region of ionotropic neurotransmitter receptors is a 15 amino acid cystine loop. We have addressed whether the cystine loop is involved in AChR subunit assembly. AChR subunit assembly was assayed using an ^{35}S-methionine pulse-chase protocol. With the addition of 5 mM DTT to reduce disulfide bonds on all 4 subunits, the subunits assemble into αβγ trimers, but all subsequent steps in assembly are blocked. When α subunit loop cysteines are mutated to serines, assembly is blocked at the same step as with DTT. However, when homologous β subunit cysteines are mutated to serines, assembly proceeds further and is blocked at a later step, between the assembly of αβγδ tetramers and $\alpha_2\beta\gamma\delta$ pentamers. Using a monoclonal antibody (mAb) that recognizes only the intact α subunit cystine loop (from J. Lindstrom, U. Penn.), we demonstrate that the α subunit cystine loop forms before the block of assembly by DTT or the α subunit mutation. The mAb precipitated αβγ trimers at early chase times, after which the epitope disappeared and the subunits could not be precipitated at later chase times. The disappearance of the epitope is a conformational change that buries the epitope since the epitope reemerges when the subunits are denatured. The cystine loop conformational change occurs at the same step in assembly as the block by DTT and the α subunit mutation indicating that this step in assembly requires a conformational change of the cystine loop. The data suggest that the δ subunit recognition site forms after a series of folding events key to which is the cystine loop conformational change. Supported by NSF grant IBN-9319656 & a Council for Tobacco Research Scholar Award.

603.5

FORMATION OF THE DIFFERENT ACETYLCHOLINE (ACH) BINDING SITES DURING ACH RECEPTOR ASSEMBLY. C.P. Wanamaker, M.A. Gertz & W.N. Green*. Dept. Pharm. & Physiol. Sci., University of Chicago, Chicago IL 60637

The 2 ACh binding sites of the ACh receptor (AChR) are located on different α subunits at or near the interface with either the γ (αγ) or δ (αδ) subunits. To determine when each of the two ACh binding sites form during assembly, subunit complexes were precipitated using bromoACh affinity resin or α-Bungarotoxin (BuTx)-sepharose. The first ACh binding site forms at about the same time as the first BuTx site and the epitope for monoclonal antibody (mAb) 14, which indicates that this site forms on αβγ trimers just before the addition of the δ subunit. The second ACh site forms soon after the addition of the second α subunit to the αβγδ tetramers. Pharmacological and immunological probes were used to distinguish the two ACh sites. D-tubocurare (dTC) binds to the αγ ACh site with a higher affinity than to the αδ ACh site. Based on dTC competion of ^{125}I-BuTx binding, subunit complexes that assemble early (trimers and tetramers) have the high affinity dTC site while $\alpha_2\beta\gamma\delta$ pentamers have both the high and low affinity dTC site. Using a mAb specific for the αγ site (mAb 247g from R. Fairclough, U.C. Davis), we immunoprecipitated αβγ trimers and αβγδ tetramers. We conclude that the αγ ACh site is the first site to form and does so on αβγ trimers just prior to the additon of the δ subunit. The αδ ACh site appears later soon after $\alpha_2\beta\gamma\delta$ pentamers assemble. The order of ACh site formation provides evidence for the subunit arrangement of the trimers and tetramers and places the δ subunit recognition site at the γ-β subunit interface of the αβγ trimer. Supported by NSF grant IBN-9319656 & a Council for Tobacco Research Scholar Award.

603.6

THE C-TERMINAL REGION OF THE γ SUBUNIT IS NEEDED FOR FULL ASSEMBLY OF THE ACETYLCHOLINE RECEPTOR. A.L. Eertmoed* and W.N. Green. Committee on Neurobiology, University of Chicago, Chicago, IL 60637.

Fast excitatory transmission is mediated by a family of hetero-oligomeric neurotransmitter-gated ion channels, of which the nicotinic acetylcholine receptor (AChR) is the best characterized member. We are attempting to identify regions of the AChR subunits important for oligomerization by creating chimeric subunits and examining their ability to associate with wild-type subunits. A chimeric subunit (δγ) consisting of the extracellular N-terminus of the δ subunit fused to the region of the γ subunit from the first transmembrane domain (M1) to the C-terminus is able to associate with the α, β, and γ subunits to form cell surface receptors which bind the AChR antagonist, α-bungarotoxin (BuTx). These results suggest that the N-terminus of δ is sufficient to direct assembly of this subunit. In contrast, the reverse chimera (γδ), which consists of the N-terminus of the γ subunit followed by the region from M1 to the C-terminus of δ, does not associate with α, β, and δ subunits to form cell-surface complexes that bind BuTx. The γδ chimera associates with the α and β subunits, but does not form BuTx binding sites, and in fact prevents the formation of these sites when cotransfected with all four wild-type subunits. Thus, the C-terminal region of γ is needed for the complete assembly of the AChR. Without this region assembly is blocked. The block occurs after αβγ trimers assemble and before formation of the BuTx binding site and the addition of the δ subunit. These results suggest that the C-terminal half of the γ subunit is involved in the folding events occurring during the formation of the δ subunit recognition site. Supported by NSF grant IBN-9319656, a Council for Tobacco Research Scholar Award (W.N.G), and a Myasthenia Gravis Foundation Viets Fellowship (A.L.E).

603.7

FUNCTIONAL ROLE OF THE α5 SUBUNIT IN THE HUMAN RECOMBINANT α3 ACHRS.
F. Wang*, V. Gerzanich, and J. Lindstrom. Dept. of Neuroscience, Univ. of Pennsylvania Med. Sch., Philadelphia, PA, 19104-6074.

cRNA's encoding α3,β2, and α5 or α3, β4, and α5 nicotinic acetylcholine receptor (AChR) subunits yielded functional AChRs containing α5 subunits in *Xenopus* oocytes. The epitope for mAb 142 was inserted at the C-terminus of α5 to immunotag this subunit. Efficient coassembly of the α5 subunit with α3β2 or α3β4 combinations was proven by immunoisolating α5-containing AChRs with mAb 142-coupled Actigel. Surface expression of the α5-containing AChRs was shown by surface labeling with mAb 142. No functional AChRs were detected after expression of α5 alone or in pairwise combinations with α3,β2, andβ4 subunits. The presence of α5 subunits increased the rate of the desensitization of AChRs containing both α3 and β2 or α3 and β4 subunits. The presence of the α5 did not change voltage-dependence of α3β2 or α3β4 AChRs. α5 caused only subtle changes in the activation potencies or equilibrium binding affinities of α3 AChRs for ACh, nicotine, cytisine or DMPP. However, addition of α5 to α3 and β2 subunits increased the efficacy for nicotine from 50% to 100%, and for DMPP from 107% to 183%. By contrast, addition of α5 to α3 and β4 subunits decreased the efficacy for nicotine from 100% to 90%, and for DMPP from 100% to 13%. The pharmacological properties of α3β2, α3β2α5, α3β4 and α3β4α5 AChRs were compared to native α3 AChRs from the human neuroblastoma cell line IMR-32.
Supported by grants to J.L. from the NIH, the Smokeless Tobacco Research Council, Inc., the MDA, and the Council for Tobacco Research, USA

603.8

α6 NICOTINIC AChR SUBUNIT CAN FORM A FUNCTIONAL HETEROMERIC ACETYLCHOLINE RECEPTOR . V. Gerzanich, A. Kuryatov, R. Anand, and J. Lindstrom*. Departments of Neuroscience and Pharmacology, Univ. of Pennsylvania Med. Sch., Philadelphia, PA, 19104-6074.

Previously, a rat brain cDNA was reported which was designated α6 because of its homology with nicotinic acetylcholine receptor (AChR) α subunits. α6 is especially homologous to α3, yet no ACh-gated cation channels were detected when it was expressed in *Xenopus* oocytes alone or in combination with other known rat AChR subunits. We cloned chicken α6 and human β4 AChR subunits and tested for ACh-gated cation channels with α6 by expression in *Xenopus* oocytes alone or in pairwise combination with chicken α3, β2, β4 or human α3, β2, β4 AChR subunits. Chicken α6 formed detectable functional AChRs only when expressed together with the human β4 subunit. The α6β4 AChR mediated currents showing strong inward rectification and dependence on extracellular Ca^{++}. It exhibited a distinct pharmacological profile with an EC_{50} for ACh of 28 μM, for (+)-epibatidine of 24 nM, cytisine of 6.6 μM and DMPP of 15 μM. Both cytisine and DMPP behaved as partial (~30 %) agonists. Nicotine (EC_{50} = 22 μM) was an even weaker partial agonist (~ 18%) and had a relatively long lasting inhibitory effect. Coexpression of the previously cloned rat α6 subunit with the human the β4 subunit also resulted in functional α6β4 AChRs with properties resembling those of the chicken/human α6β4 AChRs. Thus, α6 can function as part of AChRs with unusual pharmacological properties.
Supported by grants to J.L. from the NIH, the Smokeless Tobacco Research Council, Inc., the MDA, and the Council for Tobacco Research, USA

603.9

Subunit dependent desensitization of neuronal nicotinic receptors at low concentrations of nicotine. Robin A.J. Lester * and Michael W. Quick. Neurobiology Research Center and the Department of Physiology & Biophysics, University of Alabama at Birmingham, Birmingham AL 35294.

Both activation and desensitization of CNS neuronal nicotinic acetylcholine receptors (nAChRs) have critical roles in nicotine addiction. Because these functional properties are determined by the subunit composition, and subtypes of nAChRs show differential expression in various brain regions, information regarding the subunit specific interactions between nicotine and the receptor will be important. We have compared the activation (EC_{50}) and desensitization (IC_{50}) dose-responses for various combinations of nAChR subunits expressed in *Xenopus* oocytes. Desensitization properties were determined from the change in the amplitude of a test pulse (5-10 s) of acetylcholine (10-300 μM). The data imply that the effective concentrations of nicotine for nAChR desensitization and the time course of its onset and recovery are highly dependent on the receptor composition.

			desensitization			
subunit type	EC_{50} (μM)	IC_{50} (μM)	block at 1 μM	max. block	onset (min)	recovery (min)
α7	127	4.0	0.13	1.0	~3	~5
α3β2	60	0.2	0.63	0.75	~4	~12
α3β4	48	1.5*	0.45	1.0	~50	~40

*estimated by extrapolation of an exponential to the desensitization onset

Supported by NIH grant R29 NS31669 and the W.W.Keck Foundation 931360

603.10

Ca^{+2} MODULATION OF α4β2 AND α4α5β2 COMBINATIONS OF NEURONAL NICOTINIC RECEPTORS.

C. Yu*, J.A. Ramirez-Latorre and L.Role. Center for Neurobiology & Behavior, Columbia University. 722W 168th St. New York, NY 10032.

We have studied the modulatory effects of Ca^{+2} on the α4β2 and α5α4β2 combinations. With increasing Ca^{+2}_{Ext} to mM levels, we observe a decrease in single channel conductance and an increase in open probability. If Ca^{+2}_{Ext} is maintained at μM levels, multiple transitions within openings of individual channels i.e. The openings transit between the normal conducting state and two or more additional states. Increasing Ca^{+2}_{Ext} to mM concentration reduces the conductance states to one.

Ca^{+2} produces a potentiation on the α4β2 combination of similar magnitude to that produced by MTSEA, a positively charged sulfyhdryl reagent . We have also observed that MTSEA interferes with the potentiating effects of Ca^{+2} in the α4β2 combinations. At the single channel level, Ca^{+2} and MTSEA increase the open time of the channel, decrease the single channel conductance and affect the rate of desensitization at concentrations of ACh ranging from 1 to 100 μM. In outside out patches, α4β2 channels reacted with MTSEA have lower apparent permeability to Ca^{+2}. The presence of α5 in the complex affects the permeability and the kinetics caused by alteration of external Ca^{+2} concentrations. Mutational analysis of the α4 sequence implicates a Cys residue in the intracellular M2 domain as being part of a modulatory Ca^{+2} site. We are currently examining whether comparable Cys residues in either α5 or in the β subunit are similarly implicated in Ca^{+2} modulation. Supported by NS22061.

603.11

LEUCINE MUTATIONS AT THE 9' POSITIONS OF THE M2 DOMAIN IN ACHR CHANGE THE CHANNEL GATING AND SIMULATED SYNAPTIC RESPONSE. H. Zhang*, C. Labarca, P. Deshpande, Y. Zhang and H. A. Lester. Division of Biology, 156-29, Caltech, Pasadena, CA 91125

Mutation of a highly conserved Leucine residue to Serine at the 9' position of the M2 transmembrane domain of mouse muscle nicotinic ACh receptors (nAChR) produces a ~10-fold leftward shift in the ACh dose-response relation for each subunit mutated. We expressed nAChRs with varying numbers (m^*_s) of Leu9'Ser mutated subunits in *Xenopus* oocytes and studied single-channel properties in outside-out patches with symmetrical KCl solutions. ACh-induced openings at mutated AChRs have (1) longer openings and bursts, (2) briefer closed times, and (3) more frequent spontaneous openings. These effects increase with m^*_s. For example, average channel open times for wild type $\alpha_2\beta\gamma\delta$ and for $\alpha^*_2\beta^*\gamma\delta^*$ (m^*_s = 4) receptors are 1.8±0.3 ms and 40±5 ms, respectively. The spontaneous openings, however, have the average open time of 0.24±0.02 ms for all $m^*_s > 0$. Synthesized postsynaptic currents, produced with a piezoelectric micromanipulator that delivered ACh pulses, decayed as expected from channel burst durations: for $\alpha^*_2\beta^*\gamma\delta^*$ (m^*_s = 4), the time constant is 2,200±500 ms, ≥100 fold higher than for wild type. Thus (1) both longer and more frequent openings contribute to the $\geq 10^4$ fold shift in EC_{50} between wild type and m^*_s = 5; and (2) the highly conserved 9' Leucine is crucial for appropriately brief synaptic events.

Supported by NIH and CA TRDRP.

603.12

SUSTAINED NICOTINE EXPOSURE INCREASES PHOSPHORYLATION OF THE α4 SUBUNIT OF α4β2 NEURONAL NICOTINIC RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES BY BOTH CYCLIC AMP-DEPENDENT PROTEIN KINASE AND A TYROSINE KINASE. Y-N. Hsu, S.C. Edwards, and L. Wecker*. Dept. Pharmacol., Univ. South Florida Coll. of Med., Tampa, FL 33612.

Studies have shown that the chronic administration of nicotine to rats increases the density of nicotinic receptors, but decreases the ability of nicotine to release acetylcholine (ACh) from brain slices. In addition, we have demonstrated that nicotine-induced currents in *Xenopus* oocytes expressing α4β2 receptors are significantly reduced following 24 hour exposure to 1-50 nM nicotine. To determine whether this nicotine-induced loss of function involved alterations in the state of phosphorylation of the receptor, the cRNAs for α4 and β2 subunits were coinjected into oocytes, and cells were incubated for 24 hours in media in the absence or presence of 50 nM nicotine. Receptors were isolated from a detergent-solubilized membrane fraction using an ACh-AffiGel affinity column and immunoprecipitated with a β2 monoclonal antibody (mAb270, J. Lindstrom) and protein G Sepharose. When receptors were either: a) treated with the catalytic subunit of PKA and ^{32}P-labeled ATP, separated by SDS PAGE, and visualized by autoradiography; or b) subject to western blot analysis with an antiphosphotyrosine antibody, results indicated that phosphorylation the α4 subunit by both PKA and a phosphotyrosine kinase was increased significantly by sustained exposure to nicotine. Results support the idea that nicotine-induced receptor phosphorylation may mediate decreased receptor function. (Supported by grant 0411 from the STRC, Inc.)

603.13

STABLE FUNCTIONAL EXPRESSION OF THE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR α3β4 IN MEL (MURINE ERYTHROLEUKEMIA) CELLS: A NOVEL EXPRESSION SYSTEM FOR LIGAND GATED ION CHANNELS. M. Garcia-Alonso*[1], SJ Dunbar[1], JD Windass[1], M Needham[2], M Criado[3]. [1] ZENECA Agrochemicals, Jealotts Hill, Bracknell, Berkshire, RG42 6ET, United Kingdom. [2] ZENECA Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TJ. [3]Universidad de Alicante, Dpto. de Neuroquimica, Campus de San Juan, Ap. correos 374, 03080, Alicante, Spain.

MEL cells are erythroid progenitor cells which have been infected with the Friend virus complex and thus arrested at the pro-erythroblast stage. In recent years a novel method for high level of expression of heterologous proteins has been developed at ZENECA using MEL cells (Needham *et al.* 1992 Nucleic Acid Research **20** 997-1003). The overall expression levels were increased using the human β-globin promoter, the β-globin gene (which provides mRNA processing and maturation signals) and the locus control region (LCR) which confers position-independent expression of stably transfected genes linked in *cis*. Here we describe the use of this system for the expression of a neuronal nicotinic acetylcholine receptor α3(bovine)/β4(rat). MEL cells were transfected with equal amounts of both cDNAs, confirmed by Northern Blot analysis. Clones containing both sub-units were studied electrophysiologically using whole cell patch-clamp and pressure application of the agonist DMPP (100μM). Rapidly desensitising currents were detected, indicating that expression of the receptor was functional. The simplicity and speed of the MEL/LCR system affords advantages over many other expression systems, such as *Xenopus* oocytes, for the detection of functional activity of receptors and ion channels.

Funding for this project was provided by ZENECA Agrochemicals as a PhD grant

603.14

HEK293 CELLS STABLY EXPRESSING RECOMBINANT HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS α3β2 AND α4β2 DISPLAY DIFFERENTIAL SENSITIVITY TO EXTERNAL Ca^{2+}. L.S. Mahaffy, K.A. Stauderman*, L.E. Chavez-Noriega, J. Crona, and J. Corey-Naeve. SIBIA Neurosciences, Inc., La Jolla, CA 92037.

We examined the influence of external Ca^{2+} on agonist-induced responses in HEK293 cells stably transfected with cDNAs encoding full-length human neuronal nicotinic acetylcholine receptor (nAChR) subunit combinations α3β2 (A3B2.1) and α4β2 (A4B2.1; see also Chavez-Noriega *et al.*, this meeting). In whole-cell recordings, application of ACh, DMPP, or nicotine evoked inward currents in both A4B2.1 and A3B2.1 cells that were 2-3 fold larger when external $[Ca^{2+}]$ was raised from 2 to 20 mM. In comparison to the electrophysiology, single-cell imaging of nicotine-evoked increases of cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) revealed that elevating external Ca^{2+} from 2 to 20 mM increased the percentage of A4B2.1 cells responding to nicotine from 68% to 96%, whereas the percentage of responding A3B2.1 cells increased from 5% to 97%. Furthermore, the magnitudes of nicotine-evoked $[Ca^{2+}]_i$ signals were 3.6- and 23-fold larger in 20 mM external Ca^{2+} in the A4B2.1 and A3B2.1 cells, respectively. Consistent with the single-cell results, nicotine-stimulated increases of $[Ca^{2+}]_i$ in a population of cells were enhanced 4-fold in A4B2.1 cells and 8- to 16-fold in A3B2.1 cells when external Ca^{2+} was raised from 1.8 to 21.8 mM. In contrast , raising the external $[Ca^{2+}]$ from 1.8 to 21.8 mM caused only a 2-fold increase in nicotine-stimulated $[Ca^{2+}]_i$ increases in cells expressing human α3β4 receptors (A3B4.1 cells). These data suggest that Ca^{2+} flux through human α3β2 receptors is more sensitive to changes in external Ca^{2+} than either α4β2 or α3β4 receptors. Changes in external $[Ca^{2+}]$ may, therefore, differentially regulate the activity of nAChR subtypes in neurons.

603.15

CHARACTERIZATION OF HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) α4β2 and α3β2 STABLY EXPRESSED IN HEK293 CELLS. L.E. Chavez-Noriega*, N. Zahl, L.S. Mahaffy, J. Crona, R. Reid, P. Adams, K.J. Elliott, K. Berckhan, K.A. Stauderman and J. Corey-Naeve. SIBIA Neurosciences, Inc., La Jolla, CA 92037.

HEK293 cells have been stably transfected with cDNAs encoding full-length nAChR subunit combinations α4β2 (A4B2.1) or α3β2 (A3B2.1; see also Mahaffy *et al.*, this meeting). Northern blot analysis demonstrated mRNA encoding each subunit. ^{3}H-epibatidine bound to A4B2.1 and A3B2.1 cells with a K_d value of 103 ± 14 pM (mean ± SD) and 7.5 ± 2.4 pM, respectively, and B_{max} values of 13,100 ± 1,600 and 498 ± 79 fmol/mg protein, respectively. Increases in cytosolic [Ca^{2+}], measured with fluo-3, were elicited by nicotine (NIC), DMPP or cytisine with EC_{50} values of 2.1 ± 1.3, 2.5 ± 0.6 and 1.6 ± 0.9 μM for A4B2.1 cells, and 8.3 ± 3.7, 3.2 ± 2.1 and 32.8 ± 25.0 μM for A3B2.1 cells, respectively. Responses are stable for at least 35 passages in A4B2.1 and 50 passages in A3B2.1 cells. Agonist-induced responses were blocked by DHβE, d-tubocurarine and mecamylamine with IC_{50} values of 0.7 ± 0.4, 4.1 ± 3.8 and 0.3 ± 0.1 μM for A4B2.1 cells, and 14.3 ± 5.2, 0.2 ± 0.2 and 1.0 ± 1.4 μM for A3B2.1 cells, respectively. Agonist-induced inward currents were observed in whole-cell voltage clamped (V_h=-80 mV) A4B2.1 cells (538 ± 357 pA, n=13) and A3B2.1 cells (1,113 ± 2,643 pA, n=20) in response to ACh, NIC or DMPP (30-100 μM). A3B2.1 cells displayed faster desensitization kinetics than A4B2.1 cells. Agonist-induced currents from both cell lines exhibited strong inward rectification in the current-voltage (I-V) relation with an E_{rev} near 0 mV. Our results indicate that stably expressed nAChRs α4β2 and α3β2 are pharmacologically diverse and are valuable tools for the discovery of subtype-selective nAChR compounds with potential therapeutic utility.

603.16

6-HYDROXYDOPAMINE LESION OF RAT NIGROSTRIATAL DOPAMINERGIC NEURONS DIFFERENTIALLY AFFECTS NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT mRNA EXPRESSION. K.J. Elliott*, A.I. Sacaan, T.S. Rao, G.K. Lloyd and J. Corey-Naeve. SIBIA Neurosciences, Inc., La Jolla, CA 92037.

Parkinson's disease is characterized by degeneration of the dopamine (DA) neurons originating in the substantia nigra (SN) and ventral tegmental area. Since neuronal nicotinic acetylcholine receptors (nAChRs) participate in the control of DA release *in vitro* and *in vivo*, the subunit composition of nAChRs in the DA-ergic neurons of the SN is of interest. We have initiated a study using RT-PCR to examine the effect of unilateral 6-hydroxydopamine (6-OHDA) lesioning of rat nigrostriatal DA-ergic neurons on nAChR subunit mRNA expression levels. 6-OHDA was injected into male SD rats according to the atlas of Paxinos and Watsons at (A/P = -4.8; D/V = -8; ML = +1.9) (Zivic Miller). After 6 months, tissues were collected bilaterally from various brain regions and total RNA was isolated. Semi-quantitative RT-PCR was performed with oligonucleotide primers specific for glyceraldehyde-6-phosphate dehydrogenase (GAPDH), glutamic acid decarboxylase (GAD) and nAChR α5, α6 and β2 subunit mRNAs over a linear amplification range as determined by template titration. RT-PCR products were separated on an ethidium bromide-stained agarose gel and signal intensities were normalized to GAPDH. The results indicate significantly reduced levels of α5 and α6 but not β2 mRNAs in lesioned compared to unlesioned SN. GAD mRNA levels appeared to be unaffected by lesioning, confirming the integrity of the GABA-ergic neurons in the SN. The treatment did not affect α5, α6 or β2 mRNA levels in hippocampus, prefrontal cortex or thalamus, which are known to express nAChR transcripts. The results suggest that nAChR α5 and α6 mRNAs are expressed in SN DA-ergic neurons, while β2 mRNA is more heterogeneously expressed in the SN.

603.17

PERMEABILITY OF DIVALENT CATIONS IN RAT α7 NICOTINIC AChR ION CHANNEL. M. Dabrowski[1], K. Eskesen*[2], P. Bennekou[1]. [1]August Krogh Institute, Universitetsparken 13, DK 2100 Ø, Denmark. [2]Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark.

In this study we examined the relative permeabilities of divalent cations on mature *Xenopus laevis* oocytes expressing the rat α7 homooligomeric nicotinic AChR. Whole-cell current responses induced by 10 and 30 μM (-)-nicotine from oocytes voltage-clamped at -80 and -60 mV was obtained. The endogenous Ca^{2+}- activated Cl^- - channel was blocked by flufenamic or niflumic acid to less than 5% of the unblocked response. The potentiation and regulatory effects of Ca^{2+} were minimized by using a constant background of 100 μM Ca^{2+} solution when, obtaining concentration-current curves for the other divalent ions. We found the relative permeability sequence for the Group 2A ions: $Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$. The sequence is monotonic in radius and the selectivity ratio is near 10 for Ba^{2+}/Mg^{2+}, which suggests a weak-site electrostatic effect in the permeation. We also examined some of the transition elements and found: $Mn^{2+} > Co^{2+} > Ni^{2+}$ and Cd^{2+}. Ni^{2+} and Cd^{2+} both completely block the channel (at 1 mM), whereas the Co^{2+} current is comparable to Mg^{2+} and Mn^{2+} even more permeable than Mg^{2+} at low concentration (1 mM). This is not a simple electrostatic effect: Co^{2+} has a radius comparable to Ni^{2+} and hence similar hydration-energies and water-substitution rates but Co^{2+} is somewhat permeable whereas Ni^{2+} carries no current. All ions except Ca^{2+} show decreasing currents with increasing concentrations (from 1 mM). This could be explained by competition at receptor regulatory site(s), rather than a sudden block by a normally permeant ion.

603.18

REGULATION OF RECOMBINANT HUMAN α7 NICOTINIC RECEPTORS BY ACTIVATOR AND ANTAGONIST LIGANDS. M. Gopalakrishnan*, O. Delbono, E. J. Molinari, M. Renganathan, L. Messi, S.P. Arneric and J.P. Sullivan. Neuroscience Research (D-47W), Abbott Laboratories, Abbott Park, IL 60064-3500 and Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1083.

Although (-)-nicotine has been shown to regulate the neuronal α4β2 nAChRs following chronic treatment, very little is known about changes at the level of the α-bungarotoxin [BgT]-sensitive nAChRs. In this study, we examined changes in the expression and functional characteristics of the human α7 nAChRs stably expressed in HEK-293 cells following treatment with nicotinic cholinergic ligands. Treatment of cells for 120 h with activator ligands such as (-)-nicotine, (±)-epibatidine and DMPP elicited significant upregulation of [^{125}I]α-BgT binding sites although the concentrations required for upregulation were at least 100-fold higher than those required at the α4β2 nAChRs [see Molinari *et al.*, this meeting]. (-)-Nicotine [100 μM] treatment increased the Bmax values of [^{125}I]α-BgT binding 3-4 fold over untreated cells with no change in ligand affinities. A concentration-dependent upregulation of binding sites was also observed following treatment of transfected cells with the selective α7 nAChR antagonist, methyllycaconitine. Functionally, the peak amplitude of the whole-cell currents recorded by fast application of (-)-nicotine after treatment of cells for 120 h with 100 μM (-)-nicotine [495±17 pA] and 10 μM methyllycaconitine [1450±150 pA] was significantly larger than those observed in untreated cells [298±35 pA; n = 5-6]. The single channel properties including unitary current amplitude [5 pA at -100 mV], conductance [65 pS] and dwell time distributions were unaltered under such conditions. These studies suggest that α7 nAChRs are upregulated by activator and antagonist ligands and that regulation at the level of the human α7 nAChR may be important in the long-term therapy with cholinergic channel modulators. *(Supported by Abbott Laboratories).*

603.19

UPREGULATION OF RECOMBINANT HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS BY CHOLINERGIC CHANNEL LIGANDS: A ROLE FOR PROTEIN KINASES. E. Molinari, M. Gopalakrishnan and J. P. Sullivan* Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064-3500.

Alterations at the level of neuronal nicotinic acetylcholine receptors (nAChRs) have been implicated in a variety of homologous and heterologous influences including neuropathologic conditions. This study was designed to examine the regulation of the human α4β2 nAChRs by cholinergic channel ligands and to address the underlying mechanisms. Significant increases in [^{3}H]cytisine binding sites were observed following treatment of cells stably expressing the human α4β2 nAChRs for 24 - 162 h by activator ligands including (-)-nicotine, (-)-cytisine, DMPP, ABT-418, ABT-089 and (±)-epibatidine, and by antagonists including dihydro-β-erythroidine (DHβE), d-tubocurarine and methyllycaconitine. A good correlation was observed between the K_I values for binding inhibition and the EC_{50} values for receptor upregulation. (-)-Nicotine-evoked increase in [^{3}H]cytisine binding sites was maximal at 10 μM [15-fold], steroselective, rapid ($t_{0.5}$ = 4.0±0.5 h] and totally reversible [$t_{0.5}$ = 11.7±0.1 h]. Mecamylamine failed to upregulate nAChRs and did not alter (-)-nicotine-evoked effects. Upregulation was blocked by cycloheximide suggesting a role for protein synthesis. Functional studies showed that chronic treatment with (-)-nicotine or DHβE resulted in a differential enhancement in the maximal functional efficacy of ACh to activate cation efflux. Upregulation was be mimicked by treatment of cells with activators of protein kinase C and protein kinase A. Treatment of cells with phorbol myristate acetate (100 nM) or forskolin (10 μM) increased [^{3}H]cytisine binding by 95±19% and 79±9% respectively and increased the functional efficacy of ACh to enhance cation efflux by 108±39 % and 54±9 % [n=4] respectively. These studies demonstrate that human α4β2 nAChRs are rapidly upregulated by both activator and competitive antagonist ligands and that protein kinases may play a role in receptor upregulation. *(Supported by Abbott Laboratories).*

603.20

MOLECULAR AND FUNCTIONAL EXPRESSION OF THE NICOTINIC α_7 ACETYLCHOLINE RECEPTOR (nAChR) IN RAT SPINAL CORD MOTONEURON. M.L.Messi, M. Renganathan, E. S. Grigorenko and O. Delbono*. Bowman Gray School of Medicine, Dept. of Physiology-Pharmacology and Internal Medicine (Gerontology), Winston-Salem, NC 27157.

Spinal cord motoneurons (MN) undergo early and late onset developmental and pathological modifications. As α_7 nAChR has been related with neuronal trophism due to the high Ca^{2+} permeability, we investigated the receptor expression on a purified embryonic (E15) spinal cord MN culture. The α_7 subtype was identified in single MN using antisense RNA amplification technique combined with PCR. Fast (-)-nicotine application elicited inward currents that were blocked by 2 nM α-bungarotoxin (α-BgT)- or 3 nM methyllycaconitine. (-)-Nicotine-induced currents were associated with a significant increase in [Ca^{2+}]$_i$ (cell body, 475 ± 51 nM; neurites, 780 ± 65; n = 5). Single channel activity induced by 100 μM (-)-nicotine showed two mean open time distributions of 0.193 ms and 2.13 ms, corresponding to α_7 nAChR and $\alpha_4\beta_2$, respectively. Brief and long single channel events were blocked by preincubation in 2 nM α-BgT or in 10 nM dihydro-ß-erythroidine, respectively. (-)-Nicotine was applied chronically to MN cultures using basically two schemes. A pulsed protocol (50 μM, 4 times/day for 1 min) significantly increased (3 fold) MN survival 3 days after plating. This effect was precluded by 2 nM α-BgT. A steady state (-)-nicotine application protocol (1-100μM, 6-12 hs/day) did not show significant increase in MN survival respect to control. These studies demonstrate that activation of the α_7 nAChR promotes a Ca^{2+}-mediated increase in spinal cord MN survival. (Supported by MDA and NIH).

603.21

THE MONGOOSE ACETYLCHOLINE RECEPTOR α-SUBUNIT IS INSENSITIVE TO α-BUNGAROTOXIN AND IS HIGHLY SENSITIVE TO ACETYLCHOLINE. O. Asher[1], M. Lupu-Meiri[2], B. S. Jensen[1], T. Paperna[1], Y. Oron[2] and S. Fuchs[1]*. [1]Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel, and [2]Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel.

In studying the ligand binding site of the nicotinic acetylcholine receptor (AChR), we have cloned recently the mongoose AChR α-subunit. This subunit is highly homologous to the mouse and rat AChR α-subunit and has only six amino acid substitutions at positions that are conserved in animal species that bind α-bungarotoxin (α-BTX). Four of the six substitutions are present in the ligand binding region of AChR and one of these (Asn-187) is a putative N-glycosylation site. Indeed, the glycosylated form of the mongoose α-subunit has a higher molecular mass (46kD) than that of the glycosylated rat α-subunit (44kD). Expression of the mongoose α-subunit together with the rat β, γ & δ subunits in *Xenopus* oocytes, results in the assembly of functional AChR channels. The affinity of the expressed mongoose/rat hybrid AChR to α-BTX is three orders of magnitude lower than that of the rat AChR, whereas its affinity to ACh (EC_{50} 1.1 ±0.31μM) is higher than that of the rat AChR (EC_{50} 10.2 ± 2.13 μM). Thus, substitution of just a few amino acid residues in the mongoose AChR α-subunit appears to be sufficient for determining the resistance to α-BTX, and may also alter the sensitivity to ACh.

Supported by The Israel Academy of Sciences and Humanities, The Muscular Dystrophy Association of America (MDA) and The Association Francaise Contre les Myopathies (AFM).

EXCITATORY AMINO ACIDS: PHARMACOLOGY I

604.1

STRUCTURAL DETERMINATES OF AGONIST/ANTAGONIST ACTIVITY AT THE AMPA RECEPTOR. D.M. Weinstein*, G. Shams, D.M. Miller, R.A. Hill, N.J. Uretsky, and L.J. Wallace. College of Pharmacy, The Ohio State University, Columbus, OH 43210.

Glutamate, the most abundant excitatory neurotransmitter in the central nervous system, mediates excitatory activity in neurons through its activation of three families of receptors: N-methyl-D-aspartate, AMPA/kainate, and metabotropic. Our work is focused on agents that interact with the AMPA receptor, with the goal of finding potent agents that show selectivity for AMPA and for kainate mediated effects. From a variety of compounds examined using radioligand binding analysis and an in vitro bioassay, two series with interesting properties have been discovered. In one series, the addition of an N(1)-linked alanine residue to the parent quinoxaline-2,4-dione produced a potent, stereoselective AMPA receptor agonist (isomeric EC_{50} values = 3 uM vs. > 1000 uM). Placing a 6,7-di-methyl substitution on these molecules resulted in non-stereoselective AMPA antagonists (isomeric IC_{50} values = 144 uM vs 156 uM). This suggests differences in binding sites/orientation between agonists and antagonists. In the other series, (RS)-5-nitrowillardiine behaves as a potent agonist, while the compounds (RS)-3,5-dinitro-*m*-tyrosine and (RS)-2,4-dinitro-*o*-tyrosine behave as weak antagonists. These results suggest that differences in substituent patterns and/or electronic properties in the heterocyclic ring systems have a profound effect on receptor interactions. (NIH grants DA06776 and DA7722)

604.2

IN VITRO CHARACTERIZATION OF YM872: A SELECTIVE, POTENT AND HIGHLY WATER-SOLUBLE AMPA RECEPTOR ANTAGONIST. A. Kohara, M. Okada, K. Ohno, S. Sakamoto, J. Shishikura, H. Inami, M. Shimizu-Sasamata†*and T. Yamaguchi. Neuroscience and Gastrointestinal Res. Lab., Institute for Drug Discovery Res., Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan †Stroke Res. Lab., M.G.H., Harvard Medical School, Boston, MA, USA.

AMPA receptor antagonists are potentially useful as novel therapeutic agents for the treatment of cerebral ischemia. We have discovered a competitive AMPA receptor antagonist, YM872 ([2,3-dioxo-7-(1*H*-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxalinyl]-acetic acid monohydrate), that is a highly water-soluble agent. We have characterized the pharmacological properties of YM872 *in vitro*.

YM872 has a potent inhibitory effect on [^{3}H]-AMPA binding with the Ki value of 0.095 μM. In contrast, YM872 has very low affinity for other ionotropic glutamate receptors, using [^{3}H]-CGS19755 (NMDA receptor glutamate binding site), [^{3}H]-glycine (NMDA receptor glycine binding site) and [^{3}H]-kainate (high affinity kainate binding site) as a ligand (Ki = 100 μM, >100 μM and 2.2 μM, respectively). YM872 has an inhibitory effect on 300 μM kainate-induced currents with an IC_{50} value of 0.24 μM in *Xenopus Laevis Oocytes* expressing AMPA receptors by injecting rat brain poly(A)+RNA. In rat hippocampal primary cultures, YM872 blocked 300 μM kainate-induced neurotoxicity with an IC_{50} value of 1.1 μM.

These data indicate that YM872 is a potent and selective AMPA receptor antagonist, and YM872 may be useful in the treatment of cerebral ischemia.

604.3

GLUTAMATE RECEPTOR PROFILE OF THE AMPA/KAINATE RECEPTOR ANTAGONIST LY293558. D. Bleakman*, [1]B. Ballyk, [1]K. Hoo, [2]P. Ornstein, [1]R. Kamboj & [2]D.D. Schoepp. Eli Lilly and Co., Lilly Research Centre, Windlesham, Surrey, U.K., [1]Allelix Biopharmaceuticals Inc., Mississauga, Ontario, Canada and [2]Lilly Research Laboratories, Indianapolis, Indiana, U.S.A.

The activity of the competitive non-NMDA ionotropic glutamate receptor antagonist, LY293558 ((3*S*,4a*R*,6*R*,8a*R*)-6-[2-(1(2)H-tetrazole-5-yl)ethyl]decahydroisoquinoline-3-carboxylic acid), has been examined on HEK293 cells expressing cloned human homomeric and heteromeric AMPA and kainate (KA) receptors. Ligand binding studies using either ^{3}H-AMPA or ^{3}H-KA as ligands revealed that LY293558 was active at GluR1, GluR2, GluR4 and GluR 5 receptors, but did not compete for binding at GluR6 receptors (approximate Ki values: GluR2, 3.2μM; GluR5, 4.8μM; GluR1, 9.2μM; GluR4, 50.5μM and GluR6, >1mM). LY293558 also showed relatively weak activity at GluR7 and KA2 receptors. In support of the binding studies we have been able to demonstrate functional antagonism of agonist-induced currents for the cloned AMPA (GluR1,2 and 4) and KA receptor subtypes (GluR5) expressed in HEK293 cells using whole-cell voltage clamp electrophysiology. In addition, LY293558 showed no antagonism of agonist-induced currents for either homomeric GluR6 or heteromeric GluR6/KA2 KA receptors expressed in HEK293 cells. We have also compared the functional activity of LY293558 at human cloned AMPA/KA receptors to responses in native AMPA receptor and KA receptor-mediated responses in acutely isolated rat cerebellar Purkinje cells and rat dorsal root ganglion neurons, respectively. These studies illustrate the pharmacological profile of LY293558 at AMPA/KA receptor subtypes and the potential of LY293558 as a pharmacological tool for the discrimination of AMPA and KA receptors.

604.4

NON-PEPTIDE *CONUS* VENOM COMPONENTS: EFFECTS ON KAINATE/AMPA RECEPTORS AND OTHER CONDUCTANCES IN SQUID GIANT FIBER LOBE NEURONS. D.J. Steel*[1], D. Piper[2], J.P. Danaceau[2], S.S. Mitchell[3], C.M. Ireland[3], L.J. Cruz[1], B.M. Olivera[1], and M.T. Lucero.[2] Depts. of Biology[1], Physiology[2], and Medicinal Chemistry,[3] University of Utah, Salt Lake City, UT 84112

Cone snails are members of a class of predatory marine snails which immobilize their prey by injection of a potent venom. Study of these venoms has lead to the discovery of highly selective peptides for targets including Ca channels, Na channels, nACh receptors, and NMDA receptors. We examined the venom of *Conus marmoreus*, a mollusc-hunting cone snail, for novel substances which might affect neuronal activity in the nervous system of molluscs. RP-HPLC-purified fractions of 0.1% TFA-extracted venom were examined for effects on squid giant fiber lobe (GFL) neurons. Two separate activities were identified. Both elute under highly polar conditions, and subsequent analysis established that they were non-peptide, small organic molecules. We have identified one of these substances as betaine, a molecule known to be an osmoregulator in the molluscan nervous system. In squid GFL neurons, betaine activates a large conductance that washes out rapidly, suggesting that the response is second messenger-mediated. The second molecule has a distinctive UV absorbance maximum of 335 nm. Electrophysiological studies in squid GFL neurons indicate that this molecule activates an ionotropic kainate/AMPA receptor. Thus, *Conus* venoms contain a number of small molecules capable of selectively interacting with unique target sites. Supported by NIH NS09771 and DC02587.

604.5

IN-VITRO AND IN-VIVO PHARMACOLOGY OF SYM 2206 & SYM 2207: A NOVEL SELECTIVE NON-COMPETITIVE INHIBITOR OF THE AMPA RECEPTORS, David P. Hesson, Jeffrey C. Pelletier, Kenneth A. Jones*, Ana-Maria Costa. Symphony Pharmaceuticals, 76 Great Valley Parkway, Malvern, PA 19355

As part of our effort to find selective modulators of ionotropic glutamate receptors, we discovered a series of novel 1,2-dihydrophthalazines which selectively antagonize AMPA receptors. The lead compounds, SYM 2206 SYM 2207, are active in animal models which suggests it could be a useful antiepileptic and neuroprotectant. SYM 2206 and SYM 2207 antagonizes AMPA receptor mediated currents in recordings of rat cortical neurons (IC50 = 2.3 μM & 1.8 μM) but do not antagonize Kainate receptors (GluR6) expressed in HEK293 cells. Neither compound inhibits binding of [3H]-CGS 19755, [3H]-glycine, [3H]-AMPA, or [3H]-kainic acid. Therefore, the inhibitory action on AMPA receptors occurs at a site separate from the glutamate binding site. SYM 2206 is protective in the MES seizure model when tested in mice (ED50 = 30 mg/kg ip) and rats (ED50 = 39 mg/kg po). SYM 2207 protects all of the animals at 30 mg/kg ip. The therapeutic index for SYM 2206, measured by comparison of rat MES and rat rotorod, is approximately 2 while the therapeutic index of SYM 2207 is significantly greater than 2. Neither compound is effective in inhibiting subcutaneously injected metrazol in mice but also did not increase seizure activity in the intravenous metrazol model. SYM 2206 is not active in a rat hippocampal kindled rat model. Both compounds at 30 μM show 100% protection of rat hippocampal neurons in an in vitro model of ischemia. In additional receptor binding models SYM 2206 was shown not to bind to the GABA receptor when compared GYKI 52466. The anticonvulsant activity and safety profiles suggests that these compounds makes them candidates for further development.

604.6

PHARMACOLOGY OF ZK200775 AND ZK202000, COMPETITIVE NON-NMDA GLUTAMATE RECEPTOR ANTAGONISTS. L. Turski[1]*, A. Huth[1], M.J. Sheardown[2], P. Jacobsen[2], and E. Ottow[1]. [1]Research Laboratories of Schering AG, Müllerstr. 178, D-13342 Berlin, Germany and [2]Novo Nordisk AS, Novo Nordisk Park, DK-2760 Copenhagen, Denmark.

AMPA receptor antagonists have been shown to have marked neuroprotective effects in a variety of animal models of cerebral ischemia and trauma which indicates that this class of compounds may have clinical potential for the treatment of stroke or head trauma in humans. The major drawback of currently available AMPA/KA receptor antagonists is their poor solubility in water. Therefore we investigated pharmacological and neuroprotective properties of ZK200775 and ZK202000, water-soluble quinoxaline-phosphonate derivatives in rodents. ZK200775 and ZK202000 specifically inhibit ^{3}H-AMPA binding (IC50 0.15 μM and 0.44 μM) and have no affinity to non-glutamate receptors. The biochemical profile of both compounds is reflected in vitro by selective antagonism of non-NMDA receptor mediated spreading depression in chicken retina and quisqualate-induced depolarisation in the rat cortical slice preparation. In vivo pharmacology shows selective elevation of seizure threshold for non-NMDA receptor agonists. In models of neurodegeneration, ZK200775 and ZK202000 protect against neuronal cell loss induced by global ischemia in rodents, reduce the infarct volume in mouse models of focal ischemia, and mitigate traumatic brain damage in rats. The neuroprotective action of ZK200775 is seen in focal ischemia models when the drug is administered up to 4 h post occlusion. These data indicate that ZK200775 is a potent and selective AMPA/KA receptor antagonist and neuroprotective drug with a wide therapeutic time-window in animal models of neurodegeneration. ZK200775 may therefore prove useful in the therapy of stroke and head trauma.

604.7

PHARMACOLOGY OF NMDA RECEPTOR SUBTYPES. V.J. Andaloro[1], H.W. Tse[2], D.E. Jane[2], J.C. Watkins[2], and D.T. Monaghan*[1]. [1] Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198-6260 [2] Department of Pharmacology, University of Bristol, Bristol BS8 1TD, UK

Previously, we have shown that at least four pharmacologically distinct NMDA receptor subtypes exist (agonist- and antagonist-preferring, cerebellar, and midline thalamic receptors), and presently identify several structural features of competitive NMDA receptor antagonists that lend to receptor subtype selectivity. Using quantitative receptor autoradiography, we have examined the ability of over 75 compounds to compete with [^{3}H]-glutamate binding to NMDA receptors in horizontal rat brain sections. The compounds we have studied fall into six major structural classes: straight chain amino acid derivatives, piperidine carboxylates, piperazine carboxylates, decahydroisoquinolinates, quinolinates, and 5-phosphono-methyl-1,1'-biphenyl-3-alkylcarboxylates. We report here that compounds containing a straight-chain structure are the least subtype-selective inhibitors of [^{3}H]-glutamate binding to NMDA receptors. Piperazines and piperidines all tend to be more potent in antagonist preferring regions, while sparing receptors in agonist-preferring regions and cerebellum. Decahydroisoquinolinates and 5-phosphono-methyl-1,1'-biphenyl-3-alkylcarboxylates also display lower affinity for agonist-preferring and cerebellar receptors, but have a unique pharmacology of selective potency for midline-thalamus, a region known to be enriched in NMDAR-2D receptor subunit. Quinolinate derivatives spare both cerebellar and midline thalamic receptors. Extension of a 5 carbon bond-length compound to a 7 carbon homolog results in a significant and specific decrease in only cerebellar NMDA receptor affinity. Lastly, compounds containing a phosphonate or tetrazole moiety in the ω-acidic position are more selective for antagonist-preferring regions than the corresponding carboxylates. These data reveal several chemical structural features that confer selectivity between NMDA receptor subtypes. Supported by US-DOD contract DAMD 17-94-C-4050

604.8

CHRONIC ANTIPSYCHOTIC TREATMENT AFFECTS NMDA RECEPTOR GENE EXPRESSION. F. Tascedda*[a], E. Lovati[a], J.M.C. Blom[a], G. Racagni[a], and M.A. Riva[a,b]. [a]Center of Neuropharmacology, Univ. of Milan, 20133 Milan, Italy; [b]Di.Bi.T., San Raffaele Hospital, Milan, Italy.

The neurochemical mechanisms underlying the effects of antipsychotic drugs (APD's) are not well understood. The majority of these drugs share one common pharmacological property, namely the blockade of dopamine D-2 receptors, although many ADP's interact with other neurotransmitter receptors as well. More recently, based on anatomical evidence and functional interactions between dopamine and glutamate, new hypothesis on their mechanisms of action have been put forward. In particular, the cloning of different glutamate receptors has given the opportunity to investigate in more detail the regulation of this system. The N-methyl-D-aspartate (NMDA) receptor is a glutamate-gated cation-specific ion channel which plays a key role in many forms of neuronal plasticity. Using a sensitive RNase protection assay, the gene expression of two families of NMDA receptors (NR-1 and NR-2) was evaluated in different brain areas following 21 day ip treatment of young adult male rats (Sprague-Dawley) with typical (haloperidol 1mg/kg/day) and atypical (clozapine 30 mg/kg/day) neuroleptics. Haloperidol produced a significant increase in mRNA levels of NR-1 and NR-2 in striatum, whereas clozapine, consistent with its relative weaker influence on nigro-striatal dopamine function, did not affect the expression of NR subunits in this region. Furthermore, both ADP's decreased the expression of NR-2 subunit mRNA in the hypothalamus, but not in the hippocampus. The regulation of these neurotransmitter systems and of NMDA receptor subunits by ADP's in different brain structures may provide useful information to discriminate between therapeutical and side effects of these drugs, and may represent a novel and important mechanism through which ADP's affect the brain.

604.9

SPECIES DIFFERENCES IN VACUOLIZATION OF CORTICAL NEURONS AFTER ADMINISTRATION OF THE NMDA RECEPTOR ANTAGONIST DIZOCILPINE. Patrick Raboisson[a,b], Karin Flood[a], Anders Lehmann[c] and Odd-Geir Berge[a]*. [a]Dept. Pharmacol. Astra Pain Control AB, S-151 85 Södertälje, Sweden, [b]Lab. Physiol. Oro-Faciale, Faculté de Chirurgie Dentaire, 63000 Clermont-Ferrand, France and [c]Gastrointestinal Pharmacol., Astra Hässle AB, S-431 83 Mölndal, Sweden.

In rats, NMDA receptor antagonists may cause transient vacuole formation in neurons of the posterior cingulate and retrosplenial (PC/RS) cortex and, after higher doses, necrosis in the same regions. We have now investigated whether vacuolization occurs in the guinea pig.

Dizocilpine ((+)-MK-801) or saline were given subcutaneously to adult female Dunkin-Hartley guinea pigs (age 15-18 weeks; 1, 4 and 12 mg/kg) and female Sprague-Dawley rats (age 16 weeks; 1 mg/kg). After four hours, the animals were anesthetized and transcardially perfused. Brains were embedded in plastic, thin-sectioned, stained with toluidine blue and examined by light microscopy.

In the guinea pig, only occasional vacuolated neurons were identified in the PC/RC cortex after dizocilpine but additional affected neurons, mainly large pyramidal cells of layer V, where found in neocortical areas lateral to the anterior cingulate cortex. This reaction was evident at 1 mg/kg and reached a maximum at 4 mg/kg. Rats injected with 1 mg/kg dizocilpine showed an intense vaculolar reaction in layers III and IV of the PC/RS cortex but no affected neurons were noted in the neocortical areas. Vacuoles were not seen in any of the saline injected animals.

We conclude that there is a significant species difference in intensity and location of vacuolization induced by NMDA receptor antagonists.

604.10

POTENTIAL NMDA RECEPTOR LIGANDS: TWO NEW SLEEP-INDUCING PEPTIDES FROM *CONUS RADIATUS* VENOM. E. Jimenez[1], F. Abogadie[2], B. M. Olivera[2], J. Rivier[3] and L. J. Cruz.[1,2]* Marine Science Inst.[1], Univ. of the Philippines, Diliman, Quezon City, 1011, PI; Dept. of Biology[2], Univ. of Utah, Salt Lake City, UT, 84112; Clayton Foundation Laboratories for Peptide Biology[3], Salk Institute, La Jolla, CA, 92186.

Conantokin-G, the first peptide ligand known to inhibit NMDA receptors induced a sleep-like state in mice. To identify other potential NMDA receptor ligands, we examined the venom of *Conus radiatus* for fractions which induce sleep; two peptides were purified and characterized. The first, conantokin-R, is homologous to conantokin-G and contains the unusual amino acid γ-carboxyglutamate (Gla); however, conantokin-R is much longer (27 AA residues vs. 17 in conantokin-G) and has a disulfide bond. The other sleep-inducing peptide of *C. radiatus* venom exhibits no homology to the conantokins; this 33 AA peptide has 3 disulfide bonds and 4 Gla residues. Whereas conantokins induce sleep upon i.c. injection in young mice and hyperactivity in older mice, the novel *C. radiatus* peptide causes sleep in both age groups. The peptide is very potent in 9-day-old mice, causing sleep at 10-15 pmol/g body weight. (Supported by GM PO1 48677 and Philippine DOST-ESP Program (E. J.).)

604.11

FELBAMATE INHIBITION OF RECOMBINANT N-METHYL-D-ASPARTATE RECEPTORS EXPRESSED IN ***XENOPUS*** OOCYTES. J. C. Glazewski, C. C. Chen, T. D. Moscrip, and N. W. Kleckner*. Bates College, Lewiston, ME 04240.

The anticonvulsant effects of felbamate are thought to be mediated through an interaction with N-methyl-D-aspartate (NMDA) receptors. The site on NMDA receptors with which felbamate interacts is unknown, although both the glycine and channel sites have been suggested. The purpose of this study was to determine the site of action of felbamate, and its specificity of action against recombinant NMDA receptors comprised of different receptor subunits. NMDA receptors comprised of the NR1a subunit and either the NR2A or NR2C subunits were expressed in ***Xenopus laevis*** oocytes. The oocytes were voltage-clamped with two microelectrodes, and the currents measured in response to perfusion of NMDA and glycine in the absence and presence of felbamate. For both NR1a-NR2A and NR1a-NR2C receptors, the maximum current measured during glycine and NMDA dose-response curves in the presence of felbamate was reduced compared to the currents measured in the absence of felbamate. Furthermore, the EC_{50} values for glycine and NMDA were unchanged by felbamate. These data suggest that felbamate is not a competitive inhibitor of either site. Felbamate dose-response analysis showed felbamate to be slightly more potent for NR1a-NR2C receptors (IC_{50} ~ 2 mM) than for NR1a-NR2A receptors (IC_{50} ~ 8 mM). In addition, NR2A-containing receptors required the pre-perfusion of felbamate for effective (but highly variable) inhibition, suggesting that the access to felbamate on NMDA receptors containing NR2A subunits is limited compared to NR2C-containing receptors, and that the open channel may not be required for inhibition. The lower potency of NR2A-containing receptors is surprising, in that they have been postulated to be the antagonist-preferring receptors (Buller, et al. 1994). These findings may be useful for the design of new anticonvulsants with fewer side-effects than felbamate. (Supported by Grants to N.K., T.M. & C.C. from the Howard Hughes Medical Institute through Bates College)

604.12

GLYCINE DOES NOT REVERSE ETHANOL-INDUCED INHIBITION OF NMDA RECEPTORS IN CEREBELLAR GRANULE CELLS. S. Liljequist*, G. Cebers, A. Cebere and A. Zharkovsky. Dept of Clinical Neuroscience, Div Drug Dependence Research, Karolinska Institute, S-17176 Stockholm, Sweden.

The effects of ethanol and/or glycine on NMDA-induced enhancement of cytoplasmic free Ca^{2+} $[Ca^{2+}]_i$, $^{45}Ca^{2+}$ uptake, 4-β- [^{3}H]phorbol-12,13-dibutyrate ([^{3}H]PDBu) binding, and neuronal necrosis in cultured rat cortical and cerebellar granule neurons were examined. In microfluorimetric assays with rapid perfusion of single brain neurons, we found that ethanol (50 mM; in the presence of 10 μM glycine) inhibited the effects of NMDA in some, but not all, cortical and cerebellar granule neurons. In monolayers of cortical and cerebellar granule cells, glycine (10 μM) was a necessary prerequisite to unmask inhibitory actions of ethanol on $^{45}Ca^{2+}$ uptake induced by NMDA. Moreover, NMDA-induced stimulation of [^{3}H]PDBu binding to monolayers of intact cerebellar granule cells was concentration-dependently inhibited by ethanol (20-100 mM). Finally, ethanol caused a concentration-dependent inhibition of NMDA-induced necrotic cell death, assessed by measuring the ability of cerebellar granule cells to transform MTT into formazan. In none of these assays, the ethanol-induced inhibition of NMDA receptors was reversed by glycine (up to 100 μM). In contrast to earlier reports, our data suggest that ethanol and glycine produce their effects by acting at different regulatory sites within the NMDA receptor system in brain neurons.

604.13

AN ANTAGONIST AT THE STRYCHNINE-INSENSITIVE GLYCINE SITE DIFFERENTIALLY AFFECTS HYPERACTIVITY AND THE DISRUPTION OF PREPULSE INHIBITION (PPI) INDUCED BY PHENCYCLIDINE. Y. Furuya, T. Kagaya, Y. Nishizawa* and H. Ogura. Tsukuba Research Lab. Eisai Co., Ltd. Tsukuba, Ibaraki 300-26 Japan

The amplitude of the acoustic startle response is reduced by a preceding weak stimulation, which by itself does not elicit the startle response. This phenomenon has been named prepulse inhibition (PPI) and is thought to reflect the operation of the sensorimotor gating system, which is deficient in schizophrenic patients. It has been reported that (+)-HA-966, an antagonist at the strychnine-insensitive glycine site, antagonizes phencyclidine (PCP)-induced hyperactivity and has an atypical neuroleptic profile. We examined the effect of (+)-HA-966 on PCP-induced (3 mg/kg, s.c.) hyperactivity and disruption of prepulse inhibition in rats, and compared the effects with that of haloperidol, a typical neuroleptic. The antagonism of (+)-HA-966 on PCP-induced hyperactivity was ascertained. However, (+)-HA-966 did not antagonize the PCP-induced disruption of PPI, which is thought to be a model of refractory symptoms in schizophrenia. Haloperidol exhibited the same results as (+)-HA-966. Moreover, (+)-HA-966 slightly disrupted PPI at 30 mg/kg, s.c., and intracerebroventricularly administered 5,7-dichlorokynurenate, an antagonist at the strychnine-insensitive glycine site, disrupted PPI. These results indicate that (+)-HA-966 antagonizes only the dopamine-dependent effects (hyperactivity) but not dopamine-independent effects (disruption of PPI) of phencyclidine. Furthermore, it is apparent that an antagonist at the strychnine-insensitive glycine site disrupts PPI, although the disruptive effect is weaker than that of PCP.

604.14

BLOCKADE OF PHENCYCLIDINE- AND MK801-INDUCED REGIONAL zif268 mRNA EXPRESSION WITH D-CYCLOSERINE AND NE-100. X.-M. Gao*, L.-W. Chen and C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore MD 21228

Phencyclidine (PCP)-induced neurochemical changes in CNS may provide a useful paradigm for studying the neurochemistry of psychosis. We have already studied several pharmacologic features of PCP pharmacology in animals and reported time-dependent effects of PCP and MK801 on glucose utilization, NMDA-sensitive glutamate receptor density, its subunit mRNA and on the expression of zif268 and cfos mRNA in rat brain (Gao, et al., Neurosci. Abstr., 1995). In cortical areas, different doses of PCP and MK801 produced early induction (3h) and delayed suppression (24h) of the zif268 mRNA. The pharmacology of this immediate early gene could suggest neuronal mechanisms associated with PCP's other acute and delayed neuropharmacological effects. In the present study, we tested the ability of D-cycloserine, a partial agonist at the glycine site of the NMDA-sensitive glutamate receptor, and NE100, a sigma receptor antagonist, to block PCP- and MK801-induced zif268 mRNA expression at 3h and 24h in rat brain. We employed *in situ* hybridization and quantified the film densities autoradiographically. D-cycloserine (5 mg/kg) significantly attenuated the PCP (1 mg/kg) stimulated induction of zif268 mRNA at 3h in medial prefrontal (MPC) and dorsolateral frontal (DFC) cortices. In hippocampal areas (CA_1, CA_2, CA_3 and DG), D-cycloserine significantly increased the zif268 mRNA at 3h compared with either PCP or MK801 alone. NE100 (1 mg/kg) failed to block any of the PCP- or MK801-induced zif268 mRNA changes at 3 h. We will report the effects of D-cycloserine and NE100 on the actions of PCP/MK801 on zif268 mRNA at 24h. Research supported by NIMH (CRC MH40279).

604.15

SUBUNIT SPECIFIC EFFECTS OF D-CYCLOSERINE ON NMDA RECEPTORS EXPRESSED IN XENOPUS OOCYTES. A. J. O'Connor, G. Vlachogiannis, J. Moskal*, S. R. Kelso. Dept. of Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL 60607, and Chicago Inst. of Neurosurgery and Neuroresearch, Chic., IL 60614.

D-cycloserine (DCS) is a partial agonist at the glycine binding site of the NMDA receptor. Administration of DCS in both nonhuman and human subjects suggests it may act to enhance learning and memory in certain cognitive tasks. However, this enhancement is lost when DCS is administered chronically or at high doses. To better understand how DCS interacts with the NMDA receptor, we studied the effects of DCS on several heteromeric receptors. Using in vitro transcription, RNA was made from clones for the zeta1 and epsilon 1- 3 subtypes of murine NMDA receptors. The RNA was injected into Xenopus oocytes and recordings were made using standard voltage clamp techniques. Dose response curves indicate that the efficacy of DCS is different for the different subtypes. It is a partial agonist for z1/e1 and z1/e2 heteromers (with 38% and 56% the efficacy of 10 uM glycine). By conrtrast, for z1/e3, DCS was more effective than glycine (130% compared to 10 uM glycine).

These results may explain why DCS is effective at enhancing only certain cognitive tasks and may prove to be helpful in understanding the mechanism whereby chronic administration of DCS leads to impairment of cognition.

Supported by Neurotherapeutics and the UIC Epilepsy Center.

604.16

NOVEL SYSTEMICALLY ACTIVE ANTAGONISTS OF THE GLYCINE SITE OF NMDA RECEPTOR - BEHAVIOURAL CHARACTERISATION

W. Danysz*[1], C.G. Parsons[1], M. Karcz-Kubicha[1], M. Gold[1], I. Kalvinch[2], I. Piskunova[2] and E. Rozhkov[2], Dept. Pharmacol., Merz + Co, 60318 Frankfurt / Main, Germany[1], Institute of Organic Synthesis, 1006 Riga, Latvia[2].

Although a number of uncompetitive and competitive NMDA receptor antagonists are already used clinically or are at a late phase of development, less is known about the therapeutic potential of antagonists acting at the strychnine-insensitive glycine recognition site of the NMDA receptor ($glycine_B$). Suggested better therapeutic index of such compounds was mainly based on central administration of compounds and needs verification using systemically active agents.

Several $glycine_B$ antagonists - pyridazine quinoline derivatives (see Parsons et al. and Chizh et al. - this meeting) - have been tested in behavioural tests. They inhibited PTZ-, NMDA- and MES-induced convulsions in mice with ED_{50}s ranging from 8 to 20 mg/kg i.p. The duration of anticonvulsive action was rather short (30-40 min.) but was prolonged by the organic acid transport inhibitor probenecid (100 mg/kg). At doses within the anticonvulsive range, myorelaxation (traction test) and ataxia (rotarod test) were observed. In rats in the open field test the same $glycine_B$ antagonist produced sedation and attenuated the hyperlocomotion induced by both PCP and amphetamine. This antagonism was specific at moderate, non-ataxic doses as evidenced by a significant interaction factor in ANOVA. These data indicate a lack of psychotomimetic potential and possibly even antipsychotic activity of Merz $glycine_B$ antagonists. Merz $glycine_B$ antagonists also attenuated haloperidol-induced catalepsy but failed to produce reliable anxiolytic activity either in the elevated plus maze or the Vogel test. At higher doses all Merz $glycine_B$ antagonists tested induced anterograde amnesia in the passive avoidance test.

Hence, the agents tested represent a novel class of systemically active $glycine_B$ antagonists with potential antipsychotic and antiparkinsonian activity.

604.17

NOVEL ANTAGONISTS OF THE GLYCINE SITE OF THE NMDA RECEPTOR - ELECTROPHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISATION

C.G. Parsons*[1], W. Danysz[1], S. Hartmann[1], A. Bartmann[1], M. Gold[1], I. Kalvinch[2], I. Piskunova[2] and E. Rozhkov[2], Dept. Pharmacol., Merz + Co, 60318 Frankfurt / Main, Germany[1], Institute of Organic Synthesis, 1006 Riga, Latvia[2].

A series of novel pyridazine quinoline derivatives was tested for antagonistic effects at the strychnine-insensitive glycine modulatory site of the NMDA receptor complex ($glycine_B$). All compounds displaced [^{3}H]-5,7-dichlorokynurenic acid (10nM) and [^{3}H]-glycine (20nM) binding to rat cortical membranes with IC_{50}s of between 10nM and 1μM. Steady-state inward current responses of cultured superior colliculus neurones to NMDA (200μM with glycine 1μM) were antagonized by these same compounds with IC_{50}s of 0.2-2.0μM. The antagonism observed was typical for $glycine_B$ antagonists i.e. they induced desensitisation and their effects were not use- or voltage-dependent. Moreover, increasing concentrations of glycine were able to decrease their apparent potency e.g. Mrz 2/502 had IC_{50}s of 0.28, 1.06 and 3.08μM in the presence of glycine 1, 3 and 10μM respectively. Much higher concentrations (>100μM) were required to antagonise steady-state inward current responses to AMPA (100μM) and, in this case, the antagonism observed was reminiscent of a competitive interaction i.e. peak responses were more affected than steady state responses. These $glycine_B$ antagonists were also tested against NMDA (500μM) induced neurotoxicity in cultured cortical neurones and provided near complete protection at 5μM in the presence of 1μM glycine. These compounds are also NMDA receptor antagonists in the CNS following systemic administration and are active in a variety of *in vivo* behavioural models where glutamate is known to play a pivotal role (Chizh et al., and Danysz et al. this meeting). Hence, these pyridazine quinoline derivatives represent a novel class of systemically active $glycine_B$ antagonists and should prove to be useful tools to elucidate the therapeutic potential of this class of NMDA receptor antagonist.

604.18

AN *IN VIVO* STUDY OF THE POTENCY AND SELECTIVITY OF NOVEL SYSTEMICALLY ACTIVE ANTAGONISTS AT THE GLYCINE SITE OF THE NMDA RECEPTOR.

B.A. Chizh[1], M. McClean[1], C.G. Parsons[2] and P.M. Headley[1] [SPON: Brain Research Association*], Dept. Physiol., Univ. Bristol, Bristol BS8 1TD, U.K.[1] and Dept. Pharmacol., Merz+Co., 60318 Frankfurt/Main, Germany[2]

A new series of pyridazine quinoline derivatives has been tested for activity as antagonists of NMDA and of nociceptive responses in the rat spinal cord after i.v. administration in α-chloralose-anaesthetized spinalized rats *in vivo*. They are selective antagonists at the NMDA receptor glycine ($glycine_B$) site and are active as anticonvulsants in mice (Parsons et al. and Danysz et al., this meeting). Compounds were tested on responses of dorsal horn (DH) neurones to iontophoretic NMDA and AMPA. The most potent and selective NMDA antagonists were Mrz 2/502, Mrz 2/576 and Mrz 2/570 (ID_{50} ±s.e.m. 1.6±0.3, 2.8±0.7 and 4.5±0.7 mg/kg i.v., n=6-7, cf 1.3±0.3 mg/kg i.v. for ketamine, n=9). At the maximal doses tested, responses to NMDA were reduced by Mrz 2/502 (2-4, mean 3 mg/kg) to 17±6% control (AMPA 76±8%); by Mrz 2/576 (2-8, mean 4 mg/kg) to 30±7% control (AMPA 88±6%); by Mrz 2/570 (4-16, mean 9 mg/kg) to 29±4% control (AMPA 100±9%). On the same cells, ketamine (1-4, mean 3 mg/kg, n=17) reduced NMDA to 15±3% control (AMPA 91±4%). MRZ 2/502 and MRZ 2/576 were also tested i.v. on responses of hindlimb flexor muscle single motor units to noxious pinch of one foot. These responses had a strong NMDA receptor mediated component (ketamine ID_{50} for inhibiting nociceptive responses 0.9±0.2 mg/kg, n=11). The potency rank order and ID_{50} values were similar to those obtained on NMDA responses of DH neurones: Mrz 2/502 1.0±0.3 mg/kg (n=10), Mrz 2/576 3.3±0.4 mg/kg (n=5). In both kinds of tests the effects of all compounds were short-lived ($t_{1/2}$ 8-12 min). These compounds are therefore selective, and systemically active, antagonists of NMDA receptor mediated events.

Supported by Merz+Co.

604.19

IN VIVO SIGNIFICANCE OF THE DIFFERENT VOLTAGE DEPENDENCE OF UNCOMPETITIVE AND $GLYCINE_B$ NMDA ANTAGONISTS.

M. McClean[1], B.A. Chizh[1], M.W. Jones[1], C.G. Parsons[2] and P.M. Headley[1] [SPON: Brain Research Association]. Dept. Physiology, University of Bristol, BS8 1TD, UK[1] and Dept. Pharmacology, Merz + Co, 60318 Frankfurt/Main, Germany[2]

In isolated tissues, uncompetitive NMDA antagonists show voltage dependence, whereas NMDA receptor associated $glycine_B$ antagoinsts do not. The extent to which this property affects their actions on synaptic responses under *in vivo* conditions is not established. We have now examined two uncompetitive channel-blocking NMDA antagonists and two $glycine_B$ NMDA antagonists (Chizh et al., Parsons et al., this meeting). In α-chloralose anaesthetized spinalized rats, cumulative doses were tested on spinal mixed NMDA/non-NMDA receptor mediated responses to calibrated noxious pinch stimuli to the hindfoot (on motoneurones), and to iontophoretic NMDA (on dorsal horn neurones). The log of antagonist doses reducing responses to 50% control (mg/kg i.v.) was plotted against control response amplitude (mean spike discharge during pre-drug responses, taken as a measure of membrane depolarization) by linear regression. The rank order of these slopes (with Pearson product moment correlation values) was: memantine (r=0.73, n=12) > ketamine (r=0.58, n=20) > Mrz 2/502 (r=0.73, n=18) = Mrz 2/576 (r=0.95, n=8). Equivalent data for responses to iontophoretic NMDA were: memantine (r=0.86, n=12) = ketamine (r=0.68, n=17) >> Mrz 2/502 (r=0.28, n=8) = Mrz 2/576 (r=-0.21, n=9). This rank order matches the degree of voltage dependence determined *in vitro*. The results suggest that, as predicted, greater voltage dependence results in lower effectiveness on those synaptic responses generating higher levels of spike discharge. NMDA antagonists having greater voltage dependence are therefore less likely to affect strong synaptic signals than low level NMDA receptor mediated activity, and so should have less serious side effect profiles.

Supported by Merz + Co.

EXCITATORY AMINO ACIDS: PHARMACOLOGY II

605.1

PRETREATMENT WITH NMDA RECEPTOR ANTAGONIST MK801 IMPROVES NEUROPHYSIOLOGICAL OUTCOME AFTER AN ACUTE SPINAL CORD INJURY.

S.S. Haghighi*, G.C. Johnson, C.F. de Vergel, and B.J. Vergel Rivas. Dept. of Neurosurgery, MCPHU, Phila.,PA.

The posttraumatic release of excitatory amino acids (EAA) and their actions on N-methyl-D-aspartate (NMDA) receptors plays a major role in the spinal cord secondary injury process. The neuronal damage caused by the release of EAA may be reduced by NMDA-receptor channel blockers. To investigate the involvement of NMDA receptors in spinal cord injury (SCI), we pretreated animals with the non-competitive NMDA antagonist MK801 (1.0 mg/kg) before a compressive acute SCI. Pretreated animals with MK801 significantly (p=0.038) improved the recovery of function as measured by evoked potential activities. Morphologically specimens from rats treated with MK801 were characterized by milder and more localized hemorrhage in the gray matter. Immunohistochemical staining for glial fibrillary acidic protein (GFAP) and neurofilament (NF) histochemistry showed leakage of these antigens in traumatized cord while characteristic staining of astrocytes and neurons and their processes was observed in morphologically preserved tissue. The loss of NF immunoreactivity was reduced by MK801 treatment.

605.2

MK-801 CAUSES TERMINAL DEGENERATION IN WIDESPREAD AREAS OF RAT BRAIN.

S. Sparenborg*, R.C. Switzer III, J. Forster and M.G. Filbert. FDA, Rockville, MD, 20857, NeuroScience Associates, Knoxville, TN, 37922, and U.S. Army Med. Res. Inst. of Chemical Defense, Aberdeen Proving Ground, MD, 21010

Single sc injections of the NMDA antagonist MK-801 (0.1, 0.3, or 0.5 mg/kg) were given to female rats 6, 14, 24 or 96 hrs before sacrifice. Neuronal degeneration was evaluated in one brain hemisphere with the cupric-silver method. A single 1-micron-thick methacrylate section from the other hemisphere, containing the retrosplenial cortex, was stained with toluidine blue for visualization of neuronal vacuoles.

Rats sacrificed after 6 hrs had vacuoles in the cytoplasm of retrosplenial neurons (0, 0, 10, and 30 neurons w/vacuoles, respectively, in the control, low, mid and high dose groups). Vacuoles were not found in rats with longer survival times. After 6 hrs, synaptic terminal degeneration (TD), induced by MK-801, appeared in the superficial (I-IV) layers of dysgranular retrosplenial cortex (Rsd) and in its efferent cortical targets, including granular retrosplenial cortex (Rsg) , occipital and parietal areas processing vision and audition, and frontal (motor) association areas. In the hippocampus, TD was mild to severe in the molecular layer of the dentate gyrus and in stratum lacunosum moleculare of CA1. These effects were noted at all dose levels and generally increased in severity with longer survival times. Dead neurons were noted in the piriform cortex of some rats given the low dose of MK-801 and sacrificed 14 or 24 hrs later. However, silver staining was virtually absent from all brain areas of low-dose rats sacrificed after 96 hrs, indicating that degenerated terminals and soma had been cleared from the brain.

Many dead cell bodies were visible in Rsg and Rsd of mid- and high-dose rats sacrificed after 24 and 96 hrs, and were occasionally found in piriform and entorhinal cortices. TD represents the permanent loss of interneuronal communication through the affected synapses. Widespread TD and the loss of cells in the piriform cortex indicate that MK-801 produced a long-lasting toxic effect at a dose lower than that which produced vacuoles.

Supported by USAMRICD

605.3

CGS-19755 FULLY GENERALIZES TO THE DISCRIMINATIVE STIMULUS PROPERTIES OF PHENCYCLIDINE WHEN ADMINISTERED INTRAVENOUSLY IN RATS. M.J. Piesla and K.L. Marquis*, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

When studying glutamate antagonists, these drugs should be tested in animal models that predict a potential for psychotomimetic side effects. In the current study, phencyclidine (PCP) and CGS-19755 were compared following intraperitoneal or intravenous administration in male Sprague-Dawley rats trained to discriminate 1.25 mg/kg i.p. PCP from saline in a food-reinforced operant procedure. PCP fully generalized to the PCP discriminative stimulus when administered 15 minutes prior to the test session (ED_{50} = 0.79 mg/kg i.p. and 77% of the animals selected the drug appropriate lever at 0.54 mg/kg i.v.). CGS-19755 partially generalized when administered either 15 minutes (maximum effect = 45% of the animals selected the PCP lever; ED_{50} > 54 mg/kg i.p.) or 90 minutes (maximum effect = 64%; ED_{50} = 7.0 mg/kg i.p.) prior to the test session in i.p. dose response studies. CGS-19755 fully generalized when administered i.v. 90 minutes prior to the test session (maximum effect = 83%; ED_{50} = 11.1 mg/kg i.v.). CGS-19755 (30 mg/kg i.p.) fully generalized (> 70%) 60 and 120 minutes following i.p. administration. CGS-19755 partially generalized at other time points tested ranging from 45 minutes to 6 hours (i.p. and i.v.). These data indicate that the competitive NMDA antagonist CGS-19755 has the potential to produce PCP-like stimulus properties in some subjects at high doses. When conditions are optimized in animals by altering pretreatment time or route of administration, full generalization to the PCP cue can be observed. As PCP discriminative stimulus properties predict psychotomimetic potential in humans, these data indicate a risk for these side effects in some individuals following administration of CGS-19755. These results are consistent with recent reports of psychotomimetic side effects induced by CGS-19755 in clinical trials (Grotta *et al.*, 1995).

605.4

ABSENCE OF [^{3}H]MK-801 BINDING IN COS-7 CELLS TRANSIENTLY EXPRESSING THE NMDAR1 GLUTAMATE RECEPTOR SUBUNIT. J.E. Both*, P.H. Franklin, T.F. Murray and M. Leid, College of Pharmacy, Oregon State University, Corvallis, OR 97331.

To increase our understanding of the functional significance of recombinant homomeric NMDAR1 receptors, COS cells transiently expressing NR1-1a were tested for their ability to bind the noncompetitive NMDA antagonist [^{3}H]MK-801. Rat forebrain membranes and membranes derived from COS cells electroporated with either NR1 or pTL1 (empty vector) were incubated with increasing concentrations of [^{3}H]MK-801 for 4 hrs. at 22°C in the presence of glycine (10 μM) and glutamate (10 μM). A centrifugation assay was employed to separate bound from free radioligand. [^{3}H]MK-801 bound saturably and with high affinity (K_D = 2 nM, B_{max} = 3 pmol/mg protein) to rat forebrain membranes as detected using either 1 μM PCP or 10 μM MK-801 to define nonspecific binding. However, [^{3}H]MK-801 binding as defined by 1 μM PCP was not detected in membranes derived from COS cells transfected with NR1 even though these membranes specifically bound the glycine site antagonist [^{3}H]5,7-dichlorokynurenic acid and contained high levels of NR1 protein as determined by immunoblot and immunocytochemical analysis. Interestingly, the use of 10 μM unlabelled MK-801 to define nonspecific binding allowed detection of displaceable [^{3}H]MK-801 binding in membranes derived from COS cells transfected with NR1. [^{3}H]MK-801 labelled a population of binding sites with an apparent K_D of 54 nM and B_{max} of 11 pmol/mg protein. However, [^{3}H]MK-801 labelled a similar population of binding sites in control membranes transfected with the expression vector pTL1, suggesting that this [^{3}H]MK-801 binding is not dependent on NR1 expression and therefore not associated with occupancy of NMDAR1. These findings caution against the use of a self displacement strategy when using this radioligand.

Supported by DA00217 to T.F.M.

605.5

AMPA MODULATES NITRIC OXIDE RELEASE IN THE STRIATUM AS MEASURED BY BRAIN MICRODIALYSIS. K. Sakai[1], K. Kashihara[1]*, T. Tsuji[1], K. Akiyama[2], and T. Shohmori[1] 1)Department of Neurology 2)Department of Neuropsychiatry, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700

An effect of amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), a specific agonist of AMPA receptor, on the striatal nitric oxide release was investigated using in vivo microdialysis technique in the freely moving rat. Each dialysis probe was implanted into the left anterior dorsal striatum. The probe was perfused with artificial cerebrospinal fluid and perfusates were collected every 20 min.

The perfusion of the AMPA 1.0 mM increased concentration of extracellular nitric oxide oxidation products nitrite and nitrate. The perfusion of the AMPA 0.1 mM did not change the extracellular nitrite and nitrate levels.

The present results demonstrate that AMPA receptor modulates the release of nitric oxide in the striatum.

AMPA receptor may be involved in the release of nitric oxide in the central nervous system.

605.6

N-METHYL-D-ASPARTATE ADMINISTRATION IN RAT HIPPOCAMPUS AND STRIATUM INCREASES NITRITE AND NITRATE LEVELS: A MEASURE OF NITRIC OXIDE RELEASE. Edward Spangler*, Donald K. Ingram and Hideki Kametani. Gerontology Research Center, NIA, NIH, Baltimore, MD 21224, and Fukuoka Prefectural University, Fukuoka, JAPAN

Nitric oxide (NO) in the central nervous system has been linked to long-term potentiation, possibly the cellular basis for learning, and brain dysfunction including ischemia and aging. Stimulation of the N-methyl-D-asparate (NMDA) receptor results in release of NO, which serves as a retrograde messenger, that binds to cGMP to induce glutamate release. Wistar Kyoto rats 4-mo old were anesthetized, and a probe stereotaxically implanted unilaterally into striatum (STR:AP 0.7;ML 3.0;DV -4.5) and hippocampus (HC:AP-3.8;M/L2.O;D/V-2.2). Using an in vivo microdialysis procedure on the following two days, we first perfused HC and STR, counterbalancing side and day, with artificial cerebrospinal fluid (aCSF) for approximately 90 min and evaluated basal levels of oxidized byproducts of NO, nitrite (NO_2) and nitrate (NO_3), using an automated NO analyzer (Eicom; Kyoto, Japan). Then, 1 mM N-methyl-D-aspartic acid, a potent NMDA agonist, was added to aCSF and perfused for 20 min, and NO_2 and NO_3, evaluated in the same way followed by 1 hr of aCSF. This injection was followed by 5mM and 10mM NMDA using the same procedure. NO_2 and NO_3 levels were increased 20-50% above basal levels at each dose of NMDA in STR and HC. Increases above baseline levels were maintained for 20 min after NMDA administration at each dose. In each rat, we observed marked behavioral changes associated with NMDA stimulation including wet dog shakes, increased movement and grooming. In vivo levels of NO_2 and NO_3 thus appear to represent reliable measures of NO release in brain and may be determined using automated equipment, such as the NO analyzer.

605.7

THE EFFECTS OF DIRECT INFUSION OF NMDA INTO THE PREFRONTAL CORTEX ON NUCLEUS ACCUMBENS AND VENTRAL TEGMENTAL AREA DOPAMINE: AN IN VIVO MICRODIALYSIS STUDY USING SIMULTANEOUS SAMPLING. **J.J. Panos, K.J. Thompson, T.E. Egan, and R. E. Steinpreis*. Department of Psychology, The University of Wisconsin-Milwaukee, 53211.**

NMDA was infused directly into the prefrontal cortex and the effects on extracellular dopamine in the nucleus accumbens and the ventral tegmental area were measured using simultaneous microdialysis sampling. Male Sprague Dawley rats had indwelling unilateral cannula surgically implanted in the prefrontal cortex. Microdialysis probe guides were implanted in the nucleus accumbens and the ventral tegmental area. Three days later microdialysis samples were collected simultaneously from the nucleus accumbens and the ventral tegmental area for two hours pre-NMDA infusion into the prefrontal cortex and for six hours post injection. Dialysis samples were analyzed using HPLC. Infusion of NMDA resulted in elevations of extracellular dopamine in the nucleus accumbens and the ventral tegmental area. These elevations corresponded to increases in locomotor activity.

605.8

NMDA-STIMULATED NOREPINEPHRINE (NE) RELEASE IN SPINAL CORD: EFFECTS OF SCIATIC NERVE INJURY. D.W. Glasser and D.J. Jones*, Depts Pharmacol. and Anesth., Univ. of TX Hlth. Sci. Ctr., San Antonio, TX 78284.

The N-Methyl-D-Aspartate (NMDA) subtype of glutamate (GLU) receptor has previously been shown to be involved in transmission of sensory impulses to the spinal cord. Since NMDA also stimulates the release of norepinephrine (NE) from spinal cord slices, the purpose of the present studies was to determine if NMDA-stimulated NE release may play a role in the spinal integration of increased sensory input using a sciatic nerve cryoneurolysis (SNC) as a model of chronic pain.

Male Sprague-Dawley rats were anesthetized and the right sciatic nerve exposed and frozen with a nitrous oxide cryoprobe. Sham-operated animals served as controls. After appearance of pain-related behaviors (autotomy and tactile hyperalgesia), animals were sacrificed and spinal cords removed for [^{3}H]NE release experiments or frozen for [^{3}H]MK-801 binding assays. Fractional [^{3}H]NE release was measured from perfused regional spinal cord slices preloaded with [^{3}H]NE (0.05μM).

In the absence of Mg^{++}, NMDA increased NE release from spinal cord slices in a concentration-dependent manner. NMDA released more NE from dorsal spinal cord slices than ventral, whereas non-NMDA stimulation showed no difference between dorsal and ventral regions. In SNC animals, NMDA-stimulated release of NE from dorsal lumbar spinal cord was significantly reduced compared to sham animals, but was unchanged in other regions. K^+- or non-NMDA stimulated release of NE was likewise unchanged. [^{3}H]MK-801 binding was decreased in SNC animals but not controls. These findings suggest that interactions between spinal GLU and NE are altered when afferent input to the spinal cord is changed, possibly due to changes in the number or open state of NMDA receptors. Supported by S/IUCRC - CEBBI.

605.9

ASPARTATE AND GLUTAMATE RELEASE IN THE VENTRAL TEGMENTAL AREA INCREASES BIPHASICALLY FOLLOWING N-METHYL-D-ASPARTATE PERFUSION IN RAT PREFRONTAL CORTEX. A DUAL MICRODIALYSIS PROBE STUDY IN THE AWAKE RAT. K. Frantz*, U. Ungerstedt and W.T. O'Connor[1]. Department of Physiology and Pharmacology, Division of Pharmacology, Karolinska Institute, Stockholm S171-77, Sweden and [1]Department of Human Anatomy and Physiology, Neuroscience Division, University College, Dublin, Ireland.

The effect of local perfusion with the glutamate receptor agonist N-Methyl-D-Aspartate (NMDA) in the left medial prefrontal cortex (mPFC) on aspartate and glutamate release locally in the mPFC and in the ventral tegmental area (VTA) was investigated using the dual microdialysis probe approach in the awake freely moving rat. One probe was implanted in the mPFC and a second probe in the ipsilateral VTA. Perfusion with NMDA into the mPFC at 100, 300 and 600μM elicited concentration-related increases in local aspartate and glutamate release. There was a strong correlation in the NMDA-induced increases in aspartate and glutamate release. In the VTA, a biphasic change in aspartate and glutamate release occurred, such that the 300μM dose of NMDA was associated with the strongest increases. Following perfusion with the noncompetitive NMDA receptor antagonist, MK801 (10μM), the NMDA (300μM)-induced increase in aspartate and glutamate release was abolished in the mPFC and attenuated in the VTA .

The data suggest that NMDA receptors are located on aspartate- and glutamate-containing neurons in the mPFC. The results also demonstrate an excitatory mPFC projection to the VTA and indicate that this pathway displays a biphasic response to mPFC NMDA receptor stimulation *i.e.* maximal activation at the 300μM concentration of NMDA and reduced activation at lower and higher concentrations of NMDA. Thus, *in vivo* functional evidence is provided for a dynamic regulation of VTA transmission by cortical aspartate and glutamate.

Sponsored by Swedish Medical Research Council Grant B96-21X-11641-01A to WTO.

605.10

PENTOBARBITAL INHIBITS N-METHYL-D-ASPARTATE-EVOKED RELEASE OF DOPAMINE IN RAT STRIATUM. Megumi Sugahara, Shinji Shibanoki and Koichi Ishikawa*. Dept. of Pharmacol., Nihon Univ. Sch. of Med., Tokyo 173, Japan.

The effect of pentobarbital on N-methyl-D-aspartate (NMDA)-evoked dopamine release in the rat striatum was investigated by in vivo microdialysis combined with high-performance liquid chromatography and electrochemical detection. The basal output of dopamine was slightly decreased when pentobarbital (50 mg/kg) was injected intraperitoneally. Under pentobarbital anesthesia, the effect of continuous NMDA (1 mM), which causes an increase in extracellular dopamine concentration accompanied by decreases in its metabolite (3,4-dihydroxyphenylacetic acid and homovanillic acid) concentrations, was significantly inhibited. However, the effect of NMDA on dopamine was not inhibited by other intravenous anesthetics such as urethane (400 mg/kg, i.p.). The inhibitory effect of pentobarbital on the NMDA-evoked dopamine release was reduced by increasing the perfusate concentration of Ca^{2+}. On the other hand, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (non-NMDA agonist)-evoked release of dopamine was not inhibited by the treatment with pentobarbital. High K^+ (50 mM KCl)-evoked release of dopamine also remained under pentobarbital anesthesia. These results suggest that pentobarbital selectively inhibits the extracellular release of dopamine by activation of NMDA receptors, but not non-NMDA receptors in the striatum, and the inhibition of neurotransmitter release may, in part, contribute to the protective effect of pentobarbital on brain damage induced by ischemic brain injury.

605.11

INHIBITION OF GLUTAMATE TRANSPORTER ACTIVITY BY SUBSTANCE P. R. Ganel[1] and C. E. Crosson*[1,2], Departments of Pharmacology[1] and Ophthalmology and Visual Sciences[2], Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The termination of synaptic glutamate transmission and the prevention of excitotoxic damage depends on the fast removal of the glutamate by high-affinity transport systems. This study examines the effects of the neuropeptide substance P (SP) on a human glutamate transporter (EAAT2) expressed by Y-79 retinoblastoma cells. Retinoblastoma cell cultures were maintained in RPMI-1640 media containing 10% fetal calf serum. On the experiment day, cells were washed and then incubated in a modified Ringer's solution containing 2 μM tritiated L-glutamate (5 μCi) for 2, 4, 6 and 8 minutes. The uptake of the labeled glutamate was measured in control (untreated) cells and in cells pre-incubated for 10 minutes with 0.1 nM to 100 nM SP. Control uptake of 2 μM glutamate by retinoblastoma cells was found to be 1.3 ± 0.16 pmoles/min/million cells (n = 10). Following a 10-minute pre-incubation with 10^{-7} M SP, a 61% reduction in glutamate uptake was measured. Analysis of the dose-response curves revealed an IC_{50} of 22 nM for the SP-induced inhibition of glutamate uptake. Preincubation with the NK1 antagonist CP-96345 or the NK2 antagonist SR 48,968 did not alter the SP-induced inhibition of glutamate uptake. Analysis of glutamate uptake kinetics demonstrated that preincubation with 100 nM SP significantly reduced the Vmax for glutamate uptake from 2.5 ± 0.19 to 0.6 ± 0.13 pmoles/min/million cells. However, no significant change in Km was observed. This study demonstrates that the EAAT2 glutamate transporter expressed by human retinoblastoma cells is inhibited by the neuropeptide SP in a non-competitive manner. However, this response is not due to the activation of NK1 or NK2 receptors.

NIH (EY09741)

605.12

DEVELOPMENTALLY REGULATED RNA EDITING OF GLUR5 AND GLUR6 TRANSCRIPTS IN CEREBELLAR GRANULE CELLS. S.M. Belcher and J.R. Howe*. Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06520-8066.

RT-PCR analysis combined with patch-clamp techniques were used to compare the extent of RNA editing of the kainate receptor subunits GluR5 and GluR6 in developing rat cerebellar granule cells.

During the first two postnatal weeks the proportion of transcripts edited at the Q/R site increased significantly for both GluR5 and GluR6. GluR5(R) transcripts increased from 9% at postnatal day 1 (P1) to 13% ($P<0.01$) at postnatal day 15 (P15); the corresponding values for GluR6(R) were 61% and 86% ($P<0.01$), respectively.

The extent of editing at the I/V and C/Y sites in TM1 of GluR6 was also determined for P1, P7 and P15. The extent of editing was least at the I/V site. As for the Q/R site, the proportion of edited transcripts increased for both TM1 sites during the first two postnatal weeks.

Single-cell RT-PCR analysis showed that individual cerebellar granule cells express both Q and R forms of GluR5 and GluR6, although GluR5(Q) and GluR6(R) transcripts are most abundant. Northern analysis of RNA from acutely isolated granule cells showed that GluR5 and GluR6 mRNAs are expressed at similar levels .

The levels of each mRNA encoding the double-stranded RNA deaminases DRADA and RED1 were found to increase approximately two-fold between P1 and P15 indicating that either or both of these enzymes may contribute to the developmental increase in editing of GluR5 and GluR6. (Supported by NS 30996).

605.13

COMPARISON OF [^{3}H]-(S)-FLUOROWILLARDIINE AND [^{3}H]-(S)-AMPA BINDING IN RAT BRAIN MEMBRANES. Stephen A. Espitia, D. Scott Wieland* and Jon E. Hawkinson. CoCensys, Inc., 213 Technology Dr., Irvine, CA 92618

[^{3}H]-(S)-Fluorowillardiine binding in the presence and absence of the chaotopic agent KSCN was evaluated relative to [^{3}H]-(S)-AMPA binding in the presence of KSCN in rat brain membranes. AMPA receptor assays were conducted with 10 nM radioligand in a 96-well plate format and terminated by filtration. In contrast to [^{3}H]-(S)-AMPA (specific binding 80-90%, ~0.7 pmol/mg protein), [^{3}H]-(S)-fluorowillardiine displays acceptable specific binding in the absence of KSCN (specific binding 85-90%; ~0.7 pmol/mg protein). KSCN (50 mM) increases [^{3}H]-(S)-fluorowillardiine specific binding to 90-95% with ~2 pmol/mg protein. The rank order potency for displacement of radioligand binding by selected agonists and antagonists was similar with both ligands and conditions. However, quisqualate is less potent in the [^{3}H]-(S)-fluorowillardiine assay in the absence of KSCN, possibly because KSCN increases the affinity of [^{3}H]-(S)-fluorowillardiine to a greater extent than quisqualate. The antagonist ligands NBQX, DNQX and CNQX were consistently more potent in the absence than in the presence of KSCN in the [^{3}H]-(S)-fluorowillardiine assay, possibly due to a decrease in affinity of [^{3}H]-(S)-fluorowillardiine in the absence of KSCN. In conclusion, [^{3}H]-(S)-fluorowillardiine displays a similar pharmacological profile to [^{3}H]-(S)-AMPA, provides higher specific binding than [^{3}H]-(S)-AMPA, can be used in filtration assays in the absence of KSCN, and provides a useful tool to evaluate the effects of KSCN on the potency of AMPA receptor agonists and antagonists.

605.14

AGING AND GLUTAMATERGIC EFFECTS OF ANTIPSYCHOTIC DRUGS. E. Yablonsky-Alter*, T.I. Lidsky, S.P. Banerjee. Sophie Davis School of Biomedical Education, CUNY, NY.

The present study investigated the basis of antipsychotic drug (AP) effects as a function of aging. Previous work from this laboratory indicated that APs have unique action on NMDA receptors. At low, clinically relevant concentrations, APs facilitate activity at NMDA receptors while at higher concentrations activity is suppressed. In the current study, a comparison was made of the characteristics of NMDA receptors in mature (13 month old) and aging (26 month old) adult mice. Specific binding of [^{3}H]MK-801 in the presence of glutamate and glycine was significantly higher in older animals suggesting an increase in the functional activity of NMDA receptors. In contrast, affinity at the antagonist site was significantly decreased. This may suggest that a population of NMDA receptors with different characteristics emerges with aging In accord with this suggestion is the additional finding that the stimulatory effect of nanomolar concentrations of APs disappears with aging. The significance of these findings, particularly with respect to APs' increased risk of motor side effects in older patients, remains to be determined.

605.15

EFFECT OF ARGININE ADMINISTRATION ON PLASMA AND BRAIN LEVELS OF ARGININE AND VARIOUS AMINO COMPOUNDS IN RATS. I. Buchmann, L. Milakofsky, N. Harris, J. M. Hofford and W. H. Vogel*. Penn State Univ., Berks Campus, Reading, PA 19610 and Thomas Jefferson Univ., Dept. of Pharmacology, Phila., PA 19107.

Arginine (ARG) administration can cause many pharmacological effects in the periphery and CNS. Little information is available on the accumulation of ARG in rat brain and its effect on related amino compounds in this tissue after ARG administration. Thus, we studied the levels of ARG and related compounds via an HPLC-fluorometric method in rat plasma and 4 brain areas in the morning and afternoon in control rats and in rats injected with ARG, 0.8 g/kg,ip. In control rats, brain values for ARG and some amino compounds are lower in the afternoon than in the morning. After ARG injection, ARG levels increase about 10 fold in plasma and 2 to 3 fold in the brain areas. Brain ARG levels follow plasma levels. Elevated ARG levels affect a number of related amino compounds both in the plasma and all brain areas (i.e. ornithine, phosphoserine, glycine, GABA and ammonia). Increased citrulline after ARG injection suggests ARG stimulated nitric oxide formation in the midbrain. Thus, ARG shows a daily rhythm in the plasma and brain and its administration increases ARG brain levels. Also, ARG alters other amino compounds (GABA, glycine, ornithine and ammonia) indicating that some pharmacological effects seen after ARG injection might be caused by elevated levels of ARG and/or changes in other amino compounds. Supported by the Sanders Medical Research Foundation, Wilmington, DE.

EXCITATORY AMINO ACID RECEPTORS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION I

606.1

CHARACTERIZATION OF GLUTAMATE RECEPTOR AGONIST-INDUCED NITRIC OXIDE PRODUCTION IN THE RAT CEREBELLUM IN VIVO. K. Yamada* and T. Nabeshima. Dept. of Neuropsychopharmacology and Hospital Pharmacy, Nagoya Univ. Sch. of Medicine, Nagoya 466, Japan.

We examined the mechanisms of nitric oxide (NO) production in vivo by measuring levels of nitrite (NO_2^-) and nitrate (NO_3^-) in the dialysate of the cerebellum in awake rats, by using an in vivo brain microdialysis technique. The levels of both NO_2^- and NO_3^- were decreased by the i.p. injection of N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase, whereas D-NAME had no effect. L-Arginine by itself increased NO_2^- and NO_3^- levels, and diminished the reduction of their levels caused by L-NAME. Direct infusion of L-glutamate (L-Glu), NMDA, AMPA or trans-(±)-ACPD into the cerebellum through a dialysis probe resulted in a dose-dependent increase in NO_2^- and NO_3^- levels. The stimulatory effects of both L-Glu and NMDA were inhibited by (±)-CPP, an NMDA receptor antagonist, while the effect of trans-(±)-ACPD was antagonized by L(+)-AP-3, a metabotropic glutamate receptor antagonist. NMDA failed to increase NO_2^- and NO_3^- levels in rats which had been previously treated with L-NAME. On the other hand, AMPA and trans-(±)-ACPD caused an increase in NO_2^- and NO_3^- levels in the L-NAME-pretreated rats. These results suggest that NO_2^- and NO_3^- levels are related to NO production in vivo. Further it is suggested that although NMDA increases NO production through the activation of NO synthase, NO production caused by AMPA and trans-(±)-ACPD may be independent on NO synthase activity in the cerebellum in vivo.
Supported by the grant from the Ichiro Kanehara Foundation.

606.2

REGIONAL EFFECTS OF CHRONIC LEAD EXPOSURE ON MK-801 AND AMPA BINDING IN RAT BRAIN. Eric K. Richfield[1]*, L. M. McCoy[2] and D.A. Cory-Slechta[3]. Departments of Neurology[1], Psychiatry[2], and Neurobiology and Anatomy[3], University of Rochester School of Medicine & Dentistry, Rochester, NY 14642

Prefrontal cortical glutamate modulates subcortical dopamine (DA) function in striatum, in part through the NMDA receptor/ion channel. Insults at any link in this prefrontal cortex-nucleus accumbens-thalamo-cortical circuit can result in effects on the entire circuit. Lead (Pb) exposure disrupts DA systems, decreasing DA binding preferentially in ventral striatum after as little as 2 weeks of postweaning Pb exposure. The present study sought to determine whether such changes might be an indirect consequence of changes in NMDA or non-NMDA receptor binding. ^{3}H-MK-801 and ^{3}H-AMPA binding in medial frontal cortex and dorsal and ventral striatum were measured autoradiographically. The ^{3}H-MK-801 assay allowed the combined and individual assessment of glutamate, glycine and spermidine activation of the PCP site on the NMDA receptor/ion channel (McCoy and Richfield, 1995). Rats were treated with levels of 0, 50 or 150 ppm Pb acetate in drinking water for either 2 weeks or 8 mos. After 2 weeks of Pb exposure, AMPA binding in striatum, and glycine-activated MK-801 binding in ventral striatum were preferentially increased, particularly at 50 ppm, whereas decreases were seen in all regions at 150 ppm under condition of non- or maximally stimulated MK-801 binding. At 8 mos, notable Pb -related decreases in both AMPA and MK-801 sites were observed at both 50 and 150 ppm, with more pronounced decreases in striatal regions than in cortex. These results demonstrate that chronic Pb exposure impacts both NMDA and non-NMDA receptors. Comparison of Pb effect levels on glutamate and DA systems raises the possibility that DA system dysfunction may arise indirectly from initial Pb alterations in glutamate systems since effects of 50 ppm Pb on DA were generally not evident. *ES05017, ES05903, ES01247, MH18911, MH40381 and the Tourette Syndrome Association, Inc.*

606.3

GLUTAMATE MODULATES RELEASE OF SOMATOSTATIN FROM CULTURED HIPPOCAMPAL NEURONS THROUGH THE ACTIVATION OF NMDA AND AMPA RECEPTORS. G. Fontana, L. Valenti, A. Gemignani, M. Marchi* and M. Raiteri. Inst. of Pharmacology and Pharmacognosy, University of Genoa, Italy

L-Glutamate, NMDA, D,L-α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate (KA) increased the release of somatostatin-like immunoreactivity (SRIF-LI) from primary cultures of rat hippocampal neurons. In Mg^{2+}-containing medium, the maximal effects (reached at about 100 μM) amounted to 737% (KA), 722% (glutamate), 488% (NMDA) and 374% (AMPA); the apparent affinities were 22 μM (AMPA); 39 μM (glutamate); 41 μM (KA) and 70 μM (NMDA). The metabotropic receptor agonist trans-1-aminocyclopentane-1,3-dicarboxylate did not affect SRIF-LI release. The release evoked by glutamate (100 μM) was abolished by 10 μM dizocilpine (MK-801) plus 30 μM 1-aminophenyl-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466). Moreover, the maximal effect of glutamate was mimicked by a mixture of NMDA + AMPA. The release elicited by NMDA was sensitive to MK-801 but insensitive to GYKI 52466. The AMPA- and KA-evoked releases were blocked by 6,7-dinitroquinoxaline-2,3-dione (DNQX) or by GYKI 52466 but were insensitive to MK-801. The release of SRIF-LI elicited by all four agonists was Ca^{2+}-dependent while only the NMDA-evoked release was prevented by tetrodotoxin. Removal of Mg^{2+} caused increase of basal SRIF-LI release, an effect abolished by MK-801. Thus glutamate can stimulate somatostatin release through ionotropic NMDA and AMPA/KA receptors. Receptors of the kainate type (AMPA-insensitive) or metabotropic receptors seem not to be involved. Supported by grants from the Italian MURST and CNR.

606.4

REGULATION OF ACETYLCHOLINE AND GABA RELEASE BY GLUTAMATERGIC AND DOPAMINERGIC AGONISTS IN RAT STRIATUM. **T. Hanania and K. M. Johnson*.** *Dept. of Pharmacol. and Toxicol., Univ. of Texas Med Branch, Galveston,TX 77555-1031*

Phencyclidine (PCP) is thought to act by blocking NMDA receptors and monoamine transporters. PCP-induced alterations in rat motor behavior is critically dependent on descending glutamatergic input as well as ascending dopaminergic input to the striatum. In this study, radioactivity released from striatal slices loaded with [^{14}C]GABA and [^{3}H]choline were used as markers of GABA and acetylcholine (ACh) release in order to understand how dopaminergic and glutamatergic receptors interact to regulate striatal function. NMDA increased ACh and GABA release in a dose-dependent and TTX sensitive manner. The duration of the release of ACh by NMDA was much greater than that of GABA, suggesting that different NMDA subunits mediate these responses as previously suggested by others. NMDA-induced GABA release is also PCP and nipecotic acid-sensitive. The D1 agonists SKF82958 and SKF38393 potentiated NMDA-induced GABA release. The D1 antagonist SCH23390 and the protein kinase A inhibitor, H-89, blocked this potentiation. Experiments with D1 agonists on ACh release gave inconclusive results. The D2 agonist quinpirole inhibited NMDA-stimulated GABA and ACh release, and this effect was reversed by the D2 antagonist sulpiride. The mGluR agonist 1S,3R-ACPD potentiated NMDA-stimulated GABA release, but had no effect on ACh release. The D1 agonist SKF82958 together with 1S,3R-ACPD, did not produce an additive effect on GABA release, suggesting the possibility that both agonists may act through G_s-mediated increases in cAMP. These data help to provide a better framework for understanding the mechanisms by which PCP alters striatal functions. Supported by DA 02073.

606.5

THE ACUTE SYSTEMIC ADMINISTRATION OF MK-801 INCREASES LOCOMOTOR ACTIVITY AND DOPAMINE METABOLISM IN RAT NUCLEUS ACCUMBENS. D. Jolly*, J. Brose and P. Vezina. Department of Psychiatry, University of Chicago, Chicago, IL 60637.

The present experiment assessed the effects of acute systemic injections of the noncompetitive N-methyl-D-aspartate receptor antagonist, MK-801, on locomotor activity and extracellular levels of dopamine (DA) and its metabolites, DOPAC and HVA, in the nucleus accumbens (NAcc). Each dose of MK-801 tested (0, 0.05, 0.10, 0.30 and 0.50 mg/kg, i.p.) was administered to rats in separate groups. Measurement of NAcc DA and its metabolites was accomplished with *in vivo* microdialysis in chambers designed to simultaneously measure locomotion. The DA, DOPAC and HVA recovered in dialysates during twelve 20 minute samples (three baseline and nine post-injection) were quantitated by HPLC-EC. MK-801 produced dose-dependent increases in locomotor activity that were observed throughout the three hours of testing following injection. While consistent elevations in extracellular DA were not observed, levels of DOPAC and HVA showed dose-dependent increases, with the highest levels attained after the 0.30 and 0.50 mg/kg doses of MK-801. These locomotor and neurochemical results suggest that MK-801 activates DA neurons projecting to the NAcc and are consistent with electrophysiological data reported by others indicating increased firing of A10 DA cells after the systemic administration of this noncompetitive receptor antagonist.

This work was supported by a Brain Research Foundation Grant to P.V.

606.6

REGULATION OF DOPAMINE RELEASE BY N-METHYL-D-ASPARTATE IN THE STRIATUM OF RATS WITH PARTIAL DOPAMINE-NIGROSTRIATAL LESION. M.E. Andrés, K.Gysling and G. Bustos*. Lab. Biochemical-Pharmacology, Dept. of Cell and Molecular Biology, Faculty of Biological Sciences. Catholic University of Chile, Santiago, CHILE.

The role of N-methyl-D-aspartate (NMDA) in the regulation of striatal dopamine (DA) release, was studied with *in vivo* microdialysis, in rats with partial lesions of the DA-nigrostriatal system. Dopaminergic nigrostriatal input to the right striatum was partially lesioned with 6-hydroxydopamine (2-8 µg/ 4µl) injections. Seven days later a microdialysis probe was implanted in the right striatum and after a stabilization period, two stimuli period were performed, the first with 500 µM NMDA and the second with 40 mM K^+.

In sham-treated rats, DA release induced by NMDA was higher than DA released by K^+. In rats with 20-80% of striatal DA depletion, DA release induced by NMDA was significantly diminished, whereas DA released by K^+ was maintained. In rats with 80-95% of DA depletion, both stimuli induced a significant minor DA release, and the ratio between both stimuli was reverted. In contrast, rats with >95% of striatal DA depletion, exhibited a relatively high fractional DA release evoked by NMDA.

These results suggest that partial destruction of nigrostriatal pathway in rats reduce the stimulatory regulation induced by NMDA on DA release. They also suggest that extreme lesions may produce a compensatory response that accounts for the fractional DA release induced by NMDA observed under this condition. (Supported by grants FONDECYT 2950009 to M.E.A., 1930555 and 1960329 to G.B).

606.7

INTERACTIONS OF THE GLYCINE (NMDA) ANTAGONIST, MDL 103,371 WITH MONOAMINERGIC SYSTEMS: EVIDENCE FOR ANTIPSYCHOTIC-LIKE ACTIVITY. C.J. Schmidt, S. F. Chaney, G.M. Fadayel, T.C. McCloskey, Y. Senyah, V.L. Taylor, and J.H. Kehne* Hoechst Marion Roussel, Inc., 2110 E. Galbraith Rd., Cincinnati, OH 45215.

The clinical utility of antagonists of the NMDA-glutamate receptor for neurodegenerative states such as ischemic stroke or trauma has been severely limited by their psychotogenic activity. Results in models of psychotomimetic potential suggest that blockade of the NMDA-associated glycine site may offer a means of reducing NMDA receptor mediated damage without the side-effect liability observed for competitive and uncompetitive agents. MDL 103,371, *E*-3-[2-(3-aminophenyl)-2-carboxyethenyl]-4,6-dichloro-1*H*-indol-2-carboxylic acid, is a centrally active, highly selective glycine site antagonist with efficacy in several animal models of ischemic brain damage. Pharmacological studies of MDL 103,371 compared its effects on the dopaminergic (DA) system to those of other psychotomimetic agents such as MK-801 or the amphetamine analogue, MDMA. Unlike these agents, MDL 103,371 did not disrupt prepulse inhibition nor did it enhance *in vivo* DA synthesis in rats. Paradoxically, MDL 103,371 antagonized the stimulation of DA synthesis and release produced by MK-801. This functional anti-dopaminergic activity was also observed behaviorally in that MDL 103,371 significantly reduced amphetamine stimulated locomotion in mice. These results suggest that glycine site antagonists may provide a means of reducing NMDA receptor function without incurring significant psychotomimetic risk. In fact, the pharmacological profile of MDL 103,371 is in many ways predictive of antipsychotic activity.

606.8

CYCLOTHIAZIDE ENHANCEMENT OF NMDA- VS NON-NMDA-INDUCED RELEASE OF [^{3}H]NE FROM SPINAL CORD SLICES: RECEPTOR DESENSITIZATION. D. Jones, G. Woodlee, R. Suchdev, M. Albin,* D. Glasser, Dept. Anesth, Univ TX Hlth Sci Ctr, San Antonio, TX 78284

Previous studies have demonstrated rapid desensitization with activation of AMPA and kainic acid subtypes of the glutamate receptor. Cyclothiazide (CYCLO) and concanavalin A (CON A) have been demonstrated to modulate this desensitization in various regions of the CNS. Since desensitization plays a role in the overall efficacy of glutamate responses, the purpose of the present study was to determine the contribution of this process to [^{3}H]NE release from spinal cord slices induced by agonists at subtypes of the ionotropic glutamate receptor.

Rat spinal cord slices (dorsal region; 0.3 µm) were preloaded with [^{3}H]NE (0.05 µM). Slices were perfused (0.5 ml/min) and agonist-induced release expressed as % of total remaining [^{3}H]. CYCLO and CON A were added to the perfusion 10 min before agonists were added.

4-AP, K^+, NMDA, AMPA and kainic acid all produced concentration-dependent release of [^{3}H]NE from spinal cord slices. Only the NMDA response was Mg^{++}-dependent. Both AMPA- and kainate-induced release of [^{3}H]NE was enhanced by CYCLO in a concentration-dependent manner. Neither NMDA- nor 4-AP-induced release was enhanced by CYCLO. In the presence of DNQX (10 µM) which completely blocked kainate-induced release, CYCLO was still able to enhance release in a concentration-dependent manner. In contrast to the effects of CYCLO, the lectin CON A did not enhance either AMPA- or kainate-induced release.

The results demonstrate that glutamate-receptor subtypes modulate [^{3}H]NE release in spinal cord. Moreover desensitization of the receptor in the presence of agonists may play a role in modulating glutamate signal strength in this region. Supported by the S/IUCRC - CEBBI Fund.

606.9

COX-2 AND FOS PROTEINS IN BRAIN AFTER STIMULATION OR INHIBITION. D.J. Knapp*, G.E. Duncan, G.R. Breese, and F.T. Crews. Ctr for Alcohol Studies, Univ. North Carolina, Chapel Hill, NC 27599

The protein product (FOS) of the immediate early gene c-fos is induced in a regionally-specific manner in the rat brain following stress, acute or chronic drug treatments, or withdrawal from chronic ethanol treatment. Presented here are results of studies to investigate the brain regional distribution of FOS and cyclooxygenase-2 (COX-2) proteins following excitatory amino acid stimulation or blockade, ethanol administration, or during ethanol withdrawal from liquid diet. Kainic acid (15 mg/kg, i.p.) or NMDA (150 mg/kg, i.p.) injection increased COX-2 and FOS in the medial prefrontal cortex (MPC) and related limbic cortical areas, but only FOS was robustly induced in the paraventricular nucleus of the hypothalamus (PVN) and central nucleus of the amygdala (CE). In the hippocampal CA1 area, kainic acid failed to induce COX-2, but intensely induced FOS, whereas both proteins were robustly induced in the dentate gyrus. Acute ethanol (2.5 g/kg, i.p.) induced FOS in the PVN, CE, and MPC, but inhibited NMDA-induced FOS in the MPC and CE. Withdrawal from chronic ethanol treatment induced a specific FOS response in brain regions thought to be important in mediating emotional responses, including the amygdala, limbic cortical regions, and PVN. Interestingly, the glutamate antagonist ketamine produced a dose-dependent FOS response in many cortical and subcortical regions of brain, even at anesthetic doses. These results indicate that 1) FOS and COX-2 are useful markers for isolating specific brain regions following drug treatments, 2) that measures of one immediate early gene may reveal regionally specific neuronal activation not revealed by another or that qualitatively different stimuli may specifically target a particular gene product in some brain regions. (Supported by NIAAA 00214, 10025, and 06069)

606.10

PATCH CLAMP AND CSD ANALYSIS OF CHOLINERGIC THETA FREQUENCY OSCILLATIONS IN NEOCORTICAL SLICES

H. S. Lukatch* and M. B. MacIver. Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

Neocortical brain slices produce theta frequency micro-EEG oscillations when exposed to carbachol (100 µM), a cholinergic agonist, and bicuculline (10 µM), a $GABA_A$ antagonist. (Lukatch and MacIver Soc. Neurosci 1994). The present study examined phase, amplitude and current source density (CSD) profiles associated with these oscillations. Cellular mechanisms underlying oscillatory activity were further elucidated with laminar transection studies and whole cell patch clamp experiments. Peak oscillation amplitudes occurred in layer 2/3, and phase reversals were observed in layers 1 and 5. Current source density analysis revealed large amplitude current sinks and sources in layers 2/3 and 5, respectively. Laminar transections localized oscillation generating circuitry to superficial cortical layers. Whole cell recordings identified three distinct cell types based on their response properties during rhythmical micro-EEG activity: oscillation-on and oscillation-off neurons, and glial cells. Oscillation-on neurons displayed theta frequency membrane potential oscillations which increased in amplitude with hyperpolarization (from -30 to -90 mV). Sustained currents associated with oscillations reversed polarity near 0 mV under voltage clamp. These results, together with previous findings that glutamate antagonists depress oscillatory micro-EEG activity, suggest that excitatory synaptic transmission in superficial cortical layers underlies cholinergically-driven synchronized neocortical oscillations.

Supported in part by USAF OSR/SCEEE and NIH GM49811.

606.11

MUSCARINIC RECEPTOR ANTAGONISTS AFFECT MEMANTINE AND PHENCYCLIDINE INDUCTION OF HEAT SHOCK PROTEIN HSP72 IN THE POSTERIOR CINGULATE CORTEX OF RAT BRAIN. S.TOMITAKA*, K. Hashimoto, N. Narita, Y. Minabe and A. Tamura. Natl. Inst. Neurosci., NCNP, Tokyo and Dept. of Psychiatry, Tokyo Women's Medical College, Tokyo, Japan.

Non-competitive NMDA receptor antagonists such as memantine (1-amino-3,5-dimethyladamantane) and phencyclidine induce heat shock protein HSP-72, a useful marker of neuronal damage, in the posterior cingulate cortex. We studied the role of muscarinic receptors on the induction of HSP-72 by memantine and phencyclidine. Memantine (25-50mg/kg) and phencyclidine (40mg/kg) were injected into male SD rats. The immunohistochemistry of HSP-72 was studied 24 hr after the treatment. The administration of memantine and phencyclidine induced HSP-72 in the posterior cingulate and retrosplenial cortex of rat brain. Pretreatment with the muscarinic receptor antagonists, scopolamie (0.1-5mg/kg) and atropine (1-5mg/kg), blocked induction of HSP-72 in the layer III of the posterior cingulate cortex. However, induction of HSP-72 in the layer V was not blocked by the muscarinic receptor antagonists. These results suggest a regional difference in the mechanism of HSP72 induction by NMDA receptor antagonists in the posterir cingulate cortex. This research was supported by the research grant from the Ministry of Health and Welfare, Japan.

606.12

MODULATION OF SENSORY AND EXCITATORY AMINO ACID (EAA) RESPONSES BY NITRIC OXIDE (NO) DONORS AND GLUTATHIONE IN THE RAT VENTROBASAL (VB) THALAMUS. P.J. Shaw and T.E. Salt*. Institute of Ophthalmology, University College London, Bath Street, London. EC1V 9EL. U.K.

The precursor of NO, L-arginine, potentiates somatosensory responses and responses to EAAs in rat VB. To further study the effects of the NO system in VB, somatosensory responses and responses to iontophoretically applied agonists were recorded extracellularly from single VB neurones in urethane anaesthetised rats. Reproducible controls were tested during iontophoretic coapplication of the NO-donors S-nitrosoglutathione (GSNO) and 3-morpholinosydnonimine (SIN1).

GSNO significantly ($p<0.05$, Wilcoxon Signed Rank; n=12) potentiated mean responses (±SEM) to air-jet stimulation of a vibrissa (10ms duration) to 124±7% of control. The response to a 1sec stimulus was increased to 126±8%; responses to NMDA and AMPA were increased to 123±12% and 123±10%. GSNO had no effect on the response to carbachol. SIN1 applied to a different sample of 6 VB neurones also potentiated responses ($p<0.05$): 10ms air-jet to 203±26% of control; 1sec to 138±15%; NMDA to 166±26%; AMPA to 176±33%.

Reduced glutathione (GSH) significantly ($p<0.05$; n=6) inhibited responses to a 10ms stimulus to 77±5% of control and responses to NMDA and AMPA to 51±7% and 48±11% respectively. Oxidised glutathione (GSSG) inhibited NMDA responses to 70±9% of control ($p<0.05$; n=10).

The results support the suggestion that NO can positively modulate sensory transmission through VB via a postsynaptic action on relay neurones, and that L-arginine acts as a precursor for this system. Furthermore, the NO system is not fully activated under normal conditions *in vivo* but could be activated under conditions of prolonged stimulation. In contrast, the effects of glutathione suggest a modulatory role distinct from that of NO.

Support: Medical Research Council & the Human Frontiers Science Program.

606.13

PROLONGED RESIDENCE TIME OF GLUTAMATE IN THE SYNAPTIC CLEFT DETERMINES EXCITATORY SYNAPTIC TRANSMISSION AT A CENTRAL SYNAPSE

S. Titz, S.W. Schwarzacher# and B.U. Keller*. Zentrum Physiologie, Humboldtallee 23 und #Zentrum Anatomie, Kreuzbergring, Universität Göttingen, 37073 Göttingen, Germany

Interneurones in the *nucleus tractus solitarii* (NTS) in the orain stem of rats were investigated with the patch clamp technique by using the thin (150um) slice preparation. Neurones were identified histologically as local interneurones by intracellular labelling with neurobiotin. Glutamate (AMPA/KA) receptor mediated synaptic transmission was investigated during stimulation of single presynaptic afferents. Unitary EPSCs displayed fast rise times and slow decay time constants (3.6±1.1ms) that did not result from dendritic filtering. Bath application of 1mM D - aspartate did not affect EPSCs, indicating that local glutamate uptake did not significantly shape their time course. Also, slow EPSC decays did not result from slow deactivation of postsynaptic AMPA receptors (0.7±0.1ms) which were investigated by rapid (<0.2ms) application of agonist to outside - out patches. Recordings in high and low extracellular calcium concentrations suggested that slow EPSC decays resulted from a prolonged residence time of glutamate in the synaptic cleft.

Supported by DFG and SFB 406.

606.14

REGULATION OF POSTSYNAPTIC PROPERTIES OF AVIAN AUDITORY NEURONS *IN VITRO* J.J. Lawrence* and L.O. Trussell. Neuroscience Training Program & Dept. of Neurophysiology, U. Wisconsin, Madison 53706.

Nucleus magnocellularis (NM) consists of largely adendritic neurons which respond to long depolarizing current stimuli with a single action potential and express fast kinetic AMPA receptors. We explored the role of innervation and cellular environment by culturing E17-18 NM neurons on flat astrocytes at low density. Most NM neurons became multipolar within a few days, developing neurites which strikingly distorted the morphology of the cell body. This neuronal morphology showed similarities to that of E10-11 NM neurons, suggesting that the cells may dedifferentiate *in vitro*. In current clamp experiments, the neurons developed the capacity to fire multiple action potentials with long depolarizing current steps. Noise analysis of whole-cell current evoked by 30 μM glutamate in the presence of 200 μM APV allowed us to to determine mean channel burst time (τ) of AMPA receptors. Noise spectra generally fit best with sums of two Lorenztian functions; our results are therefore summarized as a weighted mean time constant from the fitted curves. Acutely dissociated NM neurons from E17-18 embryos showed a mean τ of 405±212 μsec (±SD, N=5). After two weeks in vitro, the mean τ was 1905±1025 μsec (N=7). These data suggest that voltage-dependent channels and transmitter receptors that are critical to auditory function *in vivo* may be dynamically regulated by the cellular environment, possibly through innervation, soluble factors, and/or glial cell contacts. Supported by NIH DC02004.

606.15

CHRONIC ANDROGEN TREATMENT EFFECTS ON EVOKED POTENTIALS IN THE CA1 REGION OF THE HIPPOCAMPUS. W. A. Pouliot*, R. J. Handa, S. G. Beck. Departments of Cell Biology, Neurobiology & Anatomy and Pharmacology, Loyola University Medical Center, Maywood, IL 60153.

Stimulation of afferent input to CA1 neurons of the hippocampus results in the synaptic activation of the glutamate receptor subtypes, N-methyl-D-aspartate (NMDA) and non-NMDA. Area CA1 also contains a high density of androgen receptor. We examined the effects of chronic androgen treatment on synaptically evoked field potentials in the CA1 region. The treatment groups included gonadectomized (GDX) male Sprague-Dawley rats and GDX rats implanted with the non-aromatizable androgen, 5-α-dihydrotestosterone-propionate (GDX+DHTP). Hippocampal slices were prepared for electrophysiology after 3 weeks. Extracellular field potentials were recorded in the stratum pyramidale following stimulation of the Schaffer collateral pathway. Stimulus- response (S-R) curves were generated. Initial studies indicate that chronic androgen treatment shifted the S-R curve to the right and decreased the maximum amplitude of the population spike. The NMDA receptor-mediated field potential was pharmacologically isolated. Superfusion with Mg^{2+}-free buffer containing 10μM CNQX led to the development of multiple population spikes which were abolished by 40μM AP-5, an NMDA receptor antagonist. The NMDA isolated population spike amplitude recorded from GDX treated animals was decreased when compared to population spikes generated in normal buffer. In GDX+DHTP treated animals the S-R curve for the NMDA isolated population spike was shifted to the right while the maximal spike amplitude was the same magnitude as the spikes generated in normal buffer. These preliminary data indicate that androgen treatment altered normal synaptic transmission from the Schaffer collateral pathway to CA1 neurons and NMDA receptor-mediated evoked potentials.

Supported by USPHS DA05615, NS28512, MH00880, NSF IBN9408890.

606.16

PAIRED-PULSE FACILITATION (PPF) IN AN INTERNAL CAPSULSE-LATERAL AMYGDALA PATHWAY. M.G. McKernan* and P.Shinnick-Gallagher. *Dept. of Pharmacology and Toxicology, Univ. of Texas Med. Branch, Galveston, TX 77555-1031*

The amygdala is widely believed to play a role in the attachment of emotional significance to experience and memory. The medial geniculate nucleus of the thalamus projects directly to the lateral amygdala nucleus (LA) via fibers traveling through the internal capsule; this connection is crucial for fear conditioning to an auditory stimulus, a model of emotional learning.

In the present study, the patch clamp technique is utilized for whole-cell recordings of neurons in the LA. Excitatory post-synaptic currents (EPSC's) evoked by stimulation of fibers arising from the internal capsule are predominantly glutamatergic, with an N-methyl-D-aspartate (NMDA) component and an α-amino-3-hydroxy-5-methyl-isoxazole propionic acid (AMPA) component; afferent stimulation also elicited a gamma-amino-butyric-acid (GABA) component in a subset of cells.

The goal of this study is to investigate alterations in this pathway induced by fear conditioning. One means of measuring potentiation at this synapse is to evaluate paired-pulse facilitation (PPF); changes in the degree of PPF are believed to reflect presynaptically-mediated synaptic alterations. EPSCs elicited with internal capsule stimulation showed both paired-pulse facilitation and depression. At an interstimulus interval (i.s.i.) of 35 ms, facilitation is 36.4 ± 18.8% (n=5); at an i.s.i. of 250 ms, depression is 15.9 ± 8.8% (n=5). In the presence of 50 μM D-APV, which blocks the NMDA component of the EPSC, control neurons fall into two groups with respect to PPF. In group I neurons, both facilitation and depression can be recorded, with 37.5 ± 5.5 % facilitation (n=9) at 35 ms i.s.i. and 9.0 ± 3.4% depression (n=7) at 250 ms i.s.i. In group II neurons, primarily depression can be recorded, with only 12.5 ± 6.9% facilitation (n=4) at 35 ms i.s.i. and 19.1 ± 4.0% depression (n=4) at 250 ms i.s.i.

Supported by John Sealy Mem. Endowment Fund for Biomed. Res., NIMH 1 F30 MH11221-01 and PhRMA Med. Stud. Res. Fellowship in Pharm. Clinical Pharm.

606.17

MEMANTINE, A CLINICALLY-TOLERATED NMDA OPEN-CHANNEL BLOCKER, DISPLAYS RELATIVE SPARING OF EPSCs. Posina V. Rayudu, H.-S. Vincent Chen*, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Memantine, a drug used clinically in Europe for spasticity and Parkinsonism, is an uncompetitive NMDA antagonist (Bormann, 1989; Chen et al., 1992) and ameliorates focal ischemia and other forms of NMDA receptor-mediated neurotoxicity in vitro and in animal models (Seif el Nasr, et al. 1990; Erdö and Schäfer, 1991; Chen et al., 1992). Recently, the basis for the clinical safety of memantine has in part been attributed to its sparing of LTP (Stieg et al., 1993; Frankiewicz et al., 1996). Here we show that in addition to the fact that memantine inhibition is relieved to some extent by depolarization during tetanic induction of LTP, evoked excitatory postsynaptic currents (EPSCs) are relatively spared in the presence of memantine (6 μM, a clinically-achievable concentration). EPSCs were evoked at 0.2 Hz during whole-cell recording with patch electrodes from autapses of solitary hippocampal neurons grown in "micro-culture" (Segal, 1991). In this preparation, memantine has excellent access to synaptic sites. Even at -60 mV which, compared to more depolarized holding potentials, should maximize voltage-dependent blockade by memantine of NMDA channels (Chen et al., 1992), only about 40% of the late (NMDA receptor-mediated) component of the EPSC was blocked. The relative sparing of EPSCs is probably due to their transient nature, producing a high concentration of glutamate in the synaptic cleft for a short time, which is functionally equivalent to low grade activation in the constant presence of agonist.

Funded by NIH grants R01 EY05477 and P01 HD29587 (to S.A.L.).

606.18

EFFECTS OF IONTOPHORETICALLY APPLIED AMINO ACIDS ON SPONTANEOUS AND EVOKED ACTIVITY OF PRIMARY AUDITORY CORTEX NEURONS IN THE CAT: AN IN VIVO STUDY. P.L. Herrling*, C.L. Meier and T.Krucker. SANDOZ Research Institute, CH-3001 Bern, SWITZERLAND.

The present study was performed to elucidate the possible role of excitatory (EAA) and inhibitory (e.g. GABA) amino acids in neurotransmission within the primary auditory cortex (AI). The main afferent to the AI, the ipsilateral medial geniculate body (MGB), was electrically stimulated and evoked responses were recorded intra- and extracellularly in the AI region in 104 halothane-anesthetized cats. A seven-barelled iontophoresis pipette glued alongside the recording electrode allowed localized application of compounds onto the recorded neuron. Iontophoretically applied NMDA (63 of 65 cells) and AMPA (43 of 54 cells) depolarized AI neurons and evoked marked increases in firing rate. NMDA evoked depolarizations were accompanied by increases in action potential frequency and the occurrence of depolarizing shifts carrying bursts of action potentials. AMPA evoked a regular firing pattern and transformed a spontaneous bursty firing pattern into regular firing (3 cells). Coapplication of the $GABA_A$ antagonists SR95531 or bicuculline usually potentiated the effect of iontophoretically applied NMDA and AMPA (31 cells). Sustained GABA application decreased spontaneous activity in five neurons. Competitive EAA antagonists AP7 (30 cells) and CNQX (4 cells) or DNQX (2 cells) were found to substantially reduce or abolish the responses to iontophoretically applied NMDA and AMPA pulses, respectively. AP7 decreased spontaneous firing frequency significantly at ejection currents which had little or no effect on AMPA responses. The early excitatory responses to MGB stimulation were not affected by AP7 (13 of 13 cells) but inhibited by CNQX (6 of 6 cells, 50-85% inhibition) in absence of any additional experimental manipulation. The $GABA_A$ antagonist SR95531 strongly potentiated MGB-EPSPs and the augmented part was entirely abolished by AP7 (27 of 31 cells). We conclude that both NMDA and AMPA receptors can be involved with the MGB to AI synaptic transmission and that NMDA receptors also contribute to the spontaneous activity of the cortical region.

Supported by SANDOZ Research Institute Bern and SANDOZ Pharma Basel

606.19

METABOTROPIC RECEPTOR AGONIST MODIFIES AMPA/KAINATE AND NMDA MEDIATED POTENTIALS IN NEOCORTEX. A.E. Bandrowski*, V.B. Aramakis, and J.H. Ashe. Departments of Neuroscience and Psychology, University of California, Riverside, CA 92521.

The purpose of this study was to examine a possible role of metabotropic glutamate receptors in cortical auditory processing. We examined the effect of (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (ACPD), a glutamate metabotropic receptor agonist, on AMPA/Kainate (A/K) mediated early EPSP (e-EPSP), NMDA mediated late EPSP (l-EPSP), and NMDA mediated membrane depolarizations (NMDA-DPs). Responses were obtained from layer II/III pyramidal neurons in the *in vitro* rat auditory cortex using whole cell recording techniques. ACPD decreased the amplitude of both the e- and l-EPSPs. The e-EPSP was decreased (44/46 cells) in a concentration dependent manner (10-75 μM; 7±1% to 63±3%). The l-EPSP was studied in the absence of the e-EPSP following the addition of an A/K antagonist, CNQX 10-20 μM. The l-EPSP was also decreased by ACPD (8/8 cells) in a concentration dependent manner (25-50 μM; 30±12% to 49±18%), and both effects were reversible (e-EPSP n=39; l-EPSP n=5). Effects of ACPD on the synaptic potentials may involve mechanisms located pre-, post-synaptically, or both, therefore we examined the effect of ACPD on NMDA-DPs. Subthreshold, NMDA-DPs were obtained by iontophoretic application of NMDA (50 mM; 1-37 nA; 5sec). These responses were increased in amplitude by ACPD (98±5%; 7/7 cells), and maintained for over 15 min. These results suggest that metabotropic receptors may have a function in auditory neocortex, both the e-EPSP and l-EPSP can be modified by a metabotropic receptor agonist, and the mechanisms of the effects may be differentially distributed in local cortical networks. Supported by NSF (IBN 9310582).

606.20

NEURONAL CELL LINES FROM MOUSE HIPPOPCAMPUS EXPRESSING NEUROTRANSMITTER RECEPTORS AND NEURONAL-TYPE ION CHANNELS. M.Jung[1]*, E.Krämer[1], T.Müller[2], C.Flocke[2], H.Antonicek[2], and J.Trotter[1]. [1] Dept. of Neurobiology, University of Heidelberg, INF 364, D-69120 Heidelberg, F.R.G. [2] Bayer AG, ZF-F, Biotechnologie, Bayerwerk, Leverkusen, F.R.G.

In many neurological disorders neurotransmitter receptors may be involved in the final pathways leading to neuronal cell death. For the development of and initial screening of agents interfering with these destructive pathways, the availability of immortalised neural cell lines expressing receptors and channels of interest would be very advantageous. Cell lines were established from primary embryonic mouse hippocampus cultures using retroviral vectors expressing the v-myc oncogene or a hybrid protein between the EGF receptor and neu tyrosine kinase (egfr-neu). In the absence of EGF the kinase activity is down regulated permitting differentiation. Stable cell lines were generated consisting of either flat and/or bipolar cells. All cell lines expressed vimentin and nestin while some of the lines contained some cells positive for A2B5, LB1, 04, GFAP, MAP-2 and neurofilament. EGF-deprived egfr-neu-expressing cell lines developed highly elaborate branched processes and many cells expressed the neuronal markers MAP-2 and neurofilament. Using Ca^{++} imaging procedures, intracellular Ca^{++} concentrations were measured after application of several agonists and antagonists for ionotropic and metabotropic glutamate receptors. Most cell lines express metabotropic glutamate receptors as shown by application of glutamate, quisqualate, t-APCD and L-CCG. Using the patch-clamp technique, AMPA, NMDA, glutamate agonists and GABA evoked inward currents mainly in the differentiated egfr-neu-expressing cells. Many cells showed a delayed and an inward rectifier. Differentiated egfr-neu-expressing cells often displayed a sodium current, enabling the generation of action potentials under current clamp conditions. These lines are thus useful tools for investigating neuronal properties.

Financed by Bayer AG.

EXCITATORY AMINO ACID RECEPTORS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION II

607.1

D1 RECEPTOR MODULATION OF AMPA/KAINATE CURRENTS IN RAT STRIATAL NEURONS. C.J. Price* and L.A. Raymond. Division of Neurosciences, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

Dopamine receptor activation regulates intracellular cAMP levels and is critically involved in modulating neurotransmission in the striatum. Previous studies have shown that ion channel function of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)- and kainate-type glutamate receptors is potentiated by activation of cAMP-dependent protein kinase. Therefore, we investigated whether D1 receptor activation had any effect on AMPA/kainate receptor currents in cultured rat striatal neurons. Perforated patch clamp recordings from medium spiny neurons showed that the D1 receptor agonist SKF 38393 (5 μM) potentiated currents evoked by rapid application of 1 mM kainate by approximately 24%. Moreover, this action could be mimicked by direct stimulation of adenylate cyclase using forskolin (50 μM). The results of this study indicate that D1 receptor-mediated enhancement of AMPA/kainate-type glutamate receptors may, in part, contribute to dopamine's modulatory effects on striatal neuronal pathways.

Supported by the Parkinson Foundation of Canada, Medical Research Council (LAR) of Canada, and the Natural Sciences and Engineering Research Council (CJP).

607.2

NITRIC OXIDE AND cGMP MODULATE RETINAL AMPA-TYPE GLUTAMATE RECEPTORS. L.V.Ponomareva and D.G.McMahon* Department of Physiology, University of Kentucky, Lexington KY 40356-0084

Recent studies have shown that ionotropic glutamate receptors are targets for modulatory second messenger signals affecting their function (Hollman and Heinemann,1994). We have investigated the nitric oxide (NO)/cGMP pathway for modulation of retinal horizontal cell (HC) AMPA-type glutamate receptors. HCs from the hybrid bass were dissociated in cell culture using standard techniques and whole cell patch-clamp recordings were made while glutamate(200μM) or its agonist kainate-KA(10 or 50μM) were applied by pressure ejection. Modulatory agents were applied by adding to the bath. Responses to glutamate and KA were reduced by approximately 50% in the presence of NO donors 1mM sodium nitroprusside(SNP) (N=9) and 100μM S-nitroso-N-acetylpenicillamine(SNAP) (N=7).The effect of SNP was blocked by the NO scavenger hemoglobin and it could be mimicked by the application of a membrane permeable cGMP analog, 8-Br-cGMP(-63%,N=4). The NO effect was also blocked by application of the guanylate cyclase inhibitor LY-83583(N=7),and by a protein kinase G inhibitor peptide RKRARKE(N=6). In addition we found that the nitric oxide synthase stimulator L-arginine reduced KA responses in H1 type cells,while application of D-arginine had no effect. The NO receptor modulation mechanism may act in concert with other pre and post synaptic mechanisms to modify HC synaptic function according to the adaptational state of the retina and may also protect HCs from glutamate excitotoxicity. Molecular cloning of a GluR1 homolog from the bass retina performed in our laboratory indicates the presence of serine-kinase consensus sites on the C-terminal and high homology in the predicted membrane-spanning regions.The bass GluR1 predicts a 964 amino acid sequence with molecular weight of 107kDa and shows 77.8% identity to the rat GluR1. Supported by NEI EY 09256 and NS 01734.

607.3

EFFECTS OF TYPICAL AND ATYPICL ANTIPSYCHOTICS ON NON-NMDA SITE BINDING AND GENE EXPRESSION. L.McCoy*, E.K. Richfield. Depts of Psychiatry and Neurology, University of Rochester, Rochester, NY 14642-8409.

Results from human postmortem studies have suggested that binding to non-NMDA receptors is not affected by chronic treatment with antipsychotic drugs (APDs). However, more detailed animal studies on the effects of APDs are needed, since binding to non-NMDA receptors may be altered in some patients with schizophrenia. The heterogeneous anatomical distribution of ^{3}H-CNQX binding sites correlate with ^{3}H-AMPA binding to non-NMDA receptors, but binding to high and low affinity states differ between the two ligands. To determine if chronic APDs alter non-NMDA receptror site binding and receptor gene expression, we measured ^{3}H-AMPA, ^{3}H-CNQX and GluR1 and 2 (flip/flop) following chronic (21 day, i.p.) treatment with two atypical APDs, clozapine (CLZ) and risperidone (RSP) and two typical APDs, haloperidol (HAL) and pimozide (PMZ). Binding and mRNA expression densities were measured in six cortical and subcortical brain regions. With ^{3}H-AMPA , APDs as a group were different from saline treated animals and both typical APDs and atypical APDs were different from saline. The typical agents HAL and PMZ resulted in the largest increases in binding (58 and 65%, respectively) compared to the atypical agents CLZ and RSP (10 and 25%, respectively). The overall ANOVA for ^{3}H-CNQX binding showed no difference between drug treated and saline treated groups. These findings demonstrate an APD effect on the high affinity state of the AMPA receptor and that AMPA site binding may be a useful biochemical marker differentiating typical and atypical agents. The effect on AMPA receptor subunit expression and a synthesis of the data will be presented. *Supported by MH18911; MH40381 and the Tourette Syndrome Association, Inc.*

607.4

PROTON MODULATION OF AMPA-PREFERRING GLUTAMATE RECEPTORS. E. C. Ihle* and D. K. Patneau. Committee on Neurobiology, University of Chicago, Chicago, IL 60637.

Although NMDA receptors are more sensitive to changes in extracellular pH than AMPA receptors, the potential neuroprotective effects of acidification that occurs during ischemia may involve inhibition of both glutamate receptor subtypes (Giffard et al., 1990). We have examined proton modulation of AMPA-preferring glutamate receptors, to determine both the mechanism whereby protons exert their effects and the extent to which this modulation may affect neurotoxicity.

Proton modulation of native AMPA receptors was studied using fast perfusion techniques during whole-cell voltage-clamp recording of hippocampal neurons dissociated from newborn rats and maintained in primary culture. Steady-state AMPA receptor currents were unaffected by alkalinization (change from pH 7.3 to 7.8), but were significantly reduced at acidic pHs typically associated with ischemia (pH 6.5 - 6.8). This effect was dependent on agonist. At pH 6.5 currents evoked by the weakly-desensitizing agonist kainate were reduced by 15%, while those evoked by glutamate were 30% smaller than at physiological pH (7.3), suggesting that protons have a greater effect on more strongly desensitizing responses. Similarly, the effect of pH 6.5 on glutamate-evoked responses in the presence of cyclothiazide and other modulators that block desensitization were reduced by $\leq$ 5% relative to control.

Receptor subunit composition also appears to influence sensitivity to proton modulation. Attenuation of kainate-evoked responses in Type I neurons was voltage-independent, whereas inhibition of kainate-evoked currents in Type II neurons was stronger at hyperpolarized potentials.

Funded by University of Chicago, Biological Sciences Division.

607.5

AMPA/KAINATE RECEPTORS INVOLVEMENT IN CONVULSANT AND PROCONVULSANT PROPERTIES OF MITOCHONDRIAL TOXIN, 3-NITROPROPIONIC ACID IN MICE. P. Blaszczak[1], T. Saran[1], W.A. Turski[1,2], E.M. Urbanska*[1,2]. [1]Dept. Pharmacology and Toxicology, Medical University School, Lublin, [2]Dept. Clinical Toxicology, Institute of Agricultural Medicine, Lublin, Poland.

3-Nitropropionic acid (3-NPA) is a naturally occuring enzyme inhibitor. It inactivates succinate dehydrogenase of the complex II of mitochondrial electron transport chain. The clinical course of acute intoxication with 3-NPA in humans includes convulsions, sometimes leading to status epilepticus. Chronic poisoning with 3-NPA may produce neurodegenerative changes in the brain. This study investigates convulsant and proconvulsant activity of 3-NPA in mice. Albino-Swiss mice (20-25 g) were injected s.c. with 3-NPA. 3-NPA produced clonic convulsions and death of animals with an ED50 of 158 mg/kg and a LD50 of 189 mg/kg, respectively. The competitive AMPA/kainate antagonist NBQX (s.c.) blocked convulsions induced by 3-NPA (220 mg/kg) with an ED50 of 14.1 mg/kg. The competitive NMDA antagonist, D-CPPene (i.p.) and non-competitive NMDA antagonist, MK-801 were not effective at doses up to 20 and 0.4 mg/kg, respectively. 3-NPA (100 mg/kg) potentiated the convulsant properties of AMPA and KA (i.c.v.), which was reflected by lowering the ED50 doses of AMPA from 0.98 to 0.55 nmol and KA from 0.73 to 0.58 nmol, but not those of NMDA. These results may indicate that non-NMDA receptors could contribute to convulsant and proconvulsant properties of 3-NPA in mice.

Supported by Grant No. 22/96 from Medical Univ. School, Lublin, Poland.

607.6

AUTORADIOGRAPHIC LOCALIZATION AND CHARACTERIZATION OF NON- NMDA BINDING SITES IN ADULT QUAIL BRAIN. M. Martinez de la Torre, A. Mitsacos*, J. Balthazart, K. Zavitsanou and E.D. Kouvelas. Dept. Physiology, Med. Sch., Univ. Patras, Greece, Dept. Morphological Sci., Univ. Murcia, Murcia, Spain, and Lab. Biochemistry, Univ. Liege, Belgium.

The distribution of non-NMDA binding sites was studied in coronal and sagittal sections in the brain of adult Japanese quail by quantitative autoradiography, using tritiated 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), as a radioligand. Saturation binding experiments were, in addition, performed in areas showing high levels of binding and demonstrated that the binding of [^{3}H]CNQX is saturable and of high affinity. Competition studies with AMPA and kainic acid indicated that kainic acid strongly inhibits [^{3}H]CNQX binding in all brain areas, whereas AMPA is only a weak inhibitor. The highest [^{3}H]CNQX binding levels were observed in the molecular layer of cerebellum. Very high levels of binding were detected in various preoptic/hypothalamic sites, including nucleus preopticus paraventricularis, nucleus suprachiasmaticus pars medialis, nucleus infudibularis and nucleus mammillaris medialis. High levels of binding were also detected in bulbus olfactorius, nucleus striae terminalis, nucleus raphes, nucleus accumbens and nucleus commissurae pallii. In the preoptic area/hypothalamus, high levels of binding were clearly present in all areas that contain GnRH (LHRH) cells or fibers. Moderate binding was also present in nucleus tractus solitarius and nucleus motorius dorsalis, as well as, in dorsal thalamus, substantia grisea centralis and nucleus intercollicularis. Moderate levels of binding were associated with catecholaminergic areas, such as substantia nigra, area ventralis of Tsai and locus coeruleus. These anatomical data suggest a possible implication of [^{3}H]CNQX binding sites in the synthesis and/or release of both GnRH and catecholaminergic neurotransmitters that should be tested by pharmacological experiments. Supported by EU grant HCM/CHRX-CT94-0472.

607.7

EFFECTS OF A MEMORY ENHANCING DRUG ON AMPA RECEPTOR CURRENTS AND SYNAPTIC TRANSMISSION IN HIPPOCAMPUS. A. Arai*, M. Kessler, G. Rogers and G. Lynch. Center for the Neurobiology of Learning and Memory (CNLM), University of California, Irvine, CA 92717.

The benzoylpiperidine drug BDP-12 enhances the encoding of transient and stable forms of memory by rats. Results reported here show that the drug increases fast, excitatory (glutamatergic) synaptic responses in hippocampal slices by about 50% with an EC_{50} of 170 μM. Analyses using polysynaptic responses indicated that the drug has a facilitatory action at concentrations a low as 12.5 μM. BDP-12 at 1 mM did not change the resting membrane potential, input resistance or spiking threshold and it did not alter monosynaptic potentials mediated by GABA receptors; it did, however, enhance disynaptic inhibitory responses. In membrane patches excised from hippocampal neurons, BDP-12 at moderate concentrations (50 μM) increased the steady-state currents mediated by AMPA receptors and slowed the rate at which the receptors desensitize, with a much larger effect on the former; the drug delayed the closing of the AMPA receptor channel after one-millisecond agonist pulses. BDP-12 had no detectable effect on [^{3}H]AMPA binding affinity. A related and more potent analogue produced a different pattern of results in that it had about equal effects on steady-state currents and desensitization rates and significantly increased binding to AMPA receptors. These results indicate that the benzoylpiperidine family of modulators has functionally distinct subclasses. The findings also establish that BDP-12 *i)* enhances synaptic responses in the same concentration range at which it alters AMPA receptor kinetics, *ii)* has a lower apparent threshold for effects on complex network operations than on monosynaptic transmission, *iii)* does not directly influence inhibitory responses, and *iv)* is likely to modulate AMPA receptors on interneurons as well as on pyramidal neurons. (supported by 95-I-0304 from AFOSR and CP-19982 from Cortex Pharmaceuticals).

607.8

CHARACTERIZATION OF MICE LACKING THE GLUR2 AMPA RECEPTOR SUBUNIT. F. A. Taverna[1]* Z. Jia[1], W. Abramow-Newerly[1], J. Henderson[1], R. Gerlai[1], Z. Xiong[2] J. MacDonald[2] and J. Roder[1]. Samuel Lunenfeld Research Institute, Mount Sinai Hospital[1], and Dept. of Physiology, University of Toronto[2], Toronto Ontario, Canada.

Glutamate receptors constitute the major excitatory neurotransmitter receptor system in the mammalian central nervous system. The GluR2 subunit is the most widely expressed member to the AMPA receptor family and controls the calcium permeability of the receptor. We generated mice lacking the GluR2 subunit by gene targeting. Mutant mice exhibited increased mortality, without spontaneous seizures, and reduced exploration and motor coordination. Mutant mice showed grossly normal development and organization of brain nuclei, fibre tracts and dendritic arbors. The fine structure of the hippocampus appeared normal. The AMPA receptor mediated ion channel activity in the GluR2-deficient mice exhibited a 9-fold increase in calcium permeability. Western blot analysis revealed that the level of expression of other AMPA receptors, as well as representative members of kainate and NMDA receptors was similar in wild type and mutant mice. Preliminary experiments on cultured cortical neurons derived from perinatal mice suggest that there is no increase in basal excitotoxicity in the mutants. Experiments are now underway to determine if AMPA receptor-dependent excitotoxicity will be enhanced in mutant mice. In addition, we are examining the biochemical consequences of the enhanced calcium influx on second messenger mediated signal transduction pathways. Supported by the Medical Research Council of Canada.

607.9

Characterization of glutamate receptors in GluR2 null mutant mice. P. Miu*, Z.P. Jia, J. Roder, P.L. Carlen. Playfair Neuroscience unit, Toronto Western Hospital, Toronto, Ontario, Canada.

Seeburg *et al* (1995) reported that editing deficient GluR2 mice express AMPA receptors with increasing calcium permeability, and that these mice developed lethal seizures by 3 weeks of age (*Science, 270:1677*). In contrast, GluR2 null mutant mice (Traverna *et al*, this meeting) showed neither seizure-like activities nor high mortality rate. We, therefore, examined evoked synaptic responses of CA1 pyramidal neurons obtained from hippocampal slices (400 μM) of GluR2 null mutant and their sibling wild type mice using whole-cell mode of patch clamp technique.

Excitatory postsynaptic currents (EPSCs) were recorded in the presence of GABA antagonists. $GABA_A$ and $GABA_B$ were blocked by bicuculline (10 μM) and internal Cs^+ (150 mM) respectively. Under these conditions, the dual component EPSCs did not show any obvious difference among cells obtained from control and GluR2 null mutant slices. In addition, we did not observe any spontaneous seizure-like activities, despite the absence of GABAergic inputs. The current-voltage relation of the NMDA PSCs was similar in cells obtained from control and GluR2 null mutant mice; while the AMPA PSCs of the GluR2 null mutant mice showed a significant rectification at the positive holding potentials.

These results provide some evidence that in the absence of GluR2 subunit, the remaining AMPA receptor subunits are capable of forming functional channels for fast synaptic transmission. *(Supported by MRC Canada, and P. Miu is a fellow of Epilepsy Canada)*

607.10

INTRACELLULAR POLYAMINES MAY DETERMINE RECTIFICATION IN THE AMPA SUBTYPE OF GLUTAMATE RECEPTORS , C. B. McCullum*, R.I. Hume, Univ. of Michigan, Ann Arbor, MI

We have studied the mechanism of rectification in the AMPA class glutamate receptor expressed in Xenopus oocytes. When the GluR1, 3, or 4 subunits are expressed alone, the whole cell I-V curves inwardly rectify and the permeability to divalent cations such as calcium is high compared to that of monovalent cations. When the GluR1, 3, or 4 subunits are co-expressed with GluR2 subunits, the whole-cell current-voltage relationship becomes linear and the relative permeability to divalent cations is low. We previously demonstrated that the inward rectification of receptors composed of the GluR1, 3 or 4 subunits is not caused by a voltage-dependent gate, by an external negatively charged blocker, by unequal concentrations of permeant ions, or by an intrinsic property of the channel in the membrane. At the same time, we also found that when outside-out patches were pulled from oocytes expressing homomeric GluR1, 3, or 4, the inward rectification was lost within 3-5 minutes following patch excision. Subsequently, we and others found that addition of micromolar quantities of polyamines to the intracellular solution of the patch pipette was sufficient to maintain inward rectification in outside-out patches. Because oocytes as well as many neuronal types contain 100-500 μm polyamines cytoplasmically, polyamines may mediate rectification physiologically. We tested whether polyamines are necessary for inward rectification in the whole oocyte by manipulating the levels of polyamines with inhibitors of polyamine synthesis. Changes in polyamine levels were confirmed using HPLC analysis. Our results strongly support the idea that polyamines endogenously regulate ion permeation through the AMPA class of glutamate receptor. Supported by PHS NS 21043.

607.11

AMPA RECEPTOR CALCIUM FLUX AND CALCIUM PERMEABILITY IN CULTURED RAT CEREBELLAR GRANULE CELLS A.L. Utz* and T.A. Verdoorn, Dept. of Pharmacology, Vanderbilt Univ., Nashville, TN 37232

The contribution of AMPA receptor activation to calcium signaling in cerebellar granule cells was examined using both the fluorescent $[Ca^{2+}]_i$ indicator, fura-2, and whole cell patch clamp measurement of Ca^{2+} permeability. Ratiometric analysis and cobalt quench of fura-2 fluorescence were used to measure responses to glutamate receptor agonists. In 11 DIV granule cells (n=258), 30μM AMPA increased $[Ca^{2+}]_i$ 12.4±1.7nM above baseline. The addition of 50μM cyclothiazide augmented this response to 1013±84nM. Similarly, 100μM glutamate produced a $[Ca^{2+}]_i$ rise of 26.8±2.8nM that was increased to 702±65nM by 50μM cyclothiazide. Exposure to 100μM kainate increased $[Ca^{2+}]_i$ by 768±60nM. Two approaches were used to distinguish direct AMPA receptor calcium flux from indirect flux through depolarization-activated channels. Decreasing AMPA-mediated depolarization by replacing extracellular sodium chloride with N-methyl-D-glucamine decreased the AMPA/cyclothiazide response by only 55% to 460±52nM. The magnitude of cobalt flux through activated ion channels was determined by measuring the fura-2 fluorescence quench rate (from 80% to 20% of maximum fluorescence). 50mM K^+ and 30μM AMPA produced similar low rates of quench, 52.2±0.6sec (n=108) and 50.6±1.3sec (n=46), respectively. However, 30μM AMPA + 50μM cyclothiazide and 100μM kainate induced rapid cobalt quench, 31.7±1.9sec (n=47) and 33.2±1.2sec (n=115), respectively. Therefore, significant calcium flux in conditions of limited depolarization, and the selectivity of divalent-permeable AMPA receptors for cobalt suggest that sustained activation produces direct AMPA receptor calcium flux. Whole cell patch clamp of cerebellar granule cells at 11 DIV, exposed to 100μM AMPA + 30μM cyclothiazide, showed a low average P_{Ca}/P_{Na} ratio of 0.033±0.027 (n=7). These results suggest that low AMPA receptor calcium permeability does not prevent substantial calcium influx through neuronal AMPA receptors. NS30945 and American Heart Association.

607.12

TNFα TREATMENT INCREASES THE NUMBER OF CORTICAL NEURONS EXPRESSING Ca^{2+} -PERMEABLE AMPA/KAINATE RECEPTORS. D.M. Turetsky*, L.L. Dugan and D.W. Choi. Center for the Study of Nervous System Injury and Dept. of Neurology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Recent studies have identified subpopulations of neurons in hippocampus, cerebellum, spinal cord and cortex which express Ca^{2+} -permeable AMPA or kainate receptors, and exhibit selective vulnerability to death induced by kainate exposure. These neurons can be identified by a histochemical stain for kainate-stimulated cobalt uptake. We previously reported that oxidative stress - specifically, a brief sublethal exposure to H_2O_2 (60 μM, 30 min) - produced a 25% increase in the number of neurons exhibiting kainate-stimulated cobalt uptake in murine cortical cultures, as assessed 24 hr after the treatment (Dugan et al., Soc. Neurosci. Abstr. 21:353). This increase in cobalt positive neuronal number was accompanied by a two-fold increase in kainate-stimulated $^{45}Ca^{2+}$ influx, and a selective decrease in the amount of GluR B protein.

TNFα, a cytokine known to increase oxidative processes in a variety of cell types, is increased in brain after focal ischemia (Liu et al., Stroke 25:1481, 1994) or inflammation, as well as in AIDS-related dementia (Perrella et al., J Neurol 239:387, 1992). Because of our hypothesis linking oxidative stress and Ca^{2+} permeable AMPA/kainate receptor expression, we tested the effect of TNFα on cobalt positive neuronal number in our culture system. Twenty-four hour treatment with 100 ng/ml TNFα caused a 27% increase in the number of cobalt-positive cortical neurons at the end of the treatment period. Present observations raise the possibility that TNFα may alter AMPA/kainate receptor properties in certain disease states, favoring Ca^{2+} permeable forms and perhaps increasing neuronal vulnerability to excitotoxic injury. Supported by NIH NINDS grants NS 30337 (DWC) and NS 32636 (LLD).

607.13

ANTISENSE OLIGONUCLEOTIDES TARGETED TO GLUR2 INCREASE HIPPOCAMPAL NEURONAL CELL DEATH RAPIDLY AFTER KAINATE-INDUCED STATUS EPILEPTICUS IN ADULT RATS. *L.K. Friedman*, J. Velísková, S.L. Moshé, D.C. Spray, X. Zheng, T. Opitz, R.S. Zukin. Depts Neurosci ence and Neurology, Albert Einstein Coll Med, Bronx, NY* **10461**

Kainic acid (KA) induces status epilepticus and a marked reduction in GluR2 (the AMPA subunit that limits Ca^{2+} permeability) expression that precedes the selective and delayed neuronal cell death of hippocampal CA3 neurons. To investigate whether reduced GluR2 subunit expression enhances CA3 vulnerability, we administered GluR2 antisense oligodeoxynucleotides by unilateral microinfusion into the dorsal hippocampus before inducing KA status epilepticus. CA3 cell loss was accelerated and enhanced following antisense pretreatment and KA status epilepticus; rats exhibited extensive or complete loss of CA3 neurons in the ipsilateral hippocampus at 24 hrs, a time when significant cell loss is not typically observed. Immunohistochemical and Western analyses confirmed that GluR2 receptor protein was reduced ipsilaterally after the GluR2 antisense infusions in the presence and absence of seizures. Injection into a different site (thalamus) did not enhance CA3 damage suggesting that a reduction in GluR2 expression within the hippocampus is important to alter the timing and pattern of the KA seizure-induced damage. In cultured hippocampal neurons, application of GluR2 antisense oligodeoxynucleotides increased Ca^{2+} permeability through AMPA receptors as determined by ratiometric imaging with Indo 1. Hence, the expression of GluR2 receptor subunits appears necessary for the survival of CA3 neurons following status epilepticus. The "GluR2 hypothesis", that increased formation of AMPA permeable Ca^{2+} channels enhances glutamate pathogenicity is further supported by these studies.

Supported in part by the March of Dimes

607.14

GENERATION OF ANTIBODIES SPECIFIC FOR THE PHOSPHORYLATED AMPA RECEPTOR GLUR1 SUBUNIT. K. Kameyama, A. L. Mammen, K. W. Roche and R. L. Huganir*, Dept. of Neuroscience, Howard Hughes Med. Inst., Johns Hopkins Univ. School of Med., Baltimore, MD 21205

Protein phosphorylation has been shown to be an important mechanism for modulating ionotropic glutamate receptors. We have shown previously that protein kinase A and C directly phosphorylate the AMPA receptor GluR1 subunit on serine residues 845 and 831 respectively, in a heterologous expression system. To study the phosphorylation state of this receptor *in vivo* and *in vitro*, we have generated anti-phosphopeptide antibodies recognizing the GluR1 protein only when it is phosphorylated on either serine residues 831 or 845. These antibodies are very specific and recognize GluR1 only when it is phosphorylated on the appropriate serine residue. Immunoblot experiments with these antibodies indicate that GluR1 is basally phosphorylated on these sites in hippocampal slice preparations. Interestingly, the basal phosphorylation of these sites is much less in primary cultures of cortical neurons than in the hippocampal slice. The phosphorylation at serine 831 or 845 is increased by preincubation of slices or primary culture neurons with phorbol dibutyrate to activate PKC or forskolin to activate PKA, respectively. These antibodies should prove useful for monitoring the phosphorylation states of GluR1 during a variety of neuronal processes, such as LTP or LTD.

607.15

ACTIVATION OF GLIAL GLUTAMATE RECEPTORS MEDIATES THE TRANSFER OF THE NITRIC OXIDE PRECURSOR ARGININE FROM GLIA TO NEURON

G.Grima*, B.Benz and K.Q. DO; Brain Research Institute, University of Zurich, 8029 Zurich, Switzerland

Arginine (Arg), the nitric oxide (NO) precursor, has been reported to be predominantly localized in glial cells, whereas NO synthase, the NO producing enzyme, is mainly found in neurons. Therefore, a transfer of Arg from glial cells to neurons is necessary to replenish the neuronal precursor pool. This is further supported by the finding that Arg is released upon selective pathway stimulation both in vitro (Hansel et al., 1992) and in vivo (Do et al., 1994). We investigated the mechanism underlying this glial-neuronal interaction by analyzing the effect of glutamate (Glu) agonists on the extracellular [^{3}-H]-Arg level in cerebellar slices and in cultures of cortical astrocytes and neurons. In cerebellar slices, Glu, in a TTX-insensitive manner, AMPA, kainate and NMDA increased significantly the extracellular level of labeled Arg. This effect was blocked by the selective antagonists, CNQX and CPP respectively. Moreover, tACPD induced a delayed rise of the Arg release, whereas L-AP4 and L-CCG1 were ineffective. In astrocytes, Glu, AMPA, kainate (effect blocked by CNQX) and the Ca^{2+}-ionophore A23187 caused an increase in the extracellular Arg level. NMDA was ineffective in this system. Glu showed no effect in neurons. These results together with the glial localization of Arg support the hypothesis of an Arg transfer from glia to neurons in order to supply NO synthase with its substrate. The activation of ionotropic non-NMDA receptors present on astrocytes mediates this Arg transfer probably through a rise in intracellular [Ca^{2+}]. Thus, NO synthesis and NO transmission may be based on the glial-neuronal transfer of Arg which is dependent on activation of excitatory amino acid receptors on glial cells. This interaction represents a novel glial-neuronal signaling which could play a role in the synaptic transmission. (Swiss National Foundation (NF31-39681.93) and Human Frontier Science Program)

607.16

INTEGRINS INHIBIT THE KAINATE-INDUCED CALCIUM WAVE IN HIPPOCAMPAL ASTROCYTE CULTURES, A.H. Cornell-Bell[1]*, W. Kim[2], M.G. Rioult[2], R.M. Villalba[1] and V.Trinkaus-Randall[3]. [1]Viatech Imaging, Ivoryton, CT 06442; [2]Yale School of Medicine, New Haven CT 06510, [3]BU School of Medicine, Boston, MA 02118.

Kainate superfusion (100uM) overwhelms the Na^+-buffering capacity of astrocytes within 100 sec resulting in reversal of the Na^+/Ca^{2+} exchanger. Ca^{2+} entering the cytoplasm contributes to long-distance regenerative waves which travels at 20 um/sec without decrement. Na^+_{in} is regulated by the Na^+/Ca^{2+} exchanger; TTX has no effect on the Kainate wave. Ca^{2+} enters through reversal of the exchanger; voltage-gated Ca^{2+} channels are not involved since Nimodipine has no effect on the Kainate wave. Ca^{2+} release from stores does not contribute to the kainate wave; MCPG the metabotropic (IP3) receptor inhibitor has no effect on the kainate wave. Removal of Na^+ or Ca^{2+} from outside and the specific Na^+/Ca^{2+} exchange inhibitor benzamil (10 uM) inhibit the kainate wave. We speculated that the only requirement for the regenerative kainate wave was closely aligned cell membranes containing the Na^+/Ca^{2+} exchanger. This would imply that Ca^{2+} or IP3 need not pass through the gap junction, but reversal of the exchanger would propel the Ca^{2+} wave at the cell surface. Uncoupling gap junctions with octanol (Finkbeiner, 1993; Kim et al, 1995; Lee et al., 1995) inhibits the kainate wave which may result from membrane-membrane changes. Alpha6 Integrin antibodies immuno-fluorescently stain astrocyte edges reflecting their role in cell-cell communication.Time-lapse confocal imaging was used to show that a functiona antibody to alpha6-Integrin inhibits the spread of the kainate wave in a dose and time-dependent manner. FRAP analysis shows gap junctions remain open in the presence of anti-alpha6. Viatech Imaging and NIHEY06000.

607.17

LONG-TERM EXPOSURE TO GLUTAMATE RECEPTOR LIGANDS ALTERS THE SUSCEPTIBILITY TO EXCITOTOXIC DAMAGE AND GLUTAMATE RECEPTOR SUBUNIT EXPRESSION IN HIPPOCAMPAL SLICE CULTURES, J. Zimmer*, B. Jakobsen, N. H. Diemer. PharmaBiotec, Dept. of Anat. & Cell Biol., Odense Univ. and Dept. of Neuropathol., Copenhagen Univ., Denmark.

Long-term treatment of developing rat hippocampal slice cultures by glutamate receptor agonists was used to explore inducible changes in glutamate receptor mediated neurotoxicity and receptor subunit expression.

Slices of 5 day old rat hippcampi were cultured by the roller-drum method with preservation of the normal, basic cellular and connective organisation. When 4 week old slice cultures were acutely exposed to a high dose of KA (10 μM), there was a selective loss of CA3 pyramidal cells. Similar cultures exposed to a low dose of KA (2 μM) from day 4 and onwards appeared normal, but had become resistant to the acute high dose of KA (10 μM). The long-term treatment with KA also resulted in downregulation of GluR6 mRNA in CA3ab and KA2 mRNA in CA3c, as revealed by quantitative *in situ* hybridization.

Preliminary studies of cultures long-term treated with NS102, a AMPA/KA antagonist developed by NeuroSearch, DK, showed loss of CA3 pyramidal cells by an acute, otherwise sublethal dose of KA.

We conclude that the susceptibility of hippocampal slice cultures to KA is modifiable by long-term exposure to NS102 and KA, and that the KA exposure is parallelled by a decrease in glutamate receptor subunit mRNA expression.

607.18

THE PROBABILITY OF OPENING OF HOMOMERIC GLUR6 RECEPTORS. Philip Wahl*, David Mott, and Stephen F. Traynelis. Dept Pharmacology, Emory University, Atlanta, GA 30322 and NovoNordisk A/S, DK-2880 Bagsværd, Denmark

GluR6 glutamate receptor subunits have been suggested to be involved in the development of the central nervous system. We have used rapid agonist application and nonstationary variance analysis to examine the single channel conductance and the probability of channel opening for rat GluR6 homomeric channels expressed in HEK293 cells and activated by the endogenous neurotransmitter, glutamate. Glutamate (0.15-10 mM) was applied to excised membrane patches (Vhold=-80 mV) at 15 s intervals, and 35-240 current responses recorded at 50 kHz. The mean risetime (10-90%) for responses to 10 mM glutamate was 0.5 ms; desensitization was well described by a single exponential function with tau of 3.6 ms (n=10 patches), and recovered with a time constant of 2 s (n=11). The weighted mean slope conductance of single channel openings was 15 pS (n=6) for receptors unedited in the M2 region but fully edited in TM1; unitary current determined at -80 mV was independent of agonist concentration (0.15 - 10 mM; n=27). RNA editing in the M2 region (Q621R) reduced unitary conductance by more than 25-fold (n=3). Popen (0.6; n=15) was independent of voltage and RNA editing in M2 region, but varied with agonist concentration. The EC50 value (0.2 mM) for maximal Popen was similar to that obtained for the macroscopic current (0.5 mM). The Hill slope was 1.0. Inclusion of the catalytic subunit of PKA in the intracellular solution caused a 33% increase in Popen ($p<0.5$; n=24). We conclude that binding of a single molecule of glutamate to homomeric GluR6 receptors is associated with a high probability of channel opening, and this probability of receptor opening can be modified by intracellular kinases.

This work was generously supported by the John Merck Fund and NovoNordisk A/S.

607.19

ACTIVATION AND DESENSITIZATION OF KAINATE RECEPTORS IN CULTURED HIPPOCAMPAL NEURONS. T.J. Wilding and J.E. Huettner* Dept of Cell Biology & Physiology, Washington Univ, St Louis, MO 63110

We have used whole-cell recording and rapid agonist applications in the presence of MK-801 (2 μM) and GYKI 53655 (100 μM) to characterize the physiological properties of kainate receptors expressed by rat hippocampal neurons in dissociated cell culture. GYKI 53655 is a potent, non-competitive inhibitor of AMPA-preferring receptors (IC50 ~ 1 μM), with greater than 200 fold selectivity for the AMPA versus kainate subtype.

In the presence of GYKI, both kainate (EC50 ~ 35 μM) and glutamate (EC50 ~ 400 μM) evoked desensitizing currents. Maximal responses were roughly 10% as large as the current elicited without GYKI. In contrast to current mediated by AMPA receptors, kainate currents recorded in GYKI were blocked potently by lanthanum (IC50 ~ 3 μM) and were desensitized strongly by 1 μM 2S, 4R-4-methylglutamate. Coapplication of either 5 μM AMPA or 500 μM aspartate had little or no effect on responses to kainate, although 1 mM AMPA elicited a small current in some cells. Glutamate currents desensitized nearly to baseline with a τ of approximately 50 msec, while in most cells, kainate currents decayed along two exponentials (τ ~ 30 and 460 msec) to a steady state level roughly 20 to 60% of the peak. Recovery from desensitization to kainate and glutamate followed 2 exponentials with τs of 4-6 and 30-40 sec.

Collectively, these results confirm that kainate-preferring receptors underlie the currents evoked by kainate or glutamate in the presence of GYKI 53655; they are not mediated by electrogenic transport or by AMPA-preferring receptors that are simply insensitive to GYKI. In contrast to previous work on native and recombinant kainate receptors, however, exposure of hippocampal neurons to Con A reduced the peak current amplitude but had little effect on the amplitude at steady-state.

NIH NS30888, McDonnell Center for Cellular & Molecular Neurobiology

607.20

THE KAINATE BINDING PROTEIN FROM *RANA PIPIENS* HAS A FUNCTIONAL ION CHANNEL DOMAIN. C. Villmann* and M. Hollmann. Glutamate Receptor Laboratory, Max-Planck-Institute for Experimental Medicine, D-37075 Göttingen, Germany.

Kainate binding proteins (KBPs) have been cloned from several species (frog, chick, duck, and goldfish). They share significant sequence homology with ionotropic glutamate receptors, particularly kainate and AMPA receptors.. Also, their transmembrane topology is believed to be identical to that of glutamate receptors. However, none of the KBPs forms functional ion channels upon heterologous expression in *Xenopus* oocytes or mammalian cells. The observed lack of ion channel function could be due to lack of protein expression, lack of ligand binding, a non-functional ion pore domain, or a defect in the mechanism translating ligand binding into opening of the pore. Since ligand binding can readily be measured in heterologous systems, lack of ion channel function can neither be attributed to lack of protein expression nor failure of the agonist to bind.

Therefore, we set out to investigate whether the hypothetical ion pore domain of the KBPs in principle is capable of conducting ionic currents. We excised the ion pore domain of *Rana pipiens* KBP (kindly provided by Drs. Keiji Wada and Bob Wenthold) and inserted it into the kainate receptor GluR6 and the AMPA receptor GluR1. In both cases the chimeric receptors upon expression in *Xenopus* oocytes formed functional ion channels activated by kainate and glutamate. We conclude that the KBPs have an ion pore domain capable of conducting current. Lack of function in homomerically expressed KBPs may thus be due to a failure to communicate agonist binding to the ion pore.

Supported by the Deutsche Forschungsgemeinschaft, and SFB 406.

607.21

POTENTIATION OF THE KAINATE RECEPTOR GluR6 BY LECTINS APPARENTLY INVOLVES MULTIPLE CARBOHYDRATE SIDE CHAINS. I. Becker and M. Hollmann*, Glutamate Receptor Laboratory, Max-Planck-Institute for Experimental Medicine, D-37075 Göttingen, Germany.

Lectins such as Concanavalin A (ConA) have long been known to potentiate currents at certain glutamate receptors. This effect is thought to be due to inhibition of desensitization, and is most prominent with kainate receptors (up to 4000-fold increase in currents at GluR6). However, the molecular mechanisms underlying this effect are entirely unknown. Since ConA acts by binding to carbohydrates, we investigated whether the ability of ConA to potentiate GluR6 responses is mediated by the carbohydrate side chains present on the protein. Expression of GluR6 in *Xenopus* oocytes injected with tunicamycin to block all N-glycosylation totally abolished the ConA effect while receptor function was retained. We next analyzed whether any particular carbohydrate side chain attached to one of the 10 consensus N-glycosylation sites present on GluR6 might be responsible for the ConA effect. Only 9 of these sites are extracellular, and each of them was eliminated by site-directed mutagenesis. All mutants were still functional, although in some cases current amplitudes were significantly smaller than in wildtype GluR6. Importantly, though, all mutants still showed ConA-potentiation. Thus, there is no single carbohydrate side chain which mediates the effect of ConA. Presumably, ConA interacts simultaneously with several such side chains. Combinations of side-chain mutations are currently under investigation.

Supported by the Deutsche Forschungsgemeinschaft, and SFB 406.

EXCITATORY AMINO ACID RECEPTORS: PHYSIOLOGY, PHARMACOLOGY, AND MODULATION III

608.1

IN VIVO RELEASE OF HOMOCYSTEATE FROM RAT THALAMUS FOLLOWING STIMULATION OF SOMATOSENSORY AFFERENTS

T.E. Salt[1], B. Benz, K.E. Binns[1], S .A. Eaton[1] and K.Q.Do*. Brain Research Institute, Zurich University, CH-8029 Zurich, Switzerland; [1]Institute of Ophthalmology, University College London, London EC1V 9EL, UK.

The sulphur-containing amino acid homocysteic acid (HCA) is present in and released from nervous tissue and is a potent neuronal excitant, predominantly activating NMDA receptors. However, HCA is localized not in neurons but in glial cells (Grandes et al., 1991), and activation of a nervous pathway *in vitro* leads to delayed efflux of HCA (Do et al., 1992). We now report the *in vivo* release of HCA from ventrobasal (VB) thalamus following natural stimulation of somatosensory afferents arising from the mustachial vibrissae of the rat. Simultaneously with electrophysiological recording, [^{35}-S]-methionine, a HCA precursor, was perfused (15 µl/min) through a push-pull cannula in VB thalamus of anaesthetized rats. One minute effluent perfusates were collected before, during and after 4 minute stimulation of the vibrissal afferents with an air jet. A marked release of radiolabeled HCA was observed during and after the stimulation. In separate experiments, the excitatory actions of iontophoretically applied L-HCA on VB neurons were inhibited by the NMDA receptor antagonist CPP, but not by the non-NMDA antagonist CNQX. These results, combined with the HCA release induced by noradrenaline from glial cells (Do et al., 1994) suggest a gliotransmitter role for HCA in VB thalamus. The release of HCA from glia might exert a direct postsynaptic response or modulate responses to other neurotransmitters, thus enhancing excitatory processes. These studies also indicate that the communication between glia and neurone is indeed part of their physiological activity and is induced by pathway stimulation both *in vitro* and, for the first time, *in vivo* under physiological conditions.

(Work supported by the Human Frontier Science Program).

608.2

PREFERENTIAL INHIBITION BY BARBITURATES OF CALCIUM-DEPENDENT SYNAPTOSOMAL RESPONSES INDUCED BY NMDA RECEPTOR ACTIVATION. K. Fink*, W. Meder and M. Göthert, Inst. Pharmacol. Univ. Bonn, Reuterstrasse 2b, 53113 Bonn, Germany

Barbiturates inhibit neuronal function by increasing Cl^- conductance of the $GABA_A$ receptor channel. In addition, they may inhibit other ligand- and voltage-gated ion channels, thus decreasing Ca^{2+}-dependent neurotransmitter (NT) release from axon terminals. To test this hypothesis, rat brain cortex synaptosomes were used to examine the influence of methohexital (1) on NT release induced by activation of NMDA receptors, voltage-gated Na^+ channels (by veratridine) or voltage-gated Ca^{2+} channels (by high K^+) and (2) on depolarization-induced increase in intrasynaptosomal Ca^{2+} concentration. Synaptosomes preloaded with [^{3}H]noradrenaline ([^{3}H]NA) or [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT) were superfused with Krebs' buffer. [^{3}H]NT release was induced by addition of veratridine (1 µM), NMDA (0.3 or 1 mM) or 15 mM K^+. FURA 2-loaded synaptosomes (in Hepes buffer) were transferred into a fluorometer; the increase in $[Ca^{2+}]_i$ induced by K^+ (30 mM) or veratridine (1 µM) was evaluated on the basis of 340/380 nm fluorescence ratios. The NT release induced by NMDA- or veratridine was almost completely Ca^{2+}-dependent. The increase in $[Ca^{2+}]_i$ induced by high K^+ or veratridine depended mainly on extracellular Ca^{2+} and was inhibited by 0.1 µM ω-conotoxin GVIA (by 33% and 24%, respectively), but not by 1 µM nifedipine. Methohexital inhibited the veratridine-induced release of [^{3}H]NA or [^{3}H]5-HT (pIC_{50} = 3.40 or 3.67, respectively), the increase in $[Ca^{2+}]_i$ induced by veratridine (pIC_{50} = 3.30), the Ca^{2+}-induced [^{3}H]NA release (pIC_{50} = 3.47) and the increase in $[Ca^{2+}]_i$ induced by high K^+ (pIC_{50} = 3.65) at similar potencies. However, the NMDA-evoked [^{3}H]NA release was more potently inhibited (pIC_{50} = 4.01). It is concluded (1) that in nerve terminals methohexital inhibits both the depolarization-induced increase in $[Ca^{2+}]_i$ and the depolarisation-induced NT release and (2) that methohexital inhibits the NMDA receptor channel at higher potency than voltage-gated Na^+ and Ca^{2+} channels. (Supported by a grant of the DFG)

608.3

CLONING AND EXPRESSION STUDIES OF KYNURENINE/α-AMINOADIPATE AMINOTRANSFERASE FROM WHOLE HUMAN BRAIN.

S.Gatti, L.Cozzi, B.Valsasina, M.Mauriello, J.Breton, N.Avanzi, E.Okuno+, C.Speciale++* and S.Toma. Pharmacia & Upjohn, Bioscience Center and ++CNS Research, Nerviano (Mi), Italy; +Wakayama Medical College, Wakayama 640, Japan.

In the brain, kynurenic acid (KYNA), an endogenous antagonist at the excitatory amino acid receptors, is produced from L-kynurenine by two different kynurenine aminotransferases (E.C.2.6.1.7.), KAT1 and KAT2. These enzymes, by regulating brain KYNA content, may play a role in neurobiology. Recently, KAT2 has been described as kynurenine/α-aminoadipate aminotransferase and its clone has been obtained from rat brain (Buchli et al., JBC, 270:29330, 1995). We have now partially purified hKAT2 from whole brain and sequenced 11 peptides fragments obtained by tryptic digestion of the SDS-PAGE band (44KDa). The hKAT2 cDNA sequence was obtained by RT-PCR using polyA$^+$ from human hippocampus, and by screening of a λZapII human striatum cDNA library. The analysis of the nucleotidic sequence showed the presence of a single open reading frame coding for a 425AA long protein. The deduced primary amino acid sequence of human KAT2 was found to have 78% homology to the rat kynurenine/α-aminoadipate aminotransferase protein sequence. Northern blot analysis showed the constitutive presence of a 2000bp long mature KAT2 transcript in human liver and kidney. In the brain, the constitutive expression of hKAT2 mRNA was detectable in hippocampus, nucleus caudatus, hypothalamus, thalamus, subthalamic nucleus, substantia nigra, amigdala, and corpus callosum. The enzymatic activity of the recombinant protein expressed in COS1 cells tested as kynurenine aminotransferase and α-aminoadipate aminotransferase is currently ongoing. Molecular studies related to KYNA synthesising enzymes in the human brain will provide insightful information on the role of this endogenous compound in physiology and pathology in the central nervous system.

608.4

EXPRESSION CLONING OF RAT KIDNEY KYNURENINE-3-HYDROXYLASE.

S.Magagnin*, R.Bormetti, C.Speciale, M.Cini and L. Benatti. Pharmacia & Upjohn, CNS Research, Nerviano (MI), Italy.

Several lines of evidence suggest that an enhancement of kynurenic acid formation and a reduction of quinolinic acid production by pharmacological means may offer new opportunities to develop novel neuroprotective molecules. Following this strategy, kynurenine-3-hydroxylase (KYN 3-OHase), catalysing the conversion of kynurenine to 3-hydroxykynurenine has been proposed as the major target for the pharmacological manipulation of the kynurenine pathway (Speciale et al., this meeting). Since attempts for its purification have been so far unsuccessful, an expression cloning strategy was designed. *Xenopus laevis* oocytes were first injected with poly (A)$^+$RNA isolated from rat kidney, a tissue in which the activity of the enzyme was reported to be particularly high. KYN 3-OHase activity was present neither in water-injected oocytes nor in oocytes injected with poly(A)$^+$ RNA isolated from rat heart (a tissue in which the activity of the enzyme is very low). On the other hand, the oocytes injected with rat kidney mRNA showed an activity 6 folds over background. A directional cDNA library was therefore constructed from rat kidney poly(A)$^+$ RNA. The unamplified library (about 50,000 independent clones) was initially subdivided in 25 pools of about 2,000 colonies each. cRNA from each pool was injected in *X.* oocytes and positive clones selected on the basis of KYN 3-OHase expressed activity. Following the same criterion, in the last round of screening we had a single positive clone that showed an activity about 400 folds higher than that induced by injection of total rat kidney poly(A)$^+$ RNA. Identity of the isolated clone was further verified comparing the kinetic parameters of the expressed activity with those of the native kidney enzyme. Isolation and characterization of KYN 3-OHase cDNA will help to understand its role in physiology and pathology and to design more specific inhibitors possibly bearing neuroprotective potential.

608.5

INHIBITION OF KYNURENIC ACID SYNTHESIS BY AMMONIA IN RAT BRAIN SLICES. W.A. Turski[1,2*], J. Albrecht[3], T. Kocki[1], T. Saran[1], E.M. Urbanska[1,2]. [1]Department of Pharmacology and Toxicology, Medical University School, Lublin, Poland, [2]Department of Clinical Toxicology, Institute of Agricultural Medicine, Lublin, Poland, [3]Department of Neurotoxicology, Medical Research Centre, Polish Academy of Science, Warsaw, Poland.

Kynurenic acid (KYNA) is an endogenous neuroprotectant which acts on all three ionotropic excitatory amino acid receptors. Its synthesis from L-kynurenine is catalyzed by aminotransferase located preferentially in astrocytes. Astrocytes are the primary metabolic target of ammonia in the brain. This prompted us to investigate the effect of ammonia on KYNA synthesis. Rat cortical slices were incubated in the presence of L-kynurenine (10 μM) and different concentrations of ammonium acetate. KYNA was quantified by HPLC. Ammonia at the subtoxic to toxic concentration range from 1 mM to 10 mM produced a dose-dependent inhibition of KYNA synthesis by 21% and 76%, respectively. Subthreshold concentration of ammonia (0.5 mM) influenced neither KYNA synthesis under standard conditions nor the inhibition of KYNA synthesis evoked by L-glutamate. The results point to impaired neuroprotection by KYNA as a potential astroglia-mediated aspect of ammonia neurotoxicity observed in hyperammonemia.
Supported by KBN Research Grant No. 4.PO5A.027.09

608.6

GLUCOSE DEPRIVATION RESULTS IN DECREASED KYNURENIC ACID PRODUCTION IN RAT BRAIN SLICES: EFFECTS OF LACTATE SUBSTITUTION. P.S. Hodgkins*, A. Rassoulpour and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kynurenic acid (KYNA) is an endogenous metabolite of tryptophan which is produced in the brain and is an established neuroprotective compound. As demonstrated previously using brain tissue slices, synthesis of KYNA from its immediate precursor L-kynurenine (KYN) is glucose-dependent (J. Neurochem. 52: 1629, 1989). This effect of glucose deprivation is not due to an impaired transport of KYN into the cell (J. Neurochem. 54: 156, 1990). Follow-up studies were now performed using tissue slices from male rats (routinely 200-220 g) and a physiological concentration of KYN (2 μM). Under standard incubation conditions [cortical slices, Krebs-Ringer buffer (KRB); 5 mM glucose], approx. 4 pmol KYNA/mg protein/h were produced and recovered extracellularly. Removal of glucose from the KRB decreased KYNA production to 32.7 $\pm$ 2.6% (SEM) of control levels (N=6; $P < 0.001$). No effect of glucose removal was observed in liver slices incubated under identical conditions. Similarly, KYNA production was glucose-independent in striatal slices from animals which had been lesioned 7 days previously with 300 nmoles quinolinic acid.

Lactate substitution partially reversed the effect of glucose deprivation, with 10 mM lactate restoring KYNA production in cortical slices from adult rats to 68.3 $\pm$ 3.7% of controls (N=7; $P < 0.001$) (ED_{50}: 0.5 mM). In cortical slices from 14 day-old rats pups, glucose deprivation caused KYNA synthesis to decrease only to 61.7 $\pm$ 2.5%, and addition of 10 mM lactate returned KYNA production to normal levels (N=3; $P < 0.05$ vs. no glucose). These data suggest a role of cellular energy metabolism in the production of KYNA and may be of relevance for hypoglycemia-induced excitotoxic damage. Supported by USPHS grants NS 16102 and NS 28236.

608.7

FACILITATED BRAIN UPTAKE OF HALOGENATED KYNURENINES VIA THE LARGE NEUTRAL AMINO ACID TRANSPORTER IN RATS. [1]C.L. Eastman*, M. Hokari, [1]H.-Q. Wu, [1]R. Schwarcz and Q.R. Smith. Lab. Neurosci., Nat. Inst. Aging, NIH, Bethesda, MD 20892 and [1]Maryland Psych. Res. Ctr., Baltimore, MD 21228.

7-Chlorokynurenic acid (7-Cl-KYNA) is of therapeutic interest as a potent NMDA receptor antagonist and neuroprotective agent. However, 7-Cl-KYNA crosses the blood-brain barrier only poorly following systemic administration. We therefore examined the brain uptake and metabolic conversion of L-4-chlorokynurenine (4-Cl-KYN), the immediate precursor of 7-Cl-KYNA (J. Med. Chem. 37: 334, 1994), using an *in situ* rat brain perfusion method (Am. J. Physiol. 247: H484, 1984) and 100, 200 or 500 μM of the prodrug. 4-Cl-KYN was shown to be taken up readily into brain by the large neutral amino acid transporter of the blood-brain barrier (K_m = 101 $\pm$ 12 μM, v_{max} = 18.4 $\pm$ 1.6 nmol/min/g tissue) and to reach concentrations comparable to that of endogenous KYN after 20 s. During the same period, some 4-Cl-KYN was also converted to 7-Cl-KYNA which was recovered in concentrations similar to those of endogenous cerebral KYNA (i.e. 15-25 pmoles/g tissue). Essentially identical tissue contents of 4-Cl-KYN and 7-Cl-KYNA, respectively, were measured in cortex, striatum and hippocampus. Parallel experiments using the precursor of 5,7-dichlorokynurenic acid, L-4,6-dichlorokynurenine, revealed an inferior affinity of the di-halogenated compound for the neutral amino acid transporter (K_m = 410 $\pm$ 18 μM; n=6). These results suggest that 4-Cl-KYN may be a useful prodrug for brain delivery of 7-Cl-KYNA.

Supported in part by USPHS grants NS 16102 and NS 28236.

608.8

EXTRACELLULAR CONTENT OF KYNURENIC ACID IN THE RODENT HIPPOCAMPUS: EFFECTS OF SYSTEMICALLY ADMINISTERED KYNURENINE 3-HYDROXYLASE INHIBITORS. N. Carfagna*, H.-Q. Wu[1], C. Speciale, C. Post, M. Varasi and R. Schwarcz[1]. Pharmacia & Upjohn, CNS Research, Nerviano (MI), Italy and [1]Maryland Psychiatric Research Center, Baltimore, MD 21228, USA.

Kynurenic acid (KYNA) is a brain metabolite and broad spectrum antagonist of ionotropic glutamate receptors. We have studied by microdialysis the effects of the p.o. or i.p. administration of two potent and selective kynurenine 3-hydroxylase inhibitors, (R,S)-3,4 dichlorobenzoylalanine (FCE 28833A) and m-nitrobenzoylalanine (mNBA), on KYNA levels in the hippocampus of unanesthetized rats and mice. These compounds divert kynurenine pathway metabolism to increase the formation of endogenous KYNA in the brain. The basal concentrations of extracellular KYNA were 13.0 nM and 5.3 nM, respectively, for rats and mice. P.o. administration of FCE 28833A (400 mg/kg) to rats or mice resulted in a substantial increase in KYNA, reaching a peak of 10-12 times control levels by 6 hrs and reverting to baseline by 12-18 hrs. P.o. treatment with the same dose of mNBA caused a maximal KYNA increase of 4-fold, peaking at 4 hrs and returning to baseline by 11 hrs. After i.p. injection in rats (400 mg/kg each), mNBA caused a peak increase of 10 times basal levels at 4-5 hrs, whereas FCE 28833A treatment resulted in a peak 80-fold increase above control levels after 9 to 11 hrs. In mice, repeated p.o. administration (400 mg/kg every 12 hrs for 3 days) failed to indicate tolerance to FCE 28833A-induced KYNA enhancement. These data confirm that FCE 28833A is the most potent KYNA-enhancing agent described so far. FCE 28833A-induced elevations of brain KYNA levels may be related to the compound's neuroprotective properties (cf. Speciale et al., this meeting). Supported by USPHS grant NS 16102.

608.9

FCE 28833A, A POTENT INHIBITOR OF KYNURENINE 3-HYDROXYLASE, ENHANCES BRAIN KYNURENIC ACID AND IS NEUROPROTECTIVE IN THE GERBIL ISCHEMIA MODEL. C.Speciale, P.Salvati, M.Cini, L.Benatti, M.Tamburin, A.Molinari, B.Rosa, C.Allievi[1], C.Caccia, M.Varasi and C.Post*. Pharmacia & Upjohn, CNS Research and [1]Pharm. & Metab., Nerviano (MI), Italy.

The presence of the neuroprotectant kynurenic acid (KYNA) in the mammals brain offers opportunities for pharmacological intervention in neurodegeneration. Inhibitors of kynurenine 3-hydroxylase (KYN 3-OHase), the first enzyme involved in kynurenine degradation, are able to enhance brain KYNA content. FCE 28833A, (R,S)-3,4-dichlorobenzoylalanine (Molinari et al., Soc. Neurosci., 1995), is the most potent and selective inhibitor (K_i=130 nM) described so far, and upon oral administration in gerbils (400 mg/kg) increases KYNA in brain tissue over 60-fold. In order to examine the possibility that the KYNA rise results in neuroprotection, we tested FCE 28833A in the gerbil model of global ischemia. FCE 28833A (400 mg/kg) was given orally 3 hours before 5 min ischemic insult. 7 days later neuronal loss per mm CA1 was evaluated by image analysis. FCE 28833A-treated gerbils showed a significantly larger number of living neurons (95.8±13.2, n=31, $p<0.01$), in comparison with the vehicle-treated group (41.3±10.1, n=41). Excitatory amino acid (EAA) receptor antagonists can prevent the induction of immediate early genes (IEG) mRNA expression evoked by excitotoxic events. To investigate the link between increased KYNA in the brain and EAA receptor activation, the effect of FCE 28833A on ischemia-induced c-fos and c-jun mRNA was studied in gerbils by *in situ* hybridization in the dentate gyrus. Having ruled out any direct effect of FCE 28833A on the ionotropic EAA receptors, the significant attenuation of the ischemia-induced mRNA expression observed in the drug-treated group in comparison to controls, was consistent with the hypothesis that endogenous KYNA may cause EAA receptor blockade. Our results strongly support the notion that modulation of endogenous kynurenines constitutes a viable strategy for the development of neuroprotective therapies.

608.10

FCE 28833A, A NOVEL KYNURENINE 3-HYDROXYLASE INHIBITOR: *IN VITRO* AND *IN VIVO* CHARACTERIZATION OF THE TWO ENANTIOMERS. M.Varasi*, C.Speciale, M.Cini, M.Marconi, M.Breda[1], A.Giordani, R.Bormetti and C.Post. Pharmacia & Upjohn, CNS Research and [1]Pharmacokinetic & Metabolism, Nerviano, (MI), Italy.

Modulation of the synthesis of endogenous neuroactive kynurenines has been proposed as a viable strategy for neuroprotection. In particular, inhibition of kynurenine 3-hydroxylase (KYN 3-OHase) has been shown to enhance the levels of endogenous kynurenic acid (KYNA) in the brain, and may thus increase the physiological defensive mechanisms of the brain. Indeed, FCE 28833A [(R,S)-3,4-dichlorobenzoylalanine], the most potent KYN 3-OHase inhibitor described so far (K_i=0.13 μM), provides neuroprotection in the gerbil model of global ischemia (Speciale et al., this meeting). When administered orally to gerbils or rats at 400 mg/kg, brain tissue levels of KYNA showed peak increases of 60- and 14-fold, respectively, by 8 hrs after the treatment. The competitive mechanism of enzyme inhibition was demonstrated by kinetic analysis. The enantiomeric (R)- and (S)-forms of FCE 28833A, termed FCE 29196A and FCE 29191A, respectively, inhibited KYN 3-OHase activity in vitro with K_i values of 1.8 and 0.068 μM. Subsequently, the two enantiomers were separately administered orally to gerbils at a dose of 400 mg/kg, and the time curve of increases in brain KYNA levels was examined. Whereas the effect of FCE 29196A paralled the one obtained with the racemate, the increase evoked by the S-enantiomer was shorter-lasting. Consistently, drug distribution studies in plasma and brain, performed by assessing the amount of the R- and the S-form after treatment with FCE 28833A, revealed a prolonged presence of the R-enantiomer in blood and brain. Full understanding of the *in vivo* effects of currently available KYN 3-OHase inhibitors provide insightful information for the design of future molecules.

608.11

FURTHER CHARACTERIZATION OF KYNURENIC ACID-SYNTHESIZING ENZYMES IN THE RAT BRAIN. P. Guidetti* and R. Schwarcz. Maryland Psych. Res. Ctr., Baltimore, Maryland 21228.

The endogenous neuroprotectant kynurenic acid (KYNA) is produced by irreversible transamination of L-kynurenine (KYN). In the brain, two distinct kynurenine aminotransferases (KAT I and KAT II) appear to be responsible for the neosynthesis of KYNA. The present experiments were designed to examine the respective roles of the two KATs in KYNA production in the normal rat brain. To this end, the two enzymes were partially purified, and their characteristics were examined. KAT I (identical with glutamine transminase K) had an optimal pH of 9.5, preferred pyruvate as a co-substrate and was potently inhibited by glutamine. KAT II (identical with α-aminoadipate transaminase) had a neutral optimal pH, showed no preference for pyruvate and was essentially insensitive to inhibition by glutamine. Notably, KAT II was selectively inhibited by quisqualic acid (IC_{50}=450 μM). The endogenous substrate 3-hydroxykynurenine had an approximately ten-fold preference for KAT II. These enzyme properties enabled us to measure brain KAT I and KAT II in parallel using dialyzed tissue homogenate (to remove interfering endogenous amino acids). Under these conditions, both enzymes presented the same apparent K_m values as the partially purified enzymes. Using dialyzed tissue and employing the selective inhibitors glutamine and quisqualate as tools, the respective roles of the two enzymes were examined at different pH values. The data indicated that under physiological conditions the majority of brain KYNA derives from KAT II. However, at more alkaline pH KAT I might play an increasingly important role in cerebral biosynthesis of KYNA.

Supported by USPHS grant NS 28236.

608.12

KYNURENIC ACID PRODUCTION IN THE RAT STRIATUM: ACUTE AND CHRONIC EFFECTS OF QUINOLINATE LESIONS. G. Ceresoli*, P. Guidetti and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, Maryland 21228.

Intrastriatal injection of quinolinic acid (QUIN) in rats provides an animal model for Huntington's disease (HD). We have used this model to study the short- and long-term effects of the QUIN-induced lesion on the production of the endogenous neuroprotectant kynurenic acid (KYNA). Tissue levels of KYNA and its bioprecursor L-kynurenine (KYN), and the activities of kynurenine aminotransferases (KAT) I and II, were measured in the striatum after the focal injection of 300 nmoles QUIN. 2 hrs after the QUIN injection, KYNA levels were increased by 53%, whereas KYN, KAT I and KAT II remained unchanged. Two days later, both KYNA and KYN levels were elevated (to 630% and 173% of controls, respectively), KAT I activity was unchanged and KAT II activity was decreased by 33%. By 7 days after the QUIN injection, there were no changes in KYN levels and KAT II activity, a two-fold increase in KYNA levels and a 29% reduction in KAT I activity. At 1, 2, and 5 months after the lesion, 2 - 2.5-fold increases were observed in both KYNA levels and KAT II activity. KAT I activity was decreased by 24% and KYN levels were unchanged. These data show that multiple mechanisms are in place at different stages of the degenerative process to effect an elevation in the concentration of brain KYNA. The results also indicate that both KAT I and KAT II are present in neuronal *and* non-neuronal cells, and that KAT II is primarily responsible for the observed KYNA increases in the chronically lesioned striatum. Since this contrasts with the *decreases* in KYNA and KAT II activity seen in the HD striatum (J. Neurol. Sci. 130: 39, 1995), KAT II may play a role in the pathogenesis of HD.

Supported by USPHS grant NS 28236.

608.13

PROPOFOL ACTIONS ON TWO FORMS OF GABA-MEDIATED INHIBITION IN HIPPOCAMPAL CA1 NEURONS

S. Pittson and M. B. MacIver*. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

Anesthetics have been shown to enhance GABA-mediated synaptic inhibition, but it is not known whether both feedforward and feedback inhibition contribute to the depressant effects. The GABA receptor antagonist, bicuculline, was used to probe involvement of enhanced inhibition in the depressant effects produced by halothane and propofol. Anesthetic effects on excitatory postsynaptic potential (EPSP) and paired pulse population spike (PS) responses were studied to determine effects on feedforward or feedback inhibition, respectively. Both anesthetics depressed PS responses to ~ 100% at clinical concentrations of 250 μM (1.0 rat MAC) and 30 μM for halothane and propofol, respectively. EPSP responses were depressed by ~ 30 % at these concentrations. Bicuculline (10 μM) reversed the halothane-induced PS depression by > 20 %, but did not reverse EPSP effects. In contrast, the propofol-induced depression of PS responses was reversed by > 70 % and EPSP responses by > 80 % in the presence of bicuculline. These results suggest that halothane preferentially enhances feedback inhibition while propofol enhances both forms of GABA-mediated inhibition equally.

This study was supported in part by NIH grant GM49811.

608.14

STEROIDS MODULATE KAINATE-INDUCED ELECTRICAL RESPONSES IN OXYTOCINERGIC SUPRAOPTIC NEURONS THROUGHOUT LACTATION IN RATS. J.M. Israel, H. Breton, D. Theodosis* and D. Poulain. INSERM 378, Université Bordeaux 2, 33076 Bordeaux, France.

To study the role of glutamatergic inputs on the electrical properties of oxytocinergic (OT) neurons, intracellular recordings were performed on immunocytochemically identified neurons in rat hypothalamic slices (450 μm), obtained from rats at an early (5 days, EL) or late stage of lactation (20 days, LL). During long recording periods (4-6 h) most basal electrical properties were similar at the 2 stages of lactation. At both stages, OT neurons spontaneously displayed slow (or silent), continuous and bursting patterns of electrical activity; some cells switched from one pattern to the other spontaneously. All cells exhibited after-hyperpolarizations (AHP) and/or depolarizing after potentials (DAP). However, in LL cells, an additional outward rectification was observed when depolarizing from hyperpolarized potentials. In EL cells, a spike frequency adaptation was noted during depolarizing current pulses. All neurons responded to the glutamate agonist, kainate, with a depolarization, which triggered or accelerated firing. Nevertheless, EL cells appeared more sensitive to kainate since they responded at concentrations of 10^{-5} M, versus 10^{-4} M for LL cells. 17-β-estradiol (10^{-7} M, 20 min) did not modify the membrane potential, despite a weak increase in membrane resistance. However, it potentiated kainate-induced responses in LL, but not in EL, neurons, an effect which was reversible after washing. These results show that responses to glutamate in OT cells changes in the course of lactation, and that these changes may be partly modulated by estradiol.

608.15

EFFECTS OF HALOPERIDOL ON RAT EXCITATORY AMINO ACID RECEPTORS: COMPARING THE XENOPUS OOCYTE EXPRESSION SYSTEM WITH THE CORTICAL WEDGE PREPARATION. H.L. Sudan, K.S.J. Thompson, I. Phillips, P.L. Needham, K.F. Martin*, and D.J. Heal. Knoll Pharmaceuticals Research and Development, Nottingham NG2 3AA, U.K.

Glutamatergic systems have been implicated in schizophrenia and are potential targets for neuroleptics. We have compared the effects of haloperidol (HAL) on glutamate receptors expressed in *Xenopus* oocytes injected with rat brain RNA with those found occuring naturally in the rat brain cortex.

In voltage-clamped *Xenopus* oocytes, NMDA responses were potentiated by HAL in some oocytes (n=7), but concentration-dependently inhibited (10^{-9}-10^{-3}M HAL) in others (n=14; IC_{50}=1.8"0.9:M); however, complete inhibition was not achieved. Antagonism by HAL was voltage-dependent (7/8 oocytes) and only partially reversible by washing, suggesting that HAL does not readily dissociate from the NMDA receptor. Kainate responses were weakly inhibited by HAL and failed to achieve 50% inhibition (IC_{50}>10^{-3}M); the inhibition was concentration-dependent, non-competitive and readily reversed by washing. In the cortical wedge preparation, HAL (1-100:M) had no effect on NMDA responses. However, kainate responses were reduced by HAL (1-100:M) in a concentration-dependent manner although complete inhibition was not observed.

The concentration of HAL effective in the oocyte experiments compares well with its affinity for the NMDA receptor-ion channel F site (IC_{50}=1.9:M), suggesting the oocyte is a valid model for assessing functional receptor interactions. However HAL's lack of effect in the rat cortical wedge raises important questions of the pharmacological relevance of these interactions.

Financial support provided by Knoll Pharmaceuticals.

608.16

HALOTHANE ENHANCES SPONTANEOUS GABA RELEASE

V. A. Doze*, H. S. Lukatch, F. A. Monroe and M. B. MacIver. Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

This study investigated the effects of the volatile anesthetic, halothane, on spontaneous GABA release. Hippocampal brain slices (500 μm thick) were prepared from male Sprague-Dawley rats (80 to 120 gm) using an experimental protocol approved by the institutional animal use committee. Whole cell voltage-clamp recordings from CA1 pyramidal cells were used to measure the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) arising from local circuit GABAergic interneurons. Hippocampal interneuron and pyramidal cell discharge activity were also studied with whole-cell and cell-attached patch recordings, respectively. The volatile anesthetic, halothane, at clinically relevant concentrations (1.2 vol. %, 1 rat MAC) caused a >100% increase in IPSC frequency (5 ± 3 Hz, control; 12 ± 5 Hz, halothane; n = 5, $p < 0.01$). The effect of halothane persisted in the presence of glutamate receptor antagonists (8.5 μM CNQX and 50 μM APV; n = 5), suggesting a direct action on GABAergic interneurons. In a whole-cell recording from a hippocampal interneuron, halothane at 1.2 vol. % caused a membrane depolarization (>15 mV) which increased spontaneous action potential discharge frequency from ~1 Hz in control to ~4 Hz in the presence of halothane. In contrast, in cell-attached patch recordings from pyramidal cells (n = 3), administration of halothane was associated with an cessation of all spontaneous discharge activity. All effects of halothane were fully reversible upon washout. These results suggest that halothane may act directly on hippocampal interneurons to increase their discharge frequency, leading to enhanced GABA release. This excitatory action of halothane on surrounding inhibitory interneurons may contribute to the halothane-induced decrease in the spontaneous activity of excitatory pyramidal neurons and the potent anesthetic actions of halothane observed *in vivo*.

This study was supported in part by NIH grant GM49811.

608.17

DIFFERENTIAL EFFECTS OF A NOVEL GSH ADDUCT ON GSH AND NMDA RECEPTORS IN CORTEX. C.A. Shaw[1,2,3*], B.A.Pasqualotto[3], K. Curry[5], S.U. Kim[4], and M. Langmuir[6]. Depts.: Ophthalmology[1], Neuroscience[2], Physiology[3], Medicine and Neurology[4], Univ. of British Columbia, BC, Canada; Precision Biochemicals[5], Vancouver; Covalent Associates[6], Woburn, Mass.

We have used a variety of methods to examine the characteristics and distribution of a fluorescent adduct to GSH (GSHR-1) in an *in vitro* cortical slice preparation and in cortical neurons and astrocytes grown in culture. Field potential recordings from adult rat cortical slices showed typical responses to EAA agonists NMDA and AMPA and large depolar-izing potentials to GSH and cysteine (Shaw et al., NeuroReport, 1996; Pasqualotto et al., this meeting). NMDA and cysteine responses were reversibly blocked by AP5 and the GSHR-1 while the GSH response was unaffected, suggesting that GSHR-1 was exerting its effects on the cysteine modulatory site of the NMDA receptor. In human fetal cortical astrocytes and neurons in culture, GSHR-1 preferentially labelled GSH binding sites but had a low affinity for NMDA or other EAA receptors. The present data suggest similarities between NMDA and GSH binding sites which may have an age-dependent component. GSHR-1 is also likely to prove a useful tool for localizing the cysteine binding site of NMDA receptors in various cell preparations. Supported by NSERC Canada (CAS) and NIH (ML).

608.18

EXCITATORY ACTIONS OF GSH IN NEOCORTEX. B. A. Pasqualotto*[2], K. Curry[4], and C. A. Shaw[1,2,3], Depts. of Ophthalmology[1], Physiology[2] and Neuroscience Program[3], University of British Columbia and Precision Biochemicals Inc.[4], Vancouver, BC, Canada.

The reduced form of the tripeptide glutathione (GSH) serves as a free radical scavenger and antioxidant defense mechanism and is involved in the detoxification of xenobiotics. In addition to these functions GSH may also act as an excitatory neuropeptide. Application of GSH to cortical slices produces a large, rapid depolarizing membrane potential shift in the cortical wedge recording preparation. This depolarization is concentration dependent and is not blocked by antagonists of the excitatory amino acid glutamate. GSH-induced depolarization is not attenuated by calcium or potassium removal from the extracellular medium but is blocked by sodium removal. GSH-induced depolarization does not appear to be mediated indirectly by breakdown into its constituent amino acids since neither glutamate, cysteine, nor glycine produce qualitatively or quantitatively similar effects. In contrast to GSH, the oxidized form of glutathione (GSSG) had no effect on membrane potential. These results demonstrate a novel role for GSH as an excitatory neuropeptide and indicate the existence of a novel GSH-gated, Na^+-permeable ionotropic receptor. Supported by a Natural Sciences and Engineering Research Council of Canada grant to C.S.

608.19

RECEPTORS RESPONSIBLE FOR DEPOLARIZATIONS INDUCED BY BRIEF PULSES OF KAINIC ACID IN SLICES OF GUINEA PIG HIPPOCAMPUS. C. Yamamoto, S. Sawada, T. Ohno-Shosaku, N. Kobayashi and Y. Fukuda*. Dept. of Physiology, Fac. of Med. Kanazawa Univ., Kanazawa 920, Japan.

A brief pulse of kainic acid (KA) administered to proximal portions of apical dendrites of CA3 pyramidal neurons induces a fast depolarization and a following slow depolarization in these neurons. The purpose of this study was to determine subtypes of excitatory amino acid receptors responsible for these depolarizations. Transverse slices of the hippocampus were prepared from guinea pigs anesthetized with ether. Membrane potentials were recorded intracellularly from pyramidal neurons in the subfield CA3, and brief pulses (100-150 ms duration) of L-glutamate (Glu) or KA were ejected in the stratum lucidum at the spots where Glu pulses induced large depolarizing potentials in impaled neurons. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) suppressed both the fast and slow depolarizations induced by KA. The action of CNQX was more potent on the fast response than on the slow one. While aniracetam augmented Glu-induced depolarizations, it had no effects on the fast and the slow depolarizations induced by KA. These findings suggest that the fast and slow KA-responses result from activation of AMPA-specific and KA-specific receptors, respectively. This work was supported by a grant from the Ministry of Education, Science and Culture of Japan.

608.20

NEW PHOTOLABILE CAGED PRECURSORS OF KAINATE AND NMDA, INERT PRIOR TO FLASH PHOTOLYSIS K.R. Gee,[1] L. Niu,[2] K. Schaper,[2] C. Grewer,[2] and G.P. Hess.[2]* [1]Molecular Probes, Inc., Eugene, OR 97402 and [2]Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853.

Photolabile ("caged") precursors of neurotransmitters have proven useful in the study of receptor activation mechanisms, receptor localization, and mapping of synaptic connections. Pulsed photolysis rapidly generates free neurotransmitter with better temporal and spatial resolution than is possible using simple diffusion of neurotransmitter solutions. We report here synthesis and photochemical characterization of a new caged version of kainic acid, in which the γ-carboxylate is protected as a photolabile ester of 2-methoxy-5-nitrophenol. The caged compound does not induce any response on cultured rat hippocampal neurons before photolysis, nor does it potentiate or inhibit the response to free kainate. UV photolysis generates free kainate in the nanosecond time region, with a photolysis quantum yield of 0.21. A new caged version of NMDA has also been prepared, in which the β-carboxylate is protected as a photolabile ester of 2,2'-dinitrobenzhydrol. Again, the caged compound does not induce any response on cells before photolysis, nor does it potentiate or inhibit the response to free NMDA. These results represent the first caged NMDA that is inert before photolysis. UV photolysis generates free NMDA with a time constant of 6 μs and a quantum yield of 0.18. Supported by Molecular Probes, Inc., a DFG Fellowship (K.S.), an A. Humboldt Fellowship (C.G.), and NIH GM 04842 (G.P.H.).

CRF RECEPTORS: STRUCTURE AND FUNCTION

609.1

EFFECT OF CRH, α-hCRH AND CRH_1 ANTAGONISTS ON SOCIAL INTERACTION IN RATS. K. W. Rohrbach*, A.J. Cocuzza, R. Bakthavatchalam, W. J. Keim, J.F. Cawley, C. W. Johnson. Central Nervous System Diseases Research, The DuPont Merck Pharmaceutical Company, Wilmington DE 19880-0400.

CRH has been implicated in the pathophysiology of anxiety. CRH levels are increased in anxious conditions in humans and CRH produces effects in experimental animals consistent with increased anxiety and known anxiolytic agents decrease these effects. The social interaction test in rats has been used as an experimental animal test of anxiety, and has been shown to be sensitive to a range of anxiolytic treatments. CRH has been shown to produce an anxiogenic action in the social interaction test (Dunn and File, 1987). The following experiments were carried out to further characterize the effect of CRH in the social interaction test and to examine the effect of α-hCRH and selective CRH_1 antagonists on CRH and bright light-induced effects on social interaction. CRH, when administered i.c.v., produced a dose related decrease in social interaction. Light produced an intensity related decrease in social interaction. Both of these effects were reversed by chlordiazepoxide and α-hCRF. The previously reported selective CRH_1 receptor antagonist YY941 antagonized both CRH and light-induced decreases in social interaction as did the newly reported selective CRH_1 receptor antagonist SC241. The data are consistent with the interpretation that CRH induces a state of anxiety in rats, that the decrease in social interaction induced by bright light is mediated by CRH and that a selective CRH_1 receptor antagonist may have utility as an anti-anxiety agent in humans.

609.2

COMPARISON OF A CRH RECEPTOR ANTAGONIST AND KNOWN ANXIOLYTIC AGENTS ON DEFENSIVE WITHDRAWAL AND LOCOMOTOR ACTIVITY BEHAVIOR IN RATS. K.A. Weidemann, K.L. Zeller*, A.L. Fetterman and J.F. McElroy CNS Diseases Research, The DuPont Merck Pharmaceutical Co., Experimental Station, P.O. Box 80400, Wilmington, DE 19880-0400.

Rats placed in a small darkened chamber located in an unfamiliar open field spend most of their time within the small chamber (i.e., defensive withdrawal), an action consistent with a state of heightened anxiety. Rats pretreated systemically (ip) with the anxiolytic agent chlordiazepoxide (CDP) readily emerge from the chamber and explore the open field. This effect is dose-dependent, and the 50% effective dose (ED_{50}) is 7.3 mg/kg. Direct intracerebroventricular (icv) administration of CDP (5 ug/rat) similarly reduced the latency to exit the chamber. This ~ 400-fold increase in potency (versus ip dosing) is consistent with a central site of CDP action. Two additional anxiolytic agents, pentobarbital (ED_{50} = 15mg/kg) and meprobamate (ED_{50} = 41 mg/kg), also reduced exit latencies in a dose-dependent manner. The anxiolytic-like action of each of these agents occurred at doses 2- to 8-fold lower than doses required to reduce open-field locomotor activity, a behavioral effect employed as an index of non-specific drug action. The anxiolytic agent buspirone was inactive in the defensive withdrawal paradigm at all doses tested (0.1-30 mg/kg), including doses that markedly decreased locomotor activity (3-30 mg/kg).

Corticotropin Releasing Hormone (CRH), a 41 amino acid peptide widely distributed throughout the CNS, has been implicated in anxiety and stress related disorders. Similar to CDP, pentobarbital, and meprobamate, the CRH antagonist α-helical CRH_{9-41} (20 ug/rat, icv) reduces exit latencies in defensive withdrawal. Results suggest that α-helical CRH_{9-41}, or preferably a non-peptide CRH antagonist, might represent a useful anxiolytic agent. Funding source: commercial.

609.3

CORTICOTROPIN RELEASING FACTOR (CRF) MEDIATES ACTH RELEASE AND HYPOTENSION BY TWO PHARMACOLOGICALLY DISTINCT CRF RECEPTOR SUBTYPES. D.R. Luthin, K.L. Youngblood, A.Rabinovich, M.R.Brown* and J.M. May. Alanex Corporation, San Diego, CA 92121.

Multiple CRF receptor subtypes with distinct brain and peripheral localization have recently been cloned and characterized. Using several peptide and non-peptide antagonists, we have compared the pharmacological profile of the CRF receptor subtypes mediating ACTH release and hypotension with the ability of compounds to displace ^{125}I-CRF binding to rat brain membranes and to recombinant CRF1 and CRF2β receptors.

Compound	CRF1 IC_{50} (nM)	CRF2β IC_{50} (nM)	Rat Brain IC_{50} (nM)	Block of CRF ↑ACTH	Block of CRF ↓BP
oCRF	2.2 ± 0.5	3.1 ± 0.2	3.6 ± 0.5	NA	NA
αH-CRF (9-41)	42 ± 3.3	3.5 ± 0.6	23.6 ± 8.1	-	+++
Tyr^{32}-Astressin[1]	3.6 ± 0.4	2.4 ± 0.4	1.4 ± 0.2	+++	+++
Compound A[2]	34 ± 2	>10000	28 ± 5.3	+	-
Compound B[3]	124 ± 44	>10000	145 ± 49	+	-
Compound C[4]	121 ± 11	5300 ± 1800	413 ± 35	++	-

[1]Gulyas *et al.*, PNAS 92:10575-10579, 1995.
[2]Example 5i, WO 94/13677, Pfizer Pharmaceuticals.
[3]Example 78, WO95/10506, DuPont Merck Pharmaceutical Co.
[4]Anti-parallel homodimeric peptide: Pmp-Arg-Lys-Cys-Cha-Asp-Ile-Ala-NH_2;Pmp=3-Mercapto-3,3-cyclopentamethylene proprionic acid; Cha = Cyclohexylalanine.

These results suggest that the CRF receptor subtypes mediating ACTH release and hypotensive responses are the CRF_1 and $CRF_{2\beta}$ receptors, respectively.

609.4

CRF_1 RECEPTOR BINDING, mRNA EXPRESSION AND SIGNAL TRANSDUCTION AFTER CHRONIC ANTIDEPRESSANT TREATMENT IN RATS. S.C. Stout*, M.J. Owens, C.B.Nemeroff; Dept. of Neuropsychopharmacology, Dept. of Psychiatry and Behavioral Sciences, Emory Univ. Sch. Med., Atlanta, GA 30322.

Corticotropin-releasing factor (CRF) is the major regulator of the hypothalamic-pituitary-adrenal (HPA) axis, and also plays a role in non-endocrine stress responses. Patients with major depression exhibit HPA axis hyperactivity including increases in hypothalamic CRF mRNA synthesis, and increases in CSF CRF concentration. Successful psychopharmacologic treatment reverses most of these alterations. Brady et al. reported that chronic antidepressant treatment redcues CRF mRNA synthesis in the paraventricular nucleus of the rat hypothalamus under basal, non-stressed conditions. We wished to examine whether antidepressants also produce CRF receptor changes in the CNS and pituitary gland. Desipramine (12 mg/kg/day), paroxetine (6 mg/kg/day) or vehicle were infused by osmotic minipump into adult, male Sprague-Dawley rats. Brains from several treatment batches were frozen, dissected, and processed for ^{125}I-oCRF binding, rCRF-stimulated adenylyl cyclase activity, and ribonuclease protection assays (n = 5 to 12 per group per assay). Although preliminary work suggested an upregulation of cortical and pituitary CRF receptors by desipramine, further investigation failed to demonstrate oCRF binding changes with either drug. 1 mM rCRF stimulation of adenylyl cyclase was unchanged in pituitary and frontal cortex. Expression of CRF_1 receptor mRNA was unchanged in pituitary, cortex, and several other brain regions, but was increased in the hypothalamus of drug-treated rats. These studies suggest that widespread alterations of CNS CRF_1 receptor binding and expression by antidepressants do not occur in non-stressed rats. Supported by NIH MH-42088 and NIH MH-51761

609.5

CRF RECEPTOR DESENSITIZATION AND RECOVERY IN HUMAN RETINOBLASTOMA Y-79 CELLS. R.L. Hauger[1,2], F.M. Dautzenberg[1], L.L. Judd*[2], and J. Spiess[1,2]. Dept. Molec. Neuroendo., Max Planck Inst. Exp. Med[1]., 37075 Goettingen & Dept. Psychiatry, UCSD[2], La Jolla, CA.

Many G-protein receptors become rapidly desensitized by their ligand concentrations in the μM range, and rapid recovery typically occurs upon agonist removal. Since the stimulatory action of CRF during stress may require rapid counterregulation, we determined the time course for CRF receptor desensitization (CRFR des) in human retinoblastoma (Y-79) cells. CRFR des was characterized by an EC_{50} of 6.34±0.78 nM ovine CRF, a rapid onset at 10 min, and a $t_{1/2}$ of 38 min. Since the ability of VIP, isoproterenol, and forskolin to increase cAMP accumulation was not diminished in Y-79 cells desensitized to CRF, the observed CRFR des is considered to be a specific homologous action of CRF. Its specificity was also confirmed by CRFR des being markedly attenuated in the presence of a CRFR antagonist which by itself was not found to desensitize CRFR. The homologous CRFR des was associated with a >60% loss of CRFR from the Y-79 cell surface after 1 h exposure to 10 nM CRF. Since CRFR, type 1 (CRFR1), was identified on the basis of cDNA data to be the predominant receptor form in Y-79 cells, it was concluded that CRFR1 was responsible for the observed CRFR des. CRFR1 mRNA levels were not altered over 24 h of CRFR des in contrast to the rapid decrease in CRFR1 mRNA reported for CRFR des in anterior pituitary cells. The $t_{1/2}$ for the recovery from CRFR des in Y-79 cells was ~13 h with a full reversal by 24 h. In conclusion, we propose that differential mechanisms mediate the homologous receptor desensitization and recovery in cells of different origin [Supported by the Max Planck Society and the UCSD Dept. of Psychiatry.]

609.6

DIFFERENTIAL REGULATION OF TYPE-1 AND TYPE-2α CORTICOTROPIN-RELEASING HORMONE RECEPTOR mRNA IN THE PARAVENTRICULAR NUCLEUS BY INTRAPERITONEAL LIPOPOLYSACCHARIDE INJECTION AND ADRENALECTOMY. T. Takemura, S. Makino*, T. Takao and K. Hashimoto, 2nd Dept of Internal Medicine, Kochi Medical School, Okoh-cho, Nankoku, Kochi 783, Japan.

Novel corticotropin-releasing hormone receptor (CRHR), designated type-2α CRHR (CRHR-2α), was recently cloned and functionally characterized. In situ hybridization study revealed that CRHR-2α mRNA had a distinct distribution from type-1 CRHR (CRHR-1) mRNA in the rat brain. Interestingly, CRHR-2α mRNA showed a relatively high expression in the hypothalamic paraventricular nucleus (PVN) even under unstressful condition. This may reflect the important role of CRHR-2α in the autoregulation of CRH secretion in the PVN. To determine the regulation of CRHR-2α mRNA expression in the PVN, we examined the alteration of CRHR-2α mRNA levels in the PVN in rats with lipopolysaccharide (LPS) injection or adrenalectomy, and compared with that of CRHR-1 mRNA, using in situ hybridization histochemistry. Intraperitoneal LPS injection (50 μg) induced a significant increase in PVN CRHR-1 mRNA at 3 and 6 hours, whereas CRHR-1 mRNA levels in the PVN in adrenalectomized rats were significantly lower than that in sham-operated rats when sacrificed 7 days after surgery. This alteration in PVN CRHR-1 mRNA is consistent with previous reports. In contrast, CRHR-2α mRNA levels in the PVN were not altered by LPS injection or adrenalectomy. These results indicate that CRHR-1 and CRHR-2α mRNA are differentially regulated in the PVN. Further study will be necessary to elucidate the CRHR-2α function in the PVN.

609.7

MOLECULAR CLONING AND EXPRESSION ANALYSIS OF HUMAN CRH RECEPTOR TYPE 2 α AND β ISOFORMS. W. Kostich, A. Chen, K. Sperle, R. A. Horlick^, J. Patterson, and B. L. Largent*; DuPont Merck Research Labs, CNS Diseases Research and ^Applied Biotechnology, Wilmington, DE 19880.

The neuropeptide Corticotropin Releasing Hormone (CRH) is an integral player in the HPA axis and is known to be present in a number of brain regions. CRH is bound by at least two G-protein linked receptor families termed type 1 and type 2 (R1 and R2). The R2 receptor was recently cloned in rat and mouse and exists in two isoforms, termed α and β, which have alternative exons at the amino terminus. We have cloned the human homologues of the R2 α and β isoforms from a human amygdala cDNA library and have characterized their expression in human tissues with quantitative RT-PCR. The human α isoform is approx. 94% identical at the amino acid level to rat α, corroborating the human CRHR2α sequence reported by Liaw et.al. (Endocrinology.(1996) 137:72-77). The human β isoform is approx. 89% identical at amino acid level to rat β. The β isoform specific exon at the amino terminus is only approx. 63% conserved between human and mouse at the nucleotide level and 55% at the amino acid level. The α isoform specific exon is much better conserved between rat and human with 92% identity at the amino acid level. Quantitative RT-PCR analysis of R2α expression in human CNS tissues shows that R2α is present in many of the same tissues as in rat. Rat septum has been noted for higher levels of R2α expression, however, in human septum we do not find high R2α expression. Also interestingly, in human heart RNA we find significant R2α expression without strong R2β expression, which is in direct opposition to what we observe for rat. Heterologous expression of human CRHR2α in HEK293EBNA cells reveals a similar pharmacological agonist profile to rat CRHR2α for stimulation of adenylate cyclase, with EC50 for sauvagine < urotensin < r/h CRH.

609.8

HUMAN CRF RECEPTOR CHIMERAS: MAPPING OF LIGAND BINDING DETERMINANTS R.L. Martone, J.M. Cook, A.W. Schmidt,#+ Y.C. Clancy, C.L. James, D.W. Schulz# & J.R. de Wet Depts. of Molecular Sciences and #Neuroscience, Pfizer Central Research, Groton, CT 06340

Multiple physiological and behavioral effects of corticotropin releasing factor (CRF) are mediated by the G protein-coupled receptor, CRF_1. CRF_1 shares approx 70% amino acid sequence identity with the CRF_2 receptor, but these receptors have significantly different pharmacological profiles in response to CRF, the related peptides sauvagine (SV) and urotensin I (UI), and to the non-peptide CRF_1-specific antagonist CP-154,526. In order to elucidate the physical basis for these differences in pharmacological behavior, we generated several CRF_1/CRF_2 and CRF_2/CRF_1 chimeras by exchange of specific receptor cDNA restriction fragments, and by using the technique of random chimeragenesis. In the latter technique, a RecA+ *Escherichia coli* strain was transformed with linearized plasmids containing tandemly arranged single copies of each receptor cDNA. Homologous recombination between the receptor subtype cDNAs resulted in the generation of circular plasmids containing single chimeric receptor cDNAs. Several receptor chimeras were transiently expressed in COS-7 cells, and binding studies were performed using the radioligand [^{125}I-Tyr^0]-Sauvagine. Subtype-specific affinities for UI and ovine CRF appear to depend primarily upon the amino acid sequence of the first extracellular loop. The presence of the first extracellular loop of CRF_1 is necessary but not sufficient for binding the antagonist CP-154,526. The contribution of adjacent receptor sequences to the CRF_1-specific antagonist binding is suggested by the lack of CP-154,526 binding in both CRF_2/CRF_1 and CRF_1/CRF_2 chimeric receptors with crossovers in TM 3. These studies should contribute to the understanding of structure-function relationships in neuropeptide receptors.

These studies were supported by Pfizer Inc

609.9

THE N-TERMINAL DOMAIN OF THE CORTICOTROPIN RELEASING FACTOR (CRF) RECEPTOR IS A MAJOR BINDING DETERMINANT FOR THE CRF ANTAGONIST, ASTRESSIN.

M.H. Perrin, S.W. Sutton, W.T. Berggren and W.W. Vale* Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037

CRF receptors belong to a family of 7 transmembrane G-protein coupled receptors, which are characterized by relatively large N-terminal domains and which include the growth hormone releasing factor receptor (GRF-R). It has been shown that the N-terminal domain of the GRF-R contains major ligand binding determinants. In order to explore the binding determinants for the rat CRF-R1, we have constructed chimeric receptors in which domains of the rat CRF-R1 and the rat GRF-R (kindly provided by K.Mayo) have been exchanged. A chimera in which amino acids 1-127 of the rCRF-R are replaced by amino acids 1-135 of the rGRF-R and transiently expressed in COSM6 cells shows no binding to tracers related to CRF including the potent CRF antagonist, astressin (^{125}I-[D-Tyr^{12}]-astressin). The complementary chimera, in which amino acids 1-127 of the rCRF-R replace amino acids 1-135 of the rGRF-R (rCRF-R_N/rGRF-R) binds labeled astressin with high affinity; the K_D for astressin bound to this chimera is 16(6.2-42)nM, compared to 2.2(1.2-4.1)nM for the rCRF-R1 expressed in COSM6 cells. Both rCRF and the related novel mammalian peptide, urocortin, stimulate cAMP production in COSM6 cells expressing the rCRF-R_N/rGRF-R, but the maximal level of stimulation is reduced compared to that resulting from GRF stimulation of the rGRF-R or CRF stimulation of the rCRF-R1. Thus, determinants for binding and activation of the CRF-R reside in its N-terminal domain.

Supported by NIH DK-26741

609.10

STRUCTURAL AND MOLECULAR CHARACTERIZATION OF MAMMALIAN AND AMPHIBIAN CRF RECEPTORS

F. M. Dautzenberg, S. Sydow, A. Rühmann and J. Spiess*.
Dept. Molecular Neuroendocrinology, Max Planck Institute Exp. Med., Hermann-Rein-Str. 3, D-37075 Göttingen, Germany.

Corticotropin releasing factor (CRF) is the most important regulator of the secretion of hypophyseal ACTH and is involved in various endocrine, behavioral and autonomic responses to stress. In contrast to mammalian species, the CRF receptor (CRFR) from amphibian pituitaries does not respond to sauvagine. Thus, it was concluded that the involved receptor proteins differ structurally.

For the investigation of the molecular differences between the amphibian and the mammalian CRF receptor(s), a degenerate PCR with cDNA from various tissues of *Xenopus laevis* was performed. By subcloning of the resulting PCR products, two different partial cDNAs were identified. One cDNA encoded a protein with approximately 65% identity to human CRFR1, whereas the second cDNA encoded a polypeptide that was more than 80% identical to human CRFR2. Meanwhile, we have cloned both receptor cDNAs by a PCR-based approach. The sequence and tissue distribution of both CRF receptors will be provided.

To obtain further structural information on the receptor molecules, the N-terminus of the rat CRFR1 was overexpressed in *E. coli* and in mammalian cell lines. The N-terminal fragment which bound CRF specifically but with low affinity, is being used to characterize the binding site of the CRFR fragment cross-linked to a photoactivable CRF analog. Successively truncated forms of rat CRFR were expressed in mammalian cells to analyze the structural requirements for the formation of a CRF binding site with high affinity.

(F.M.D. and S.S. should be considered co-first authors; the work was supported by the Max Planck Society.)

609.11

REGIONAL DISTRIBUTION OF PUTATIVE CRH_2 RECEPTORS IN RAT BRAIN AS DETERMINED BY [^{125}I]SAUVAGINE RECEPTOR AUTORADIOGRAPHY.

C. M. Rominger*, W. Zhang, S. Ho, R. Zaczek, and L.W. Fitzgerald, CNS Diseases Research, The DuPont Merck Research Laboratories, Wilmington, DE 19880.

Corticotropin-releasing hormone (CRH) is a crucial mediator of the hypothalamic-pituitary-adrenal stress axis, and likely serves a central neurotransmitter role throughout the brain. CRH mediates these functions via at least two G-protein-coupled receptor subtypes (CRH_1 and CRH_2). We here describe a novel subtractive binding strategy for revealing high affinity CRH_1 and putative CRH_2 sites utilizing [^{125}I]sauvagine, which at 150 pM, labels both receptor subtypes but neglibly the CRH binding protein (reported K_i >15 nM). Adjacent brain sections were incubated with [^{125}I]sauvagine in the presence or absence of a potent triazolopyrimidine non-peptide CRH_1 antagonist which is inactive at CRH_2 receptors. Nonspecific binding of [^{125}I]sauvagine was defined in the presence of 0.5 μM α-helical CRH. [^{125}I]sauvagine, in the absence of CRH_1 blockade, produced a broad labeling pattern of cortical and subcortical regions that was concordant with previous reports using [^{125}I]oCRH. Interestingly, in the presence of CRH_1 blockade, specific labeling of the neocortex was virtually eliminated, whereas significant labeling of various subcortical regions remained. High density of residual, putative CRH_2 sites was observed in the choroid plexus and blood vessels, with moderate densities in the ventral medial hypothalamus, lateral septum, bed nucleus of the stria terminalis, and posterior cortical amygdala. These observations are very consistent with the reported distribution of CRH_2 mRNA. This strategy may be useful for studying the functional and regulatory properties of CRH_2-specific neuronal circuits in brain.

609.12

[^{125}I-TYR°]-SAUVAGINE AS A RADIOLIGAND FOR CRH-R2α RECEPTORS.

D.H. Rominger*, C.M. Rominger, A. Chen, K. Sperle, B. Largent, R. Wilk, R. Zaczek Central Nervous System Diseases Research, The Du Pont Merck Research Laboratories, Wilmington, DE 19880-0400

The *in vitro* binding properties of [^{125}I]-labeled tyr°-sauvagine, were examined in HEK 293 cells expressing the CRH-r2 alpha receptor. Non-specific [^{125}I-tyr°]-sauvagine binding is defined using 500 nM α-helical-CRH_{9-41}. Specific binding at 23°C is linear up to 100 μg protein. Specific binding is saturable and reversible with addition of excess α-helical-CRH_{9-41}. The cell line exhibits two populations of sites. A high-affinity site posseses an apparent K_d of 157 pM and a B_{max} of 67.4 *f*mol/mg protein. A low-affinity site posseses an apparent K_d of 5 nM and a B_{max} of 790 *f*mol/mg protein. [^{125}I-tyr°]-sauvagine binding to CRH-r2α receptors is completely inhibited by sauvagine, urotensin, α-helical-CRH_{9-41}, r/h-CRH and o-CRH. In contrast, novel non-peptide compounds, which are potent and specific antagonists for the CRH-r1 receptor subtype, do not inhibit [^{125}I-tyr°]-sauvagine CRH-r2α binding up to 10 μM. We also describe the use of [^{125}I-tyr°]-sauvagine, in the presence of these CRH-r1 antagonists, to specifically label CRH-r2α receptors in rat brain homogenates. These data demonstrate the utility of [^{125}I-tyr°]-sauvagine to specifically label and examine CRH-r2α binding in transfected cell lines and to distinguish between CRH-r1 and CRH-r2 selective antagonists.

609.13

IDENTIFICATION OF POTENT AND SELECTIVE NON-PEPTIDE ANTAGONISTS FOR THE HUMAN CORTICOTROPIN-RELEASING HORMONE$_1$ (CRH_1) RECEPTOR.

L.W. Fitzgerald*, P. Gilligan[2], S. Culp, R. Horlick, B. Largent, G. Aguilera[3], S. Keim, C. Krause, A. Marshall, J. Patterson, C. Rominger, D. Rominger, A. Logue, R. Bakthavatchalam[2], A. Arvanitis[2], T. Christos[2], A. Cocuzza[2], R. Zaczek, CNS Diseases Research, [2]Chemical & Physical Sciences, The DuPont Merck Research Laboratories, Wilmington, DE 19880, and [3]NICHD, NIH, Bethesda, MD 20892.

CRH plays an important integrative role in regulating the endocrine, autonomic, immunological, and behavioral responses to stress. Moreover, CRH has been implicated in the pathophysiology of anxiety and other neuropsychiatric disorders. We describe the identification of novel triazolopyrimidine and anilinotriazine CRH_1 antagonists that potently inhibit (Ki's=4-50 nM) the binding of [^{125}I]oCRH to hCRH_1 receptors stably expressed in HEK 293 cells and native receptors in rat cortex. These compounds as well as α-helical CRH inhibited CRH-stimulated adenylyl cyclase activity in HEK 293 cells and rat brain, and CRH-stimulated ACTH release in AtT20 cells, with rank-ordered potencies predicted by their binding affinities. Additionally, these compounds displayed selectivity for the CRH_1 receptor subtype as they did not inhibit [^{125}I]sauvagine binding to expressed $CRH_{2\alpha}$ receptors, or [^{125}I]r/hCRH binding to the CRH binding protein (35-40 kDa) derived from human plasma as determined by SDS-PAGE. Novel non-peptide antagonists for CRH receptors may have unique utility in the treatment of anxiety, depression, and other disorders in humans.

609.14

NON-PEPTIDE CORTICOTROPIN-RELEASING HORMONE (CRH) RECEPTOR ANTAGONIST: CHARACTERIZATION OF BINDING.

E. L. Webster*, D. Lewis, D. Torpy, K. Zachman, K. Rice, G. Chrousos, Pediatric Endo. Sect., NICHD and Med. Chem. Br., NIDDK, NIH, Bethesda, MD 20892.

CRH is a major regulator of the hypothalamic-pituitary-adrenal (HPA) axis and principal coordinator of the stress response, including its neuroendocrine, autonomic, immune, and behavioral components. Specific membrane receptors bind to and mediate the actions of CRH throughout the central nervous system and periphery. Recently, subtypes of the CRH receptor have been cloned, and characterized in terms of their pharmacological specificity and regional localization. Existing peptide antagonists for the CRH receptors cannot be given parenterally, are rapidly degraded, have limited bioavailability and, therefore, limited research and therapeutic usefulness. Recently, we prepared a nonpeptide CRH receptor antagonist compound, trimethyl-7-(2,4,6-trimethyl-phenyl-7H-pyrrolo[2,3-d]N-butyl-N-ethyl[2,5,6-pyrimidin 4-yl] amine, for use as a research tool to further characterize specific membrane receptor proteins, which interact with CRH to produce biological actions associated with stress. The affinity and potency of the antagonist compound were analyzed in competitive displacement studies in tissue homogenates from rat frontal cortex, pituitary, cerebellum, and heart. The trimethylpyrrolamine compound was potent in displacing ^{125}I-oCRH binding and exhibited Ki values of 4.2 ± 0.8, 4.6 ± 1.7, and 7.1 ± 1.9 nM (mean ± SEM) in pituitary, cerebellum, and frontal cortex homogenates, respectively. Approximately 87% of the specific binding of ^{125}I-oCRH observed with 1μM rat/human CRH, was displaced in pituitary, cerebellum, and frontal cortex homogenates. Interestingly, this compound was not effective in displacing ^{125}I-oCRH binding from rat heart homogenates, a tissue expressing subtype 2 but not subtype 1 CRH receptors. Administration of the compound to intact rats for over 2 weeks revealed no apparent toxicity. We conclude that the nonpeptide CRH antagonist tested here is specific for type 1 CRH receptors and promises to enhance our understanding of the actions of CRH in normal and pathological states and in potentially providing a therapeutic tool.

Source of funding: NICHD Intramural

610.1

THE MURINE NPY-Y1 GENE PROMOTER DIRECTS EXPRESSION OF E.COLI *LACZ* TO BLOOD VESSELS IN THE NERVOUS SYSTEM OF TRANSGENIC MICE. Alessandra Oberto, Rossella Brusa, Emanuela Tolosano*, Fiorella Altruda*, Nicoletta Aste* and Carola Eva. Dept. Anatomy, Pharmacology and Forensic Medicine, Pharmacology Section, University of Torino and * Dept. Genetics, Biology and Medical Chemistry, University of Torino, Torino, Italy. .

The Y1 receptor subtype plays important roles in mediating NPY-induced control of several functions, including cardiovascular system activity, neuroendocrine secretion, food intake and nociception.

1.3 kb of 5' flanking of the murine Y1 receptor gene has been ligated upstream the bacterial *lacZ* gene and used to generate transgenic mice. Expression of the transgene has been detected in two independent transgenic lines that show overlapping pattern of ß-gal activity. In the embryo, at day 13 and 16 after fertilization, the transgene is expressed specifically in the posterior and lateral choroid plexi, in the otic vesicles and in the hypophysis in blood vessel structures. In the posterior region of the embryo body ß-gal staining is detectable at the level of the spinal cord.

Further analysis to define the transgenic expression during development and adult mice is in progress.

610.2

ISOLATION AND CHROMOSOMAL LOCALIZATION OF NOVEL HUMAN MEMBERS OF THE G PROTEIN-COUPLED RECEPTOR GENE (GPCR) FAMILY. B. O'Dowd*, T. Nguyen, B. Jung, A. Marchese, R. Cheng, H. Heng, L.F. Kolakowski, K. Lynch and S.R. George. Add. Res. Fdn., Dept. of Pharm., Univ. of Toronto; Hosp. for Sick Children, Toronto, ONT, Canada; Dept. of Pharm., Univ. of Texas, TX 78284, Dept. of Pharm., Univ. of Virginia, VA 22908.

The characterization of novel receptor systems in the brain continues in parallel with the discovery of endogenous peptides, however the ligands for many of these receptors, remain to be identified. Our research plan has been to identify GPCR genes involved in drug addiction, in particular opioid-like receptors which may bind opioid and related peptides. Our search for novel GPCRs has revealed 13 genes (at least 6 are expressed in the CNS) that presently have not been identified by pharmacological means: APJ, GPR1, GPR2, GPR3, GPR4, GPR5, GPR6, GPR7, GPR8, GPR9, GPR10, GPR14, GPR15. We now report the cloning of additional four genes named GPR16, GPR17, GPR18 and GPR19, each of which contain an intronless open reading frame encoding GPCRs. GPR16 was isolated by amplifying DNA with primers based on the opioid, opioid-like and somatostatin receptor genes. Searching of a human EST data base revealed cDNA sequences that partially encoded two novel GPCRs, and a third EST cDNA, encoding a novel GPCR, was identified by searching the GenBank data base. Each of these DNA clones were used as probes for screening a genomic library in order to isolate the full-length coding regions. A search of the GenBank data base with amino acid sequences of the receptors encoded by GPR16, 17, 18 and 19 revealed that the receptors were most closely related to the proteinase-activated receptor-2, the β1-adrenergic receptor, the cholecystokinin-B receptor, and the purinergic P2Y5 receptor, respectively. Northern blot analysis revealed expression of GPR16 and GPR18 in human brain regions and GPR16 in liver. Fluorescence in-situ hybridization was used to map GPR16 to chromosome 8, GPR17 to chromosome 9, GPR18 to chromosome 17, and GPR19 mapped to chromosome X. (Supported by MRC)

610.3

IDENTIFICATION AND REGULATION OF A NOVEL G - PROTEIN COUPLED RECEPTOR EXPRESSED IN GLIAL CELLS M.E. CHARLTON* and R.S. DUMAN. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508

Previously we described the identification of several PCR products which encoded fragments of novel putative G-protein coupled receptors. One of these fragments, VTR 15-20, isolated from the rat ventral tegmental area was used for hybridization screening of several cDNA libraries. A partial fragment isolated from a spleen library was ligated to a 3' RACE PCR product to form a full length VTR 15-20 clone. Sequence analysis revealed the highest overall amino acid homology with an orphan clone and lower homology to the PAF receptor. The 1.8 Kb VTR 15-20 transcript was detected in peripheral regions with highest levels of expression in the spleen as determined by northern blot. Lower levels were apparent throughout the CNS. In addition, RNA encoding VTR 15-20 was detected in several cells of glial (microglia and astrocytes) and hematopoietic (HL-60, murine B-cell line, and human monocytes) origin. The detection of VTR 15-20 expression in glial and blood elements suggests that this receptor may contribute to the function of the neuro-immune axis. Using several paradigms we have shown the differential regulation of VTR 15-20 expression. Chronic stress results in a 30% reduction of spleen mRNA levels as shown by northern blot. However, in a model of reactive gliosis, the expression of mRNA encoding VTR 15-20 increased approximately 5 fold in astrocyte and microglial cultures. We are currently investigating the regulation of VTR 15-20 by additional *in vitro* and *in vivo* models of neuronal insult. These studies suggest that VTR 15-20 may play a role in mediating the CNS response to injury / insult.
(Supported by DA 08227 Program Project Grant)

610.4

NEUROPEPTIDE Y-FAMILY RECEPTOR CLONES FOR Y1, Y2, AND PP1/Y4 IN THE GUINEA PIG. D. Larhammar*, H. Eriksson, S. Holmberg, and M. Berglund. Department of Medical Pharmacology, Uppsala University, Box 593, S-751 24 Uppsala, Sweden.

Neuropeptide Y (NPY) forms a family of structurally related peptides together with peptide YY (PYY) and pancreatic polypeptide (PP). Three receptors for these peptides have been cloned, namely Y1, Y2 and PP1/Y4, all of which couple to G proteins. Surprisingly, the Y2 sequence was found to differ dramatically from Y1 and PP1. Whereas the Y1 and Y2 sequences are highly conserved between species, the PP1 sequence is the most divergent heptahelix receptor sequence so far observed between man and rat. In addition, the PP1 receptor differs in pharmacological properties as well as tissue distribution between these species. To study these intriguing evolutionary differences, we decided to isolate receptor clones from various species including the guinea pig.

Clones were isolated from a genomic library by homology screening. The guinea pig PP1 sequence has approximately 82% overall identity to the human sequence but only 75% identity to the rat sequence. The latter displays 75% identity also to human PP1. Thus, although rat and human are evolutionarily more closely related to one another than either is to the guinea pig, the rat PP1 receptor is the most divergent. This agrees with the rat having the most divergent sequence for the receptor's ligand, PP, and suggests that receptor and ligand coevolve at a rapid rate. Further studies will show whether guinea pig PP1 is more similar to the human receptor also in pharmacology and tissue distribution.

Supported by the Swedish Natural Science Research Council.

610.5

CLONING OF NEUROPEPTIDE Y RECEPTORS FROM THE ZEBRAFISH. I. Lundell*, M. Ringvall, P. Starbäck, E. Salaneck, M. Berglund, A. G. Blomqvist, D. Larhammar. Department of Medical Pharmacology, Uppsala Univ., Box 593, S-75124 Uppsala, Sweden.

Neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP) form a family of structurally related peptides. As we have previously islolated clones for NPY and PYY from zebrafish (C.Söderberg et al. unpublished), we wished to isolate NPY receptor clones from this species to allow correlation of ligand and receptor expression in studies of tissue distribution and development.

The Y1 receptor concensus sequence from several species was used to design degenerate primers for PCR. This approach gave two different subtypes of receptors with homology to the NPY family of receptors. A third subtype was obtained with degenerate primers based on the new sequences. The zebrafish receptors, tentatively designated zY*a*, zY*b* and zY*c* display equally high identity to the Y1 receptor as to the recently cloned PP1 receptor with an amino acid sequence identity of approximately 55% in the transmembrane regions. Like the PP1 receptor, they lack the intron after TM5 that is found in Y1. The *a* and *b* subtypes are also about 55% identical to one another, while the *c* receptor is more closely related to the *b* subtype. In agreement with the structural similarities, zY*b* and zY*c* display a binding profile that is more reminiscent of Y1 binding than Y2. Affinities for NPY and PYY are in the picomolar range and the affinity for PP is considerably lower. These observations show that the zebrafish receptors are related to Y1 but most probably represent distinct subtypes that are likely to be present also in mammals. These receptors will help elucidate the evolution of the NPY receptor family. Work is in progress to map the tissue localization in the zebrafish and to clone the corresponding mammalian receptors.

Supported by the Swedish Natural Science Research Council.

610.6

CLONING OF A NOVEL MEMBER OF THE NPY/PYY/PP RECEPTOR FAMILY. M. Matsumoto, T. Nomura, Y. Ikeda, S. Kawabata* and T. Yamaguchi. Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki, 305, Japan

Neuropeptide Y (NPY), peptide YY (PYY), and pancreatic peptide (PP) belong to a family of structurally related 36-amino acid peptides. These peptide functions in both neural and endocrine signaling are mediated through specific membrane receptors. Although recent molecular cloning studies revealed the structure of Y1, Y2, and Y4/PP1 receptors, previous pharmacological study indicated the existence of additional type of receptors for this NPY/PYY/PP family, e.g. Y3, PYY-preferring and feeding receptor.

Using low stringency hybridization method, we cloned a cDNA encoding novel receptor which shares significant homology with the receptors for this peptide family. The cloned cDNA from rabbit encodes a heptahelix receptor of 371 amino acids sharing the greatest identity with Y1 (50%) followed by Y4/PP1 (40%) and Y2 (25%) receptors. The mRNA was detected in brain, skeletal muscle and small intestine by RT-PCR. We also cloned a cDNA of human homologue, which shares 87% identity in nucleotide sequence with rabbit's. In human tissues the mRNA was detected at 3 kb, abundantly in heart and skeletal muscle. Lower level of mRNA was also detected in the small intestine, prostate, spleen, kidney, testis and colon. The pharmacological study is currently in progress to compare the profile of this novel receptor with that of proposed NPY/PYY/PP receptors.

610.7

Expression of Neuropeptide Y/Peptide YY Receptor Subtypes in the Human Cerebral Cortex and Hypothalamus. M.A. Statnick*, D.A. Schober, J.P. Burnett, N.G. Mayne, and D.R. Gehlert. Lilly Research Labs, Lilly Corporate Center, Eli Lilly and Company, Indianapolis, IN 46285.

Neuropeptide Y (NPY) is a 36 amino acid C-terminally amidated peptide that is synthesized by peripheral and central nervous system neurons. NPY modulates neuronal activity through an interaction with at least three different receptor subtypes (Y1, Y2 and Y3 receptors). Recently, species related differences in the distribution of NPY receptors were described in the rat and human brain. From these studies it appears that the rat brain expresses a mixture of Y1 and Y2 receptors while the human brain is predominantly Y2. Because species differences in the distribution of NPY receptors could have functional consequences in man, we examined the expression of Y1 and Y2 receptors in the human cerebral cortex and hypothalamus by radioligand binding and competitive PCR (PATTY). Using saturation analysis with [^{125}I][Leu^{31},Pro^{34}]-PYY (Y1 selective) and [^{125}I]-PYY$_{3-36}$ (Y2 selective), similar densities of Y1 and Y2 binding sites were found in the cerebral cortex, with estimated B_{max} values of 313 and 458 fmol/mg protein, respectively. In preliminary experiments using hypothalamus homogenates, [^{125}I]-PYY$_{3-36}$ binding to Y2 receptors was twice that of [^{125}I][Leu^{31},Pro^{34}]-PYY (Y1) binding. Using PATTY, we estimated the relative ratio of Y1 to Y2 mRNA levels to be ~200:1 in the human cerebral cortex. Conversely, the relative amount of Y2 mRNA was 5-10 times higher than Y1 mRNA levels in the human hypothalamus. Collectively, these data support our hypothesis that the human brain contains a mixture of Y1 and Y2 receptors. We attribute previous underestimation's of Y1 binding sites in the human brain to preferential labeling of Y2 receptors with low concentrations of [^{125}I]-PYY. Therefore, to avoid underestimation's in receptor density, when studying tissues with a mixed or unknown receptor population, it is critical to use subtype selective ligands.

610.8

DIFFERENCES IN CATION SENSITIVITY OF LIGAND BINDING TO Y_1 AND Y_2 SUBTYPE OF NEUROPEPTIDE Y (NPY) RECEPTOR OF RAT BRAIN. M.S. Parker[2/], W.R. Crowley[1/] and S.L. Parker*[1/], Dept. of Pharmacology, University of Tennessee College of Medicine[1/], and Dept. of Biology[2/], The University of Memphis, Memphis, TN 38163.

The high-affinity ligand binding to the Y_1 subtype of NPY receptor in rat brain particulates is increased three- to four-fold by Ca^{2+}, and also stimulated by Sr^{2+}, but reversibly reduced by Ba^{2+}, Mg^{2+}, Mn^{2+}, by the organic polycations neomycin and spermidine, and by chelators EGTA and EDTA. The alkali metal monovalent cations inhibit the Ca^{2+}-enabled Y_1 subtype binding with some selectivity ($Cs^+ >= NH_4^+ > Li^+ > Na^+, K^+$), with IC_{50} values between 70-120 mM at 1.67 mM Ca^{2+}. Inhibition of the calcium-promoted Y_1 binding by alkali cations is essentially allosteric, since the half-maximal effect of Ca^{2+} remains in the range of 0.3 - 0.6 mM between 0-140 mM of Na^+ or K^+. The specific Y_2 subtype binding is enhanced about threefold by all alkaline earth divalent cations, Mn^{2+}, neomycin or spermidine in the range of 0.1 - 10 mM, and also stimulated by Na_3EGTA and Na_3EDTA at 3-20 mM. The Y_2 binding is biphasically stimulated by alkali cations at up to 100 mM, and inhibited with IC_{50} values in the range of 170 - 250 mM, with Na^+ being the least efficacious inhibitor. The large differential in cation sensitivity points to important differences in structure and properties of the binding sites of the Y_1 and Y_2 receptors. The selective promotion of the Y_1 binding by Ca^{2+} might be linked to calcium chelation of essential aspartate residues in extracellular loops of the Y_1 receptor. The strong influence of monovalent cations on Y_1 binding could relate to the LSFSD motif in the second transmembrane segment of the receptor (similar to motifs known to allosterically modulate agonist binding to other monovalent cation-sensitive G-protein linked receptors). The low cation sensitivity and selectivity of the Y_2 binding could reflect use of the entire NPY(24-36) C-terminal stretch, LRHYINLITRQRY amide (invariant in all known NPY molecules, and largely conserved in all peptide YY variants) as a 'super-epitope' of the Y_2 site. Supported by HD-13703.

610.9

PHARMACOLOGY OF RAT VS. HUMAN Y-TYPE RECEPTORS FOR NEUROPEPTIDE Y, PEPTIDE YY AND PANCREATIC POLYPEPTIDE, INCLUDING A NOVEL RECEPTOR CLONE. P.J.-J. Vaysse*, M.W. Walker, C. Gerald, K.E. Smith, T.L. Laz, J.A. Bard, Z. Shaposhnik, N. Rogacki, R.L. Weinshank and T.A. Branchek. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

The pancreatic polypeptide family includes neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP). These peptides bind to and activate membrane bound receptors. Amino acid identity between cloned human and rat receptors is 93.7 % for Y1, 94.5% for Y2 and 74.7% for Y4. We evaluated the binding affinity of a series of compounds for the cloned human and rat homologs of Y1, Y2, Y4 and a novel Y-type receptor, transiently expressed in COS-7 cells. Membrane preparations were incubated with ^{125}I-PYY for competitive displacement assays. Some species-dependent differences in binding were observed. Consider as an example the Y1 antagonist BIBP 3226. This compound was described as having Y1 selectivity based on its ability to preferentially bind SK-N-MC neuroblastoma cells (a human Y1 model) vs. SMS-KAN neuroblastoma cells (a human Y2 model). The pK_i values that we measured for BIBP 3226 binding to human Y1 and Y2 clones (8.07 and 4.51, respectively) are consistent with those reported previously for the neuroblastoma cells, while the pK_i value for the human Y4 (5.20) provides new information not previously available in the absence of a Y4 model cell line. The pK_i values that we measured for BIBP 3226 binding to rat Y1, Y2 and Y4 clones (7.70, 4.18 and 6.38, respectively) indicate a narrower window of selectivity for rat Y1 vs. Y4 receptors than for human Y1 vs. Y4. These data emphasize the need to characterize compounds not only with human receptor subtypes but also with receptor homologs appropriate for the animal model of choice. A comparative pharmacological analysis of the novel Y-type receptor homologs will be presented, as outlined for the Y1, Y2 and Y4 receptors.

This work was supported by Ciba-Geigy Limited and Synaptic Pharmaceutical Corporation.

610.10

DELINEATION OF AGONIST BINDING DOMAINS IN THE HUMAN NPY1 AND NPY4 RECEPTOR SUBTYPES. J.A. Tamm, Y. Shifman, N. Adham, C. Hou, M.W. Walker, J.A. Bard, P.J.-J. Vaysse, R.L. Weinshank, T.A. Branchek, C. Gluchowski, W.E. Heydorn* and J.A. Salon. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

The isolation of pharmacologically distinct members of the neuropeptide Y (NPY) receptor family has facilitated the analysis of receptor chimeras to identify the domains responsible for subtype-specific agonist binding. The human Y1 subtype binds NPY with 15 times greater affinity than does the human Y4 subtype. Conversely, the binding of pancreatic polypeptide (PP) is almost 1000-fold higher at the Y4 subtype than at the Y1 subtype. By systematically exchanging sequences between the Y1 and Y4 genes, we have shown that any chimera containing extracellular loop 2 and/or TM5 from the Y1 subtype binds NPY with high affinity. The remaining regions of the Y1 receptor appear to have little direct impact on the selective binding of NPY. Similarly, our data suggest that discontinuous regions of the Y4 receptor specify high affinity binding of PP. Constructions that carry TM6 from the Y4 subtype generally bind PP with the highest affinity. In addition, the extracellular loops of the Y4 subtype, especially loops 2 and 3, promote improved binding of PP when exchanged with the corresponding loops in the Y1 receptor. Our results show that these two closely related receptors use different domains to selectively bind similar agonists.

This work was supported in part by NIH grant R44NS31843-03 and in part by Synaptic Pharmaceutical Corporation.

610.11

BINDING OF PANCREATIC POLYPEPTIDES AT A CONSERVED HYDROPHOBIC POCKET IN THE Y1, Y2 AND Y4 RECEPTOR SUBTYPES. J.A. Salon*, J.A. Tamm, P. Du, N. Adham, M.W. Walker, W. Cui, P.J.-J. Vaysse, B. Dowling, N. Boyle, T.A. Branchek, R.L. Weinshank, D.L. Linemeyer and C. Gluchowski. Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652.

The pancreatic polypeptide family consists of NPY, PYY and PP. These peptides, together with their respective receptors Y1, Y2 and Y4, comprise one of the most important neurohormone signaling systems in the body. The PP family of peptides are structurally homologous and the possibility of similar modes of interaction between these peptide ligands and their receptors seems plausible. Accordingly, we have explored the binding interactions of NPY, PYY and PP to a conserved hydrophobic pocket formed by tyrosine residues in TMs I and II of the Y1, Y2 and Y4 receptors. The receptor residues were modified by site-directed mutagenesis and the ability of the mutated receptors to bind the peptides was assessed by measuring IC_{50} values in competitive displacement assays using ^{125}I-PYY as the displaceable radioligand. Results of these binding studies do not support a common mode of interaction between the conserved bioactive portions of NPY, PYY and PP with this conserved aromatic pocket found in the Y receptor family. Exploration of alternate scenarios for docking each peptide with its respective receptor is suggested.

This work was supported in part by NIH grant R44NS31843-03 and in part by Synaptic Pharmaceutical Corporation.

610.12

AUTORADIOGRAPHIC DISTRIBUTION OF NEUROPEPTIDE Y RECEPTOR SUBTYPES IN THE MONKEY BRAIN. J.A. St-Pierre*, Y. Dumont and R. Quirion. Douglas Hospital Res Ctr, Depts. Psychiatry, and Neurological Sciences, McGill Univ, Montréal, Qc, Canada, H4H 1R3.

The distribution and pharmacological characteristics of neuropeptide Y (NPY) Y_1 and Y_2 receptor subtypes are well established in the rat brain (for example, Dumont et al., Synapse, 22: 139, 1996). In contrast, the postmortem human brain contained only low to very low levels of [^{125}I][Leu^{31},Pro^{34}]PYY/Y_1-like labeling in all brain structures except in the dentate gyrus (Widdowson, Brain Res., 631: 27, 1993). It is thus clear that major species differences exist in the discrete localization of brain NPY receptors. In orther to investigate further this issue, we studied here the respective distribution of the Y_1 and Y_2 receptor subtypes in the brain of two strains of primates using [^{125}I][Leu^{31},Pro^{34}]PYY and [^{125}I]PYY$_{3-36}$ as radioligands. Adjacent coronal brain sections were incubated with 25-35 pM of either of the radiolabeled probes in the presence and absence of 1 µM pNPY to determine specific binding levels. The marmoset (*Callithrix jacchus*) brain contained significant levels of both Y_1- and Y_2-like binding sites in various cortical areas. Very high amounts of [^{125}I]PYY$_{3-36}$/Y_2-like labeling were detected in superficial cortical laminae while moderate levels of [^{125}I][Leu^{31},Pro^{34}]PYY/Y_1-like sites were observed in superficial and deep layers of the cortex. Moreover, the marmoset brain is highly enriched with specific Y_1- and Y_2-like binding sites in the cerebellum and various brainstem nuclei, respectively. Additionally, [^{125}I][Leu^{31},Pro^{34}]PYY binding is highly sensitive to the antagonist, BIBP3226, demonstrating further its belonging to the Y_1 subtype. In contrast, in the vervet monkey (*Cercopithecus pygerythrus*) brain, Y_1 and Y_2 binding sites are distributed as in the human brain with only low levels of Y_1 sites seen in most brain areas. Preliminary data also suggest that only low to very low levels of [^{125}I]hPP/Y_4-like binding sites are present in the marmoset brain. Taken together, these results suggest that the respective distribution of the Y_1 and Y_2 receptor subtypes is rather similar in the vervet monkey and human brain while being different in the marmoset. Supported by the MRC of Canada.

610.13

ULTRASTRUCTURAL LOCALIZATION OF NEUROPEPTIDE Y AND THE Y1 RECEPTOR IN RAT NUCLEUS ACCUMBENS. J. Chan*, A. Beck-Sickinger, H.A. Wieland, and V.M. Pickel. Dept. of Neurology and Neuroscience, Cornell University Medical College, New York, Dept. of Pharmacy, ETH, Zurich, Switzerland, and Dept. of Biological Research Cardiovascular Pharmacology I, Dr. Karl Thomae GmbH, Germany.

The mRNA and ligands for neuropeptide Y (Y1) receptors are known to be present in the nucleus accumbens septi (NAS) where many large aspiny neurons also contain neuropeptide Y (NPY). To further assess the functional sites for the Y1 and its relation to NPY, we examined the electron microscopic immunocytochemical dual labeling of antipeptide antisera against sequences within the Y1-receptor and NPY in single sections through the rat brain NAS. The Y1-like immunoreactivity (Y1-LI) was seen mainly in dendrites and dendritic spines, but was also detetected in axon terminals and astrocytic processes many of which were near blood vessels. Within both neuronal and glial processes, Y1-LI was most often seen near the plasma membrane or associated with cytoplasmic organelles, especially smooth endoplasmic reticulum and vesicles. In dendrites, Y1-LI was also prominently localized within and near asymmetric, excitatory-type synapses on spines, and in larger aspiny dendrites some of which also contained NPY. Terminals containing Y1-LI were both with and without NPY and many formed asymmetric synapses with spines. This distribution implicates the Y1 receptor in the NAS in (1) modulation of the post- and pre-synaptic effects of excitatory transmitters, (2) autoregulation of NPY-containing neurons, and (3) shape or metabolic changes in astrocytes. (Supported by grants MH00078; MH40342; and HL18974).

610.14

NEUROPEPTIDE Y RECEPTORS IN THE HUMAN BRAIN: GENE EXPRESSION, ANATOMICAL LOCALIZATION AND POSSIBLE ALTERATION IN ALZHEIMER'S DISEASE. D. Jacques[1*], Y. Dumont[1], Y. Tong[1,2], S. H. Shen[2] and R. Quirion[1]. [1]Douglas Hospital Res. Ctr., Dept. Psychiatry, McGill University, Montréal, Canada, H4H 1R3; [2]National Research Council Canada, Biotechnology Research Institute, 6100 Royalmount Ave., Montréal, Canada, H4P 2R2.

We recently reported on the apparent paucity of the Y_1 receptor subtype in the human brain, including in cortical areas known to be highly enriched with this receptor class in the rodent brain (Dumont et al., J. Neurosci. 13:73, 1993). Interestingly, the Y_1 receptor mRNA is expressed at a somewhat higher level than the translated protein as revealed using in situ hybridization and quantitative receptor autoradiography (Jacques et al., Neuroreport, in press) In the present study, we investigated the comparative, discrete localization of the Y_1 and Y_2 receptors in the normal and Alzheimer's (AD) human brain, post-mortem. This is particularly relevant since earlier studies reported decreases in NPY-like immunoreactivity (Chan-Palay et al., J. Comp. Neurol. 238:390, 1985; ibid, 248: 376, 1986) and [^{3}H]NPY binding sites (Martel et al., Brain Res. 519:228, 1990) in the AD brain. As expected, the normal human brain is enriched with [^{125}I]PYY$_{3\text{-}36}$/Y_2-like receptors while [^{125}I][Leu^{31},Pro^{34}]PYY/Y_1-like receptors are rare and mostly concentrated in the dentate gyrus. In the AD brain, the level of Y_2 binding sites is significantly decreased in temporal and frontal cortices, and in basal forebrain nuclei. Specific Y_1-like binding in the granular layer of the dentate gyrus is also apparently decreased in the AD brain. It thus appears that the amount of both Y_1-like and Y_2-like binding sites may be decreased in the Alzheimer's brain albeit in different areas. Supported by MRCC and FRSQ.

610.15

EVALUATION OF VARIOUS SUBSTITUTIONS IN THE C-TERMINAL PORTION OF NEUROPEPTIDE Y IN Y_1- AND Y_2-LIKE BINDING ASSAYS: POSITION 29 IS CRITICAL FOR Y_1 VS Y_2 RECEPTOR RECOGNITION. T. Trinh*, Y. Dumont[1], A. Fournier[2], S. St-Pierre[2] and R. Quirion[1]. [1]Douglas Hosp. Res. Ctr. and Depts. Psychiatry, and Pharmacology and Therapeutics , McGill University, Montréal, Qc. Canada, H4H 1R3. [2]INRS-Santé, Université du Québec, Pointe-Claire, Qc, Canada, H9R 1G6.

The existence of at least four, and possibly more, neuropeptide Y (NPY) receptor subtypes has clearly been demonstrated, at least three (Y_1, Y_2 and Y_3/PP_1) having recently been cloned. Accordingly, the development of highly selective agonists and antagonists for each of these receptor subtypes is critical in order to properly assess their respective functional relevance. Highly selective non-peptide Y_1 receptor antagonist such as BIBP 3226 (Rudolf et al.,Eur. J. Pharmacol., 271:R11, 1994) have recently been developed; such tools not being available for the other receptor classes. Regarding agonists, while early studies suggested that [Leu^{31}, Pro^{34}]NPY and [Leu^{31}, Pro^{34}]PYY behaved as highly selective Y_1 receptor agonists, more recent data revealed their affinities for the Y_3 and Y_3/PP_1 receptor subtype, respectively (Dumont et al., Mol. Brain Res., 26:3220-3324, 1994. Gehlert et al., Eur. J. Pharmacol., in press). Accordingly, we are pursuing extensive structure-activity studies aimed at developing highly selective agonists for each receptor subtype and have recently focused on the C-terminal portion of the NPY molecule. Mono-substitution of residues in position 27 (Tyr), 29 (Asn), 34 (Gln) and 36 (Tyr) were studied here. Analogues were tested for their affinities in Y_1-like/[^{125}I][Leu^{31},Pro^{34}]PYY and Y_2-like/[^{125}I]PYY$_{3\text{-}36}$ binding assays (Dumont et al., JPET, 272:673-680, 1995). Interestingly, substitution of Asn^{29} by Asp ([Asp^{29}]NPY) generated an analogue having high selectivity for the Y_1 subtype (IC_{50}=4nM for Y_1, >>1000nM for Y_2). This substitution in NPY$_{13\text{-}36}$ ([Asp^{29}]NPY$_{13\text{-}36}$) completely reversed the selectivity profile of the fragment which became a preferential Y_1 (IC_{50}=46nM) vs Y_2 (IC_{50}>>1000nM) agonist. These results suggest that in addition to the glutamine residue in position 34 (Fuhlendorff et al., PNAS, 78:182-186, 1990), the asparagine residue in position 29 is critical for Y_1 vs Y_2 receptor selectivity. Supported by the MRC.

610.16

NEUROPEPTIDE Y Y_3/PP_1 RECEPTOR IN THE RAT BRAIN: A COMBINED IN SITU HYBRIDIZATION AND RECEPTOR AUTORADIOGRAPHY STUDY. Y. Dumont[1*], Y. Tong[2], S.H. Shen[2], I. Lundell[3], D. Larhammar[3] and R. Quirion[1] (1) Douglas Hosp Res Ctr and Dept Psychiatry, McGill Univ, Montréal, Qc. Canada. (2) Biotech Res Inst, Montréal, Qc, Canada. (3) Dept Pharmacol Uppsala Univ, Uppsala, Sweden.

Neuropeptide (NPY) Y_1 Y_2 and Y_3/PP_1 receptor subtypes have been cloned. The respective distribution and pharmacological characteristics of the Y_1-like and Y_2-like receptor subtypes are well established in the rat brain. On the other hand, little is currently known on the discrete localization of the newly cloned Y_3/PP_1 receptor subtype in the mammalian CNS. To target this unique receptor, human PP (hPP) and rat PP (rPP) were iodinated using the chloramine T method as previously described (Dumont et al., JPET, 272:673, 1995). Autoradiographic studies were performed on adjacent coronal rat brain sections using 35 pM of either [^{125}I]hPP or [^{125}I]rPP. Non-specific binding was determined by adding 1 µM of unlabelled hPP or rPP in the incubation medium and represented less than 10 % of total binding. Very high to high levels of specific [^{125}I]hPP binding sites were detected in various brainstem nuclei including the interpenduncular nucleus, vestibular nuclei, nucleus tractus soliratius, area postrema and dorsal vagal nucleus. Moderate amounts of [^{125}I]hPP labeling were found in the medial preoptic area and the paraventricular nucleus of the hypothalamus. Few other areas such as the external plexiform layer of the olfactory bulb, the anterior olfactory nucleus and the lateral septum contained significant levels of [^{125}I]hPP binding sites while very low amounts of labeling were detected in cortical areas and the hippocampal formation. Specific [^{125}I]rPP binding was similarly distributed. Competition binding studies confirmed that hPP is at least 10 times more potent than rPP to compete for specific [^{125}I]hPP binding with a rank order of potency hPP > rPP > [Leu^{31},Pro^{34}]PYY >>> BIBP3226. Preliminary data obtained using in situ hybridization revealed that the Y_3/PP_1 receptor mRNA is present in the anterior olfactory nucleus, pyramidal layer of the olfactory tubercle, vestibular nuclei and area postrema. Hence, Y_3/PP_1 receptors are broadly but discretely distributed in the rat brain. Clear differences with the localization of the Y_1 and Y_2 subtypes are evident, especially at the level of the paraventricular hypothalamic nucleus, a key structure in appetite behaviors. Supported by the MRC of Canada.

610.17

DISTRIBUTION AND mRNA EXPRESSION OF NEUROPEPTIDE Y (NPY) RECEPTORS IN HUMAN BRAIN VESSELS, ASTROCYTES AND VASCULAR CELLS IN CULTURE. R. Abounader[1]*, D. Jacques[2], Y. Tong[2], R. Quirion[2] & E. Hamel[1], Mont. Neurol. Inst., Dept. Neurol. & Neurosurg.[1] and Douglas. Hosp. Res. Ctr., Dept. Psychiat.[2], McGill Univ., Montréal, QC, Canada

NPY is a potent constrictor of extra- and intracerebral blood vessels where it may act through Y1 receptors (*Br. J. Pharmacol.* **116**, 2245-2250, 1995). However, four receptors (Y1, Y2, Y3 and Y4) for NPY have been pharmacologically identified and three (Y1, Y2 and Y4) have recently been cloned in man. Here, we report the distribution and mRNA expression of various NPY receptors in human brain vessels, astrocytes and vascular cells in culture using quantitative binding radioautography, *in situ* hybridization (ISH) and/or reverse transcriptase-polymerase chain reaction (RT-PCR). Sections of human pial vessels were incubated with [^{125}I]-[Leu^{31}, Pro^{34}]-PYY or [^{125}I]-PYY$_{3\text{-}36}$, respective radioligands for Y1 and Y2 receptors, and competition studies were performed with (10^{-6}-10^{-9} M) NPY, PYY, [Leu^{31}, Pro^{34}]-NPY, NPY$_{13\text{-}36}$ or the Y1 specific antagonist BIBP 3226. The cellular distribution of Y1, Y2 and Y4 receptor mRNAs in human brain vessels was studied by ISH with ^{35}S labelled cRNA probes, and Y1 mRNA expression was further analyzed in intracerebral microvessels and cultures of astrocytes, smooth muscle and endothelial cells by RT-PCR. [^{125}I]-[Leu^{31}, Pro^{34}]-PYY binding was potently competed by NPY, PYY and the selective Y1 receptor antagonist BIBP 3226, indicating the presence of Y1 receptors on human brain vessels. In contrast, [^{125}I]-PYY$_{3\text{-}36}$ binding could not be competed by Y1 (NPY and [Leu^{31}, Pro^{34}]-PYY) or Y2 (NPY$_{13\text{-}36}$) receptor agonists, suggesting the abscence of specific Y2 binding sites in brain vessels. By ISH, a strong signal for Y1 mRNA was present in the smooth muscle layer of vessels. Preliminary observations also suggest the presence of a positive signal for the Y4, but not for the Y2 receptor mRNA. PCR products of the expected size for the Y1 receptor were detected in pial vessels, microvessels, capillaries, astrocytes and smooth muscle cells but not in endothelial cells. We conclude that Y1 receptor protein and mRNA are present on the smooth muscle of human extra- and intracerebral blood vessels. Supported by Dr Karl Thomae GmbH/Bio-Mega and the MRC of Canada through a University-Industry grant.

611.1

SUBSTANCE P IN THE TWO MAJOR SUBPOPULATIONS OF PROJECTION NEURONS WITHIN THE NUCLEUS ACCUMBENS. X.-Y. Lu*, M.B. Ghasemzadeh, L. Churchill and P.W. Kalivas. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

Substance P, an endogenous tachykinin, was previously found to be primarily expressed in the striatal efferent projection to the substantia nigra rather than in the striatal efferent projection to the globus pallidus. The nucleus accumbens has projections analogous to those of the striatum with outputs to the ventral tegmental area and the ventral pallidum. To determine whether a similar substance P expression pattern holds for the nucleus accumbens, in situ hybridization for mRNA of the substance P prohormone, β-preprotachykinin, was combined with Fluoro-Gold retrograde labeling. Substance P-containing neurons were found in the nucleus accumbens projecting to both the ventral tegmental area and the ventral pallidum. Following iontophoretic deposit of Fluoro-Gold in the ventral tegmental area, up to 67% of retrogradely labeled neurons in the shell of the nucleus accumbens expressed substance P prohormone mRNA. Following Fluoro-Gold deposit in the ventral pallidum, up to 41% of the retrogradely labeled neurons in the shell and up to 29% in the core of the nucleus accumbens also contained substance P. This study demonstrated that substance P-containing neurons within the nucleus accumbens project to both the ventral mesencephalon and pallidum which differs from those neurons in the striatum. These data suggest that the projection from the nucleus accumbens to the ventral tegmental area may be similar to the striatonigral projection, whereas the projection from the nucleus accumbens to the ventral pallidum may be more complex than the striatopallidal projection. This work was supported by USPHS grants DA03906, MH40817 and DA00158 to P.W. Kalivas.

611.2

MODULATION OF SYNAPTIC TRANSMISSION AND EXCITABILITY OF MOUSE HIPPOCAMPAL NEURONES BY SUBSTANCE P. M. Kouznetsova, A. Nistri*, Biophys. Lab., Int. Sch. Adv. Studies (SISSA), 34013, Trieste, Italy.

In order to investigate the effects of substance P (SP), a peptide physiologically present in the hippocampus, intracellular recordings with K-acetate microelectrodes were performed on pyramidal neurones of the CA1 region of the mouse hippocampal slice preparation. Synaptic responses were elicited by focal electrical stimulation of stratum lacunosum moleculare. In 12 cells (resting potential -68 ± 1 mV, input resistance 131± 13 MΩ, mean ± s.e.m.) 7 min application of SP methylester (SPMeO; 1.2 - 5 μM), a selective NK1 agonist, elicited almost no change in membrane potential and input resistance; evoked potentials were however suppressed: EPSPs by 32 ± 9% (55% cells), IPSPs by 39 ± 9% (82% cells). Intracellularly applied depolarising steps (500 ms) were used to monitor spike current rheobase which in 7/8 neurones was decreased by 24 ± 6%. The latency for the first spike was diminished in 50% cells. In 67% cells there was also a reduction in AHP amplitude. In addition to the increase in membrane excitability a large enhancement in frequency of spontaneous postsynaptic potentials was obvious in all neurones. This phenomenon had slow onset (>10 min) and a very slow recovery. These findings demonstrated that SPMeO has a complex effect on hippocampal CA1 neurones. Although SPMeO produced no changes in resting membrane properties, it modulated synaptic responses and some voltage-dependent conductances. It remains to be established whether it was a direct effect by the neuropeptide or an indirect one via modulation of other transmitter pathways. Supported by CNR and INFM.

611.3

NEUROPEPTIDE-MEDIATED MODULATION OF SPINAL REFLEXES IN THE LAMPREY. M. Ullström, D. Parker, N. Bussières*, and S. Grillner. Nobel Institute for Neurophysiology, Dept. of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

Neuromodulators change the output of the essential 'hard-wired' neural circuits by modifying cellular and synaptic properties. We have investigated the effects of the tachykinin substance P and NPY, both which are contained in terminals making close appositions on sensory axonal processes.

Reflex activation of trunk motoneurons can be elicited by stimulating the tail fin electrically. These reflexes are analysed *in vitro* by using an isolated cord preparation with the tail fin left attached. The cord is split into three baths. Peptides are added to the caudal bath, which includes the tail fin and afferent synapses. Reflex activity is recorded (ventral roots) in the rostral bath, the middle bath contains Ringer with high concentration of Mg^{2+} and Ca^{2+} to block synaptic transmission. Thus, activity in the rostral bath is due to activity in the ascending axons of the sensory neurons and interneurons from the caudal bath. The cellular and synaptic basis of the modulation was studied by making intracellular recordings from sensory neurons and interneurons. Substance P potentiated synaptic input to sensory interneurons, depolarised and increased the excitability of the sensory neurons. NPY presynaptically inhibits synaptic transmission to the sensory interneurons (Parker et al., *Eur. J. Neurosci.*, in press). Neuropeptides thus have opposite modulatory effects on reflex responses in lamprey.

Application of substance P to the caudal bath potentiates reflex responses, shown by enhanced ventral root response following tail stimulation. Preliminary results suggest that NPY reduces ventral root responses following tail stimulation.

(Swedish MRC 3026)

611.4

TACHYKININ-MEDIATED MODULATION OF MECHANO-SENSORY AFFERENTS IN LAMPREY. E. Svensson, D. Parker and S. Grillner*, Dept. of Neuroscience, Division for Neurophysiology, Karolinska institute, S-171 77 Stockholm, Sweden.

Bath application of the tachykinin substance P modulates mechanosensory afferents (dorsal cells) in lamprey, by increasing the excitability and spike duration and reducing the afterhyperpolarisation. The effects are due to the reduction of a 4-aminopyridine-sensitive potassium channel. The endogenous source of tachykinins was investigated by Lucifer yellow filling of dorsal cells combined with immunohistochemistry against substance P. Tachykinin immunoreactive fibers were found in close apposition to the dorsal cell axons and occasionally close to their somata. Tachykinin immunoreactive fibers were also found in the dorsal root and dorsal root ganglion. Tachykinin immunoreactive cell bodies that project towards the dorsal region of the spinal cord were found in the grey matter, and also peripheral to the dorsal root ganglion.

The intracellular pathway responsible for the tachykinin-mediated spike broadening was analysed using G-protein and protein kinase activators and inhibitors. The tachykinin-induced spike broadening was blocked by injection of 10 mM GDPβS but not by preincubation with pertussis toxin (300 ng/ml, 36h). Preincubation with 10 μM H7, a protein kinase C (PKC) and protein kinase A (PKA) antagonist and with chelerythine, a specific PKC antagonist blocked the effects of substance P. Bath application of the PKC-activating phorbol ester PDBu (10 μM) mimicked the effect of substance P. The PKA and protein kinase G antagonist H8 (20 μM) did not block the effect of substance P. These results suggest that substance P modulates the action potential via a pertussis toxin-insensitive G-protein and through activation of PKC. The endogenous release of tachykinins onto dorsal cell axons will thus modulate mechanosensory input in the lamprey. (Swedish MRC 3026)

611.5

NK_1 RECEPTOR EXPRESSION IN DEVELOPING RAT SPINAL CORD AND IN CULTURE. C.J. Lee, T.B. Allen, M.J.S. Heath, A.B. MacDermott*. Depts of Physio. and Cell. Biophys., Anesthesiology, and the Center for Neurobiology & Behavior, Columbia University, New York, NY 10032.

In spinal cord dorsal horn, substance P is released from small diameter primary afferents and acts as a neurotransmitter/neuromodulator by activating neurokinin(NK) receptors. Using a polyclonal antibody raised against the C-terminal peptide of NK_1 receptor (Vigna et. al, 1994), we studied the time course and localization of NK_1 receptor expression in rat cervical spinal cord from ages E16 to P25. Expression in the dorsal horn started at around birth (P0-P2) in laminae I, III and IV and continued through P25. Staining was absent from lamina II as previously reported (Bleazard et al. 1994) throughout the ages tested. Staining of the floor plate, which has been shown to start to express NK_1 receptor at E10-E11 (Heath et. al, 1995), persisted until P10. In ventral horn, NK_1 receptor was strongly expressed before birth (E16-E20) then the level of expression decreased subsequently. NK_1 receptor immunoreactivity appeared in the region of the autonomic preganglionic nuclei at E17, and was present on fibers that projected between the nuclei and the region of central canal.

NK_1 receptor expression in the dorsal horn was confirmed by fura-2 Ca^{2+} imaging of dissociated dorsal horn neurons growing in co-culture with dorsal root ganglion neurons. When applied in the presence of CNQX, APV, bicuculline, and strychnine, 100 nM substance P elicited a Ca^{2+} response (n=9). This response was mimicked by the synthetic NK_1 agonist, Sar^9-$Met(O_2)^{11}$-substance P (n=2). On these neurons, Ca^{2+} responses to substance P were blocked by tetrodotoxin (n=3) and 30 μM lanthanum (n=1), suggesting that NK_1 receptor activation causes firing of action potentials and subsequent entry of Ca^{2+} through voltage-gated Ca^{2+} channels. These data indicate that NK_1 receptors show a specific developmental pattern of expression in the spinal cord and that their presence in lamina I after P0 is consistent with the well established role of substance P in pain transmission and modulation. Supported by NIH.

611.6

CHARACTERIZATION OF AN NK3-RECEPTOR MEDIATED NONSPECIFIC CATION CONDUCTANCE IN GUINEA PIG CARDIAC NEURONS. J.C. Hardwick*, G.M. Mawe, and R.L. Parsons. Department of Anatomy & Neurobiology, University of Vermont, Burlington, VT 05405.

The parasympathetic postganglionic neurons of the guinea pig cardiac ganglia appear to receive inputs from substance P (SP)-immunoreactive afferent fibers. Previous studies demonstrated that application of exogenous SP produced a slow, long-lasting depolarization in these neurons. The present studies demonstrate that this depolarization is mediated by NK3 receptor activation. Whole mounts of guinea pig atria were dissected and continuously superfused with Krebs solution at 35-37°C and gassed with 95%O_2/5%CO_2. Individual neurons within a ganglia were impaled with 2 M KCl filled microelectrodes for current clamp recording. Dose response curves were generated by determining the amplitude of depolarization in response to 15 sec (~2 mL) superfusions of either SP, neurokinin A (NKA), or senktide (an NK3 agonist) at concentrations ranging from 10^{-10} to 10^{-5}M. The rank order of potency (senktide > NKA > SP) is consistent with an NK3 receptor. Superfusion with the specific NK3 antagonist SR142801 (30 nM) produced a 67% inhibition in the response to 10^{-6}M senktide. To determine the potential ionic currents underlying the NK3-induced depolarization, we examined the amplitude of the depolarization induced with a brief (1 sec, 10psi) pressure ejection application of senktide (10^{-4}M) with and without specific inhibitors added to the Krebs solution. The senktide-induced depolarization was unaffected by Cl^--current inhibitors (100 μM niflumic acid or mefenamic acid), Cl^--substitution, 1-2 mM Ba^{++}, 1 mM Cs^+, 0.3 μM TTX, or 5 mM TEA. However, replacement of 50% of the external Na^+ with NMG resulted in an approximate 50% reduction in the amplitude of the senktide-induced depolarization. We conclude that the tachykinin-induced depolarization of cardiac neurons is mediated by NK3 receptors which lead to activation of a nonspecific cation conductance. Supported by NS 23978 (RLP), NS 26995, and DK 45410 (GMM).

611.7

THE MECHANISM OF TACHYKININ NK-3 RECEPTOR-INDUCED RELAXATION IN RAT MESENTERIC ARTERY. H. Kamiya, A. Mizuta, S. Tanaka, K. Honda, R. Saito, and Y. Takano*. Dept. of Pharmacol., Fac. of Pharmaceut. Sci., Fukuoka Univ., Fukuoka 814-80, Japan.

Tachykinin peptides show a variety of pharmacological effects via three tachykinin receptors, NK-1, NK-2 and NK-3. Substance P (SP) has been shown to cause endothelium-dependent relaxation of precontracted arteries and coronary arteries in some mammalian species. In addition, binding studies have demonstrated that the endothelial cells of porcine aorta have NK-1 receptors which are coupled to G-protein (Saito et al., 1990). Although there is much evidence for vasodilation mediated by NK-1 receptors, little is known about NK-3 receptor-mediated relaxation of arteries. In this study, we examined the mechanism of vasodilation mediated by tachykinin peptides in isolated mesenteric arteries of rats, and obtained the results as follows: (1) Neurokinin B and senktide, a selective NK-3 agonist, elicited potent endothelium-dependent relaxation of rat mesenteric arteries precontracted with phenylephrine (10^{-5} M), but an NK-1 agonist did not. (2) A non-peptide NK-3 antagonist, SR 142801, inhibited senktide-induced relaxation. (3) N^{ω}-nitro-L-arginine (L-NA), a nitric oxide synthesis inhibitor, markedly attenuated the relaxant response to senktide. (4) Electrical field stimulation (EFS) of the precontracted-artery produced a vasodilation in the presence of guanethidine. This EFS-evoked vasodilation was attenuated by tetrodotoxin and SR 142801. These results suggest that neurokinin B may play a role in the neurogenic vasodilation in the rat mesenteric artery, whose endothelium possesses tachykinin NK-3 receptors, and that the vasodilation is mediated by release of nitric oxide (NO) from the endothelium.

611.8

Molecular cloning of the mouse Hippocampal cholinergic neurostimulating peptide(HCNP) precursor protein and cellular distribution of HCNP mRNA in the mouse brain by non-isotopic in situ hybridization.

N,Matsukawa[1,2] K,Ojika[1]* H,Okada[3] N,Tohdoh[4] T,Yamamoto[2] and R,Ueda[1] ; 1: 2nd Department of Int. Med. 3: Department of Molecular Biology, Nagoya City Univ. , Nagoya, 467 ; 2: Choju Medical Institute, Noyori-Fukushimura Hospital, Toyohashi, 441 ; 4: Discovery Research Laboratories. Sumitomo Pharmaceutical Research Center,Osaka, 554, Japan.

We have previously reported that rat Hippocampal Cholinergic Neurostimulating Peptide, an undecapeptide purified from the hippocampus of 10-12 day old rats, specially enhances acetylcholine synthesis in medial septal nuclei in vitro by inducing the production of choline acetyltransferase activity (K,Ojika et, al,. 1992, Brain Res., 572, 164- 171). In this study, we have cloned a cDNA which encoded for mouse HCNP precursor protein. A mouse brain cDNA library Uni-ZAP™ XR Vector (Stratagene, CA) was screened by the rat HCNP precursor protein cDNA as a probe. The deduced amino acid sequence is composed of the same 186 amino acid residues as rat and human HCNP precursor protein (N, Tohdoh. 1995, Mol Brain Res., 30, 381-384). Mouse HCNP proper aligned at the N-terminal domain of precursor protein is the same structure as that of rat HCNP. Subsequently,we studied HCNP precursor protein mRNA concentration and cellular distribution by Northern blot analysis and non-sotopic in situ hybridization method in the adult mouse brain. The expression was significantly increased in the neuronal cells in the cerebral cortex, entorhinal cortex, hippocampus and Purkinje cells in the cerebellum

611.9

ENDOTHELIN MEDIATES Ca^{2+} SIGNALING IN ENTERIC GLIA VIA ACTIVATION OF ENDOTHELIN B RECEPTOR COUPLED TO PHOSPHOLIPASE C. WZ Zhang*, DC Barnhart, GA Sarosi, MW Mulholland. Dept. of Surgery, University of Michigan Medical Center, Ann Arbor, MI 48109

The ability of enteric glia to respond to endothelins (ET) was examined using fura-2-based digital microscopy in glial cells derived from guinea pig taenia coli. Each isoform of ET (ET1,ET2,ET3) evoked dose-dependent and equipotent increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$) and in percentage of cells responding. ET3 (100nM) caused a biphasic $[Ca^{2+}]_i$ response, characterized by an initial peak (541±16nM) followed by secondary, sustained elevation in $[Ca^{2+}]_i$ in 100% of cells (n=117). 4alaET1, a specific ETB receptor (ETB) agonist, elicited similar $[Ca^{2+}]_i$ increments (540±17 nM, 100% response at 1μM, n=84). BQ788 (100nM), an ETB antagonist, abolished $[Ca^{2+}]_i$ responses to low doses of ET3 (0.01-1nM), and inhibited $[Ca^{2+}]_i$ increments to high doses ET3 (10 and 100nM) by 79% and 71%, respectively (n=542). BQ610 (1μM), an ETA antagonist, did not demonstrate significant inhibition (4% ±3%,n=159). Preincubation of glia with U-73122 (10μM), a PLC inhibitor, abolished the initial $[Ca^{2+}]_i$ response to ET3 exposure (100nM). Thapsigargin (1μM), an inhibitor of endoplasmic reticulum Ca^{2+} ATPase, also eliminated initial ET3-evoked Ca^{2+} signaling. The IP3 receptor antagonist, heparin, introduced into glial cells by radiofrequency electroporation, decreased peak $[Ca^{2+}]_i$ response to ET3 (100 nM) by 63% (n=49). The effect of extracellular Ca^{2+} on the secondary response was studied by superfusion of glia with Ca^{2+}-free buffer or Ni^{2+}. The secondary elevation in $[Ca^{2+}]_i$ caused by ET3 was abolished by removal of Ca^{2+} from the buffer, and inhibited 80% by Ni^{2+} (1mM, n=108). Preincubation of glia with 100nM PMA (16 hr) also inhibited the secondary response by 82% (n=87). The presence of IP3 receptors in enteric glia was confirmed by immunofluorescent confocal microscopy. This study demonstrates that (1) enteric glia possess ETB receptors which mediate $[Ca^{2+}]_i$ signaling; (2) ET3 acts via PLC to release IP3-sensitive $[Ca^{2+}]_i$ stores with, (3) subsequent influx of extracellular Ca^{2+}. Supported by R01 DK41204.

611.10

ENDOTHELIN RECEPTOR SUBTYPES MAP DIFFERENTIALLY IN THE RAT BRAIN. Nashat Latib[1], Bo Yoo[1], Emilio C. Tayag*[1], Arco Y. Jeng[2], John C. Lehmann[1], 1. Dept. Neurosurgery MS 407, Medical College of Pennsylvania and Hahnemann University, Philadelphia PA 19102-1192; 2. Research Dept., Ciba-Geigy Corp., Summit NJ 07901-1398.

Two clearly defined receptor subtypes for endothelin (ET), the ET_A- and ET_B -subtypes, were found to have a differential regional distribution in the brain. Cryostat sections were incubated with [125I]ET-1 with or without ET_A antagonist BQ123 (1 uM), ET_B antagonist BQ788 (1 uM), or both ligands. In general, the ET_A receptor was particularly concentrated in white matter, but the ET_B receptor was by far predominant in the gray matter. ET_B receptors were much more enriched compared to ET_A receptors in the striatum, the nucleus of the diagonal band, the medial septum, the superior colliculus, the CA1 and dentate gyrus of the hippocampus, the fimbria, and the dorsal tegmental decussation. The choroid plexus contained both receptors. Some ventricular ependymal surfaces were labeled, e.g., the lateral surface of the lateral ventricles, but not the superior or medial surfaces, or, for instance, the periaqueductal gray. The pineal gland contained one of the highest densities of ET_B receptors and also contained a substantial density of ET_A receptors.

Ciba-Geigy Corp.

611.11

VASOTOCINERGIC INNERVATION IN A MALE PASSERINE BIRD (*Junco hyemalis*): EFFECT OF PHOTOPERIODIC CONDITION. P. Deviche[1]*, E. Garcia-Ojeda[2], L. Plumari[2], and G.C. Panzica[2]. [1]Inst. Arctic Biology, Univ. Alaska Fairbanks, Fairbanks, AK 99775; [2]Dept. Anatomy, Pharmacol. For. Med., Univ. Torino, Italy.

In domesticated birds, vasotocinergic (VT) fibers innervate brain regions that play an important role in the control of reproduction, and there is evidence that VT innervation is modulated by testosterone (T). The present investigation compared the distribution of immunoreactive VT innervation in brains of intact adult male dark-eyed juncos that were photosensitive and either exposed to short photoperiods (nonphotostimulated; low plasma T concentrations; **LO-T**) or naturally stimulated by long vernal photoperiods (high plasma T concentrations; **HI-T**). VT-containing cells were observed in regions where this peptide was previously found in another passerine bird (canary: paraventricular and supraoptic n., n. stria terminalis [nST]). Also similar to canaries, VT-containing fibers were widespread across many brain regions, being particularly abundant in the medial preoptic area (POM), the basal region of the septum, and the hypothalamic-neurohypophysial tract. Brain region-specific differences in the distribution of VT immunoreactivity between LO-T and HI-T males were observed. Specifically, LO-T males had markedly less VT-containing nST cells and less VT-containing POM and lateral septum fibers than HI-T males. Thus, VT expression in brain regions that are presumably involved in controlling reproduction was photoperiodic condition-dependent. Based on other studies, it appears that this dependency may be gonadal steroid-mediated. Work supported by E.U. (CT94-0472), C.N.R. (94.02462), and M.U.R.S.T. Awards.

612.1

ABUNDANT NEUROPEPTIDE Y (NPY) Y1 mRNA EXPRESSION IN THE HUMAN FOREBRAIN. L. Caberlotto, K. Fuxe, J.M. Rimland*, G. Sedvall, and Y.L. Hurd. Karolinska Institute, Depts. of Neuroscience and Clinical Neuroscience, Stockholm, Sweden. Dept. Pharmacology, GlaxoWellcome Spa, Medicine Research Centre, Verona, Italy.

Though many neurobiological functions have been ascribed to the NPY Y1 receptor subtype, autoradiographic analysis has failed to detect Y1 binding sites in most areas of the human brain in contrast to the rat. In the present study, we have analyzed the regional distribution of NPY Y1 mRNA-containing cells in the human brain to clarify if there exists a major discrepancy between the rat and the human brain in terms of the existence and distribution of NPY Y1 receptors in the telencephalon, in particular the striatum and cortex. *In situ* hybridization experiments revealed a widespread distribution of the Y1 mRNA signal in all layers of most limbic and neocortical regions with the predominant labeling localized to layer IV (most cortical regions) and layer VI. The striatum showed moderate levels of Y1 receptor mRNA expression with intensely expressing cells localized to the nucleus accumbens. Overall, the highest expression of the Y1 receptor mRNA was within the dentate gyrus, with the lowest in the subiculum, parahippocampal gyrus, cerebellum, and thalamus. Moderate levels were also evident in the amygdala. *In vitro* autoradiography using [^{125}I]PYY with NPY (13-36) or Leu^{31}Pro^{34}-NPY, to mask Y2 or Y1 receptors, respectively, confirmed the low presence of Y1 binding in the human brain despite abundant Y1 mRNA expression. However, using a rat model of the human autopsy process, we found that the inability to reveal high Y1 vs Y2 receptors in the human brain is related in part to significant reductions of the Y1 receptor even within a 4 hr postmortem delay in comparison to Y2.

The work was supported by GlaxoWellcome Spa, Verona, Italy

612.2

MODULATION OF SYNAPTIC TRANSMISSION BY MULTIPLE NEUROPEPTIDE Y (NPY) RECEPTORS IN THE ARCUATE NUCLEUS OF THE RAT. H. Rhim* and R.J. Miller. Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

NPY is a 36 amino acid peptide that it is widely distributed in the central and peripheral nervous systems. Recently, NPY has been the focus of attention due to its dramatic effects on feeding behavior. It is highly concentrated in key regulatory feeding centers in the hypothalamus. Among these, the arcuate nucleus contains not only large numbers of NPY fibers but also high concentrations of NPY-immunoreactive cell bodies. We therefore examined the effects of NPY and its analogs in the arcuate nucleus using whole-cell patch clamp recordings in hypothalamic slices. Under voltage clamp conditions, the Y1 receptor agonist Leu^{31}-Pro^{34} NPY (100 nM) produced a outward current at Vh = -60 mV and this postsynaptic effect recovered within 10-15 min after washout. This effect was blocked by the selective Y1 antagonist BIBP 3226 (1 μM). Leu^{31}-Pro^{34} NPY also inhibited evoked glutamate-mediated excitatory postsynaptic currents (EPSCs, 54.0±8.5% inhibition of control, n=7) as well as spontaneous EPSCs. This presynaptic effect was not blocked by BIBP 3226 and reversed only slowly. Leu^{31}-Pro^{34} NPY was very potent, inhibiting the EPSCs at 1 nM. The post- and presynaptic effects of Leu^{31}-Pro^{34} NPY were mimicked by NPY itself. Pancreatic polypeptide only produced weak effects. The Y2 receptor agonist NPY13-36 (100 nM) inhibited evoked EPSCs (75.8±8.8%, n=4) without changing the holding current. Interestingly, another NPY analog, D-Trp^{32}-NPY (100 nM) also produced inhibition of the evoked EPSCs (53.8±7.8%, n=7) with no change in holding current. However, D-Trp^{32}-NPY had little effect on GIRK1 channels in *Xenopus* oocytes in which rat Y1, 2 or 4 receptors were expressed (See L. Sun et al. this meeting). These data suggest that NPY receptors of different types regulate synaptic transmission in the arcuate nucleus. These include Y1 and 2 receptors and possibly another receptor with novel pharmacological properties (Y1-like). (PHS grants DA-02575, DA-02121, MH-40165 and NS-33502 to R.J.M.).

612.3

REGULATION OF G-PROTEIN LINKED K AND CA CHANNELS BY A FAMILY OF NEUROPEPTIDE Y RECEPTORS. L. Sun*, L.H. Philipson[1] and R.J. Miller. Dept. Pharmacol. and Physiol. Sci. and [1]Medicine, University of Chicago, Chicago, IL 60637.

Neuropeptide Y (NPY) is a 36 amino acid peptide neurotransmitter member of the pancreatic polypeptide (PP) family that is widely distributed in both the central and peripheral nervous systems. NPY has frequently been shown to produce presynaptic inhibition, an effect which may involve its ability to regulate neuronal Ca and K channels. Here we show that 3 different NPY receptors can all regulate inwardly rectifying K channels and N type Ca channels. We expressed rat Y1, 2 and 4 receptors (Synaptic Pharmaceutical Corp.) in *Xenopus* oocytes together with the proteins GIRK1 and CIR. Addition of NPY and structural analogues produced large inwardly rectifying K currents in these oocytes. The rank order of potency was NPY> Leu^{31}Pro^{34}NPY≈PYY>PP≈NPY13-36>D-Trp^{32}NPY for Y1; NPY>PYY>NPY13-36>Leu^{31}Pro^{34}NPY≈PP≈D-Trp^{32}NPY for Y2 and PP>PYY>Leu^{31}Pro^{34}NPY>NPY> NPY13-36≈D-Trp^{32}NPY for Y4 receptors. The effects of NPY on the Y1 receptors were antagonized by the compound BIBP 3226 in a competitive fashion. However, the effects of agonists on Y2 receptors were not antagonized. The effects of agonists at all 3 types of receptors were absent if the oocytes were treated with pertussis toxin. We also expressed Y1, 2 and 4 receptors in HEK 293 cells that stably expressed N type Ca channels. Following expression these cells bound ^{125}I NPY with high affinity. Activation of Y1, 2 and 4 receptors in these cells produced robust inhibition of N type Ca currents evoked by depolarization. The effects of all of these agonists were absent in cells treated with pertussis toxin. These results show that all of the known NPY receptors are capable of increasing G-protein linked K channels and decreasing N type Ca channels and that these effects may underlie their synaptic modulatory effects. (This work was supported by PHS grants DA-02575, DA-02121, MH-40165 and NS-33502 to R.J.M.).

612.4

ACTIONS OF NEUROPEPTIDE Y RECEPTORS IN PARAVENTRICULAR NUCLEUS OF RAT HYPOTHALAMUS

N. Pronchuk & W.F. Colmers*. Dept. Pharmacol., Univ. Alberta, Edmonton, AB

Injection of Neuropeptide Y (NPY) into hypothalamic nuclei causes a profound increase in carbohydrate-selective food intake. The receptor and neuronal mechanisms underlying this response are the subject of this report.

Slices containing the paraventricular nucleus (400 μm) were prepared with a vibratome from brains of male Sprague-Dawley rats (5-7 weeks old) according to an approved protocol. Slices from approximately 1.30 and 2.12 mm posterior to bregma were used (based on Paxinos and Watson, 1986). Slices were kept bubbled with 95% O_2/5% CO_2 at 30 °C for at least 1h, then maintained in a 500 μL recording chamber submerged in a continuous flow (2.5 ml/min) of ACSF at 34 ± 0.5 °C. Whole-cell patch clamp recordings were made with pipettes containing (in mM): K^+ -gluconate 120; KCl 2; HEPES 5; MgATP 5; BAPTA 1.1, $CaCl_2$ 0.1; 290 mOsm, Ph 7.25, while perforated patch recording were made with K^+-gluconate 135, BAPTA 0.1, HEPES 10, $MgCl_2$ 5, with 450 μg/ml nystatin (286 mOsm, pH 7.35). Stimulating electrodes were placed slightly ventral to PVN.

Postsynaptic: Recordings were made from > 40 neurons in the perforated patch configuration. Neurons exhibited spontaneous activity. Application of a centrally-truncated Y_1 receptor agonist (300 nM-1μM) caused a reduction in firing rate in 9/14 cells, which reversed in 5, while 5 cells showed no response. There was no change in conductance associated with the change in activity.

Synaptic: Whole-cell recordings made from > 20 neurons. Stimulation elicited short-latency synaptic responses that were sensitive to either NBQX/APV (EPSC) or picrotoxin (IPSC). Application of Y_1 agonists reversibly reduced EPSC amplitudes in 6/8 neurons and IPSC's in 10/15 cells. Y_2 agonists reversibly reduced EPSC's in 7/9 neurons and IPSC's in 5/10 cells. No changes in postsynaptic conductances were associated with the observed effects on synaptic activity.

In PVN, both Y_1 and Y_2 receptors mediate effects on synaptic inputs to neurons, in addition to affecting their spontaneous activity. The presence of two receptors, if their actions are synergistic, may explain the "atypical Y_1" response in feeding. Supported by Eli Lilly Canada. WFC is an AHFMR Senior Scholar.

612.5

HYPOTHALAMIC NPY mRNA LEVELS IN OB/OB MICE DURING BODY WEIGHT LOSS INDUCED BY DOPAMINERGIC AGONIST TREATMENT. M. Jakubowski*, M. Lubkin E. Tozzo and A.H. Cincotta. Ergo Science Development Corp., Charlestown, MA 02129

We have previously shown that dopamine agonists can produce dramatic anti-diabetic and anti-obesity effects in man and animals. For the genetically obese ob/ob mouse, we have used a mixture of D1/D2 dopamine agonists that markedly decreases insulin resistance, blood glucose levels, food intake and body fat stores. Here, we examined whether those changes are associated with decreased hypothalamic levels of the mRNA encoding neuropeptide Y (NPY) which is known to promote insulin resistance and food intake. Female ob/ob mice (39-52 g) and lean C57BL/6J counterparts (19-23 g) were housed under a 12:12 h light:dark schedule. Obese mice were treated daily at 1 h after the onset of light with i.p. injections of 10 mg/kg SKF 38393 (a D1 agonist) and bromocriptine (a D2 agonist). Obese mice receiving corresponding injections of vehicles alone, as well as non-treated lean counterparts served as controls. Mice were sacrificed either 6 h before or 4 h after light onset, yielding no significant night-daytime differences for any of the parameters measured. After 14 days of treatment, obese mice lost 15% of their body weight ($p < 0.001$) compared to obese controls. Cumulative food intake in the treated obese mice plunged 43% ($p < 0.001$) and 21% ($p < 0.05$) compared to obese and lean controls, respectively. Blood glucose levels were elevated 3-fold in obese controls ($p < 0.001$) and normalized in the treated obese mice, compared to lean controls. NPY mRNA levels in the arcuate nucleus, measured by *in situ* hybridization, were 3-fold higher in obese controls than in lean counterparts ($p < 0.001$). NPY mRNA levels decreased 31% in the treated obese mice compared to obese controls ($p < 0.05$), but remained twice as high as in lean controls ($p < 0.05$). We conclude that the improvement of metabolic function in the ob/ob mouse using D1/D2 agonist treatment may be mediated in part by reduced hypothalamic NPY activity. Notwithstanding, our treatment induced hypophagia and euglycemia in the ob/ob mouse in the face of sustained heightened level of NPY gene expression. Ergo Science Development Corp.

612.6

LOCALIZATION OF THE NEUROPEPTIDE Y (Y4) / PANCREATIC POLYPEPTIDE-RECEPTOR IN RAT PARAVENTRICULAR NUCLEUS (PVN) AND EFFECT OF INTRA-PVN INJECTION OF PP IN FED RATS. P. Kristensen[1]*, J. S. Rasmussen[2] and P. J. Larsen[3]. [1]Histology and [2]Molecular Biology, Health Care Discovery, Novo Nordisk A/S, Bagsværd and [3]Department of Medical Anatomy, University of Copenhagen, Denmark.

The recent isolation of the Y4 Neuropeptide Y (NPY) receptor subtype has prompted our interest into the potential role of this receptor during initiation of feeding behaviour. Within the hypothalamus, Y4 mRNA expression was exclusively found in the paraventricular nucleus. The highest level of expression was found in the lateral parvocellular subdivision and the ventral part of the medial parvocellular subdivision. The Y4 receptor binds PP-fold peptides with a rank order of PP > PYY >= NPY. Autoradiographic analysis of rat 125-I labelled PP binding (50 pM) showed specific binding (complete displacement by 1 μM cold rat PP) to the medial and lateral parvocellular subdivision of the PVN. Finally, local application in the PVN demonstrated that at low and moderate doses (2.5 - 250 pmol) PP and NPY equipotent at inducing food intake in fed rats. At higher dose, however, NPY more potently stimulated the feeding response. These findings point to a potential role for the Y4/PP-receptor subtype in the induction of feeding behaviour.

612.7

EFFECTS OF NEUROPEPTIDE Y_1 RECEPTOR ACTIVATION ON THE RESPONSES OF CELLS IN THE RAT'S VENTRAL POSTEROMEDIAL THALAMIC NUCLEUS. Y. Zhang*, N.L. Chiaia, R.D. Mooney and R.W. Rhoades. Dept. Of Anatomy and Neurobiology, Medical College of Ohio, Toledo, OH 43699

Neuropeptide Y (NPY) is contained along with norepinephrine in many axons that project from the locus ceruleus to the ventral posteromedial thalamic nucleus (VPM) and both NPY Y_1 and Y_2 receptors are present in this region. We previously reported that application of NPY altered the responses of vibrissae-sensitive neurons in VPM and this study was undertaken to determine the contribution of Y_1 receptor activation to these changes. The effects of the selective Y_1 receptor agonist, [Leu^{31}][Pro^{34}], were tested on 35 vibrissae-sensitive VPM cells. It reduced the responses to principal whisker stimulation for 11 of these cells (31%) by ≥30% and increased the responses of only one of these neurons (3%) by at least this amount. Similar effects were observed for responses to surround whisker stimulation; 18% of cells had their responses decreased by ≥30% and only 6% had their responses increased by at least this amount during [Leu^{31}][Pro^{34}] administration. Application of the Y_1 receptor agonist also reduced responses to electrical stimulation of trigeminal nucleus principalis by ≥30% in 54% of cells. No PrV-evoked responses were increased by application of [Leu^{31}][Pro^{34}]. Similarly, delivery of [Leu^{31}][Pro^{34}] decreased the responses of 38% of VPM cells to electrical stimulation of trigeminal subnucleus interpolaris (SpI) by ≥30%. Here again, no SpI-evoked responses were increased by this amount during [Leu^{31}][Pro^{34}] application. These results indicate that activation of the Y_1 receptor suppresses responses of vibrissae-sensitive VPM neurons to excitatory trigeminal brainstem input. Supported by DE 08971and DE 07734.

612.8

EFFECTS OF NEUROPEPTIDE Y_2 RECEPTOR ACTIVATION ON THE RESPONSES OF VIBRISSAE-SENSITIVE NEURONS IN THE RAT'S VENTRAL POSTEROMEDIAL THALAMIC NUCLEUS. R.D. Mooney*, Y. Zhang, N.L. Chiaia, and R.W. Rhoades. Dept. Of Anatomy and Neurobiology, Medical College of Ohio, Toledo, OH 43699

The ventral posteromedial thalamic nucleus (VPM) in the rat contains high concentrations of neuropeptide Y (NPY) receptors and the responses of vibrissae-sensitive thalamic neurons are strongly influenced by both NPY and the Y_1 receptor agonist, [Leu^{31}][Pro^{34}]. The present study was undertaken to determine the effects of selective activation of the Y_2 receptor with NPY_{13-36} on the responses of VPM neurons evoked by vibrissae deflection and electrical stimulation of either trigeminal nucleus principalis (PrV) or trigeminal subnucleus interpolaris (SpI). The effects of the selective Y_2 receptor agonist, NPY_{13-36}, were tested on 18 vibrissae-sensitive VPM cells. It reduced the responses to principal whisker stimulation for 4 of these cells (22%) by ≥30% and increased the responses of 3 neurons (17%) by at least this amount. Responses to surround whisker stimulation were decreased by ≥30% in 29% of cells tested and increased by at least this amount in 14%. Administration of NPY_{13-36} reduced responses to electrical stimulation of PrV by ≥30% in 20% of cells tested and increased responses by at least this amount for 30%. Delivery of NPY_{13-36} decreased the responses of 63% of VPM cells to electrical stimulation of SpI by ≥30% and increased the responses of only 13% of cells tested by this amount. These results indicate that activation of the Y_2 receptor has generally suppressive effects upon the responses of vibrissae-sensitive VPM cells. However, it also appears that activation of this receptor has differential effects on responses to stimulation of PrV and SpI. Supported by DE 08971 and DE 07734.

612.9

EFFECTS OF ACTIVATION OF NEUROPEPTIDE Y_1 AND Y_2 RECEPTOR ACTIVATION ON THE RESPONSES OF VENTRAL POSTEROMEDIAL THALAMIC NEURONS *IN VITRO*. M. Chen*, N.L. Chiaia, R.D. Mooney, and R.W. Rhoades. Dept. Of Anatomy and Neurobiology, Medical College of Ohio, Toledo, OH 43699

Radioligand binding and *in vivo* electrophysiological experiments have shown that neuropeptide Y_1 and Y_2 receptors are present in the rat's ventral posteromedial thalamic nucleus (VPM) and that selective activation of either of these receptors influences the sensory responses of VPM neurons. The present *in vitro* study was undertaken to assess the effects of the selective Y_1 and Y_2 agonists, [Leu^{31}][Pro^{34}] NPY and NPY_{13-36}, on the membrane potentials and responses to medial lemniscus stimulation of VPM neurons. Both agonists had mixed effects on both the membrane potentials and epsps. Administration of the Y_1 agonist resulted in hyperpolarization of 65% of 20 cells tested (mean change -5.23 ± 3.29 mV) and depolarization of 30% (mean change 2.33 ± 1.11 mV). Application of [Leu^{31}][Pro^{34}] reduced the epsps of 45% of cells tested by ≥20% (average reduction -3.0 ± 1.58 mV) and increased the epsps of 20% by at least this amount (average increase 2.88 ± 1.08 mV). Administration of the Y_2 agonist resulted in hyperpolarization of 80% of 10 cells tested (mean change -3.88 ± 2.57 mV) and depolarization of 10% (mean change 10 ± 0 mV). Application of NPY_{13-36} reduced the epsps of 30% of cells tested by ≥20% (average reduction -2.33 ± 0.62 mV) and increased the epsps of 20% of cells by at least this amount (average increase 2.5 ± 1.0 mV). The heterogeneity of the effects of selective Y_1 and Y_2 receptor activation in VPM is surprising given previous observations in several structures that activation of the former receptor results in postsynaptic excitation and stimulation of the latter is associated with presynaptic inhibition. Supported by DE 08971 and DE 07734.

612.10

NPY INHIBITS GABA NEUROTRANSMISSION AND WHOLE-CELL CALCIUM CURRENTS THROUGH BOTH Y1 AND Y2 RECEPTORS IN SCN NEURONS. G. Chen*, and A. N. van den Pol. Sect. Neurosurg., Yale Univ. Sch. Med., New Haven, CT 06520.

The suprachiasmatic nucleus (SCN) plays a critical role in the entrainment of circadian rhythms. GABAergic neurons dominate in SCN. Microinjection of NPY into SCN phase shifts the circadian rhythm, suggesting a modulatory role of NPY in SCN function. Whole-cell voltage clamp and current clamp methods were employed to study the modulation of NPY on synaptic release and barium currents in SCN cultures. NPY (80 nM) reduced the frequency of miniature spontaneous IPSPs to half of the control while having little effect on the postsynaptic GABA response, suggesting a presynaptic mechanism of NPY inhibition. Similar to NPY, [Leu^{31}, Pro^{34}]-NPY (80 nM) , a preferential Y1 agonist, and NPY 13-36 (80 nM) , a preferential Y2 agonist, presynaptically inhibited the spontaneous GABA activity. To further characterize the NPY action, single-neuron microcultures in which a single neuron forms synapses with itself was used. The single autaptic neuron allowed us to record a presynaptic release induced response and a GABA application evoked response in the same sweep. Application of NPY inhibited the autaptic release while leaving the postsynaptic response unaltered, confirming the presynaptic mechanism of NPY action. In addition, application of Y1- and Y2-selective agonists to the same autaptic neuron also significantly inhibited the autaptic GABA release, suggesting the coexistence of Y1 and Y2 receptors in the presynaptic nerve terminals of SCN neurons. NPY, as well as [Leu^{31}, Pro^{34}]-NPY and NPY 13-36, also inhibited whole-cell barium currents, indicating an expression of both Y1 and Y2 receptors in neuronal soma. The inhibition of presynaptic GABA release paralleled the inhibition of barium currents in the same autaptic neuron, suggesting an inhibition of calcium channels may underlie the NPY inhibition of GABA release. These results demonstrate that NPY Y1 and Y2 receptors coexist in both the cell body and axon terminals of a single neuron and mediate the inhibition of calcium channels and synaptic GABA release in SCN neurons.

612.11

NEUROPEPTIDE-Y DIFFERENCES BETWEEN FEMALE AND MALE RAT BRAIN: LOWER BASELINE AND DECREASED RESPONSE TO ELECTROCONVULSIVE TREATMENT IN FEMALES. BIOLOGICAL AND CLINICAL IMPLICATIONS. P.A. Jiménez, D.H. Overstreet[1], and A.A. Mathe*. Dept. of Clinical and Neuroscience, Karolinska Institute, 112 81 Stockholm, Sweden and [1]Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, NC.

Accumulated data indicate that neuropeptides, in particular neuropeptide-Y (NPY), may be involved in depression. The present study examined the concentrations of NPY-like immunoreactivity in several brain regions in three strains of rats (the Flinders Sensitive Line (FSL), Flinders Resistant Line (FRL), and outbred Sprague-Dawley (SD) rats) after a series of 8 ECT or sham ECT given every other day. Following sacrifice with focused high energy microwave irradiation, the brains were dissected, and the peptides extracted and measured with radioimmunoassays. Marked "baseline" (sham ECT treated) differences in NPY-LI concentrations in frontal cortex, occipital cortex, and hippocampus between the female and male rats of all three strains were observed. In addition, within the gender, the sham ECT treated SD males had higher values than FRL males in the frontal and occipital cortices and SD females had higher values than both FRL and FSL females in the frontal cortex. The results of ECT varied with strain, gender and brain region. ECT increased NPY-LI in the striatum of only the male SD rats. In contrast, there was an increase in the frontal cortex in all groups except the FSL female rats. The FSL rats (both genders) also were resistant to the increases induced by ECT in the occipital cortex. The largest increases in NPY-LI were seen in the hippocampus, with all genders and strains exhibiting significant changes. However, as with the other brain regions, the changes in NPY-LI were smaller in the FSL rats. In conclusion, significant NPY regional gender differences may have implications for gender biology and, together with the different response to ECT, suggest that NPY may be involved in affective disorders.

612.12

DIRECT EVIDENCE FOR THE INVOLVEMENT OF NEUROPEPTIDE Y (NPY) IN SYMPATHETIC NERVE-MEDIATED VASOCONSTRICTION S.P. Han*, C.L. Yang, X.L. Chen, L. Naes and T.C. Westfall Dept. Pharmacol. and Physiol. Sci., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

NPY is a vasoconstrictor peptide and a cotransmitter with norepinephrine (NE) and epinephrine in sympathetic nerve terminals and the adrenal medulla, respectively. Several lines of evidence suggest that, in addition to NE, NPY may also mediate sympathetic nerve activation-induced vasoconstriction. Using BIBP3226, a newly synthesized Y_1 receptor selective antagonist, we examined this hypothesis in the isolated and perfused mesenteric vascular bed of the rat. Our results indicate that NPY plays an important role in mediating sympathetic nerve activation-induced vasoconstriction. Periarterial nerve stimulation produced a frequency-dependent increase in perfusion pressure in the isolated and perfused rat mesenteric vascular bed indicative of vasoconstriction. There were concomitant increases in NE and NPY immunoreactivity (NPYir) overflow in the perfusate determined by HPLC-electrochemical detection and radioimmunoassay, respectively. Vasoconstriction induced by NE or nerve stimulation was potentiated by [Leu^{31}, Pro^{34}] NPY, a selective Y_1 receptor agonist. The potentiation was prevented by pretreating the vascular bed with BIBP3226. In addition, nerve stimulation-induced vasoconstriction was significantly attenuated by BIBP3226. These results strongly suggest that NPY is released and is involved in mediating sympathetic nerve stimulation-induced vasoconstriction in resistance vessels. Supported by NIH HL-26319 and HL-35202.

612.13

VASCULAR EXPRESSION OF NEUROPEPTIDE Y (NPY) AND NPY RECEPTORS: MECHANISMS OF ANGIOGENESIS. E. Karwatowska-Prokopczuk[1], W. Rose[2], D.S. Grant[2], E. K. Dayao[1], G. J. Hauser[1], H Ignatowska-Switalska[3], and Z. Zukowska-Grojec[1]*. [1]Dept. Physiology, Georgetown Univ, Washington DC, [2]Cardeza Found. Hematol. Res., Jefferson U., Philadelphia, PA, and [3]Dept. Hypertension, Med. Acad., Warsaw, Poland.

Previously, we reported that NPY, a sympathetic neurotransmitter and a vasoconstrictor, is angiogenic *in vivo* and *in vitro*. All components of the NPY system - the peptide, its receptors, and dipeptidyl peptidase IV (DPPIV), an enzyme which converts NPY to NPY_{3-36} (a Y2 selective agonist) - appear to be present in endothelial cells (ECs). We now attempted to determine 1) whether ECs synthesize and release NPY, 2) what is the angiogenic receptor subtype and its signaling; and 3) whether cell growth and/or differentiation alters the expression of NPY or Y1 receptors. Quiescent human umbillical vein endothelial cells (HUVECs) possess sub-pM NPY-immunoreactivity (-ir, ELISA) and undetectable NPY mRNA (Northern blot) levels. During growth on plastic and differentiation on Matrigel, HUVECs up-regulate NPY mRNA and Y1 receptor mRNA (6-fold) by 6-20 hrs, and release NPY-ir into the media by 24 hrs. NPY and NPY_{3-36} increased up to 2-fold the capillary tube formation by HUVECs on Matrigel by 18 hrs, whereas $Leu^{31}Pro^{34}NPY$ (Y1 agonist) was less active; NPY antiserum blocked these effects. Y1 receptor antagonist, (R)-N2-(diphenacyl)-N-[(4-hydroxyphenyl)methyl]-D-argininеamide, BIBP3226 (1 μM), potentiated tube formation in control and NPY-treated cells by up to 2-fold. The Y2 antagonist, T4-$[NPY_{33-36}]$4 (1μM), abolished the NPY_{3-36}-induced tube formation and inhibited the NPY effect. NO synthase inhibitor, L-NAME (0.1 mM), 50% inhibited the NPY-induced tube formation and N-alpha-benzoyl-L-arginine ethyl ester reversed this action. Thus, during EC growth, NPY and Y1 receptor expression increase, NPY is released and stimulates angiogenesis. The NPY-mediated angiogenesis is mainly dependent on activation of Y2 receptors with some involvement of Y1 receptors and NO.

612.14

ORGANIZATION AND LESION-INDUCED CHANGES IN GALANIN RECEPTORS IN THE RAT'S TRIGEMINAL SYSTEM. R.W. Rhoades*, D. Bodie, C.A. Bennett-Clarke, and N.L. Chiaia. Dept. Of Anatomy and Neurobiology, Medical College of Ohio, Toledo, OH 43699

Previous immunocytochemical experiments have shown that the neuropeptide, galanin, is dramatically upregulated in a vibrissae-related pattern in the brainstem after perinatal damage to either the infraorbital nerve or individual vibrissae follicles. The present study was undertaken to determine changes in the density of receptors for this peptide following such damage. Galanin receptors were labelled with ^{125}I-galanin in rats that sustained unilateral transection of the ION either at birth (P-0) or in adulthood. Rats that sustained lesions on P-0 were killed either 7 or > 60 days after nerve transection while rats that sustained nerve damage as adults were killed 7 days after the ION transections. Autoradiograms of tissue sections through trigeminal nucleus principalis (PrV), subnucleus interpolaris (SpI), and subnucleus caudalis (SpC) were evaluated using quantitative densitometric methods. Transection of the ION in adulthood resulted in significant ($P<0.05$) reductions in binding density in PrV (-27 ± 7%), SpI (-40 ± 15%), and the laminated portion of SpC (-68 ± 15%). Transection of the ION on P-0 produced markedly different results. Animals killed on P-7 had slight (-18 ± 6% for PrV, -21 ± 9% for SpI, and -12 ± 2% for SpC) but significant ($p<0.05$) reductions in binding density, while those killed in adulthood exhibited significant ($p<0.05$) increases (69 ± 12% for PrV, 44 ± 10% for SpI, and 13 ± 1% for SpC) in the density of galanin binding sites. Supported by NS 28888 and DE 07734.

612.15

ELECTROPHYSIOLOGICAL EFFECTS OF CRF AND CRF ANTAGONISTS ON SELECTED BRAIN REGIONS AND PITUITARY OF THE RAT IN VITRO. M.D. Davis* and C.E. Curtis. Psychiatric Disorders Therapeutics, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., 2800 Plymouth Rd., Ann Arbor, MI 48105.

Corticotropin releasing factor (CRF) is a peptide which plays a key regulatory role in the hypothalamic-pituitary-adrenal stress axis, a system which is perturbed in human depression. Since intracerebral administration of CRF evokes anxiogenic and depressive types of behavior in animals, it may also participate in the extra-hypothalamic central response to stress. Here, we examined the effects of CRF on the activity of several brain and pituitary sites known to have high levels of CRF binding or receptor mRNA.

Tissue slices were obtained from Sprague-Dawley rats (14-21 days old) and superfused in vitro. Standard extra-cellular unit recordings were made while either vehicle, CRF1-41, a-helical-CRF9-41 and/or D-Phe-CRF12-41 were bath applied (≤1uM). CRF produced an elevated firing rate in the parabrachial, pontine, solitary, inferior colliculus, ventromedial hypothalamic and lateral septal nuclei and the pituitary intermediate lobe. These effects were blocked by the CRF antagonists, a-helical-CRF9-41 or D-Phe-CRF12-41. Partial tachyphylaxis was seen on repeated testing with CRF in some regions. The excitatory effects of CRF were not seen in the locus coeruleus, substantia nigra or raphe nuclei. These results demonstrate a wide distribution of CRF-sensitive neurons in the brain and contrast, in some cases, with previous reports using in vivo methods.

(Supported by Warner-Lambert)

612.16

CENTRAL CRF ADMINISTRATION PREFERENTIALLY ACTIVATES NEURONS EXPRESSING TYPE I CRF RECEPTORS. J. C. Bittencourt*, R. A. Rissman and P. E. Sawchenko. The Salk Institute, La Jolla, CA 92037.

In an effort to explain frequently encountered "mismatches" between the central distributions of synaptically active molecules and their cognate receptors, proponents of parasynaptic or volume transmission have frequently pointed to the ventricular system as an avenue for the delivery of ligands to receptors of the appropriate chemical specificity. The recent cloning of cDNAs encoding two distinct CRF receptors permits analysis of the specificity with which central CRF targets neurons bearing CRF receptors. We followed the time course and distribution (relative to that of CRF receptors) of Fos-ir induction seen in response to CRF (1 µg in 10 µl saline; icv) given to awake, freely moving rats. CRF provoked widespread Fos induction that was detected within 30 min after injection, peaked at 2 hr, and abated thereafter. The pattern of induced Fos expression closely mimicked the distribution of CRF-R1 mRNA expression, including widespread induction throughout the cortical mantle, in cell groups involved in the processing of visual, auditory, vestibular and somatic sensory information, and in the cerebellar cortex and several of its major afferents and targets. Dual labeling experiments revealed extensive correspondence of CRF-stimulated Fos-ir and CRF-R1 mRNA at these and other loci. Major sites of CRF-R2 expression were lacking or impoverished in Fos-ir nuclei. CRF-induced cellular activation was observed in several cell groups that express neither CRF receptor subtype, including the central nucleus of the amygdala, the locus coeruleus and the paraventricular nucleus of the hypothalamus. Co-injection of a CRF receptor antagonist (1, 10 or 100 µg [$DPhe^{12}$, $Nle^{21,38}$] $rCRF_{12-41}$) blocked CRF-induced Fos-expression in a dose-related manner. Injection of an inactive synthetic fragment of CRF (1 µg $oCRF_{9-33}$) evoked Fos induction only in periventricular regions. It remains to be determined whether the highly preferential targeting of CRF-R1 expressing neurons by icv CRF may be explained by an initial activation of periventricular structures with subsequent recruitment of receptor bearing neurons by complex neuronal pathways, or by an ability of CRF to traverse the brain-CSF barrier and access receptor-expressing cells directly. (Supported by DK-26741 and the Brazilian CNPq)

612.17

BLOCKADE OF CRF-INDUCED EXCITATION OF LOCUS COERULEUS CELL FIRING BY CP-154,526, A NON-PEPTIDE ANTAGONIST OF CENTRAL CRF RECEPTORS. J. Braselton*, Y. Chen and J. Sprouse. Pfizer Central Research, Groton, CT 06340 USA

Various clinical findings suggest that CRF (corticotropin releasing factor) is hypersecreted in certain psychopathological states. While a CRF antagonist might be useful in treatment of these disorders, the agents currently available are peptides (e.g. α-helical CRF_{9-41}) and as such are not ideal candidates due to their inherent poor bioavailability and difficulty in penetrating the blood-brain barrier. Here we describe the effects of CP-154,526, a potent and selective non-peptide CRF antagonist, on one measure of central CRF activity, excitation of locus coeruleus cell firing.

Extracellular single unit recordings of locus coeruleus neurons were made in male Sprague-Dawley rats anesthetized with chloral hydrate. r/h CRF (3 µg / 6 µl) injected i.c.v. produced an increase in locus coeruleus cell firing (mean ± SEM = 102 ± 19%, n = 15), similar in magnitude to that previously reported (Valentino et al., Brain Res. 270, 363-367, 1983). CP-154,526 given i.v. 5 - 10 minutes prior to the CRF challenge blocked the excitation in a dose-dependent manner. The ID_{50} calculated from regression analysis of the dose response curve was 2 mg/kg i.v. Selectivity of the blockade by CP-154,526 was probed with substance P -induced excitation of cell firing. Substance P given alone (1.0 µg / 6.0 µl i.c.v.) increased firing rate by 102 ± 28 % (n = 4). When given as pretreatment, CP-154,526 at 5.6 mg/kg i.v., the highest dose tested against the CRF challenge, was ineffective at blocking the response to substance P (net excitation = 101 ± 11%). Taken together these data suggest that CP-154,526 is a selective CRF antagonist, active against central CRF receptors when given systemically in a well characterized model of CRF activation.

612.18

CORTICOTROPIN-RELEASING FACTOR MICROINFUSION INTO THE LOCUS COERULEUS: EFFECTS ON DISCHARGE RATE, CORTICAL NOREPINEPHRINE LEVELS AND CORTICAL ELECTROENCEPHALOGRAPHIC ACTIVITY. A.L. Curtis*, S.M. Florin-Lechner, L.A. Pavcovich and R.J. Valentino. Dept. Psychiatry, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19102.

Corticotropin-releasing factor (CRF) administered intracerebroventricularly (i.c.v.) activates rat noradrenergic locus coeruleus (LC) neurons. The present study used a technique for microinfusing CRF into the LC from calibrated micropipettes to characterize and quantify the effects of locally administered CRF on LC discharge in halothane-anesthetized rats. CRF (3-100 ng) microinfusion into the LC increased discharge rate in a dose-dependent manner from 28 ± 8% to 103 ± 22% above pre-infusion discharge rates. In contrast, microinfusion of vehicle had no effect. The CRF dose-response curve generated by local microinfusion was parallel to, and shifted 100-fold to the left, of that generated by i.c.v. administration with an ED_{50} of 5 ng vs. 700 ng, respectively. Microinfusion of the CRF antagonist, $DPheCRF_{12-41}$ (10 ng in 30 nl) attenuated LC activation produced by both 3 and 10 µg CRF administered i.c.v., suggesting that effects produced by i.c.v. CRF administration are primarily due to actions within the LC. In addition to increasing LC discharge rate, intracerulear CRF (60 ng) increased norepinephrine levels in prefrontal cortex measured by *in vivo* microdialysis. Finally, LC activation produced by CRF (60 ng) microinfusion into the LC was associated with cortical electroencephalographic activation, indicated by a decrease in the amplitude of low frequency activity. Taken together with previous anatomical and electrophysiological evidence for endogenous CRF interactions in the LC, the present results support the hypothesis that CRF serves as a excitatory neurotransmitter in the LC, and suggest that its actions on LC neurons are translated to enhanced norepinephrine release and an impact on cortical targets. Supported by USPHS Grants MH40008 and MH00840.

612.19

BEHAVIOURAL EFFECTS OF UROCORTIN IN RATS. D.N.C. Jones*, P.D. Slade, R. Kortekaas and J.J. Hagan. Psychiatry Res., SmithKline Beechams plc, Harlow, Essex, CM19 5AW, UK.

Urocortin, a novel member of the corticotropin releasing factor (CRF) family, has recently been reported. Compared with human/rat CRF (h/rCRF), urocortin has higher affinity for CRF_1 and CRF_{2B} receptors and greater potency to increase cAMP in cell lines expressing these receptors. Further, intravenous urocortin is a more potent inducer of ACTH release *in vivo*. (Vaughan et al., Nature, 378: 287-292, 1995). The present study determined the effects of urocortin, injected into the cerebral ventricles (ICV), upon motor activation and feeding in the rat, behaviours which are sensitive to h/rCRF and CRF-related peptides (Jones et al., J. Psychopharmacol., BAP Summer Meeting, 1996). In rats previously habituated to motor activity chambers (at least 2 separate exposures and 60 min habituation on the test day), urocortin (0.03-1µg ICV) dose-dependently increased total beam breaks over a 70 min period ($P<0.05$), eg from 855 ± 167 [means ± SEM, vehicle (VEH)] to 1304 ± 172 (0.3 µg, $P<0.05$). In a separate experiment, rats were food deprived for 23 h and then allowed access to food for 2 h. Urocortin (0.3-10µg ICV, 15-20 min prior to food access) dose-dependently decreased the amount of food eaten ($P<0.001$), eg from 15.4 ± 1.4g (VEH) to 4.5 ± 0.5g (10µg, $P<0.05$). Further, the increase in body weight during this period was reversed by urocortin ($P<0.001$), eg from 6.0 ± 1.4g (VEH) to -4.8 ± 1.3g (10µg, $P<0.05$). Therefore, centrally administered urocortin caused activation and hypophagia in the rat in a similar manner to that induced by h/rCRF and other CRF-related peptides.

This work was funded by SmithKline Beecham Pharmaceuticals plc.

PEPTIDES: ANATOMY AND PHYSIOLOGY III

613.1

ANGIOTENSIN II (AII), CHOLECYSTOKININ 1-8 (CCK) AND NEUROTENSIN 8-13 (NT) MODULATE CATIONIC CONDUCTANCE IN MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs) ISOLATED FROM SUPRAOPTIC NUCLEUS OF THE ADULT RAT. Yassar Chakfe* and Charles W Bourque. Centre for research in Neuroscience, Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4.

Whole cell recordings were obtained from 62 isolated MNCs using pipettes containing 6 mM [Cl^-], 8 mM [Na^+] and 120 mM [K^+] in order to examine the effects of various neuropeptides on membrane excitability. The perfusing solution comprised 147 mM [Cl^-], 140 mM [Na^+] and 3 mM [K^+]. Application of the peptides (0.1-10 µM; 1-30s) produced reversible depolarization and spike discharges lasting up to 10 minutes in 22 of 24 cells tested. In the presence of TTX (.5 µM) voltage ramps applied in the presence and absence of the peptides revealed increases in membrane conductance with reversal potentials (in mV) of -37±3 (AII; n=8), -33±3 (NT; n=6) and -35±3 (CCK; n=3). Increasing $[K^+]_O$ to 6 mM caused these values to shift to -15±4 (AII; n=7), -11±4 (NT; n=7) and -8±4 (CCK; n=3). Reducing $[Cl^-]_O$ to 10 mM failed to cause a depolarizing shift in reversal potential (n=4). These results indicate that postsynaptic receptors for NT, AII and CCK are present on MNCs of the supraoptic nucleus. Activation of these receptors induces excitatory responses through the prolonged activation of a cation permeable conductance. *Supported by the M.R.C. of Canada.*

613.2

ANGIOTENSIN ACTS AT AT_1 RECEPTORS TO DEPOLARIZE RAT MEDIAN PREOPTIC NUCLEUS (MnPO) NEURONS. D. Bai* and L.P. Renaud. Neuroscience, Loeb Research Institute, Ottawa Civic Hospital and University of Ottawa, Ottawa, Ontario, CANADA K1Y 4E9

The MnPO, an important area for angiotensin-induced drinking behaviour, contains a high density of angiotensin binding sites. The present study used whole cell patch clamp recordings from neurons in the ventral MnPO in adult rat sagittal brain slice preparations to characterize the effects of bath applied angiotensin II (AII). In 50% of cells, AII (10-2000nM for 40s) induced a tetrodotoxin-resistant and sustained (duration>6min) membrane depolarization associated with an increase in action potential frequency. Under voltage clamp, AII induced an inward current over a voltage range of -130 to -30mV. In neurons demonstrating spike frequency adaptation, AII appeared to reduced or abolish this property. AII responses were antagonized by the AT_1 receptor antagonist Losartan (2-10µM) but not the AT_2 receptor antagonist PD123,177 (20µM). These data support a direct neuromodulator role for AII on a population of MnPO cells. Supported by MRC and the Heart & Stroke Foundation of Canada.

613.3

WATER DEPRIVATION REGULATES AT_1 ANGIOTENSIN II RECEPTOR GENE EXPRESSION IN THE RAT SUBFORNICAL ORGAN. G.L. Sanvitto, O. Jöhren, W. Häuser and J.M. Saavedra*. Section on Pharmacology, Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

In the rat brain two pharmacological distinct Ang II receptor subtypes, AT_1 and AT_2, have been described. AT_1 receptors are involved in the regulation of fluid homeostasis and hormone release. The regulation of AT_1 receptors by water deprivation has been shown previously using binding experiments. We used semiquantitative *in situ* hybridization to analyze the regulation of Ang II receptor subtype gene expression in the subfornical organ (SFO), the paraventricular nucleus of the hypothalamus (PVN), and the median eminence (ME) of male rats after 1, 3, and 5 days of water deprivation. We found a significant increase of [^{125}I]Sar^1-Ang II binding to AT_1 receptors in the SFO after 5 days of water deprivation which was accompanied by an increase of AT_{1A} receptor mRNA. No changes were observed in the PVN and the ME or after 1 and 3 days of water deprivation. In no case we were able to detect AT_2 or AT_{1B} receptor mRNA in the SFO, the PVN or the ME. The present results show that after 5 days of water deprivation AT_1 receptors in the SFO are up regulated at the level of gene expression and that the receptor gene involved is that of the AT_{1A} but not the AT_{1B} receptor. Circulating Ang II might contribute to regulate drinking and fluid metabolism by stimulating AT_{1A} receptor synthesis at the level of the SFO.

613.4

EXPRESSION OF ANGIOTENSIN II RECEPTOR SUBTYPES IN THE OLIVOCEREBELLAR SYSTEM OF YOUNG RATS. O. Jöhren* and J.M. Saavedra. Section on Pharmacology, Lab. of Clinical Science, National Institute for Mental Health, Bethesda, MD 20892.

Both, AT_1 and AT_2 angiotensin II receptor subtypes have been shown in the molecular layer of the cerebellar cortex in 2-week-old rats by receptor autoradiography. Within the molecular layer Purkinje cells receive two major inputs, the climbing fibers which originate from the inferior olive and the parallel fibers from cerebellar granule cells. To clarify the cellular localization of angiotensin II receptor subtypes we analyzed their gene expression in the inferior olive and cerebellum of 2-week-old rats by *in situ* hybridization histochemistry. Therefore, we designed ^{35}S-labeled riboprobes complementary to specific sequences of AT_{1A}, AT_{1B}, and AT_2 receptor mRNAs. Using emulsion autoradiography we found AT_{1A} and AT_{1B} receptor mRNA expression in Purkinje cells, but not in granule cells or in the inferior olive. No AT_2 receptor mRNA was detected in any layer of the cerebellar cortex. However, we found AT_2 receptor mRNA in the inferior olive and in the interposed nucleus of the cerebellum. Our results suggest that within the molecular layer of the cerebellar cortex AT_2 receptors are localized on climbing fiber terminals, whereas AT_1 receptors are localized on dendrites of Purkinje cells. A role for angiotensin receptors in the postnatal establishment of the olivocerebellar connectivity is supposed.

613.5

ANGIOTENSIN II RECEPTOR SUBTYPES IN MOUSE BRAIN. W. Häuser[1], O. Jöhren[1], G.L. Sanvitto[1], T. Inagami[2]* and J.M. Saavedra[1]. [1]Section on Pharmacology, Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892; [2]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232.

Two pharmacological distinct Ang II receptor subtypes (AT_1 and AT_2) have been shown in rat brain. In addition, the existence of additional AT_2 receptor subtypes which could be the product of two different AT_2 receptor genes was proposed. To further explore Ang II receptor heterogeneity we characterized Ang II receptors in brains of control and AT_2 receptor-deficient mice by [^{125}I]Sar[1]-Ang II and [^{125}I]CGP 42112 binding experiments in the presence of receptor subtype-selective displacers. In control animals AT_1 receptors were found in areas involved in the regulation of fluid homeostasis and hormone release like the subfornical organ and the paraventricular nucleus. AT_2 receptors were detected in sensory and motor related areas like the superior colliculus and the inferior olive. Interestingly, AT_1 receptors were found in the caudate nucleus where no receptors are present in rats. In AT_2 receptor-deficient mice no [^{125}I]CGP 42112 binding was found indicating the absence AT_2 receptors. In the locus coeruleus which shows AT_2 receptor binding in rats [^{125}I]Sar[1]-Ang II binding was detected in both control and AT_2 receptor-deficient mice. However, here [^{125}I]Sar[1]-Ang II binding was sensitive to losartan. In summary, we revealed species-specific expression of Ang II receptors subtypes in selected brain areas. In addition, we clarified that in the mouse brain AT_2 receptors are the product of only one gene.

613.6

ORIGIN OF ANGIOTENSIN II (AII) IN THE RODENT CENTRAL EXTENDED AMYGDALA (CEA). Gong-yu Yang*, T.S. Gray, C.D. Sigmund and M.D. Cassell. Depts. Anatomy and Internal Medicine, U. of Iowa, Iowa City, IA 52242 and Dept. Cell Biol. Neurobiol. Anat., Loyola UMC, Maywood, IL 60513.

We investigated the origin of the prominent AII innervation of the rodent CEA. Lesions of the rat pontine parabrachial complex (PB) and tractotomy anterior to the PB eliminated almost all AII immunoreactivity in the CEA suggesting the PB is the major source of AII in the forebrain. Injections of HRP into the CEA retrogradely labelled numerous neurons in the PB but none were immunoreactive for AII. We speculated therefore that AII might be produced locally in the CEA from angiotensinogen (ANG) transported anterogradely by PB neurons. Because of the low expression of endogenous ANG in the rodent PB we sought to determine the localization of ANG in transgenic mice which have appropriate cell and tissue specific expression of human ANG. In situ hybridization for human ANG mRNA revealed that the cells in the PB of these transgenic mice expressing human ANG are predominantly glia. To document more fully the distribution of ANG in transgenic mice we developed several transgenic lines with a B-galactosidase reporter gene linked to the 5' flanking region of the human ANG gene. Histochemical staining confirmed the expression of human ANG in glia in the PB but demonstrated that neurons in the medial locus ceruleus, a structure that projects to the CEA, also express human ANG. We are currently undertaking retrograde tracing studies to determine if the PB or locus ceruleus provide an angiotensinogen-containing pathway to the forebrain. Supported by NIH HL48058 & HL55006.

613.7

COLOCALIZATION OF ANGIOTENSIN TYPE 2 (AT_2) RECEPTOR AND VASOPRESSIN IMMUNOREACTIVITY IN MAGNOCELLULAR NEURONS OF THE RAT HYPOTHALAMUS. L.P. Reagan*[1], L.M. Flanagan-Cato[2] and S.J. Fluharty[2]. [1]Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021 and [2]Depts. of Animal Biology, Psychology and the Institute of Neurological Sciences, University of Pennsylvania, Phila., PA 19104

Angiotensin II (AngII) is an important mediator of body fluid and cardiovascular homeostasis. One of the mechanisms through which AngII mediates these effects is by regulating the release of arginine vasopressin (AVP) from the magnocellular neurons of the hypothalamus. We have recently developed antisera selective for the AT_2 receptor subtype and have utilized these antisera to localize AT_2 receptor populations in rat brain via immunohistochemical techniques. Among the brain regions immunodetected by these antisera were the magnocellular neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON). In order to determine whether AT_2 receptor protein is colocalized with AVP, AT_2 receptor immunohistochemistry was performed concurrently with immunohistochemistry for AVP in rat hypothalamus. A subset of magnocellular neurons within the SON and PVN contained both AT_2 and AVP immunoreactivity. In addition, AVP negative cells clearly exhibited AT_2 staining, while some AVP containing neurons had undetectable expression of AT_2 receptors. These results, as well as the absence of double labelling in the presence of AT_2 preimmune serum, confirm the specificity of the immunostaining. Collectively, these results demonstrate that AT_2 immunoreactivity is colocalized with AVP containing neurons in the PVN and the SON. However, the precise role which AT_2 receptors play in the regulation of AngII-induced vasopressin release from the magnocellular neurons of the hypothalamus remains to be elucidated. L.F.C. supported by Amer. Heart Assoc., Southeastern Penn. Branch: L.P.R. and S.J.F. supported by NIH grant numbers MH43787 and NS23986.

613.8

HYPOTHALAMIC VASOPRESSIN GENE EXPRESSION IN THE INBRED POLYDIPSIC MICE. Y.Ueta, H.Yamashita, R.Nishi, M.Nomura, N.Kabashima, I.Shibuya and [1]K.Koizumi*, Dept.of Physiol., Univ. of Occup. Environ. Health, Sch. of Med., Kitakyushu, 807, Japan, [1]Dept. of Physiol., SUNY Health Sci. Ctr. at Brooklyn, NY 11203, USA

The genetically inbred polydipsic mice, STR/N strain, are characterized by extreme polydipsia without a lack of vasopressin (AVP) and its receptors. Daily water intake in the STR/N is 5 to 8 times more than that in control mice (ICR). Northern blotting analysis revealed that hypothalamic AVP gene expression in the STR/N is twice to 3 times higher than that in the ICR. Furthermore, to reveal whether abnomality of the hypothalamic AVP gene expression in the inbred polydipsic mice exist or not, AVP gene expression was examined by *in situ* hybridization histochemistry with ^{35}S-labelled oligodeoxynucleotide probe for mouse AVP. In the STR/N mice AVP gene expression level in the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON) was higher than that in ICR mice. In the anteroventral parts of the PVN AVP transcripts were observed in the STR/N. In ICR mice there were few AVP transcripts in the same area of the anteroventral parts of the PVN. These results suggest that abnormal expression of AVP gene in the hypothalamus may be involved in the mechanism of polydipsia in the genetically polydipsic mice.

613.9

VASOPRESSIN PATHWAYS IN THE BRAIN OF COMMON MARMOSETS (*CALLITHRIX JACCHUS*): STUDIES WITH IMMUNOCYTOCHEMISTRY, *IN SITU* HYBRIDIZATION AND RECEPTOR AUTORADIOGRAPHY. Z.X.Wang[1]*, D. Toloczko[1], L.J. Young[1], K. Moody[2], J.D. Newman[2], and T.R. Insel[1]. [1]Dept of Psychiatry and Behav. Sci., Emory Univ. Sch of Med., Atlanta, GA, 30322, and [2]Lab. of Comp. Ethology, NICHD, NIH, Poolesville, MD 20837

Vasopressin (AVP) pathways and receptors were examined in the forebrain in male and female marmosets (*Callithrix jacchus*). Matched AVP immunoreactive (ir) and AVP mRNA-labeled cells were found in the paraventricular, supraoptic, and suprachiasmatic nuclei, as well as in the lateral hypothalamus and the bed nucleus of the stria terminalis (BST). No AVP cells were detected in the amygdala with either technique. In the BST, males had more AVP-positive cells than females. This sexual dimorphism did not appear to be a direct result of androgen effects as AVP cells in the BST did not colocalize with androgen receptor immunoreactivity. Although AVP-ir was evident outside of the hypothalamic-neurohypophyseal tract, the sexually dimorphic plexus of fibers in the lateral septum and lateral habenula observed in the rat brain could not be detected in the marmoset. In addition, we mapped AVP receptors with ^{125}I-linear-AVP a high affinity antagonist selective for V_{1a} receptors. Specific binding was highest in the nucleus accumbens, diagonal band, lateral septum, BST, hypothalamus, and amygdala. Together these studies provide a comprehensive picture of AVP pathways in the marmoset brain. Supported by MH54368 & 54554 to ZXW and P51-RR00165 to TRI.

613.10

EFFECTS OF VASOPRESSIN ON NEURONS FROM HORIZONTAL LIMB OF THE DIAGONAL BAND OF BROCA (hDBB) IN THE RAT: IONIC MECHANISMS AND RECEPTOR PHARMACOLOGY. J.H. Jhamandas*, J.C. Easaw & B.S. Jassar. Dept. of Medicine (Neurology) and Div. of Neuroscience, Univ. of Alberta, Edmonton, Alberta, Canada.

The hDBB is an important basal forebrain region that subserves a wide variety of physiological functions. Vasopressin (VP), a putative peptidergic transmitter, is localized within fibres in the hDBB and modulates glutamate-evoked neurotransmission in this region. We have recently determined that applications of VP on hDBB neurons caused either a decrease or increase in outward postsynaptic potassium currents. In this study we further characterize the mechanisms of VP's dual actions. Whole-cell patch clamp recordings were obtained from acutely dissociated hDBB neurons. Under voltage clamp conditions, bath application of VP (300 nM) resulted in a reversible decrease in outward current between -30 to +30 mV that was blocked by the application of a V_1 receptor antagonist (n=26). In some cells (n=19), VP caused an increase in outward current that was blocked by V_2 receptor antagonist. Using the application of external medium containing 0 Ca^{++} and 50μM Cd^{++} or charybdotoxin (25nM) we determined that the actions of VP were mediated through blockade of a voltage-dependent Ca^{++}-activated potassium conductance. We also observed that application of VP did not influence calcium currents, which we recorded in these neurons using the barium ion as the charge carrier. VP may influence the excitability of hDBB neurons in a dual manner using the activation of specific receptor subtypes that share a common ionic conductance.

Supported by the MRC and AHFMR.

613.11

NEW CENTRAL NEUROPEPTIDE FF (NPFF) PROJECTIONS IN THE RAT BRAIN. K.H. Harris* and J.H. Jhamandas. Department of Medicine (Neurology) and Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada.

NPFF, a morphine modulatory peptide with opioid and anti-opioid profile, has been localized within discrete central autonomic regions. NPFF projections previously described include those originating from the medial hypothalamus to the brainstem nucleus tractus solitarius (NTS) and projections from the NTS to the pontine parabrachial nucleus (PBN). In this study, we further examined patterns of connectivity of NPFF cells within the hypothalamus and the brainstem. Under pentobarbital anesthesia, rats were injected with retrogradely transported rhodamine and FITC labelled microspheres. Target sites for injections were the caudal third of the lateral PBN and within the ventrolateral medulla (VLM) just lateral to the A1/C1 catecholaminergic cell group. These brainstem sites correspond to regions containing a high density of NPFF fibres. Immunocytochemical staining revealed the presence of NPFF neurons within the NTS that project to the PBN or VLM. We also observed cells with collateral branching projections to both the PBN or VLM. Within the hypothalamus, NPFF cells were also observed to project to the PBN and the VLM. We did not observe any collateral projections from the hypothalamus to these two brainstem sites. Our data provide further evidence of connectivity of NPFF neurons between central autonomic nuclei. These circuits may modulate autonomic- and pain-related information within the neuraxis.

Supported by the Medical Research Council of Canada

613.12

PEPTIDERGIC MODULATION OF CURRENTS THROUGH CALCIUM CHANNELS IN THE HORIZONTAL LIMB OF THE DIAGONAL BAND OF BROCA (hDBB). B.S. Jassar*, K. H. Harris, J.H. Jhamandas, Dept. of Medicine (Neurology), Univ. of Alberta, Edmonton, Alberta, Canada, T6G-2S2.

Voltage-activated calcium channels (VACC) are involved in several important physiological processes. The most commonly studied VACC include L, N and T-type. These are prime targets for modulation by various chemical transmitters, especially peptides, in different neuronal types. Anatomical studies have revealed the presence of several neuropeptides, including neurotensin (NT), vasopressin (VP), neuropeptide FF (NPFF, morphine modulatory peptide), met-enkephalin (met-enk) and angiotensin II, in fibres innervating the hDBB. The hDBB, a basal forebrain region is involved in memory and learning mechanisms, generation of theta rhythm and central cardiovascular regulation.

Whole cell patch clamp recordings of currents through calcium channels, from acutely dissociated rat hDBB neurons, were performed using Ba^{++} as the charge carrier (I_{Ba}). ω-conotoxin (100nM) irreversibly blocked >55% of whole-cell I_{Ba} An additional 30% was blocked by 10uM nimodipine. Thus N and L-type VACC carry the majority of the I_{Ba} in hDBB neurons. We tested the actions of six different peptides agonists on I_{Ba}. Maximum currents were evoked by a voltage command to -10mV or 0mV from a holding potential of -80mV. NT decreased the peak current by 30%. Application of DAGO (5μM; a μ-receptor opioid agonist), met-enkephalin (5μM; μ and δ opioid receptor agonist) and NPFF (5μM) reduced peak I_{Ba} by 35%, 25% and 20% respectively. In contrast, VP (300-500nM) and angiotensin II (1.5μM) were completely ineffective.

These results suggest that certain neuropeptides (NT, met-enk and NPFF) modulate excitability of hDBB neurons through VACC.

This work was supported by the MRC of Canada.

613.13

EXPRESSION OF NEUROPEPTIDE FF PRECURSOR IN RAT CNS. P. Panula, M. Nieminen, A.A. Aarnisalo, M. Lintunen, T. Karhunen*, F.S. Vilim, E. Ziff, K. Karlstedt. Dept. Biology, Abo Akademi Univ., Turku, Finland, Dept. Biomed. Sci., Univ. Helsinki, Helsinki, Finland and Dept. Biochem. HHMI NYU Med.Ctr. NY, USA.

The known neuropeptide FF (NPFF) and neuropeptide AF (NPAF) precursor contains one copy of active peptides NPFF and NPAF involved in pain, autonomic and neuroendocrine functions and memory. Antibodies against the rat NPAF-related peptide SLAAPQRFamide were applied to compare peptide distribution to the expression pattern of the precursor mRNA using *in situ* hybridization with cRNA and oligonucleotide probes. Hybridization signals were strongest in the nucleus of the solitary tract (nts), spinal trigeminal nucleus and superficial laminae of the spinal cord. The paraventricular and supraoptic nuclei were also reactive. Of these sites, the spinal cord, spinal trigeminal nucleus and nts displayed very strong SLAAPQRFamide immunoreactivity, whereas immunoreactivity in the supraoptic and paraventricular nuclei was limited to a few cells. A central hypothalamic nucleus between the dorsomedial and ventromedial nuclei contained a population of immunoreactive neurons after treatment with colchicine, but no hybridization signal was detected in this area in normal brain. Thus, the results suggest that at least some NPFF-like peptides involved in pain, autonomic and neuroendocrine regulation are derived from the known precursor, whereas the hypothalamic cell population with known connections to the limbic system and medulla may contain NPFF-like peptides derived from a hitherto unknown precursor.

Supported by the MRC of the Academy of Finland, Sigrid Juselius Foundation and Howard Hughes Medical Institute.

613.14

NEUROPEPTIDE FF AND PROLACTIN RELEASE; IN VITRO AND IN VIVO STUDIES. A.A.Aarnisalo[1]*,M.Nieminen[2],R. Tuominen[1], and P.Panula[2]. [1]Institute of Biomedicine, POB 9, University of Helsinki, Helsinki, Finland. [2]Dept. of Biology, Biocity, Åbo Akademi University, Turku, Finland.

Neuropeptide FF (NPFF, FLFQPQRFamide) is an opioid analgesia modulating peptide, which is found in high concentrations in the pituitary and spinal dorsal horn of the rat. NPFF-immunoreactive nerve terminals and fibers are dense in the posterior pituitary, whereas no NPFF immunoreactive structures can be seen in the anterior lobe. Specific binding sites for NPFF have been localized both in the anterior and posterior lobes. We have measured the effect of NPFF on cultured male rat anterior pituitary (AP) cells *in vitro* and male rats *in vivo* on the release of prolactin (PRL), thyroid-stimulating hormone (TSH) and growth hormone (GH). NPFF, in small nanomolar concentrations, released PRL *in vitro* and *in vivo* after intraperitoneal injections. It was more effective than TRH *in vitro* in cultured AP cells. NPFF had no effect on the release of GH or TSH from AP cells *in vitro*. One of the functions of the hypothalamo-neurohypophyseal NPFF-containing system may be regulation of the anterior lobe and release of prolactin from the lactotrophes. Our results also support the importance of multiple factors in coordinated regulation of PRL release.

Supported by the MRC of the Academy of Finland and Sigrid Juselius Foundation.

613.15

PFRF-AMIDE, A POSSIBLE AGONIST FOR NEUROPEPTIDE FF , BUT A POSSIBLE ANTAGONIST FOR FMRF-AMIDE. E. Y.-K. Huang, Á. Vehovszky, J. Bagust*, R. P. Sharma, and R. J. Walker. Dept. of Physiology and Pharmacology, University of Southampton. UK, SO16 7PX.

Using an isolated preparation of rat spinal cord we have previously shown that neuropeptide FF (NPFF; mammalian FMRFamide-like peptide) potentiated the amplitude of the monosynaptic reflex (MSR) whereas FMRFamide had no effect. In the present study we have examined the action of another peptide, PFRFamide, on the rat isolated spinal cord and the buccal retractor muscle of the snail *Helix aspersa*. Bath applied PFRFamide was tested on spinal cords isolated from two-week old rats at concentrations of 10^{-8}M to 10^{-5}M. A lumbar dorsal root was stimulated at 5 times threshold and the evoked reflex recorded from the corresponding ventral root using a suction electrode. In five experiments the MSR amplitude was significantly increased (t-test) above the control values at all doses. This effect is similar to that induced by NPFF.

Conc. PFRFamide	MSR %Control	±s.e.m	Significance
10^{-8}M	116.9	5.4	p<0.05
10^{-7}M	120.4	3.3	p<0.001
10^{-6}M	125.6	3.4	p<0.001
10^{-5}M	123.4	9.1	p<0.05

In contrast 10^{-5}M PFRFamide decreased the amplitude of the contraction of the pharyngeal retractor muscle of *Helix aspersa*, whereas 10^{-6}M FMRFamide potentiated this response. These results suggest that PFRFamide may be an agonist for NPFF in mammalian systems but act as an antagonist for FMRFamide in molluscs.

Funding source: The Wellcome Trust. UK

613.16

PROLACTIN RELEASE FOLLOWING LUTEINIZING HORMONE RELEASING HORMONE ADMINISTRATION TO RAT ANTERIOR PITUITARY GLANDS IN VITRO. W. Bryant, J. Janik and P. Callahan*, Miami Univ, Center for Neuroscience, Dept of Zoology, Oxford, OH 45056.

The purpose of these studies was to determine whether Luteinizing Hormone Releasing Hormone (LHRH) would stimulate secretion of prolactin from female rat anterior pituitary glands using a perfusion paradigm. Low doses of LHRH were administered to anterior pituitary glands collected from cycling female rats (3-5 months old). Pituitaries were minced and one half of a pituitary was placed in individual perfusion chambers. This preparation thus allows cellular interactions in the anterior pituitary gland to remain intact. Glands were allowed to equilibrate for at least 90 minutes prior to sample collection. After equilibration, samples were collected prior to any treatment and following 1, 10 or 1000 nM LHRH added in increasing concentrations. Each treatment was followed by 30 minutes recovery before the next dose of LHRH was administered. The flow rate was 0.25 ml per minute with samples collected over 5 minute intervals. Statistical analysis was accomplished utilizing area under the curve calculations.

Administration of LHRH increased total Prolactin secretion indicating that low levels of LHRH can stimulate Prolactin secretion from mature female rat anterior pituitary glands. The role of Angiotensin II in mediating this increase is currently being investigated by pretreating with Saralasin, an AII antagonist.

Supported by NIH DK48023-01 to PC.

613.17

PARATHYROID HORMONE-RELATED PROTEIN (PTHrP) BOTH ENHANCES AND DEPRESSES SYNAPTIC TRANSMISSION IN THE BASOLATERAL AMYGDALA (BLA). P. Shinnick-Gallagher*, C.W. Cooper and V. Neugebauer. DEPT. OF PHARMACOLOGY, THE UNIVERSITY OF TEXAS MEDICAL BRANCH, GALVESTON, TX 77555-1031.

Parathyroid hormone-related protein (PTHrP) shares sequence homology with parathyroid hormone (PTH) at the N-terminus but, unlike PTH, does function as a classical hormone. The fragment PTHrP(1-34) but not the receptor antagonist, PTHrP(7-34), activates the recently cloned PTH/PTHrP receptor that can couple via G-protein to cyclic AMP production, phosphoinositol hydrolysis, and cytosolic Ca^{2+} elevation. mRNA for the PTH/PTHrP receptor has been localized in the BLA. Although mRNA for PTHrP and the PTH/PTHrP receptor have been detected in brain, physiological effects on neuronal excitability and synaptic transmission have not been reported.

Using whole-cell voltage-clamp, the effects of PTHrP(1-34) and PTHrP(7-34) on BLA neurons were examined in rat brain slices. Monosynaptic EPSCs were evoked by stimulation in the lateral amygdala. Drugs were applied by superfusion.

PTHrP(1-34) had dual effects on monosynaptic EPSCs: lower concentrations (0.1 nM) potentiated EPSCs; higher concentrations (10 nM to 10 μM) depressed EPSCs dose-dependently (EC_{50} = 12 nM). The inhibitory action was accompanied by a small outward current (10 ± 4 pA) and an increase in slope conductance (152 ± 20 % of predrug). PTHrP(7-34) (0.1 μM) antagonized the potentiation, the depression, and the membrane effects evoked by PTHrP(1-34). PTHrP(7-34) itself reduced EPSCs to 83 ± 5 % of predrug without significant membrane effects.

The data suggest that both the excitatory and inhibitory effects of PTHrP reside in the N-terminal region and that different mechanisms may account for the dual effects. Furthermore, the PTHrP receptor may be activated intrinsically.

Supported by NIH (DK 35608, NS 29265) and the Deutsche Forschungsgemeinschaft.

CATECHOLAMINES: BIOSYNTHETIC ENZYMES

614.1

PURIFICATION AND CHARACTERIZATION OF A CATECHOLAMINE BINDING PROTEIN. V.D. Nair., M. Fu., E.S. Werstiuk*., and R.K. Mishra.Depts. of Psychiatry and Biomedical Sci., McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Catecholamine binding proteins are capable of covalently coupling to dopamine, ADTN, and NPA and do not exhibit biochemical, pharmacological, or distributions characteristics with known dopamine receptors or transporters in the CNS. These proteins are identified by using a specific ligand, 6-hydroxy-^{125}iodo-(N[N-2',4'-dinitrophenyl]-amino-propyl)-2-amino-1,2,3,4-tetrahydronaphthalene ([^{125}I]DATN). A 40 kDa catecholamine binding protein was purified from bovine striatal membrane and characterized. The striatal membrane was labelled with [^{125}I]DATN and separated on SDS-PAGE. The band corresponding to 40 kDa was excised out and the protein was collected after electro elution. The radioactivity associated with the band was measured and the homogeneity of the purified protein was confirmed by iso-electric focussing. Antibody was raised in rabbits against the purified protein and used to characterize the protein to elucidate the role in catecholaminergic neurotransmission. The 40 kDa protein is distributed throughout the CNS, but absent in various other tissues examined. Catecholamine depleting agent reserpine significantly decreased the concentration of the protein in rat brain, whereas, L-DOPA increased the concentration of the protein. These findings suggests that the 40 kDa protein may play an important role in the CNS function. *Supported by Medical Research Council of Canada.*

614.2

INHIBITORY EFFECTS OF ISOQUINOLINE ALKALOIDS ON CATECHOLAMINE BIOSYNTHESIS IN PC12 CELLS. M.K. Lee*, Y.H. Zhang, J.S. Shin, S.S. Lee, and H.S. Kim. Coll. of Pharmacy, Chungbuk Natl. Univ., Cheongju 361-763, Korea.

The rat pheochromocytoma PC12 cells have many properties of the adrenal medullary chromaffin cells, including the synthesis, storage and secretion of catecholamines. Inhibitory effects of 10 kinds of isoquinoline alkaloids on catecholamine biosynthesis in PC12 cells were investigated. Among them, protoberberine (berberine, palmatine), bisbenzylisoquinoline (tetrandrine, fangchinoline), phthalide isoquinoline (noscapine, hydrastine) and aporphine (bulbocapnine) alkaloids showed inhibitory effects on dopamine biosynthesis in intracellular PC12 cells. Tyrosine hydroxylase (TH) activity was also reduced by addition of isoquinoline alkaloids in PC12 cells.

It was thought that some isoquinoline alkaloids might be able to inhibit TH activity. Therefore, this hypothesis was investigated in bovine adrenal TH using a substrate L-tyrosine. Berberine, palmatine, hydrastine and bulbocapnine showed inhibitory effects on bovine adrenal TH (35-44% inhibition at concentration of 200 μM). Berberine, palmatine and hydrastine exhibited competitive inhibition, and bulbocapnine did uncompetitive inhibition on bovine adrenal TH.

These results suggest that the inhibition on TH activity by some isoquinoline alkaloids might be partially involved in the reduction of dopamine biosynthesis in PC12 cells. The intracellular mechanisms of isoquinoline alkaloids in PC12 cells need further investigation. Supported by KOSEF 951-0711-046-2.

614.3

REGULATION OF TYROSINE HYDROXYLASE (TH) ACTIVITY IN ATT-20 CELLS EXPRESSING WT- OR S40L-TH BY TYROSINE AND COFACTOR BH4. A.Garcia-España , K. Harada, K.Y. Lee and M. Goldstein*, Neurochemistry Research Laboratory, New York University Medical Center, New York, NY 10016

We have reported that replacement of serine 40 residue in TH with leucine by site-directed mutagenesis produces a mutated enzyme (S40L-TH) with high catalytic activity (Wu et al. J. Biol. Chem. 267: 25754, 1992). To study the mechanisms involved in the activation of S40L-TH, we have analyzed the changes in the catalytic activity of the enzyme by tyrosine and by sepiapterin, which is a precursor of natural cofactor, BH4. AtT-20 cells expressing wild type (WT-TH) or S40L were preincubated in tyrosine-free medium for 60 min and subsequently incubated with several concentrations of tyrosine with or without sepiapterin (0.6mM). TH activity *in situ* was assessed as dopa accumulation in the presence of an aromatic amino acid decarboxylase inhibitor, NSD-1015 (50 μM). Tyrosine (0-100 μM) inhibited dopa synthesis in a dose-dependent manner in cells expressing WT-TH, but not in cells expressing S40L-TH. Sepiapterin increased dopa synthesis slightly in cells expressing WT-TH and to a greater extent in cells expressing S40L-TH (up to +550 %). These results indicate that the mutated enzyme, S40L-TH is dysregulated and its high activity might be due to greater stimulation by BH4 and lack of inhibition by tyrosine. [supported by USPHS grants MH2717 and NS6801]

614.4

DOPAMINE COVALENTLY MODIFIES AND INACTIVATES TYROSINE HYDROXYLASE. Y.M. Xu, A.H. Stokes, B.A. Vogt*, R. Roskoski, Jr+, and K. E. Vrana. Department of Physiology and Pharmacology, Bowman Gray Sch. of Med., Winston-Salem, NC 27157-1083 and +Department of Biochemistry and Molecular Biology, LSU Medical Center, New Orleans, LA 70119.

It has been established that dopamine can autoxidize to form the reactive dopamine quinone. This oxidation can be enhanced through the enzymatic activities of tyrosinase or prostaglandin H synthase. The reactive dopamine quinone, in turn, can covalently modify nucleophilic groups in protein and DNA. We have shown in previous studies that ^{3}H-dopamine covalently modifies DNA and that this modification was increased by the enzyme tyrosinase. The present studies established that dopamine quinone, formed through the activity of tyrosinase, inactivates tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis. Co-incubation of purified TH (10μg) or crude pheochromocytoma homogenates (100-200μg protein) with dopamine (100μM) in the presence of tyrosinase (10μg/ml, 4400units/mg) resulted in a decrease in TH activity of 70% for purified enzyme and 90% for activity in crude pheochromocytoma homogenates. Moreover, inactivation was associated with covalent incorporation of radiolabelled dopamine into the enzyme as assessed by immunoprecipitation, gel filtration chromatography, and/or denaturing SDS-PAGE. Antioxidant compounds (DTT, GSH, and NADH) blocked covalent modification and inactivation. These findings suggest that there exists a third mechanism by which dopamine can inhibit TH (in addition to kinetic feedback inhibition and the formation of an inhibitory dopamine Fe^{+3} complex) providing further complexity in the regulation of catecholamine biosynthesis. *This work was supported by USPHS grant GM38931 (to K.E.V.).*

614.5

PARALLEL UPREGULATION OF CATECHOLAMINE-SYNTHESIZING ENZYMES, PAM, AND NEUROPEPTIDE Y BY SYSTEMIC NICOTINE TREATMENT IN THE RAT ADRENAL MEDULLA. T.C. Wessel*, J.W. Jahng, T.A. Houpt, and T.H. Joh. Laboratory of Molecular Neurobiology, Cornell Univ.Med.Coll. at Burke Med. Res. Inst., White Plains, NY 10605.

The expression of catecholamine (CA)-synthesizing enzymes in the adrenal medulla is upregulated in parallel by reserpine. In this study we examined whether a neuropeptide and its processing enzyme are also regulated in parallel with CA enzyme genes after drug treatment. Because the main effect of reserpine on the medulla is via splanchnic nerve stimulation of nicotinic receptors, we used nicotine to stimulate the medulla, and visualized expression of CA enzyme genes, the medullary peptide neuropeptide Y (NPY), and the neuropeptide-processing enzyme peptidylglycine alpha-amidating monoxygenase (PAM) by *in situ* hybridization.

Rats received a single injection of nicotine (0, 1, or 5 mg/kg s.c.). Six hours later, nicotine-treated rats and uninjected controls were transcardially perfused (n = 3-6/group). Adrenal glands were cut into 40 µm sections. Free-floating sections from each group together in the same vial were hybridized with ^{35}S-labeled cDNA probes for tyrosine hydroxylase (TH), dopamine beta-hydroxylase (DBH), phenylethanolamine N-methyltransferase (PNMT), PAM, and NPY. Relative optical density of digitized adreanl medulla images was quantified using NIH image.

Nicotine treatment upregulated expression of all genes in a dose-dependent fashion (5 mg/kg > 1 mg/kg >> saline > uninjected). Quantitative differences were observed in the relative induction of the different genes, however. At 1 mg/kg, induction of NPY was greatest (160 ± 12% of saline), followed by PAM (146 ± 13%) and TH (132 ± 11%). PNMT (121 ± 8%) and DBH (112 ± 5%) induction was lowest. These results suggest that common transcriptional activation mechanisms may differentially upregulate both CA synthesis and neuropeptide processing in the adrenal medulla.

(Supported in part by NIH grant MH24285)

614.6

REMOVAL OF THE SUCKLING STIMULUS RAPIDLY INCREASES EXPRESSION OF TYROSINE HYDROXYLASE AND SYNTHESIS OF ITS PROTEIN K.A. Berghorn*, S.Y. Pharrams and G.E. Hoffman. Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA, 15261.

Previous studies from our laboratory had described the suppression of tyrosine hydroxylase (TH) gene expression in neurons of the arcuate nucleus (Arc) in lactating animals and its upregulation following pup removal. Using biotin labeled riboprobes with *in situ* hybridization (ISH) immunocytochemistry (ICC), we demonstrated increases in both nuclear and cytoplasmic TH RNA as early as 6 h after pup removal, raising the question of when RNA levels first rise, and when TH protein expression increases. Lactating rats were perfused at 90 min - 48h following pup removal. The pattern of change in TH mRNA levels in lactating rats nursing 8 pups and following pup removal were detected using ISH with a biotin labeled TH riboprobe. After hybridization, biotin was detected with anti-biotin followed by ABC immunoperoxidase staining. To assess TH protein levels, separate groups of animals were decapitated and the median eminence was dissected; protein from tissue homogenates was assayed with Western blots. Arc TH mRNA levels in the lactating animals nursing their pups were at or below the limit of detection; within the first 3 h after pup removal, levels of TH mRNA in the arcuate nucleus were increased in both the nucleus and cytoplasm. These rose to a maximum 12-24 h after pup removal. Western blots revealed an increase in TH protein first detected at 90 min after pup removal that continued to rise over the next 24 h. These data indicate that the re-establishment of TH synthesis after the suckling stimulus ceases enables rapid re-establishment of dopamine's synthetic capacity.

(Supported by NIH NS 28730)

614.7

MOLECULAR MECHANISMS UNDERLYING THE SYNERGISTIC INDUCTION OF TYROSINE HYDROXYLASE GENE EXPRESSION BY ACIDIC FIBROBLAST GROWTH FACTOR AND CO-ACTIVATORS. **Z. Guo*, N.D. Stull and L. Iacovitti** Dept. of Neurobiology and Anatomy, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA. 19102

Previous studies from this laboratory have demonstrated that the catecholamine (CA) biosynthetic enzyme tyrosine hydroxylase (TH) can be induced in non-CA neurons of the striatum by the synergistic interaction of acidic fibroblast growth factor (aFGF) and a number of different co-activators (dopamine, TPA, IBMX/forskolin). In the present study, we sought to determine the underlying molecular mechanisms for this novel expression by identifying the relevant cis-acting DNA sequences and transcription factors. To do so, cultures of E14 rat striatum were grown one day in culture in defined medium prior to treatment with aFGF (100ng/ml) and/or dopamine (20µM), TPA (200nM), IBMX (0.25mM)/forskolin 50µM). Sixty minutes after stimulation, cultures were harvested, nuclear extracts were prepared and analyzed by gel shift assay. We found that protein binding to the AP-1 motif of the TH gene was significantly increased by aFGF and co-activator stimulation as compared to control (media only). To further investigate the components of the AP-1 protein complex, supershift assays using antibodies to specific transcription factors were performed. We found that, in controls, the AP-1 protein complex contained c-Jun, Fos B, Jun-D, ATF2 and CREM. Stimulation of the cultures by aFGF and all of the co-activators resulted in the novel recruitment of c-Fos; the increased expression of Fos B and Jun D, and decreased expression of ATF2 in the AP-1 complex. c-Jun and CREM expression remained unchanged by stimulation. aFGF and individual co-activators each contributed in specific but dissimilar fashion to the stimulated complex. We conclude that the AP-1 motif of the TH gene and the AP-1 protein complex, in particular, specific members of the Fos/Jun and CREB families (ie. c-Fos, c-Jun, Fos B, Jun-D, ATF2 and CREM) play a critical role in TH gene activation by aFGF and co-activators. It is likely that other sites on the gene and other transcription factors are also involved in TH induction. (Supported by NIH NS 24204).

614.8

EFFECTS OF CHRONIC PRENATAL HYPOXIA ON TH AND PNMT mRNA AND PROTEIN LEVELS IN MEDULLA OBLONGATA OF POSTNATAL RAT L.D.White*, E.E.Lawson Dept. of Pediatrics, UNC-CH

Our laboratory has demonstrated that the first two weeks of postnatal life are important for development of the respiratory neurons of the medulla oblongata (*Resp.Physiol.* 98:123). A subset of these neurons is catecholaminergic as evidenced by expression of the enzymes tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT). Perinatal chronic hypoxia caused increased TH expression in respiratory neurons and decreased PNMT expression in dorsal respiratory regions (*Soc.Neurosci.Abstr.* 233.8). To characterize the effects of prenatal hypoxia on subsequent TH and PNMT gene and protein expression, pregnant rats were placed in moderate hypoxia (10% O_2) from gestational day 18 until birth. Northern and Western analyses of dorsal (C2/A2 cells) and ventral (C1/A1 cells) medullary tissue of P0, P3, P7, P10 and P14 pups then examined changes in TH and PNMT mRNA and protein compared to normoxia-reared controls. Prenatally hypoxic animals had lower levels of TH mRNA and protein at birth in dorsal medulla and higher levels of TH mRNA and protein the first postnatal week in ventral medulla compared to controls. Further, hypoxic animals had significantly lower levels of PNMT protein at P10 in dorsal medulla than controls. Altered levels of catecholamine (CAT)-synthesizing enzymes in response to hypoxia may later be manifest by changes in function as these neurons continue to mature. Prenatally hypoxic animals may be less able to respond to an acute hypoxic challenge during early postnatal life because of altered levels of CATS. Hence, the subset of CAT neurons involved in respiratory control have the potential to be involved early postnatal respiratory dysfunction such as SIDS, congenital alveolar hypoventilation syndrome or infantile apnea. NIH Grant HL34919

614.9

CHARACTERIZATION OF THE HYPOXIA-INDUCIBLE PROTEIN COMPLEX ASSOCIATED WITH THE PYRIMIDINE-RICH REGION IN THE 3' UNTRANSLATED REGION OF TYROSINE HYDROXYLASE mRNA. M.F. Czyzyk-Krzeska and J. Lipski* Department of Molecular and Cellular Physiology, University of Cincinnati, College of Medicine, Cincinnati, OH, 45267-0576.

Stability of mRNA for tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine synthesis, is increased (30 h half life vs 10 h) in PC12 cells during hypoxia. This increase correlates with enhanced binding of cytosolic protein factors to the UCCCCU motif in the 3' untranslated region of TH mRNA. SDS-PAGE analysis of the UV-crosslinked binding reaction revealed two major specific RNA-protein complexes of 74 kD and 53 kD. The same complexes were formed with protein extracts from PC12 cells, carotid bodies (CB), superior cervical ganglia (SCG), adrenal glands (AG) and brain. The formation of the 53 kD complex was increased when extracts were obtained from PC12 cells, CB or SCG exposed to hypoxia. Formation of both complexes was completely abolished by addition of poly(C) RNA. Therefore poly(C)-RNA agarose affinity chromatography was used to isolate TH mRNA binding proteins from PC12 cells S-100 protein fraction. Protein extracts were incubated with poly(C) agarose in the binding buffer at 4°C for 1 h. Four repeats of this procedure depleted PC12 extracts of the TH mRNA binding proteins and the depleted extract did not show any binding to the TH mRNA. Bound proteins were eluted with 4 M guanidine HCl for 2 h at room temperature. The eluate was sufficient to restore formation of the binding with the TH mRNA. This binding was redox dependent and could be abolished by treatment with diamide. The work is in progress to further identify the TH mRNA binding proteins enriched by poly(C) agarose affinity purification.

(Supported by: NIH grant HL 51078, AHA Grant-in Aid 94017440, NARSAD Young Investigator Award; MFC-K is recipient of Parker B. Francis fellowship).

614.10

CYTIDINE-RICH PROTEIN-BINDING SEQUENCE IN THE 3' UNTRANSLATED REGION OF TYROSINE HYDROXYLASE mRNA AS A DETERMINANT OF THE TH mRNA STABILITY. J.E. Beresh & M.F. Czyzyk-Krzeska* Department of Molecular and Cellular Physiology, University of Cincinnati, College of Medicine, Cincinnati, OH, 45267-0576.

During exposure of PC12 cells to hypoxia the half-life of tyrosine hydroxylase (TH) mRNA increases from 10 to 30 h (*JBC, 269, 760-4, 1994*). This increase is associated with augmented binding of protein factors to the conserved 27 base long, pyrimidine-rich sequence located between bases 1552-1578 in the 3' untranslated region of the TH mRNA (JBC, 269, 9940-5, 1994). We further identified that the optimal *hypoxia inducible protein binding site* (HIPBS) is represented by the motif (U/C)(C/U)CCCU, where the core binding site is indicated by underlined cytidines. Substitutions of either one of these cytidines with purine or uridine abolished protein binding *(JBC, 271, 3293-9, 1996)*. In order to study whether the HIPBS regulates TH mRNA stability we have developed stably transfected PC12 cell lines that express wild type or mutated within the HIPBS TH mRNAs under control of the CMV promoter. This transfected TH mRNA is by 30 bases longer in the 5' untranslated end than the endogenously expressed TH mRNA. Thus both TH mRNAs can be detected using RNase protection assay with the riboprobe against the 5' region of mRNA. The stability of the transfected wild type TH mRNA is regulated during normoxia and hypoxia in the same manner as of the endogenous TH mRNA. However, mutations within the HIPBS that eliminate protein binding, destabilize the transfected TH mRNA and abolish regulation of its stability during hypoxia. These results indicate that the cytidine-rich HIPBS is a determinant of TH mRNA stability in PC12 cells.

(Supported by: NIH grant HL 51078, AHA Grant-in Aid 94017440, NARSAD Young Investigator Award; MFC-K is recipient of Parker B. Francis fellowship).

614.11

REGULATION OF TYROSINE HYDROXYLASE GENE EXPRESSION BY cAMP AND PHORBOL ESTER IS MEDIATED BY CREB. K.M. Piech* and A.W. Tank, Department of Pharmacology, University of Rochester, Rochester NY 14642.

Tyrosine hydroxylase (TH) gene expression is induced after activation of protein kinase A (PKA) or protein kinase C (PKC) signalling pathways. Previous data suggest that PKA or PKC mediated induction of TH gene expression is dependent on the cAMP-responsive element (CRE)(-45 to -38) in the TH gene promoter, but it is not known which transcription factors elicit these responses. To determine whether the cAMP response element binding protein (CREB) is involved in regulation of TH gene expression, clonal cultures of PC12 cells that were stably transfected with CREB antisense RNA were generated. CREB levels are decreased dramatically in these antisense CREB RNA-expressing PC12 cell lines.

Control PC12 cell cultures or those with diminished CREB were transiently transfected with a TH-CAT reporter gene construct and later incubated with a cAMP analog, phorbol ester or calcium ionophore. The cAMP inducibility of TH-CAT was dramatically decreased in cells expressing antisense CREB RNA. Interestingly, the PKC mediated induction of TH promoter activity in these cells was also decreased. However, the calcium-mediated induction of TH promoter activity was not diminished. Presumably, the loss of PKA and PKC mediated inducibility of TH-CAT results from a decreased interaction of CREB with the TH-CRE or from the diminished expression of CREB-regulated transcription factors. Western blotting indicates that fos family members are still inducible in the CREB diminished cell lines. These data suggest that CREB is an essential factor in PKA and PKC-mediated activation of TH gene expression. (Supported by NIDA grants 05014 and 07232 and STRC grant 0481.)

614.12

TRANSGENIC EXPRESSION OF THE CRE RECOMBINASE IN THE MOUSE CNS. P.J. Cox, M. Hamon and B. Giros*, INSERM U-288, 91, Bd. de l'Hôpital, 75013 Paris, France.

Transgenic 'knock-out' technology, i.e. the deletion of a specific gene of interest from the mouse genome, has revolutionized the study of gene/protein function. The technology although demanding has lead to many significant discoveries in a variety of disciplines including neurobiology. The major drawbacks of targeted gene deletion are:

1. the production of mice which are homozygote for the gene deletion whose survival rate precludes meaningful studies e.g. many knock-out animals do not survive beyond embryogenesis.
2. the original technology results in the deletion of a gene in all tissues which can give rise to the situation described in point one or to pleiotropic effects which complicate the interpretation of phenotype especially with regard to gene function in specific tissues.

A recent development in gene targeting technology is the use of the Cre-lox system which should allow the confinement of gene deletion to specific tissues, assuming tissue specific promoters are available to direct tissue specific expression of the Cre recombinase. The activity of the Cre-lox system within the CNS has not been determined. This poster describes the expression of the Cre recombinase under the control of a CNS specific promoter and the assessment of the Cre-lox system as a method for deletion of genes specifically within the CNS.

Concomitant with our research interests in dopaminergic systems and the development of new animal models with perturbations in these systems, we have developed a line of transgenic mice expressing the Cre recombinase under the control of the Tyrosine Hydroxylase promoter. Breeding of these mice with mice containing a floxed β-Gal/CAT transgene should allow the demonstration of Cre recombinase activity in the CNS.

614.13

ALTERNATIVE PROMOTER USAGE OF THE MOUSE AROMATIC L-AMINO ACID DECARBOXYLASE (AADC) GENE. Bruno Conti*, Jeong Won Jahng, Marco Belloni and Tong H. Joh, Laboratory of Molecular Neurobiology, Cornell University Medical College at the M. W. Burke Medical Research Institute, White Plains, NY 10605.

Our previous studies of rat AADC gene structure showed that (1) rat AADC is encoded by a single gene; (2) both alternative promoter usage and differential splicing account for neuronal and non-neuronal specific expression of the gene; (3) there are two alternatively utilized splicing acceptor sites in exon 2; and (4) exon 1a is localized in non-neuronal cells and exon 1b in neuronal cells as shown by in situ hybridization. The present studies examined the presence of exons 1 and 2 in mouse AADC gene and their pattern of differential transcription.

Using 5' primers specific for either rat exon 1a or 1b and a 3' primer specific for rat exon 2, we identified exon 1a and exon 1b of the mouse AADC gene by RT-PCR. Exon 1a was isolated from liver and exon 1b from substantia nigra RNAs. The mouse nucleotide sequence of exons 1 and 2 were nearly identical to the rat. However, the splicing acceptor site in exon 2 was the same in both mouse transcripts.

Thus, the present results indicate that, unlike the rat gene, no evidence exists for differential splicing in tissue specific expression of the mouse AADC gene. Alternative promoter usage alone likely accounts for mouse AADC mRNA processing in neuronal and non-neuronal cells. (Supported by NIH grant MH24285 to THJ)

614.14

NEONATAL LETHALITY OF PUPS BORN TO DBH-DEFICIENT MICE MAY BE DUE TO A DEFICIT IN MATERNAL BEHAVIOR. S. A. Thomas, N. M. Nathanson*, and R. D. Palmiter. Howard Hughes Med. Inst., Dept. of Biochemistry, Univ. of WA, Seattle, WA 98195

Using gene-targeting, we created mice deficient in the enzyme dopamine β-hydroxylase (DBH), which converts dopamine (DA) into norepinephrine (NE). The majority of pups (DBH +/-) born to females homozygous for the disruption (DBH -/-) die within several days after birth. Neonatal lethality is rare when the female is DBH +/-. In about 50% of the litters, pups are scattered within the bedding, suggesting that there may be a deficit in maternal behavior. However, this abandonment could be secondary to defects in the pups. To test whether the primary deficit resides in the DBH -/- females or their neonates, pups born to DBH -/- and DBH +/- females were cross-fostered to the female with the other genotype within 24 hours of birth. To our surprise, 10 of 12 litters were successfully raised by the DBH +/- females, and 11 of 12 litters were successfully raised by the DBH -/- females. These results demonstrate that DBH -/- females can nurse, and suggest that an important interaction occurs between dam and neonate during the first 24 hours. To address who harbors the primary deficit, we are testing whether restoration of NE in DBH -/- females shortly after birth will enhance neonatal survival. In conclusion, maternal NE released during birth may promote maternal behaviors that enhance appropriate neonatal behaviors such as nursing.

This work was supported by NIH grant HD 09172 and HHMI.

614.15

INVESTIGATION OF TRANSCRIPTION FACTORS WHICH MAY BE INVOLVED IN REGULATION OF DOPAMINE ß-HYDROXYLASE EXPRESSION IN PC12 CELLS AND ADRENAL MEDULLA. B. Nankova, B. Hiremagalur*, L. Serova and E.L. Sabban, Dept. Biochem. & Mol. Bio. New York Med. Coll., Valhalla, NY 10595.

Dopamine β-hydroxylase (DBH) mRNA levels are regulated by various stimuli *in vivo* in rat adrenal medulla (AM), such as repeated immobilization stress (IMO) and in PC12 cells by elevated cAMP. Binding to the DBH1 enhancer (-175 to -145) of nuclear extracts from PC12 cells with relatively high, or barely detectable levels of DBH expression, at least 4 (as opposed to 2 in AM) specific complexes were identified. The pattern was unchanged by 8-bromo cAMP. One of these complexes, absent in AM, was identified as an ATF-1/α-jun heterodimer. Although DBH1 is reportedly essential in cAMP-induced DBH transcription in PC12 cells, it did not bind CREB in any of the extracts. Extending the oligonucleotide to include the distal CRE-like element (-184 to -145) did not yield additional complexes. These results suggest that increased expression of DBH in response to cAMP analogs is unlikely to be mediated by phosphorylation of CREB and probably involves other transcription factors, perhaps ATF-1.

In AM, nuclear run-on assays revealed that IMO raised DBH transcription. Increased binding of AP1-like factors to DBH1 was observed with extracts from IMO animals. Our data revealed that proteins other than c-fos, fos B, Fra-1 or c-jun are involved in this complex. No CREB, ATF1 or YY1-immunoreactive proteins were found to be bound to DBH1. Taken together, our results suggest that CREB, ATF1 and YY1 are unlikely to be directly involved in the transcriptional activation of DBH by stress. Western blot analysis of the induction of immediate early genes by single and repeated IMO in AM suggest that jun-family members and other fos-related proteins may mediate increased expression of DBH elicited by IMO (Supported by NIH grants NS32166 and NS 28869).

614.16

OVEREXPRESSION OF BCL-2 CAUSES WEIGHT GAIN AND ADRENAL HYPERTROPHY IN DBH-BCL-2 TRANSGENIC MICE. J.W. Jahng*, T.H. Joh, C.H. Peng, E.S. Corp, T.A. Houpt, and J.H. Son, Laboratory of Molecular Neurobiology and Bourne Behav. Res. Lab., Cornell Univ. Med. Coll. at the Burke Med. Res. Inst., White Plains, NY, 10605.

Noradrenergic (NA) and adrenergic (A) cells in the locus ceruleus, brainstem, and adrenal gland (identified by dopamine B-hydroxylase (DBH) expression) play key roles in autonomic function, arousal, attention, learning, anxiety, and stress. During development, the populations of some NA and A cell groups are decreased by apoptotic cell death. To investigate possible behavioral and physiological effects of rescuing NA and A cells from developmental cell death, we made DBH-Bcl-2 transgenic mice with human Bcl-2 expression under the direction of a 4.2kb human DBH promoter. The cytoplasmic protein Bcl-2 can partially rescue cells from apoptotic death *in vitro*, but its effects *in vivo* are not well-known.

Mice from 3 representative transgenic lines and wildtype littermates were transcardially perfused (n=5 pr/line). Overexpression of Bcl-2 was localized in the brain and adrenal gland by *in situ* hybridization. Body weight, body length, and adrenal gland weight were measured. Fat pad size was qualitatively rated. Total volumes of adrenal glands were measured by 40 µ serial section reconstruction.

Different levels of Bcl-2 mRNA were detected in the adrenal medulla, locus ceruleus, cortex, and hippocampus in all 3 transgenic lines, and at very low levels in the substantia nigra. No Bcl-2 expression was observed in wildtype controls. Body weight (but not length), fat pad size, and adrenal gland weight and volume varied with the level of Bcl-2 expression in the adrenal medulla within each line. Bcl-2 may have rescued adrenal gland cells from apoptotic cell death during development. Bcl-2 overexpression also appears to increase body weight and fat mass. These behavioral and physiological effects may be due to increased neuromodulatory and hormonal function of enlarged NA and A populations in both brain and periphery. (Supported in part by NIH grant MH24285)

614.17

NOVEL CHARGE FORMS OF PNMT ASSOCIATED WITH DECREASED PNMT ACTIVITY. J.K. Stewart*, R.M. White, J.L. Andreassi, T.D. Gbadebo, and L.M. Kim. Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284-2012.

Recently we detected two closely migrating forms of rat PNMT on both Western blots and blots of non-denaturing polyacrylamide gels. In the present study we examined the electrophoretic pattern of adrenal PNMT extracted from male Sprague Dawley rats, weighing 210 - 270 g, maintained under constant conditions in our laboratory for 10 days. In 14 adrenal samples with unusually low PNMT activity (less than 50 pmol/ mg protein x 30 min), both forms of PNMT migrated more slowly on non-denaturing gels (a positive shift in charge) compared to the migration of PNMT in 26 adrenal samples with higher PNMT activity (100 - 500 pmol/mg protein x 30 min). Furthermore, in the high activity samples, 3 - 4 closely migrating charge forms of PNMT were observed, whereas only 2 charge forms were observed in low activity samples. These findings suggest that decreased PNMT activity is associated with modification of the PNMT protein. Supported by NIH Grant NS-26992.

614.18

HIGHLY SELECTIVE INHIBITORS OF PHENYLETHANOLAMINE *N*-METHYLTRANSFERASE. G. L. Grunewald*, T. M. Caldwell, K. R. Criscione, V. H. Dahanukar, R. K. Jalluri, M. Slavica. Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045.

Phenylethanolamine *N*-methyltransferase (PNMT) is the enzyme involved in the conversion of norepinephrine to epinephrine. In order to probe the role of epinephrine in the central nervous system, a potent and selective inhibitor of PNMT is desired. We will present data on a new compound which shows the highest PNMT selectivity thus far obtained. 3-Hydroxymethyl-7-aminosulfonyl-1,2,3,4-tetrahydroisoquinoline shows good PNMT inhibitory activity (PNMT K_i = 0.342 μM), while being much less active at the α_2-adrenoceptor (α_2 K_i = 1400 μM) giving a highly selective compound (α_2 / PNMT = 4100). However, this compound is too hydrophilic to penetrate the blood-brain barrier (calculated log P = -1.01). New analogues—designed using molecular modeling methods—with enhanced likelihood of entering the CNS and retaining a high degree of selectivity (α_2 / PNMT), along with preliminary data on blood-brain barrier penetration will be presented. Sponsored by NIH HL34193.

614.19

GLUCORTICOID INDUCED-AP-2 CONTROL OF THE PHENYLETHANOLAMINE N-METHYLTRANSFERASE GENE, D.L. Wong*, M.B. Ficklin and S.N. Ebert. Dept. Psych. and Beh. Sci., Stanford Univ. Sch. Med., Stanford, CA 94305-5485.

The transcription factor AP-2 is important for differentiation of neural crest-derived tissues. In RS1 cells, a cell line established from the rat adrenal medulla, a tissue of neural crest origin, AP-2 can induce phenylethanolamine N-methyltransferase (PNMT) gene expression, but only in the presence of glucocorticoids (GC) as demonstrated by transient transfection assays with a PNMT promoter-luciferase reporter gene construct (pRP863LUC) and AP-2 expression constructs. In vitro footprinting and consensus binding sequence identity suggest that within the proximal 863 bp of upstream PNMT promoter-regulatory sequences are three potential AP-2 binding sites (-648, -576 and -100 bp upstream of the site of transcription initiation, +1). A glucocorticoid response element (GRE) is located at -513 bp. When the AP-2 binding sites and GRE are deleted, AP-2/GC activation does not occur. In addition, site-directed mutagenesis of the AP-2 sites and GRE causes a marked reduction in AP-2/GC-stimulated PNMT promoter activity. AP-2/GC induction of the PNMT promoter requires activation of a type I glucocorticoid receptor (GR) as demonstrated using the type I and type II GR agonists and antagonists, dexamethasone, corticosterone, RU28362, aldosterone and RU38486. AP-2 protein is present in RS1 cell nuclei under conditions of AP-2/GC-mediated PNMT promoter activiation. In addition, the combination of AP-2 and GCs stimulates endogenous PNMT mRNA expression. These results suggest that AP-2 may be important in PNMT gene regulation and adrenal chromaffin cell differentiation, but it requires interaction with an activated glucocorticoid receptor for transcriptional activation to occur. (Support: NIDDK 51025, Nancy Pritzker and Gray Endowments, Gerschel Charitable Trust)

OTHER NEUROTRANSMITTERS

615.1

CANNABINOIDS INHIBIT VOLTAGE-DEPENDENT CALCIUM CHANNELS IN CULTURED HIPPOCAMPAL NEURONS. W. A. Twitchell* and K. P. Mackie. Departments of Anesthesiology and Physiology and Biophysics, Box 356540, University of Washington School of Medicine, Seattle WA 98195-6540.

INTRODUCTION: Cannabinoids and their analogs have been found to inhibit N and P/Q type Ca^{2+} currents in cell lines and sympathetic neurons. However, the effects of cannabinoids on Ca^{2+} currents in the CNS are largely unexplored. In this study we have investigated if these compounds and sympathetic neurons inhibit Ca^{2+} currents in cultured rat hippocampal neurons.

RESULTS: Using patch clamp electrophysiology we found that most pyramidally-shaped cells exhibited large Ba^{2+} currents (250-600 pA) that were inhibited by both 1 μM 2-chloroadenosine and 50 μM baclofen and were abolished by application of 100 μM Cd^{2+}. 100 nM WIN 55,212-2 reversibly inhibited this current by 27±2% (n=10, mean±sem) in a voltage-dependent, pertussis toxin-sensitive and concentration-dependent ($K_{1/2}$ =~30 nM) fashion. The current was not significantly effected by WIN 55,212-3 (100 nM). Maximal inhibition by the non-classical cannabinoid agonist, CP 55,940, was similar to that seen with maximal concentrations of WIN 55,212-2. The Ba^{2+} current we measured was carried by several types of Ca^{2+} channels. Of these, P/Q-(ωCTX-MVIIC sensitive) and N-type (ωCTX-GVIA sensitive) channels appeared to be the ones chiefly modulated by WIN 55,212-2.

DISCUSSION: These results extend to central neurons previous results demonstrating cannabinoid receptor-mediated inhibition of distinct Ca^{2+} currents. As the channels which underlie these currents are chiefly located presynaptically, and are required for evoked neurotransmitter release, our results suggest a major role for cannabinoids (endogenous and exogenous) in the modulation of transmitter release at selected CNS synapses.

SUPPORT: NS08174, NS01588, DA08934, and DA07278

615.2

ASSOCIATION BETWEEN THE CANNABINOID RECEPTOR GENE AND EEG AUDITORY EVOKED POTENTIALS IN SUBSTANCE ABUSERS. J. P. Johnson*, D. Muhleman, J. P. MacMurray, E. R. Verde, M. N. Ask, J. T. Kelly and D. E. Comings. Jerry L. Pettis Memorial VA Medical Center, Loma Linda, CA 92357.

EEG activity is one of the most heritable characteristics in humans. Therefore, genetic markers for receptor systems implicated in the neural generation of brain evoked potentials are ideal candidates for study of the genetic basis of the EEG. Others have suggested that cannabinoid receptors in the hippocampus mediate working memory, and the recent identification of a tri-nucleotide repeat gene for the cannabinoid receptor gene (CNR1) allowed us to examine its association with brain evoked potentials in a study of 34 abstinent non-hispanic white substance abusers. The EEG was recorded on a QSI-9000 computerized EEG with an Electro-cap using the 10-20 system of electrode placement using an auditory "oddball" paradigm. Subjects homozygous for the high molecular weight alleles of the CNR1 gene had significantly reduced P300 amplitudes recorded at Fz, Cz, and Pz ($p < 0.01$) and delayed P300 latency at Pz ($p < 0.05$) compared to those individuals with low molecular weight alleles.

Supported by NIDA RO1 DA08417 and Tobacco Related Disease Program Grant 4RT-0110.

615.3

FUNCTIONAL COUPLING OF RAT AND HUMAN CB1 CANNABINOID RECEPTORS. F. Petitet, B. Jeantaud, E. Heuillet, F. Perrot, M. Capet, P. Bertrand* and A. Doble. Rhône Poulenc Rorer, Neurochemistry Dept., CRVA, 13 quai Jules Guesde, Vitry sur Seine, France.

The major psychoactive component of *Cannabis sativa*, Δ^9-tetrahydrocannabinol (Δ^9-THC), as well as the putative endogenous ligand for the cannabinoid receptor, anandamide, mediate their effects *via* specific G protein-coupled receptors. Two cannabinoid receptors have been cloned from rat and human tissues. The CB_1 receptor is expressed mainly in the brain while the CB_2 receptor is localized exclusively in peripheral tissues. Using autoradiography, we have demonstrated that binding sites labelled by an agonist ([^{3}H]WIN55212-2) and an antagonist ([^{3}H]SR141716A) have the same localization in rat brain. [^{3}H]WIN55212-2 and [^{3}H]SR141716A binding sites, studied on rat cerebellar membranes, have similar pharmacological pattern. The affinities of the antagonist SR141716A and the agonist WIN55212-2 were shown to be the highest, while anandamide, Δ^9-THC or 11-hydroxy-Δ^9-THC had moderate affinities. WIN55212-2 (1 µM) enhanced (+ 80 %) the binding of [^{35}S]GTP-γ-S on rat cerebellar membranes whilst SR141716A, devoid of activity by itself, antagonized the action of WIN55212-2. In two human preparations; the astrocytoma cell line U373MG and CHO-CB_1 transfected cells there was also a correlation between binding affinities and activities (determined by [^{35}S]GTP-γ-S binding and cAMP measurement) of natural and synthetic cannabinoids.

615.4

CONCURRENT STIMULATION OF CANNABINOID CB1 AND DOPAMINE D2 RECEPTORS RESULTS IN AN AUGMENTATION OF FORSKOLIN STIMULATED CAMP ACCUMULATION IN STRIATAL NEURONS. M. Glass* and C.C. Felder. Laboratory of Cell Biology, National Institute of Mental Health. Bethesda 20892, MD, USA.

It is well established that cannabinoids act at the CB1 receptor to inhibit forskolin stimulated adenylate cyclase activity. This inhibition can be blocked by pertussis toxin, suggesting that it is mediated by a Gi/Go type of GTP-binding protein. Previously we have demonstrated that treatment with pertussis toxin blocked the anandamide mediated inhibition of adenylate cyclase, and unmasked a stimulatory effect of anandamide on cAMP accumulation. The anandamide mediated accumulation of cAMP could be blocked in a dose dependent manner by CB1 receptor antagonists LY320135 and SR141716A suggesting that the stimulatory response is regulated through the CB1 receptor. CB1 receptors have been demonstrated to be co-localized with dopamine D2 receptors within the striatum. In this study we have examined the interactions between these two receptors. In striatal neurons in primary culture both the CB1 agonist HU210 and the D2 agonist quinpirole inhibited forskolin stimulation of cAMP accumulation. In contrast, HU210 and quinpirole in combination resulted in an augmentation of cAMP accumulation. This augmentation was blocked by SR141716A. These results suggest that utilization of the Gi-mediated pathway by either pertussis toxin or agonist stimulation permits the unmasking of a stimulatory pathway coupled to the CB1 receptor.

M. Glass is supported by the New Zealand Health Research Council.

615.5

EFFECT OF 2-(2-BENZO-FURANYL)-2-IMIDAZOLINE ON LOCUS COERULEUS AND DORSAL RAPHE NEURONAL ACTIVITY IN RATS. L. Ugedo*, J.A. Ruiz-Ortega, R.Martín-Ruiz. Dept. of Pharmacology, Fac. of Medicine, Univ. of the Basque Country, E-48940 Leioa, Vizcaya, Spain.

Binding studies have shown that 2-(2-benzo-furanyl)-2-imidazoline (2-BFI) is a highly selective I_2-imidazoline receptor ligand (Nutt *et al.*, Ann. N.Y.Acad. Sci. 763: 125-139, 1995), and that a remarcable density of I_2-imidazoline receptors exists in locus coeruleus and dorsal raphe nuclei (MacKinnon *et al.*, Br. J. Pharmacol., 116: 1729-1736, 1995). In order to investigate a possible I_2-imidazoline receptor-mediated functional response, the effect of 2-BFI was assessed on locus coeruleus and dorsal raphe neurons using single-unit extracellular recordings in anesthetized rats. In locus coeruleus neurons local (56 pmoles) and systemic (1 mg/kg i.v.) administrations of 2-BFI increased the firing rate by 20% and 23% respectively; clonidine dose-response curve was not modified after 2-BFI indicating that this effect was not mediated by α_2-adrenoceptors. In dorsal raphe neurons local (67 pmoles) and systemic (1 mg/kg i.v.) administration of 2-BFI decreased the firing rate by a 30% and a 27% respectively. These results suggest that 2-BFI has a neuromodulatory action in central nervous system which migh be mediated by I_2-imidazoline receptors

Supported by PI 95/55. J.A.R-O. is a fellow from MEC and R.M-R. is a fellow from GV.

615.6

LOCALIZATION OF MRNA AND PROTEIN OF GLUCOCORTICOID RECEPTOR IN RAT CENTRAL NERVOUS SYSTEM. H. Ozawa, M. Morimoto , N. Morita* and M. Kawata . Department of Anatomy and Neurobiology, Kyoto Prefectural University of Medicine. Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, JAPAN

It is recognized that the glucocorticoid plays an important role in the development, the differentiation and the functional activity of the nervous system. The effects of glucocorticoids are mediated by the glucocorticoid receptor (GR).

In this study, the cellular localization of GRmRNA and GR immunoreactivity in the adult rat brain was studied by in situ hybridization and immunocytochemistry.

The expression of GR mRNA was detected in the cytoplasm of neurons and observed in the various regions of the rat brain, especially in the hippocampus, the thalamus, hypothalamus, the cerebral cortex. The distributional pattern of GR-immunoreactive cells was well-correlated with that of GR mRNA-containing cells, but in the hippocampus (CA3/CA4) discrepancy of the distribution of mRNAs and immunoreactivities.

Immunoelectron microscopy revealed that the immunoreactivity of GR was mainly localized in the nuclei of cells in the central nervous system. Small amount of GR immunoreactivity was also observed in the cytoplasm, particularly in the periphery of rough endoplasmic reticulum.

These results suggest that glucocorticod and its receptor are deeply related to the development and function of cells in the central nervous system.

615.7

SOLUBILIZATION AND CHARACTERIZATION OF A [^{3}H]CORTICOSTERONE MEMBRANE RECEPTOR FROM AMPHIBIAN BRAIN. Simon J. Evans, Frank L. Moore and Thomas F. Murray. College of Pharmacy, Zoology Department and Molecular and Cellular Biology Program, Oregon State University, Corvallis OR, 97331.

Alternative to the classical action of steroid hormones acting as nuclear transcription factors a great deal of evidence exists for plasma membrane receptors for many members of the steroid hormone family. Evidence includes modulation of Ca^{2+} currents by estradiol[1] and allopregnenalone[2] in rat brain. Also suppression of K^+ currents by estrogen[3] and Ca^{2+} currents by cortisol[4] has been shown in guinea pig brain. Progesterone has been shown to bind with high affinity to *Xenopus* oocyte membranes[5]. In addition to these reports a membrane corticosterone receptor from newt (*Taricha granulosa)* brains has been pharmacologically characterized and correlated with inhibition of sexual behavior by Orchinik et al in 1992[6]. We have extended this research with solubilization of the membrane corticosterone receptor using non-ionic detergents and further characterized radioligand interactions with the soluble form. Using this system we obtain >50% yield of binding sites with 2 fold enrichment of the receptor relative to total protein concentrations. Solubilization of the [^{3}H]corticosterone receptor will facilitate further characterization of the protein and perhaps enhance understanding of the membrane action of steroid hormones. (Supported by a research grant from ZymoGenetics, Inc., Seattle, WA)

1. *J. Neurosci.* 16(2):595-604
2. *J. Neurosci.* 16(1):130-136
3. *J.Neurosci.* 12(7):2745-2750
4. *J. Neurosci.* 15(1):903-911
5. *Biol. Reprod.* 49:980-988
6. *Science* 252:1848-1851

615.8

IMMUNOCYTOCHEMICAL STUDY ON CALBINDIN D-28KA IN THE COCHLEAR NUCLEUS OF MIDBRAIN OF THE CHINCHILLA. J.J. Kim*, Y.Y. Chung, I.Y. Chang, J.S. Moon and H.J. Kim.Dept. of Anat., Chosun Univ., Med. College 375 Suh-Seok Dong, Dong Ku Kwang Ju, Korea. 501-759.

This paper describes the distribution of structures stained with mono - and polyclonal antibodies to the calcium-binding proteins calbindin D-28Ka in the cochlear nucleus of midbrain in the chinchilla.

Calbindin D-28Ka immunoreactivity was preferentially located in the dorsal cochlear nucleus with occational examples being present in the ventral cochlear nucleus, as well as in adjacent brain stem locations.

Calbindin D-28Ka immunoreactive neurons are of very varied morphology: bitufted, bipolar, double bouguet, multipolar and horizontal cells have been described in the cochlear nucleus.

Calbindin D-28Ka immunoreactive neurons were observed with a soma size of 15 - 17um and a few under 15um in the cochlear nucleus.

Calbindin D-28Ka immunoreactive dendrites are considerably more densely arrayed than those of other cochlear nucleus cells, including the cartwheel cells of the dorsal cochlear nucleus.

615.9

LIPID METABOLISM MODIFICATION AND PHOSPHOLIPASE ACTIVATION IN NEUROBLASTOMA SK-N-BE DIFFERENTIATED WITH RETINOIC ACID. A. Petroni*, N.Papini, P. LaSpada, M. Blasevich and C. Galli. Institute of Pharmacological Sciences, University of Milan, via Balzaretti 9, 20133, Milan, Italy.

The aim of our study was to investigate if retinoic acid-differentiation could affect lipid metabolism and phospholipase D activation in the human neuroblastoma SK-N-BE. At different stages of cell differentiation, we have evaluated the modification of cell growth and morphology (neurite emission, thymidine incorporation, etc.). We have evaluated endogenous lipid synthesis after 3 and 8 days of retinoic acid-cell differentiation. Undifferentiated and differentiated SK-N-BE were labeled with [^{14}C]-acetate (2.5μCi/dish), cells were extracted and radioactivity in major lipid classes were measured. After 8 day-differentiation there was a reduction in the incorporation of radioactivity in phospholipids, associated with marked accumulation in TG, free fatty acid (FFA) and in diacylglycerol. Furthermore labeling cholesterol declined markedly compared to level after 3 days of retinoic acid differentiation. Moreover the conversion of [^{14}C]-linoleic acid was modified, major changes occured in Δ^5 desaturation step. To evaluate if retinoic acid modifies lipid homeostasis, we studied phospholipase D-activation in undifferentiated and 8 days-differentiated SK-N-BE . Cells were labeled with [^{14}C]-palmitic acid (4μCi/dish for 6h), exposed to ethanol150mM and then stimulated with Platelet-Activating Factor (100nM for 5 min). Phosphatidilethanol (PEt) production was evaluated. In differentiated cells the incorporation of palmitic acid in TG was more elevated than in undifferentiated cells. After PAF stimulation there was three times increment of PEt compared with controls. These data indicate the involvment of lipid homeostasis during different stages of neuronal differentiation.

615.10

MODULATION OF RECOMBINANT GLYCINE AND $GABA_A$ RECEPTORS BY GENERAL ANAESTHETICS: A COMPARATIVE STUDY. M. Pistis*, D. Belelli, J.A. Peters and J.J. Lambert; Neurosciences Institute, Department of Pharmacology, Dundee University. Dundee, DD1 9SY, Scotland U.K.

Many general anaesthetics are known to act as positive allosteric modulators of the $GABA_A$ receptor and this may be their prime locus of action. Here, we have determined the effects of the structurally diverse anaesthetics propofol, pentobarbitone, etomidate and δ-hexachlorocyclohexane (δ-HCH) on human recombinant $\alpha_3\beta_1\gamma_2$ $GABA_A$ receptors in comparison with their actions on the closely related glycine (GLY) receptors (human α_1, rat β) by utilizing the *Xenopus laevis* oocyte expression system and two point voltage-clamp techniques. All results reported below are the mean ± s.e.m. of 3-5 experiments. For cRNA ($GABA_A$) or cDNA (GLY) injected oocytes (-60mV) bath applied agonist evoked a concentration-dependent inward current with a EC_{50} of 102±18μM and of 70+3μM, respectively. Utilizing the appropriate EC_{10} concentration of the agonist, propofol potentiated GABA and GLY evoked currents to 108±18% and 98±6% of maximum current (I_{max}), respectively, with an EC_{50} of 3.4±0.1μM and 28±2μM, respectively . Pentobarbitone and etomidate potently enhanced GABA-evoked currents (110±17% and 75±8% of I_{max} ,respectively; EC_{50} of 44±5μM and 8.1±0.9μM, respectively), but were only partially effective on GLY induced currents (50±9% and 29±4% of I_{max} , respectively; EC_{50}: 757±39μM and 10±1μM, respectively). By comparison, δ-HCH enhanced GLY-evoked currents to 80±7% of I_{max} (EC_{50} 26±3μM) with a reduced effect on GABA induced currents (43±4% of I_{max} , EC_{50} 3.5±0.4μM).

Our results demonstrate that although some compounds are relatively selective for $GABA_A$ receptors (pentobarbitone and etomidate), a modulation of GLY receptor activity may contribute to the behavioural actions of propofol and δ-HCH.

We acknowledge the financial support of the MRC and MRC (ROPA) and thank H. Betz for the glycine receptor cDNA.

615.11

MEASUREMENT OF GLUTAMINE BY CAPILLARY ELECTROPHORESIS AND LASER INDUCED FLUORESCENCE DETECTION IN THE CEREBROSPINAL FLUID. S. Tucci*, P. Rada, L. Hernández, I. García and C. Pinto. Department of Physiology, P.O. Box 109, Mérida, 5101-A, VENEZUELA.

Glutamine is an amidated product of glutamic acid and the most abundant aminoacid in the brain. Glutamine measurements are an important diagnosis tool in certain diseases in which the metabolism of ammonia is compromised. It is helpful in differential diagnosis between bacterial and viral meningitis. Due to the medical relevance of glutamine, several assays for this aminoacid have been developed. Most of these techniques are based in the acidic or enzymatic breakdown of the glutamine molecule in glutamic acid and ammonia. The ammonia from this reaction is then measured by various methods. Recent observations have shown that glutamine concentrations in the brain inversely correlates with aging. In these experiment we developed a capillary zone electrophoresis with laser induced fluorescccence detection (CZE-LIFD) method to measure glutamine prederivatized with fluorescein isothiocyanate (FITC). First, we conducted tests to optimize the derivatization procedure mixing standard solutions of glutamine with increasing concentrations of FITC. Then, we applied this method to the measurement of glutamine in normal cerebrospinal fluid of 79 children and 20 adults. Spinal taps in these children were performed when a CNS infection was suspected, however, cytochemical analysis later revealed normal CSF. Adult CSF samples were taken from patients undergoing subdural anesthesia. The analysis showed that the sensitivity limit was 10^{-9} M. Glutamine concentrations were 3.89 ± 0.05 mmol/lt in children and 1.60 ± 0.07 mmol/lt in adults. These results show that there is a significant difference of CSF glutamine concentrations between normal children and adults and that CZE-LIFD can be used as an alternative method for glutamine analysis.

This research was supported by CDCHT-ULA

615.12

ENHANCEMENT OF HOMOMERIC GLYCINE RECEPTOR FUNCTION BY LONG CHAIN ALCOHOL AND ANESTHETICS. M.P.Mascia, T.K.Machu, and R.A.Harris*. Pharmacology Dept., Univ. of Colorado HSC and VAMC, Denver, Colorado, 80262; Pharmacology Dept., Texas Tech Univ. HSC Lubbock, TX 79430.

The effects of n-alcohols (ethanol-dodecanol) and anesthetics on strychnine-sensitive glycine receptors were studied in *Xenopus* oocytes expressing homomeric α1 or α2 glycine receptor subunits, using two electrode voltage-clamp recording. All the alcohols tested potentiated the function of homomeric α glycine receptors. Homomeric α1 and α2 receptors were potentiated to a similar extent by the n-alcohols, with the exception of the lower concentration of ethanol which showed a significantly greater effect on α1 than on α2 receptors(Mascia *et al*, Mol. Pharmacol.1996). The potencies of these alcohols to enhance glycine-receptor function increased as the charbon chain lengh was increased up to decanol. Dodecanol was no more potent than decanol and weakly potentiated the glycine receptor function when compared to the other alcohols. Among the anesthetics tested, pharmacologically relevant concentrations of halothane and enflurane markedly potentiated the function of homomeric α1 glycine-receptor. A modest potentiation was obtained with the intravenous anesthetics propofol, alphaxalone and pentobarbital, but etomidate and ketamine were ineffective. Two novel cyclobutane compounds were also tested and the anesthetic compound, 1-chloro-1,2,2-trifluorocyclobutane, enhanced the action of glycine, while the non-anesthetic analog, 1,2-dichloro-hexafluorocyclobutane, was ineffective. These results indicates a role for the strychnine-sensitive glycine receptor in the central depressant action of alcohols and anesthetics.

Supported by NIH grants GM 7818 and AA 06399.

615.13

MELATONIN BLOCKS NICOTINIC CHANNELS IN THE GUINEA-PIG SUBMUCOUS PLEXUS. B. Prieto-Gómez*[1], A.L. Peres[2], R. Espinosa-Luna[2], C. Reyes-Vázquez[1] & C. Barajas-López[2]. [1]Physiol. Depart., UNAM, México. [2]Biomed. Sci. Depart., McMaster Univ., Hamilton, ON.

To investigate the possible role of melatonin (ME) as a modulator in the enteric nervous system. Intracellular and whole-cell recordings were made in submucosal neurons to measure ME effects on their electrophysiological properties. ME did not alter the membrane potential, the membrane resistance, the noradrenergic inhibitory postsynaptic potentials and the slow excitatory postsynaptic potentials (EPSPs). However, ME (30-3000 μM) reversibly decreased the amplitude of nicotinic EPSPs in a concentration-dependent manner (IC_{50}=247 μM). Superfusion of ME also blocked the nicotinic depolarizations induced by locally applied ACh, indicating that at least part of its effects are postsynaptic. In whole-cell experiments, ME also inhibited the nicotinic inward currents induced by a maximal concentration of ACh (3 mM) in a concentration-dependent manner (IC_{50}=257 μM). ME (0.5 mM) decreased the maximal effects (3067 ± 68 to 1173 ± 80 pA) but did not affect the potency of ACh to induced the nicotinic inward current, indicating a noncompetitive antagonism. ME effects appear to be voltage-dependent because the blockage of the ACh-induced currents was larger for inward than for outward currents. These observations indicate that ME inhibits the fast EPSPs by directly blocking the cholinergic nicotinic channels. Our results also suggest that ME can be a local modulator of synaptic transmission in the enteric nervous system.

Supported by the grants MRC-MT-3491 (CBL), OMH-04500 (CBL), and DGAPA-UNAM IN205395 (BPG).

615.14

ZINC (Zn^{++}) MODULATION OF VOLTAGE-ACTIVATED IONIC CURRENTS IN THE HORIZONTAL LIMB OF THE DIAGONAL BAND OF BROCA (hDBB). J.C. Easaw *, B.S. Jassar, J.H. Jhamandas. Dept. of Medicine (Neurology), Univ. of Alberta, Edmonton, Alberta, Canada, T6G-2S2.

The hDBB, a basal forebrain region which mediates memory and learning mechanisms, is affected in Alzheimer's disease (AD). Examination of the brains of patients with AD reveals that endogenous levels of the transition metal zinc are significantly reduced (Constantinidis, Alzheimer Disease and Associated Disorders, 5(1)31-15,1991). Zn^{++}, which is present in the basal forebrain, normally subserves important roles in enzyme function and also exerts neuromodulatory actions within the CNS. In this study, we characterized the actions of Zn^{++} on voltage-dependent ionic currents in hDBB neurons.

Whole cell patch clamp recordings were performed in acutely dissociated rat hDBB neurons. With respect to K^+ conductances, application of 50μM Zn^+ increased A-current by 20%. While activation of this current was unaffected, inactivation kinetics were shifted to the right in the presence of Zn^{++}. In contrast, Zn^{++} decreased delayed rectifier-type K^+ currents by 20%. This metal was also observed to modulate other ionic currents. Zn^{++} elicited a modest reduction of sodium currents (11%) and a significant blockade (85%) of Ca^{++} currents recorded using Ba^{++} as the charge carrier.

These results demonstrate that Zn^{++} is a potent modulator of voltage-activated ionic currents in the hDBB neurons. The effects of Zn^{++} in the basal forebrain may be important in the context of the observed decrease in Zn^{++} in AD.

This work was supported by the MRC and AHFMR of Canada.

615.15

AGMATINE IS RELEASED FROM SYNAPTOSOMES AND ADRENAL CHROMAFFIN CELLS BY DEPOLARIZATION. S. Regunathan*, M. Sastre and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell University Medical College, New York, NY 10021.

Agmatine (decarboxylated arginine) and its biosynthetic enzyme, arginine decarboxylase are present in rat brain (Li et al., *Science*, 1994). Moreover, the facts that agmatine can be taken-up and stored in synaptosomes (Sastre et al. *Soc. Neurosci. Abstr.*, 1996) and is contained in perikarya of specific brain areas (Wang et al., *Soc. Neurosci. Abstr.*, 1995) suggests it has properties of a neurotransmitter/modulator. We investigated whether agmatine can also be released by depolarization from rat brain or adrenal chromaffin cells. Slices or synaptosomes were prepared from brains of rats killed by anesthetic overdose. Chromaffin cells were cultured from bovine adrenal medullae. Tissues were prelabeled by incubation with guanido ^{14}C- or ^{3}H-agmatine or ^{3}H-putrescine and release of radioactivity into the medium measured for 10 min following exposure to 55 mM KCl (brain) or nicotine (100 μM) (chromaffin cells) with or without Ca^{++}. Results were expressed as percent of total radioactivity released. Depolarization with 55 mM KCl resulted in significant ($p<0.05$; n=6) release of labeled agmatine from synaptosomes (9.9 $\pm$ 0.36 vs 12.6 $\pm$ 0.37) or slices (9.1 $\pm$ 0.78 vs 15.3 $\pm$ 2.5). Removal of calcium from the incubation medium significantly ($p<0.05$) reduced release. Since no significant release of ^{3}H-putrescine was observed, the released radioactivity cannot be attributed to metabolic conversion of agmatine to putrescine. In adrenal chromaffin cells, depolarization with KCl (55 mM) or nicotine (10 μM) also resulted in significant release of ^{3}H-agmatine. We conclude that agmatine can be released from presynaptic neurons and chromaffin cells in a calcium-dependent manner. The data provide further evidence that agmatine may be a novel neurotransmitter/modulator.

NITRIC OXIDE AND OTHER MODULATORS

616.1

ADRENERGIC INDUCED NITRIC OXIDE (NO) RELEASE FROM SENSORY NEURONS IS INHIBITED BY A SEROTONIN REUPTAKE INHIBITOR. M.S. Finkel, A.J. Kanai, K. Sugaya*, and L.A. Birder. Univ. of Pittsburgh. Depts. of Cardiology and Pharmacology, Pgh, PA 15261 and Univ. Of North Carolina Dept. Physiology, Chapel Hill, NC 27599.

Paroxetine, a selective serotonin reuptake inhibitor (SSRI) also reduces the activity of human cytochrome P_{450}. Neuronal nitric oxide synthase (nNOS) is structurally homologous to cytochrome P_{450}. The objective of this study was to examine the effects of paroxetine on nNOS activity. Lumbo-sacral dorsal root ganglia (DRG) were excised from deeply anesthetized adult rats (150-200 gms) and enzymatically dissociated. A nafion coated porphyrinic microsensor (tip dia. 0.5-1.5 μm; NO detection limit, 5 nM) was placed directly on the surface of individual neurons to measure NO release. Recordings were obtained from cells with a 30 μm cell-free field to assure that the response was coming from a single cell. The calcium ionophore, A23187 (10 μM), elicited a transient release of NO, functionally demonstrating the presence of a constitutive NOS in DRG neurons. We have previously reported that the adrenergic agonist, norepinephrine (NE; range 0.1 to 0.5 nM) evoked transient NO release (range 87 $\pm$ 11 to 310 $\pm$ 41 nM) from medium diameter (15-30 μm) DRG neurons. We now report that NE induced NO release is reversibly inhibited in a dose dependent manner by paroxetine (1-10 μM; N=8), as well as by the nNOS specific inhibitor, 7 nitroindazole (7-NI, 100 μM), in a population of medium diameter DRG cells (15-30 μm). nNOS enzyme activity which was determined by measuring the conversion of [^{14}C] citrulline by a brain cytosol homogenate, was inhibited in a concentration dependent manner by known arginine analogue NOS inhibitors and paroxetine. This suggests that the SSRI, paroxetine, inhibits NE induced NO release from a subpopulation of sensory neurons by directly inhibiting neuronal NOS activity. This work was supported by NIH grant HL53372.

616.2

PROSTAGLANDINS (PGs) CONFER NEUROPROTECTION TO THE NEWBORN POSSIBLY BY INHIBITING NITRIC OXIDE SYNTHASE EXPRESSION. I. Dumont, A. Doke, P. Hardy, O. Dembinska, K.G. Peri, S. Molotchnikoff, P. Lachapelle, S. Chemtob. Research Center, Hôpital Ste-Justine, Montreal, Canada.

PGs exert cytoprotection. PG levels in neural tissue (brain and retina) are high in the newborn (NB), but the role of these PG is not known. We postulated that high PG levels may confer neuroprotection. We used a model of NB asphyxia (Ax) to test the effects of prolonged (24 h) inhibition of PG G/H synthase (PGHS) using flurbiprofen (10 mg/kg/12 h iv, n=4), diclofenac (2.5 mg/kg/12 h iv [decreased PGs], n=10), or a combination of diclofenac and a stable analog of PGE2 (16,16 dimethyl PGE2, 6 g/kg/12 h iv; n=4), or saline (n=6), on the electroretinogram (ERG) of anesthetized (-chloralose) and paralyzed (pancuronium) ventilated newborn (NB) pigs (1 day old) before and 1 h after a 5 min period of asphyxia (interruption of ventilation). ERG was recorded before and 24 h after treatments, and repeated 1 h after Ax. The b-wave (generated by Müller and bipolar cells) amplitude decreased by 27-30% after Ax in saline-treated animals. In pigs treated with diclofenac and flurbiprofen the b-wave amplitude decreased to a much further extent by 52-66% 1 h after Ax. Addition of the PGE2 analog totally prevented the post-Ax ERG deterioration. Because PGs have recently been suggested to inhibit in cell lines expression of the inducible nitric oxide synthase (NOS), an important source of free radicals, we tested if the latter could explain the post-Ax neuro-deterioration observed after PGHS inhibition. Animals were treated with diclofenac and the relatively selective inducible NOS inhibitor, 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT, 3g/kg/8 h iv) or with a neuronal/inducible NOS blocker, 7-nitroindazole (10 g/kg/8 h iv), and ERG was recorded as above. Treatment of piglets with diclofenac and AMT totally prevented the post-Ax neuroretinal damage; 7-nitroindazole exerted minimal attenuation of the reduction in b-wave amplitude. Data suggest that PGE2 confer neuroprotection to the NB apparently by inhibiting newly expressed inducible NOS activity.
Supported by Medical Research Council of Canada

616.3

STRIATAL NITRIC OXIDE AND VOLUME TRANSMISSION. NITRIC OXIDE SYNTHASE INHIBITION MODULATES THE LOW MOLECULAR WEIGHT DEXTRAN DIFFUSION AND/OR CLEARANCE IN THE RAT NEOSTRIATUM, A. Jansson, L. Rosén, L.F. Agnati†and K. Fuxe*. Department of Neuroscience, Division of Cellular and Molecular Neurochemistry, Karolinska Institute, 171 77, Stockholm, Sweden, and †Department of Human Physiology, University of Modena, Modena, Italy

Nitric oxide acts as an intercellular messenger in the brain via diffusion in the extracellular fluid and across membranes to act on intracellular targets. In this paper we have studied basic aspects of diffusion and/or clearance in the extracellular compartment of dextran and the possible modulation of these processes by nitric oxide.

Biotinylated-dextran (molecular weight 3000), in a volume of 0.1 μ L was unilaterally microinjected into the neostriatum of halothane anaesthetized male rats. The total volume, maximal mean grey value and form factor of the dextran labelled area was estimated from serial sections of the neostriatum by means of computerized image analysis. A significant increase of the total volume of the biotinylated dextran labelling was observed with time after microinjection, without a change in the form factor. At the 30 min time-interval, nitric oxide synthase inhibition with two types of nitric oxide synthase inhibitors, L-nitro-arginine methyl ester (50mg/kg x 2, 4 days) and L-monomethyl-arginine (30mg/kg; 10 min) caused a significant reduction of total volume and maximal mean grey value of the biotinylated dextran labelling. These findings point to a possible role of nitric oxide in control of clearance and/or diffusion of chemical signals along extracellular paravascular pathways.

This study was supported by the Marianne and Marcus Wallenberg Foundation and the Swedish Medical Research Council (grant no. 04X-715)

616.4

NITRIC OXIDE SYNTHASE IN THE GUINEA PIG PELVIC GANGLIA AND THE EFFECT OF SODIUM NITROPRUSSIDE STIMULATION ON cGMP LEVELS. K. Holmberg*, Z.-Q. Xu, H. Steinbusch, L.-G. Elfvin and T. Hökfelt, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

Using the indirect immunofluorescence technique, the distribution of nitric oxide synthase (NOS)-like immunoreactivity (LI) was studied in pelvic ganglia of untreated guinea pigs and after lesion/crush of the hypogastric nerve. The results indicate that NOS-immunoreactive (IR) nerve fibers project in both directions of the hypogastric nerve. The NOS-positive neurons in the ganglia were choline acetyltransferase-positive and some contained vasoactive intestinal polypeptide, neuropeptide Y, somatostatin and calcitonin gene-related peptide. Nitric oxide (NO) stimulates soluble guanylyl cyclase and cGMP formation. In this study we have also examined the effect of the NO donor sodium nitroprusside (SNP) on cGMP in pelvic ganglia, and the effect of the guanylyl cyclase inhibitor 1H-(1,2,4)oxadiazolo(4,3-a) quioxaline-1-one (ODQ). The pelvic ganglia were exposed in vitro to SNP (10^{-4}, 10^{-6}M) or SNP+ODQ (100nM) for 10-15 min. Using immunohistochemistry and an antiserum to cGMP the stimulated ganglia showed a strong CGMP-LI in satellite cells and also in some neurons. Addition of ODQ abolished the cGMP staining. These findings suggest that both neurons and satellite cells may be targets for endogenously released NO in the guinea pig pelvic ganglia. (Supported by the Medical Research Council 2887 and 5189.)

616.5

Nitric oxide donors and cGMP analogs induce IPSCs in septohippocampal GABAergic neurons. W. Liu* and M. Alreja
Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT.

The rat septohippocampal cholinergic but not GABAergic neurons of the medial septal/diagonal band complex (MSDB) contain the enzyme nitric oxide synthase. Both α_1 and β_1 subunits of guanylate cyclase are also present in the MSDB. Electrophysiological studies on the effects of nitric oxide (NO) and/or cGMP analogs on MSDB neurons are lacking. Therefore, we tested the effects of several NO donors and cGMP analogs on electrophysiologically characterized cholinergic and GABA-type MSDB neurons in an *in vitro* rat brain slice preparation using extracellular and whole-cell recordings.

In whole-cell voltage-clamp recordings with K gluconate-containing electrodes, bath-applied NO donors (sodium nitroprusside, sodium nitrocysteine, SIN-1 and DEA NONOate) increased the frequency of bicuculline and TTX-sensitive IPSCs in a subpopulation of GABA-type (15/29) but not in cholinergic-type (34/34) MSDB neurons. In extracellular recordings, NO donors increased the firing rate of a subpopulation of MSDB neurons (59/115) some of which were antidromically confirmed to be septohippocampal projection neurons (c.v. - 1.71 ± 0.12 m/s; n=20). cGMP analogs (8-Br-cGMP, dibutyryl cGMP, 8-pCPT-cGMP and PET-cGMP) mimicked the effects of NO donors and increased both the extracellular firing rate (20/21) and induced GABAergic IPSCs (n=4). Hemoglobin (100 μM) blocked the excitatory effect of NO donors (n=7) but not of cGMP analogs. In voltage-clamp recordings, NO donors had no effect on any of the cholinergic-type neurons tested. Thus, NO functions as an intercellular messenger in the MSDB as cholinergic neurons which synthesize NO are insensitive to NO donors. In contrast, NO donors, via the cGMP pathway, produce an excitatory effect on septohippocampal GABAergic neurons. *Supported by NIH R 29 DA 09797.*

616.6

EXPRESSION OF ALTERNATIVE FORMS OF NEURONAL NITRIC OXIDE SYNTHASE (nNOS) IN BRAIN. H.-Y. Yun*[1], V. L. Dawson[1-3] and T. M. Dawson[1,2]. Departments of Neurology[1], Neuroscience[2] and Physiology[3], Johns Hopkins University School of Medicine, Baltimore, MD 21287.

Nitric oxide (NO) is a messenger molecule in central and peripheral nervous system and nNOS is thought to be a principal NO-generating enzyme in neurons. However, studies on nNOS null mutant mice showed grossly normal brain development and structure, normal hippocampal LTP and cerebellar LTD. Furthermore, we and others showed that nNOS null mice brain contains residual NOS activity (~8% of wild type level) and nNOS immunoreactive proteins (136 kDa and 125 kDa) in discrete brain areas such as striatum, hippocampus and cortex. The molecular identity of this NOS activity and proteins detected in nNOS null mice is not clear. We investigated nNOS expression in wild type and nNOS null mice with targeted deletion of exon 2 by *in situ* hybridization using an exon 3 riboprobe. nNOS transcripts were detected in cerebellum, hippocampus (dentate gyrus and CA1-CA3) and olfactory bulb in nNOS null mice, with a pattern generally similar to wild type except for relatively lower level in hypothalamic nuclei in mutant mice. However, the distribution of nNOS transcripts in nNOS mull mice brain did not parallel that of residual NOS activity and nNOS immunoreactive proteins reported previously in nNOS null mice brain. Reverse transcription-coupled PCR of nNOS null mice brain mRNA yielded many nNOS cDNA clones containing the exon 3 region. These results indicate the expression of alternative forms of nNOS transcripts in nNOS null mice brain and *in situ* hybridization using probes specific to each form of nNOS transcripts will provide the expression patterns of these transcripts in wild type mice. Supported by NIH.

616.7

EXPRESSION OF NO SYNTHASE (NOS) ISOFORMS AND GTP-CYCLOHYDROLASE (GTP-CH) IN RAT C6 GLIOMA AFTER LPS AND IFN-γ TREATMENT. L. Bezin*, P.Z. Anastasiadis[1,3], M.C. Berent, M.D. Flam, J.A. Foster, L.J. Gordon[1,3] and R.A. Levine[1,2,3]. [1]Gossett Neurology Labs, Henry Ford Hospital; [2]Dpt of Psychiatry, Wayne State U.; [3]Detroit VAMC, Detroit, MI.

The synthesis of the radical, nitric oxide (NO), an important signaling and cytotoxic molecule, is dependent on variably regulated isoforms of NOS. Three isoforms have been identified, two of them clearly being Ca^{2+} dependent. All of them require tetrahydrobiopterin as a cofactor, the biosynthesis of which is regulated by the activity of GTP-CH. We investigated the regulation of activity of NOS isoforms (with respect to Ca^{2+}-dependency) and GTP-CH 24h after treatment of C6 cells with lipopolysaccharide (LPS,1μg/mL) plus IFN-γ (100 U/mL); total NO synthesis increased 24 fold (media nitrite accumulation), and total *in vitro* NOS activity (^{3}H conversion assay) was increased by ~ +517%. Prior studies by others attributed the increase in NOS activity to the Ca^{2+}-independent isoform (macrophage isoform). However, we demonstrate that Ca^{2+} chelation by 3 mM EGTA in the NOS assay reduced the magnitude of elevation of NOS activity to only ~ +223%. These results suggest that about half of the total induction of NOS activity involves a Ca^{2+}-dependent NOS (endothelial or neuronal isoform). GTP-CH activity remained stable after treatment, suggesting that increased NO synthesis in this case does not require the elevation of GTP-CH activity. We are now characterizing the Ca^{2+}-dependent component of NOS activation following LPS+IFN-γ treatment, by the blockade of Ca^{2+}-independent isoform expression using dexamethasone pre- and co-treatment. We are also examining the subcellular localization of the different isoforms of NOS by confocal microscopy and the analysis of the expression of mRNAs for macrophage and endothelial NOS isoforms by quantitative RT-PCR.

616.8

NITRIC OXIDE SYNTHASE IN DIFFERENTIATING CULTURED CEREBELLAR GRANULE CELLS. R.W. Burry[1]* and D. S. Bredt[2].
[1]Dept. Cell Biol. Neurobiol. & Anat., The Ohio State Univ., Columbus, OH, [2]Dept. Physiol., UCSF., San Francisco, CA

Neuronal nitric oxide synthase (nNOS) is expressed in adult cerebellum, and produces nitric oxide (NO), a modulator of signaling in neurons. In the cerebellar molecular layer, immunocytochemical labeling has shown the expression of nNOS reaches adult levels by postnatal day 14 (P14). In the granule cell layer at P8, nNOS labeling was uniform, but after P14, nNOS-positive cells are found in alternating bands. This banding pattern indicates that after 14 days subpopulations of nNOS-positive granule cell bodies were separated by subpopulations of nNOS-negative granule cell bodies. We show here, in cultures from neonatal rats that nNOS is expressed only in a subpopulation of granule cells. With immunocytochemistry, nNOS was first seen in some granule cell bodies at 7 days of culture. At this and all subsequent times in culture, there was a range in levels of nNOS expressed in individual granule cells and their processes. Few of the early neurites at 7 days of culture were positive for nNOS, but at 21 days some of the growth cones and some axons were nNOS-positive. With scanning laser confocal microscopy, nNOS cytoplasmic labeling was distributed in fine, punctate regions around the nucleus and along axons. Some of these regions of label were associated with plasma membrane. Thus, in this neonatal culture system, only a subpopulation of granule cells express nNOS as punctate labeling. In early postnatal cerebellum most if not all of the granule cells have been shown to express nNOS, and subpopulations are only seen later. Expression of nNOS in a subpopulation of cultured granule cells suggests that conditions within the developing cerebellum, prior to dissociation at postnatal day 2, determine the subpopulation of granule cells that will express nNOS. (Supported by NSF, BNS-8909835 to RWB)

616.9

CHARACTERIZATION OF NITRIC OXIDE SYNTHASE (NOS) - CONTAINING NEURONS IN THE INSULAR CORTEX OF THE SYRIAN GOLDEN HAMSTER. Richard G. Wehby*, Center for Neurological Sciences, University of Connecticut Health Center, Farmington, CT 06030-3405.

We have previously documented two types of NADPH diaphorase (NADPHd)-positive neurons in the insular cortex of the Syrian golden hamster (Wehby and London, 1995, Soc. Neuroscience Abstracts, 21:1619.) We here further characterize these neurons. Animals were fixed with 4% paraformaldehyde for 45 min, and brains postfixed in the same solution for 2h. Antibodies to human NOS (rabbit polyclonal anti-nNOS and anti-iNOS; mouse monoclonal anti-eNOS; Transduction Laboratories) were incubated for 24h at 4 °C, then incubated with the appropriate biotinylated goat anti-IgG. Antibodies were visualized with an ABC reaction (Vector) using DAB histochemistry. In the insular cortex, (1) the "solid" labeled neurons seen with NADPHd histochemistry were immunoreactive for the nNOS (but not eNOS or iNOS) isozyme, and (2) the "punctate" cells labeled with NADPHd histochemistry were not labeled by antibodies to either nNOS, eNOS or iNOS isozymes. In agreement with these antibody results, when the NADPHd reaction was run with various inhibitors/chelators (e.g. EGTA, levamisole), the "solid" cells reacted with a NOS-like profile, but the "punctate" cells did not. In agreement with the NADPHd histochemistry results, nNOS-positive neurons were present primarily (>80%) in layers V and VI. Thus, the "solid" NADPHd-labeled cells contain nNOS, but the "punctate"-labeled cells do not. Supported by NIH grants T32DC00025 to R.G. Wehby and 2P50DC00168 to J.A. London.

616.10

INHIBITION OF NADPH-DIAPHORASE ACTIVITY AND FORMATION OF AGGREGATES IN RODENT HIPPOCAMPAL AND CEREBELLAR CELL CULTURES BY CLORICROMENE.

M.O. López-Figueroa[a]* and L.C.B. Rønn[b]
[a]Department for Medical Anatomy and [b]The Protein Laboratory, Panum Institute, Blegdamsvej 3, 2200 Copenhagen N, Denmark.

Nitric oxide (NO) plays a very important role in the brain as an intercellular signaling molecule. Formation of neuronal aggregates and processes may be investigated *in vitro* in primary cultures of dissociated nerve cells grown in microwells. We have used histochemical NADPH-diaphorase (NADPH-d) staining to study expression of nitric oxide synthase (NOS) in primary cell cultures from rodent hippocampus and cerebellum. After 24h in vitro, neurons had assembled to form large cell aggregates connected by fasciculated processes. Development of aggregates was correlated with an increase in NADPH-d activity. The coumarin derivative cloricromene inhibited in a concentration-dependent manner the formation of aggregates as well as the increase in NADPH-d activity. Thus, we demonstrated that cloricromene interferes with neuronal cell adhesion and indicate a role for NO in the formation of neuronal connections in the developing brain.
Source of support: Danish Research Academy

616.11

IMPAIRED INTESTINAL RELAXATION IN MICE WITH TARGETED DELETIONS OF EITHER NEURONAL NITRIC OXIDE SYNTHASE OR HEME OXYGENASE-2: NO AND CO AS POTENTIAL CO-NEUROTRANSMITTERS

R. Zakhary,[1]* K.D. Poss[2], S. R. Jaffrey[1], S. Tonegawa[2], S.H. Snyder[1]

[1]Department of Neuroscience, Johns Hopkins School of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205. [2]Howard Hughes Medical Institute, Center for Learning and Memory, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Neuronal nitric oxide synthase (nNOS) generates nitric oxide (NO) in neurons, while heme-oxygenase-2 (HO-2) synthesizes carbon monoxide (CO). Using mice with targeted deletions of nNOS or HO-2, we have evaluated the roles of NO and CO in intestinal neurotransmission. Nonadrenergic noncholinergic (NANC) relaxation and cyclic guanosine 3',5' monophosphate (cGMP) elevations evoked by neuronal depolarization are markedly diminished in both $nNOS^{-/-}$ and $HO\text{-}2^{-/-}$ mice. NOS inhibitors and HO inhibitors partially inhibit NANC relaxation in wild-type mice. In $nNOS^{-/-}$ animals, NOS inhibitors selectively lose their efficacy. Likewise, HO inhibitors have no effects in $HO\text{-}2^{-/-}$ animals. Immunohistochemical analysis demonstrates colocalization of nNOS and HO-2 in myenteric ganglia. These findings establish both NO and CO as physiologic modulators of intestinal relaxation.

This research was supported by a grant from the National Institute of Drug Abuse.

616.12

COLOCALIZATION OF CHOLINE ACETYLTRANSFERASE AND NITRIC OXIDE SYNTHASE IN CEREBRAL ARTERIES AND SPHENOPALATINE GANGLIA OF THE CAT AND PIG. T. KIMURA, J. G. YU, AND T. J. F. LEE*, Dept. of Pharmacology, Southern Illinois Univ. Sch. of Med., Springfield, IL 62794

The transmitter mechanism involved in cerebral neurogenic vasodilation is not fully clarified. The presence of cholinergic innervation in pial vessels led to the suggestion that acetylcholine (ACh) was the transmitter for cerebral vasodilation. This was questioned, however, by the findings that ACh constricts exclusively the smooth muscle in major pial arteries from several species. Accordingly, it has been hypothesized that a second transmitter substance is co-released with ACh to induce vasodilation. Recently, in pial vessels from different species the presence of dense nitric oxide synthase-immunoreactive (NOS-I) fibers originating in sphenopalatine ganglia (SPG) have been demonstrated. Results from pharmacological studies also have shown that endogenous NO mediates a major component of neurogenic vasodilator responses in isolated cerebral arteries. In this study, possible colocalization of choline acetyltransferase (ChAT) and NOS in SPG and cerebral pial arteries of the cat and pig was, therefore, examined immunohistochemically using antibody against ChAT and histochemically using nicotine adenine dinucleotide phosphate diaphorase (NADPH-d) staining. In SPG of both species, approximately half of the ganglionic cells were positive with ChAT-immunoreactivity and NADPH-d staining, while the other half of the ganglionic cells were positive with NADPH-d staining only. There were few cells showing ChAT immunoreactivity but not NADPH-d staining. In middle cerebral arteries most ChAT immunoreactive fibers and NADPH-d fibers were found to coincide with each other. These results provide a morphological evidence indicating that NOS and ChAT coexist in some SPG and cerebral perivascular neurons, and support the hypothesis that NO is co-released with ACh from "cholinergic" neurons to induce and/or regulate cerebral vasodilation. (Supported by NIH HL47574 and HL27763)

616.13

ENDOGENOUS GENERATION OF CYANIDE IN NEURONAL TISSUE: INVOLVEMENT OF A PEROXIDASE SYSTEM. G.E. Isom*, P.G. Gunasekar, J.L. Borowitz. Dept. of Medicinal Chemistry and Molecular Pharmacology, Purdue University, W. Lafayette, IN 47907.

We have shown that specific stimuli can initiate generation of cyanide in neuronal tissue. Further studies reveal that glycine, a substrate for cyanide production in lymphocytes, increased cyanide production in PC12 cells. Since myeloperoxidase generates cyanide in lymphocytes, peroxidase inhibitors (aminobenzoylhydrazide and azide) were tested and found to block cyanide production stimulated by carbachol. Furthermore H_2O_2 enhanced cyanide generation in the cells. Administration of aminobenzoylhydrazide to neonatal rats decreased endogenous cyanide after 30 min by 75% of control in cerebellum, with profound decreases also in hippocampus, medulla and hypothalamus (by 60-64% control). Cortical cyanide levels were decreased to a lesser extent by 33% of control. In adult rats, aminobenzoylhydrazide nearly abolished cyanide levels in all brain areas examined. Endogenous generation of cyanide in neural tissue appears to be related to activation of a peroxidase system linked to specific membrane bound receptors (muscarinic and mμ opiate) and glycine may serve as a substrate for the reaction. These results support the possibility that endogenous HCN generation may play a role in neuronal function. (Supported by NIH grant ES04140.)

616.14

ENDOGENOUS GENERATION OF CYANIDE IN NEURONAL TISSUE: POSSIBLE NEW NEUROMODULATOR. J.L. Borowitz, P.G. Gunasekar, C. Davis, N. Patel, G.E. Isom and R.P. Maickel*. Dept. of Medicinal Chemistry and Molecular Pharmacology, Purdue Univ., W. Lafayette, IN 47907.

The possibility that hydrogen cyanide might be a gaseous neuromodulator, as proposed for nitric oxide and carbon monoxide, was examined in isolated neural cells and in rats. To determine whether nervous tissue actually generates cyanide, HCN produced by rat pheochromocytoma (PC12) cells in culture was collected and quantitated. Small amounts of cyanide were detected (40 ng/10^6 cells/10 min) using a sensitive, specific colorimetric method. Both carbachol and a mμ opiate agonist, dilaudid, doubled cyanide generation and these effects were blocked by atropine and naloxone respectively. Calcium did not mediate the response since both high K^+ and A-23187 were without effect on cyanide generation. Cyanide production was blocked by pertussis toxin showing involvement of G-protein. Injection of dilaudid ip into adult rats increased cyanide in the cortex (4 times), medulla (1.5 times), cerebellum (1.8 times), hippocampus (1.1 times) and hypothalamus (1.7 times) after 15 min. Endogenous levels were highest in hippocampus and hypothalamus (~0.5 μg/g wet wt.). The presence of a drug-responsive cyanide generating system in rat brain and in isolated neuronal cells suggests cyanide may function to regulate neuronal activity. (Supported by NIH grant ES04140.)

616.15

ENDOGENOUS GENERATION OF CYANIDE IN NEURONAL TISSUE: HIGH LEVELS IN NEONATES AND DEMONSTRATION *IN VIVO*. P.G. Gunasekar*, J.L. Borowitz, R.S. Bitner and G.E. Isom. Dept. of Medicinal Chemistry and Molecular Pharmacology, Purdue Univ., West Lafayette, IN 47907.

To examine the possible effect of age on cyanide generation in rat brain, newborn (8-15 days) animals were compared with adults. Cyanide levels in newborn were 2.7 to 3.3 fold higher in cortex, medulla, and hippocampus. Levels in hypothalamus and hippocampus however were only 1.1 and 1.5 times the adult. Dilaudid given ip increased cerebellar cyanide 4.7 times the endogenous level in the neonate after 15 min. Increases in the hippocampus (1.7 times) and hypothalamus (1.6 times) were more modest and no increase was evident in cortex or medulla. Patterns of drug-induced cyanide generation in brain areas appear to be different in adults vs neonates. A microdialysis probe spanning the cortical-hippocampal area of pentobarbital anesthetized adult rats revealed low levels of cyanide and a ten-fold increase was evident on ip injection of 10 mg/kg of dilaudid hydrochloride. Pretreatment with naloxone blocked the effect of dilaudid. These results further support the existence of a cyanide generating system in neuronal tissue and reveal an age-related difference in cyanide generation. (Supported by NIH grant ES04140.)

616.16

INCREASED LYSOPHOSPHATIDATE LEVELS IN CSF AFTER SUBARACHNOID HEMORRHAGE

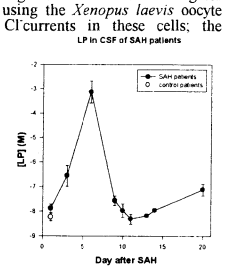
M Bavbek, GW Nietgen, C Bogaev, MB Fineman, R Polin, ZF Chen*
KS Lee, NF Kassell, ME Durieux
Departments of Neurological Surgery and Anesthesiology
University of Virginia, Charlottesville, VA 22908.

The mechanism of cerebrovascular spasm after subarachnoid hemorrhage (SAH) is still controversial, but probably multifactorial. We hypothesized that one potential mediator may be the phospholipid lysophosphatidate (LP). LP is released from activated platelets, endothelial cells, some cancer cells, and injured fibroblasts. The compound has been shown to have systemic vasoactive, smooth muscle contractile, and cerebrovascular effects at micromolar concentrations. We therefore determined LP concentrations in cerebrospinal fluid (CSF) of SAH patients.

With institutional approval, 39 CSF samples were collected from 32 SAH and 5 control patients treated at the University of Virginia. A blinded investigator determined the concentration of LP in the CSF using the *Xenopus laevis* oocyte bioassay. LP elicits oscillatory Ca^{2+}-activated Cl^-currents in these cells; the integrated amplitude of these currents is proportional to the LP concentration in the samples.

LP concentrations in control CSF samples were below the limit of sensitivity of the assay (approximately 10^{-9} M). In samples obtained after SAH LP concentrations increased over time, with a peak of approximately 10^{-3} M on the 6th day after SAH, and a return to baseline on day eleven. This corresponds closely to the onset of vasospasm in patients with SAH.

Taken together with previous evidence indicating a vasoconstrictor effect of LP, the present findings suggest that LP can play a role in the development of vasospasm after SAH.



616.17

NADPH-DIAPHORASE DISTRIBUTION IN THE GOLDFISH MAUTHNER CELL. T. Bell, A. Pereda and D.S. Faber*. Dept. of Neurobiology and Anatomy, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA. 19129.

The compound nitric oxide (NO) has been implicated as a retrograde messenger in the maintenance of hippocampal long-term potentiation. Similar sustained enhancements in synaptic efficacy have been demonstrated at identifiable excitatory and inhibitory inputs to the goldfish Mauthner (M-) cells. In an attempt to determine whether NO is also involved in this system we assayed for the presence and subcellular distribution of the enzyme nitric oxide synthase (NOS), known to catalyze the generation of NO by the selective oxidation of L-Arginine residue. For this purpose NADPH-diaphorase histochemical staining was used to localize the enzyme. Widespread labeling was observed for specific cell types throughout the goldfish brain. The M-cells appeared to stain heavily, and the reaction product was uniformly distributed along their axon, soma, and ventral and lateral dendrites (n=9). Afferent fibers which synapse with the M-cell were also NADPH-diaphorase positive they include the i) inhibitory interneurons known as PHP (passively hyperpolarizing potential) cells which are typically identified by their unmyelinated axons in the periphery of the M-cell's axon cap and ii) large myelinated club endings of eighth nerve afferents, unequivocally identified by their diameter (5-15 μm) and typical segregation to the distal part of the M-cell lateral dendrite. Interestingly, this pattern of staining was also shared by other identifiable reticulospinal neurons, consistent with the notion that they could serve as functional analogs of the M-cell.

The presence of NADPH-diaphorase in the M-cell and its afferents suggests a possible role for NO in the previously described activity-dependent long term potentiation of both the excitatory and inhibitory synapses.

Supported by NIH grant NS15335.

616.18

A P-450 METABOLITE OF ARACHIDONIC ACID MEDIATES THE DOPAMINE-INDUCED INHIBITION OF RAT ROD Na+,K+-ATPase VIA ACTIVATION OF A G PROTEIN COUPLED TO PLA_2. L.M. Shulman* and D.A. Fox. University of Houston, College of Optometry, Houston, TX 77204.

Nanomolar concentrations of dopamine (DA), acting on D_2/D_4-like receptors, inhibit ouabain-sensitive rod oxygen consumption in dark- and light-adapted rat retinas and inhibit the α3, but not α1, isozyme of Na+,K+ (Na,K)-ATPase in rod outer-inner segments (ROS-RIS) via a second messenger (Shulman and Fox, PNAS 1996). To determine the mechanisms underlying this DA-induced inhibition, pharmacological and biochemical experiments were performed. ROS-RIS were incubated with or without DA in the absence or presence of: pertussis toxin (PTX); cholera toxin (CTX); forskolin (FSK); PLC inhibitor U-73122; PKC inhibitor Calphostin C (CalC); PLA_2 inhibitors (p-bromophenacyl bromide: BPAB or Quinacrine: QUIN); BAPTA-AM; lipoxygenase inhibitor 5,8,11-eicosatetriynoic acid (ETI); or 7-ethoxyresorufin (7-ER), a P-450 inhibitor of arachidonic acid (AA). α1- and α3-isozyme activities were measured for each incubation condition. In the absence of DA, none of the drugs affected α1 and only FSK decreased α3 Na,K-ATPase activity. PTX, BPAB, QUIN, 7-ER and BAPTA completely blocked the selective inhibitory effects of DA on the α3 isozyme. In contrast, CTX, FSK, U-73122, CalC and ETI did not reduce the inhibitory effects of DA on α3. These results suggest that DA inhibits the α3-isozyme of Na,K-ATPase via: 1) a PTX-, but not CTX-, sensitive G protein: possibly Go, 2) activation of the Ca^{2+}-sensitive PLA_2 and 3) the production of a P-450 metabolite of AA. Supported by NIH Grant ES03183.

616.19

TONIC RELEASE OF NITRIC OXIDE IN ELECTROTONICALLY-COUPLED SYNCHRONISED RAT SYMPATHETIC PREGANGLIONIC NEURONES.
David Spanswick, Victoria J. Clark and Stephen D. Logan*. Department of Biomedical Sciences, Marischal College, Aberdeen, U.K.

Spontaneous membrane potential oscillations in a population of sympathetic preganglionic neurones (SPN) have been described. The presence of these oscillations gives rise to bursting or beating patterns of spike discharge. We have used whole-cell recording techniques in spinal cord slice preparations to investigate mechanisms underlying and regulating this activity in SPN. Simultaneous whole-cell recordings were obtained from 54 pairs of SPN. Of these, 9 pairs of SPN were observed to discharge action potentials and, or oscillations synchronously. The amplitude of the spikelets recorded in one neurone of a pair was larger in magnitude when the other recorded neurone discharged an action potential. In the spontaneously oscillating pairs of SPN, synchronous spikelets were detected in both neurones when recorded subthreshold for firing. Injection of hyperpolarising and depolarising current pulses into these SPN induced membrane hyperpolarising and depolarising responses respectively, in both neurones. Bath application of NO donors sodium nitroprusside (10-100μM) or DEA/NO, (10-100μM) induced concentration-dependent hyperpolarising responses in all SPN displaying spontaneous oscillations. In the presence of NO synthase inhibitors L-NAME (100-500μM), L-NOARG (50-250μM) or L-NMMA (50-200μM), the frequency of oscillations and firing in coupled SPN was increased, and in silent SPN, oscillations induced. We conclude that a subpopulation of SPN are electrotonically coupled and that spontaneous synchronised activity in these neurones is regulated by tonic release of NO.
Supported by the Wellcome Trust.

ADENOSINE AND ATP AS NEUROTRANSMITTERS

617.1

ATP, SYNAPTICALLY RELEASED IN THE BRAIN, IS NOT A CO-TRANSMITTER WITH GLUTAMATE S.J. Robertson* & F.A.Edwards Dept. Physiology, University College London, Gower St, London U.K.

In the peripheral nervous system ATP acts as a co-transmitter released with either noradrenaline or acetylcholine. We investigated, in slices of rat medial habenula nucleus, whether ATP is a co-transmitter with the dominant fast excitatory transmitter, glutamate. Several lines of evidence suggest that this is not the case.

Generally a much higher voltage of stimulation (>20V) was needed to find the sparse ATP inputs than was required to find the plentiful glutamate inputs which cover most of the slice. Consequently, in most cases, though it was clear that glutamate could be released without ATP, it was difficult to investigate directly whether ATP could be released without glutamate. In some cells however, (n=4), placement of the electrode was fortuitous and the stimulus which resulted in regular release of ATP did not result in any glutamatergic currents once CNQX was washed out, despite the presence of spontaneous glutamate currents. Thus it is clear that, in these cases, ATP is released from different axons to those which release glutamate.

Further evidence comes from the fact that various changes in stimulation parameters had different effects on failure rate of ATP- vs glutamate-mediated synaptic currents, implying that the two transmitters cannot be co-stored in the same vesicles. We then tested whether ATP and glutamate could be released from different vesicles but from a mixed population within the same bouton. If this were so, the build up of adenosine, shown in our other poster to occur at ATP-releasing synapses, should result in a progressive increase in glutamate failure rate as the stimulation frequency is increased. However glutamate release was observed to be affected by adenosine agonists and antagonists but, unlike ATP release, the sensitivity of glutamate release to adenosine antagonist, 8CPT was not frequency dependent but shifted the curve parallel throughout the frequency range (0.5-10Hz).
Support: Australian Res. Council, The Royal Soc., U.K & the Wellcome Trust.

617.2

FREQUENCY DEPENDENT INHIBITION OF SYNAPTIC ATP-RELEASE IN RAT BRAIN IS MEDIATED BY ADENOSINE RECEPTORS WITH NOVEL PHARMACOLOGY. F.A.Edwards* & S.J. Robertson Dept. Physiology, University College London, Gower St London U.K.

Synaptic ATP currents were recorded from slices of the medial habenula nucleus of 21-26 day old rats using the whole-cell patch clamp tecnique. The effect of stimulation frequency on ATP release was investigated by counting failures. Increasing the stimulation frequency from 0.5 Hz to 1, 2, 5, 10 and 100 Hz resulted in a progressive but readily reversible increase in failure rate. Thus increasing stimulus frequency results in little change in the number of quanta of ATP released per second.

This effect of frequency was dose-dependently inhibited by the adenosine antagonist 8-cyclopentyltheophylline (8CPT) but only at concentrations of ≥ 5μM (~500x the K_I for A_1-receptor binding). We thus investigated whether the effect was mediated by adenosine receptors other than A_1-receptors (which mediate most of the central inhibitory effects of adenosine in other brain areas). Surprisingly the frequency dependent effect could be mimicked by low concentrations of either the specific A_1-receptor agonist 2-Cl-N^6-cyclopentyl adenosine (CCPA 100nM) or equally the specific A_2 receptor agonist DPMA (10, 100nM). These effects were also inhibited by 8CPT. The unusual pharmacology of the receptor(s) was further confirmed when the relatively non specific agonist 2-Cl-adenosine proved to be ineffective even at concentrations as high as 1μM.

Finally, in order to test whether the endogenous adenosine mediating the frequency dependent effects came from the breakdown of released ATP, we attempted to inhibit ectoATPases with ARL67156 (Astra). This approach was however unsuccessful as, in this system, the compound appeared to have additional effects consistent with a block of adenosine uptake or a long lasting increase in adenosine release.
Support: Australian Res. Council, The Royal Society, UK & the Wellcome Trust UK

617.3

EXTRACELLULAR ATP AS A SOURCE OF ENDOGENOUS ADENOSINE IN BRAIN, T.V. Dunwiddie* and W. R. Proctor. Dept. of Pharmacology, University of Colorado Health Sciences Center, and Veterans Admin. Medical Res. Service, Denver, CO 80262.

The relative contributions of various sources to extracellular adenosine in brain are only partially understood. Adenosine is formed from the extracellular breakdown of ATP, but the rate at which this conversion can occur is unclear. To address this issue, we used infrared video-enhanced Nomarski optics to visualize CA1 neurons in hippocampal brain slices. Patch electrodes were used to make whole cell recordings from these cells, and adenosine and ATP were applied from double barrel drug application pipettes placed within 10 μm of the cell from which recordings were made. Both ATP and adenosine elicited outward currents with a moderately fast onset and long duration, which were antagonized by the competitive adenosine receptor antagonist theophylline. The amplitude and duration of the responses to ATP and adenosine under these conditions were almost indistinguishable, except that there was a delay of approximately 500 msec in the onset of the ATP response. Much faster P2 (ATP receptor)-mediated responses were observed when larger amounts of ATP were applied. Because ATP cannot activate adenosine receptors directly, we conclude that the quantitative conversion of ATP to a purine that is active at adenosine receptors occurs very rapidly (<500 msec) in the extracellular space in brain, and that the rapid formation of adenosine via this mechanism might be an important source of extracellular adenosine.
Supported by NS 29173 and the Veterans Administration Medical Research Service.

617.4

THE SOURCES OF EXTRACELLULAR ADENOSINE IN HIPPOCAMPUS AND NEOSTRIATUM OF THE RAT

M. A. Pak* and H. L. Haas. Heinrich-Heine University, Department of Physiology, Moorenstr.5, D-40225 Düsseldorf, Germany

Endogenous adenosine can enter the extracellular space either by way of a bidirectional transporter or via formation from other purines. We have compared the effects of substances blocking the transporter (dipyridamole) or the ecto-5'-nuleotidase (α-β methylene adenosine 5'-diphosphate, AOPCP) on field potentials in the hippocampus and the striatum in vitro following stimulation of the stratum radiatum or the cortico-striatal pathway respectively. Population spikes in the CA1 region and field potentials in the neostriatum were registered. AOPCP, at 40 μM, enhanced the amplitude of field potentials by 34±17.5% (mean ± SEM, n=11) in the hippocampus and by 26.5 ±14% (n=5) in the striatum. The inhibitory response to exogenously applied ATP (10μM) which is quickly broken down to adenosine, was antagonized by AOPCP (80-100 μM). Dipyridamole (1-5μM) decreased the amplitude of evoked potentials in both structures by 51± 18% (n=8). Thus both, release of adenosine from the intracellular space and, to a lesser extent, extracellular dephosphorylation of ATP contribute to the electrophysiologically effective levels of adenosine in the extracellular space in hippocampus and neostriatum.

617.5

ADENOSINE KINASE INHIBITION ENHANCES KAINIC ACID-INDUCED INCREASES IN STRIATAL ADENOSINE. D.R. Britton*, J. Mikusa, L. Lee, M. Williams and E. Kowaluk Neuroscience Research, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064-3500.

Adenosine (ADO) is released in response to nerve injury and in turn is proposed to be neuroprotective via its ability to inhibit further glutamate release. We have assessed the effects of the excitatory neurotoxin, kainic acid (KA), on extracellular ADO levels and the effects of the ADO kinase inhibitor, 5'-deoxy-5-iodotubercidin (5'd5IT) on basal and KA-stimulated ADO. Male S-D rats were anesthetized and bilateral microdialysis probes were placed in the striata. Probes were continuously perfused at a flow rate of 2.0 μl/min with artificial CSF with or without added KA. Following an immediate increase in ADO (to 20-80 μM) that accompanied insertion of the probes, levels fell over a period of 1 hr to a relatively stable baseline of approximately 1.0 μM. Pre-KA baseline samples were collected for an additional hour. Rats were then treated with saline or with 5'd5IT (2.5 then 5.0 μmol/kg) administered ip 20 min prior to and at the beginning of a unilateral 20 min exposure to 1.0 mM KA. At the end of the KA exposure the dialysis fluid was switched back to artificial CSF and the dialysis continued for an additional 60 min. ADO was assayed by HPLC using fluorometric detection of a derivatized product, ethenoadenosine. As indicated in the table below, KA caused a unilateral elevation in ADO levels. 5'd5IT significantly enhanced the ADO concentrations in the presence of KA but did not significantly alter ADO concentrations in the contralateral striatum.

Effects of 5'd5IT on Kainic Acid Induced Elevations in Striatal ADO

	Intrastriatal Saline		Intrastriatal Kainic Acid	
ip Rx	60 min. Pre-	60 min. Post-	60 min. Pre-	60 min. Post-
saline	0.7±0.2	0.6±0.2	1.2±0.1	5.1±1.6 *
5'd5T	2.1±0.5	1.8±0.4	2.4±1.4	14.0±4.9*'

* p<.05 vs Pre-Kainic Acid; 'p<.05 vs ip saline, post-kainic acid

Research funded by Abbott Laboratories.

617.6

THE EFFECTS OF ADENOSINE KINASE (AK) INHIBITORS ON ACUTE THERMAL NOCICEPTION E. A. Kowaluk *, A. Bannon, K. Gunther, K. Kohlhaas, J. Lynch, and M. F. Jarvis Neuroscience Research, D-4PM, AP10, Abbott Laboratories, Abbott Park, IL 60064

Adenosine is an inhibitory neuromodulator that can increase the nociceptive threshold in animals exposed to a variety of noxious stimuli. Inhibition of the adenosine-catabolizing enzyme, AK, provides a means of locally enhancing extracellular adenosine concentrations. In the present study, the AK inhibitors 5'-amino,5'-deoxyadenosine (NH_2dADO), 5-iodotubercidin (5-IT), and 5'-deoxy,5-iodotubercidin (5'd-5IT) were examined for their analgesic efficacy in the hot-plate model of acute somatic nociception. Control and drug-treated adult male mice were placed on a 55°C hotplate and the latency to the tenth jump recorded via a computer driven infrared-beam photo-sensor. Mice that did not respond during the 180 sec test session were removed to prevent tissue damage. All three AK inhibitors were found to significantly increase jump latencies in a dose-dependent fashion. 5'd-5IT was the most potent AK inhibitor (approx. ED_{50} value = 1 μmol/kg) followed by 5IT (ED_{50} =10 μmol/kg), and 5'NH_2dADO (ED_{50} = 100 μmol/kg). Pretreatment of mice with either theophylline (10 mg/kg) or cyclopentyltheophylline (10 mg/kg) which did not alter the jump latency of mice when administered alone, significantly attenuated the antinociceptive effects of 1 μmol/kg 5'd-5IT. The apparent efficacy of 2.5 μmol/kg of 5'd-5IT was not significantly altered following the repeated administration of this dose given twice daily for four days. The present data provide evidence for an antinociceptive action of AK inhibitors in the hotplate test, which, at least for 5'd5IT, is mediated by an enhancement of adenosine's actions at the A_1 receptor subtype and which does not exhibit tolerance following repeated administration.
Funding source: Abbott Laboratories

617.7

Axonal Adenosine Receptors in the Corpus Callosum Are Physiologically Active. S.E. Krahl*, Y.-Z. Liu, & T.H. Swanson. Departments of Neurology and Neuroscience, The Cleveland Clinic Foundation, Cleveland, Ohio.

We recently reported the presence of A_1 adenosine receptors on axons in the central nervous system. It was unclear, however, whether these receptors are functional or simply in transit from the cell body to the nerve terminal. We now present evidence that these receptors are physiologically active and are capable of modulating axonal conduction.

Coronal slices containing the corpus callosum were prepared from adult male Sprague Dawley rats for *in vitro* slice recording. Slices were placed in a recording chamber and bathed in artificial cerebral spinal fluid (ACSF). The corpus callosum was stimulated once per minute and the resultant compound action potential (CAP) recorded. After collecting 30 minutes of baseline activity, the perfusion media was changed to ACSF containing one of a variety of adenosine agonists or antagonists.

The lipophilic specific A_1 receptor agonist, N^6-cyclopentyladenosine (CPA), caused a dose-dependent decrease in the CAP. This effect was prevented by the specific A_1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). Specific A_2 receptor agonists and antagonists did not produce significant effects on the CAP.

These data are the first to demonstrate that axonal conduction can be modified by adenosine agonists, and suggest a novel mechanism of adenosine action. Such a finding supports the notion that axons are not passive transmitters of action potentials, but may be modulated by neuroactive substances.

Supported in part by a Doris Flynn Research Fellowship from the CCF (S.E.K.) and NIH grant K08-NS01705 (T.H.S.).

617.8

RAPID DESENSITIZATION OF ATP RECEPTORS OF RAT PC12 CELLS. L. Khiroug, R. Giniatullin, M. Talantova, G. J. Augustine* and A. Nistri, Int. Sch. Adv. Studies (SISSA), 34013 Trieste, Italy and Duke Univ. Med. Ctr. Durham, NC 27710, U.S.A.

Whole-cell patch clamp recording was used to study the action of ATP on PC12 cells. Membrane potential was held at -70 mV and ATP (0.5 - 5 mM) was applied from micropipettes by brief (≤50 ms) pressure pulses. ATP induced inward currents with rapid onset and rapid decay, while ADP and α,β-methylene ATP were ineffective. Applying 5 mM ATP for more than 200 ms elicited a complex current response which had a rapid peak (smaller in amplitude than the one produced by shorter ATP applications) and then largely faded. Removal of ATP caused a strong rebound current that lasted several s. The amplitudes of both peak and rebound currents were depressed in parallel by bath application of a threshold dose of ATP (25 μM). The reversal potential of the peak and rebound currents was identical. When ATP was applied to a cell clamped at a depolarised potential, no current was observed but rapid return of the membrane potential to -70 mV at the end of ATP application caused a large rebound current. Brief (20 ms) application of ATP during the onset of the rebound current strongly and transiently suppressed this current. In contrast, similar applications during the gradual decay of the rebound wave elicited a small transient inward current. Application of 2 s ATP pulses at 20 s intervals reduced equally the peak and rebound currents, which both recovered at the same rate. These present data suggest two types of desensitization for ATP receptors. The first one is characterised by fast kinetics and low agonist affinity; rapid recovery from it causes receptor reactivation and yields the rebound current. The second desensitized state has slow kinetics and high affinity for the agonist and is seen during sustained application of a low dose of ATP. We propose that desensitization may contribute to the time course of responses mediated by purinergic receptors *in situ*. Supported by INFM.

617.9

ATP is a slow transmitter in neuro-neuronal synapse.
V. A. Derkach*, Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.
Extracellular ATP directly activates ligand-gated ion channels in a variety of neurons and muscle. At both peripheral and central synapses, ATP may act as a classical fast neurotransmitter. Here I report a slow excitatory postsynaptic current (e.p.s.c.) in cultured celiac ganglion neurons that can last a second, is selectively blocked by inhibitors of P_2-purinergic receptors and is mimicked by millisecond applications of ATP to outside-out patches. Slow e.p.s.c.s alone or in combination with fast e.p.s.c.s could be evoked in the same neurons by stimulating of different inputs, indicating the presence of two populations of postsynaptic ATP receptors. Currents evoked in patches by ATP persisted in the absence of cytoplasmic GTP and were not occluded by GTPγS. Results imply that brief pulses of synaptically released ATP can have short and long lasting postsynaptic effects, both caused by activation of ligand-gated channels with different affinities to ATP. Supported by fund from Vollum Institute.

617.10

PURINE MODULATION OF EPILEPTIFORM BURSTING IN RAT HIPPOCAMPAL SLICES. T. W. Stone*, M. J. Brodie and F. M. Ross. Division of Neuroscience and Biomedical Systems, University of Glasgow, Scotland, G12 8QQ.

Hippocampal slices (450μm) perfused with a medium containing no magnesium and 4-aminopyridine (4-AP) (50μM) generate spontaneous epileptiform activity which resembles interictal activity *in vivo*. It was of interest to determine if ATP could directly modulate this activity. Adenosine and ATP (2-100μM) caused a concentration-dependent decrease in epileptiform activity which was rapid and reversible. The A_1 receptor antagonist, 8-cyclopentyl-1,3-dimethylxanthine (CPT) (100nM) significantly increased the rate of activity suggesting the existence of a tonic purine inhibition. CPT (100nM) blocked the effects of both adenosine and ATP (50μM). Adenosine deaminase (0.2 U/ml) had a non-significant tendency to increase the frequency of epileptiform activity. At a concentration that neutralised adenosine's inhibition, adenosine deaminase failed to prevent the depression of activity caused by ATP. ATP analogues were used: α, β-methyleneATP (α, β-meATP) (10μM) produced a significant rise in activity which was not affected by adenosine deaminase. 2-methylthioATP (2-meSATP) (10μM) and UTP (50μM) were ineffective. P_2 receptor antagonists suramin (10/50μM) and PPADS (5μM) had no effect on the basal rate of activity. Suramin at 10 and 50μM and PPADS failed to antagonise the inhibition produced by ATP (50μM). The increase in activity by α, β-meATP was inhibited by suramin. Thus, ATP has a dual effect on spontaneous activity in the hippocampal CA3 region causing both inhibition and excitation. The excitatory effect is produced by the activation of a P2x -like receptor which is responsive to α, β-meATP and inhibited by suramin. The inhibition is not affected by suramin or PPADS. It is unlikely that P2u receptors are involved due to the ineffectiveness of UTP. It is possible that another subtype of P2x receptor is involved in the inhibitory response or that a receptor which is activated by both nucleotides and nucleosides is responsible for the inhibitory effect.

617.11

PHORBOL ESTER REGULATION OF A1 ADENOSINE RECEPTOR mRNA LEVELS IN SH-SY5Y CELLS. R.A. Peterfreund*, E.K. Gies, J.S. Fink. Depts. of Anesthesia and Neurology, Mass. General Hospital, Boston, MA 02114

The A1 adenosine receptor (A1R) is a member of the G protein-coupled receptor (GPCR) family widely distributed in the CNS. Phorbol esters, probably acting via protein kinase C (PKC), regulate the mRNA levels of many GPCR subtypes. We tested the hypothesis that the phorbol ester TPA can regulate A1R mRNA levels in the human SH-SY5Y neuroblastoma cell line. SH-SY5Y cells maintained in monolayer culture in serum supplemented medium were treated with TPA and total RNA was prepared by standard methods. A1R mRNA levels were measured by semiquantitative Northern blot analysis under high stringency conditions. The blots were hybridized to a 32P labeled riboprobe spanning 783 bp of the hA1R cDNA including 584 bp of 3' coding and 199 bp of 3'UT sequence. The blots were rehybridized to a cDNA probe for human βactin to control for loading. Hybridization of the A1R riboprobe to SH-SY5Y RNA resulted in a single band which comigrated with that of human cerebral cortex and cerebellum RNA. Treatment with TPA (100nM) decreased (to <50% of control) the levels of A1R mRNA in a time dependent fashion with the nadir at ~4h. There was no change in the hybridization signal after incubations with the ethanol vehicle control. The selective PKC inhibitor bisindolylmaleimide (1μM) prevented the TPA effects. Stability of A1R mRNA was determined by incubation with actinomycin D in the presence or absence of TPA. TPA did not accelerate the degradation of the A1R message. We conclude that SH-SY5Y human neuroblastoma cells express A1R mRNA. Phorbol esters reduce A1R mRNA levels via a mechanism likely mediated by PKC, but which does not appear to involve alterations in A1R mRNA stability.
Supported by NSF # IBN-9319624

617.12

CO-EXPRESSION OF RAT A_1-ADENOSINE RECEPTORS AND G-PROTEIN SUBUNITS IN Sf9 CELLS. L. J. Blythe[a], M. Dennis[b] and D. R. Hampson[a]*.
[a] Faculty of Pharmacy, Univ. of Toronto, Toronto, Canada M5S 2S2.
[b] Biosignal Inc., 1744 Rue William, Montreal, Quebec, Canada H3J 1R4.

Adenosine, a purine nucleoside, modulates numerous effector systems including adenlyl cyclase, potassium channels and calcium channels. The effects of adenosine appear to be mediated by specific receptors that couple with guanosine nucleotide binding proteins (G-proteins). Of the four adenosine receptors that have been cloned, the A_1 receptor is particularly well characterized. These receptors may be involved in the pathogenesis of epilepsy and confer neuroprotective effects in cerebral ischemia. The primary objective of this project is to study and develop optimal conditions for the functional co-expression of adenosine A_1 with G-protein subunits in *Spodoptera frugiperda* (Sf9) cells using the baculovirus (BV) expression system.

In preliminary experiments A_1 receptors were expressed alone in Sf9 cells and characterized by radioligand binding analysis using the A_1 agonist [^{3}H]CCPA. Agonist binding to the receptor was characterized by relatively low affinity binding. Co-expression of the receptor with Gαi3 increased the affinity of the agonist for the receptor three fold. The effect of other G proteins on [^{3}H]CCPA binding are currently being explored. Our results indicate that Sf9 cells and the BV system provide a good expression system for studying interactions between mammalian adenosine receptors and G proteins.

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC)>

617.13

CHARACTERIZATION OF THE GENOMIC STRUCTURE OF MOUSE A2a ADENOSINE RECEPTOR GENE. J.F. Chen* and J.S. Fink. Molecular Neurobiology Lab. Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

A2a adenosine receptors play an important role in the regulation of dopaminergic function in brain, and in cardiovascular and immunological function in peripheral tissues. To analyze the A2a receptor at the level of gene expression, we characterized the genomic structure of mouse A2a receptor gene. We previously showed that a single gene encodes the mouse A2a receptors and that two exons encode the A2a receptor, with a single intron interrupting the coding sequences between transmembrane domain III and IV. In the present study, we further characterized the mouse A2a receptor gene by 5' rapid amplification of cDNA ends (RACE) and by direct sequencing of the genomic DNA upstream of the translation initiation codon of the A2a receptor gene. The study revealed an additional exon in the 5' region which encodes about 100 bp 5' untranslated sequences of A2a receptor mRNA. The identified transcription start site and promotor sequences indicate that the A2a gene promotor region are TATA-less, GC-rich and contain several characteristic transcription factor binding sites, including AP1, AP2 and Sp1 sites. This result suggests that A2a receptor gene is characterized by a TATA-less and GC-rich promotor, a common feature of several G-protein coupled neurotransmitter receptors including D1 and D2 dopamine receptors.

617.14

ADENOSINE A_3 RECEPTOR SELECTIVE ANTAGONISTS: FLAVONOID AND DIHYDROPYRIDINE DERIVATIVES.
K.A. Jacobson[a]*, A. M. van Rhee[a], J. L. Jiang[a], Y. Karton[a], X.d. Ji[a], D.K.J.E. von Lubitz[a], M.E. Olah[b], and G.L. Stiles[b] [a]Mol. Recognition, NIDDK/NIH, Bethesda, MD 20892; [b]Duke Univ. Med. Center, Durham, NC.

Adenosine A_3 receptor antagonists are sought as cerebroprotective and anti-inflammatory agents. Xanthines, the classical antagonists, have failed to provide selectivity for A_3 receptors. We have assembled and screened diverse chemical libraries and identified flavonoids (phenolics found in all vascular plants) and dihydropyridines (e.g. Ca^{2+} channel antagonists) as promising leads in displacing binding of [^{125}I]AB-MECA (N^6-(4-amino-3-iodobenzyl)adenosine-5'-N-methyluronamide) with μM affinity at cloned human and rat A_3 receptors. The flavonol galangin had affinities of 13.6 and 3.2 μM at human A_1 and A_3 receptors, respectively. The dihydropyridines nicardipine and niguldipine had K_i values of 2-3 μM at human A_3 receptors (much weaker at A_1 and A_2 receptors). We have chemically modified these two chemical classes resulting in the more potent compounds MRS1067 (a flavone) and MRS1097 (a dihydropyridine). Both compounds displayed selectivity for A_3 vs. A_1 receptors (>40-fold) and antagonized the A_3-agonist elicited inhibition of adenylyl cyclase in membranes of CHO cells transfected with rat A_3 receptors. It appears that flavonoids constitute a class of naturally occurring adenosine antagonists that are even more widespread in the diet than caffeine and other xanthines. MRS1097 did not bind to L-type Ca^{2+} channels nor to cholinergic, adrenergic, or other receptors.

617.15

PHOTOAFFINITY LABELING OF A P_3 PURINOCEPTOR-LIKE PROTEIN PURIFIED FROM RAT BRAIN MEMBRANES. Y. Saitoh*, and H. Nakata, Dept. of Mol. & Cell. Neurobiol., Tokyo Metropolitan Inst. for Neurosci., Fuchu-shi, Tokyo 183, Japan.

We have found a novel adenosine-binding protein in rat brain membranes. We partially purified this protein from rat brain membranes and characterized its properties. It can bind to both adenine nucleotides and adenosine. Based on the ligand binding specificities, the protein is likely to be classified into the P_3 purinoceptor which was recently proposed (Shinozuka et al, 1988). In the present study, we characterized the P_3 purinoceptor-like protein (P_3LP) further by photoaffinity labeling. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the labeled preparations with 8-azidoadenosine-5'-[^{32}P]triphosphate revealed that the probe was incorporated specifically into a single band with an approximate molecular size of 54kDa. We compared the photoaffinity labeling pattern with the NECA-binding activity of several fractions obtained from the purification procedures. The patterns of the 54kDa band agreed well with those of the NECA-binding activity. These results suggest that the 54kDa band is the P_3LP. This affinity labeling technique will be a useful method for the sensitive determination of P_3LP.

GLUTAMATE TRANSPORTERS I

618.1

CALCIUM REGULATED UPTAKE OF EXCITATORY AMINO ACIDS IN POSTSYNAPTIC NEURON. [1]Y. Kataoka, [2]H. Ohmori and [3]K. Kataoka*, [1]Dept. of Neurosci., Osaka Bioscience Institute, Osaka, [2]Dept. of Physiol., Kyoto Univ., Kyoto, [3]Dept. of physiol., Ehime Univ. Sch. of Med., Ehime, Japan.

Excitatory amino acids released from the presynaptic terminal are known to be removed from the synaptic cleft or other extracellular space by their uptake systems existing in the presynaptic terminal and in surrounding glial cells. The ionic current mediated by the Na^+ dependent uptake of excitatory amino acids was isolated in cultured rat cerebellar Purkinje cells voltage clamped at -65 mV with the whole cell patch clamp method. This uptake system was thought to work postsynaptically, since the uptake mediated current was induced in cell soma and in all over the dendrites. This postsynaptic uptake was accompanied by an increase in Cl^- conductance, and was facilitated approximately 3 times by the postsynaptic Ca^{2+} influx brought by membrane depolarization.

(This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, science and culture of Japan for JSPS Research Fellowships for Young Scientists)

618.2

GLUTAMATE CONTRACTS *SCHISTOSOMA MANSONI* (TREMATODA: DIGENEA) ISOLATED MUSCLE FIBERS: EVIDENCE FOR A GLUTAMATE TRANSPORTER. C.L. Miller[1]*, J.L. Bennett[1], and R.A. Pax[2]. Departments of Pharmacology & Toxicology[1] and Zoology[2], Michigan State University, East Lansing, MI 48824.

Schistosoma mansoni muscle fibers contract in response to L-glutamate in a dose-dependent manner (10^{-6}-10^{-3}M). D-Glutamate, L-aspartate and D-aspartate also cause contraction of the fibers. The glutamate receptor agonists NMDA, ibotenate, kainate, AMPA, quisqualate, ACPD, and L-AP4 produce little or no contraction at concentrations as high as 1 mM. The glutamate receptor antagonists, MK-801, CNQX, AP-5, and MCPG, did not block glutamate induced contraction of the muscle fibers. However, other amino acids such as, L-aspartate, L-cysteate, and cysteine sulfinate, known to be substrates of the high-affinity glutamate transporters were found to elicit contraction of the muscle fibers. Contractions induced by L-glutamate are dependent on extracellular Ca^{++} and are blocked by the voltage-gated Ca^{++} channel blocker nicardipine (10 and 1 μM). [^{3}H]-L-Glutamate, incubated with the muscle fiber preparation, is taken up in a dose- time- and temperature-dependent manner. Both L-glutamate induced contractions and [^{3}H]-L-glutamate uptake are Na^+-dependent, and are blocked by specific inhibitors of the high-affinity glutamate transporter, DL-threo-β-hydroxyaspartate, and L-*trans*-pyrollidine-2,4-dicarboxylic acid (THA, PDC). These data suggest that there may be an electrogenic Na^+-dependent high-affinity glutamate transporter on the schistosome muscle membrane. When glutamate is applied to schistosome muscle fibers it is possible that electrogenic transport of glutamate is causing the fiber membrane to depolarize, thereby opening nicardipine-sensitive voltage-gated Ca^{++} channels, and raising intracellular Ca^{++} concentrations leading to contraction.

Supported by NIH RO1-A130465-03, GM-07392.

618.3

INHIBITION OF SYNTHESIS OF EAAC1 GLUTAMATE TRANSPORTER ALTERS γ - AMINOBUTYRIC ACID LEVELS IN DISCRETE BRAIN REGIONS. C. U. Eccles*, M. Dykes-Hoberg, and J. D. Rothstein. Dept. of Neurology, Johns Hopkins Univ., and Dept. of Pharmaceutical Sciences, Univ. of Maryland School of Pharmacy, Baltimore, MD.

Selective knockout of individual high-affinity glutamate transporter proteins using antisense oligonucleotides have demonstrated that the astroglial transporters GLAST and GLT-1 are of primary importance in maintaining low extracellular glutamate concentrations and in protecting against excitotoxicity. The neuronal transporter, EAAC1, appears to play a lesser role in clearing extracellular glutamate because selective knockout of EAAC1 produces minimal neurotoxicity or changes in extracellular glutamate (*Neuron*, 16,675). Loss of EAAC1 protein, however, produces behavioral abnormalities which include seizures and hyperresponsiveness suggesting that loss of EAAC1 function may alter synaptic function or neuronal metabolism. Because EAAC1 has been localized to GABAergic neurons and glutamate is a precursor for GABA synthesis, we hypothesized that EAAC1 may play a role in GABA synthesis and neurotransmission. In our initial studies, we determined GABA levels in several brain regions after 7 days of treatment with EAAC1 antisense (or sense) oligonucleotide delivered by infusion into the lateral ventricle of Sprague-Dawley male rats surgically prepared with subcutaneous miniosmotic pumps and intraventricular cannulae. After 7 days of treatment, brains were removed after decapitation, dissected into 6 regions, and prepared for HPLC analysis of GABA. Preliminary studies indicate that GABA concentrations are reduced by greater than 25% in thalamus and hippocampus. GABA concentrations in anterior cortex, posterior cortex and striatum are decreased from 10-20%. No changes were detected in cerebellum which is remote from the site of infusion. These results are consistent with the possibility that inhibition of glutamate uptake may lead to limited precursor availability and decreased GABA synthesis, i.e. that a portion of GABA synthesis depends on glutamate uptake by GABAergic neurons. The behavioral abnormalities observed may be manifestations of such a decrease in GABA function. (Supported by NIH)

618.4

DIFFERENTIAL EXPRESSION OF THREE GLUTAMATE TRANSPORTER SUBTYPES IN THE RAT RETINA

[1]M.Wießner, [2]J.D.Rothstein & [1]T.Rauen*; [1]Max-Planck-Institut für Hirnforschung, 60528 Frankfurt, Germany; [2]Johns Hopkins Univ., Dept. of Neurology, Baltimore, MD 21287, USA

The major excitatory neurotransmitter in the mammalian retina is L-glutamate.

Reverse transcribed mRNA from rat retinae, amplified by polymerase chain reaction (RT-PCR) using gene-specific primers for GLAST-1, GLT-1 and EAAC-1, revealed the presence of message for all three transporter subtypes in the retina. Single cell RT-PCR of retinal dissociates demonstrated that GLAST-1 is the sole L-glutamate transporter expressed in Müller cells. Immunocytochemistry indicated that Müller glial cells and astrocytes are reactive for GLAST-1, whereas GLT-1 is preferentially expressed by different types of bipolar cells. EAAC-1 is found in horizontal cells, in amacrine cells and ganglion cells. Western blot analysis suggested the following rank order of glutamate transporter subtype expression in the retina: GLAST-1≥EAAC-1>GLT-1. Additionally, accumulation of L-[^{3}H]glutamate occurred predominately in Müller glial cells. This high capacity for glutamate uptake is compatible with our finding of relatively high amounts of GLAST-1 in Müller cell membranes surrounding synapses.

We have shown that GLAST-1, GLT-1 and EAAC-1 have specific distributions in the retina and are expressed by different cell types and thus may have different functional roles in the retina. Uptake for metabolic reasons, reuptake of synaptically released glutamate and light dependent uptake might require different transporter characteristics. Thus glutamate transporters are not only found on presynaptic terminals of glutamatergic neurons, but also in postsynaptic elements of GABAergic neurons and in glial cells.

618.5

REGIONAL EXPRESSION OF THE GLUT4 GLUCOSE TRANSPORTER IN MOUSE BRAIN. I. A. Simpson*, K. Li, E. M. Koehler-Stec, E. M. Gibbs and S. J.Vannucci. NIDDK, Bethesda, MD 20892, Pfizer Inc., Groton, CT 06340 and Dept. of Pediatrics, Hershey Med Ctr - Penn State Univ, Hershey, PA 17033.

Glucose transport into brain is mediated by a family of facilitative glucose transporter proteins (GLUT1-7). The predominant glucose transporter isoforms in brain are: GLUT1, blood-brain barrier (55 kDa) and glia (45kDa); GLUT3, neurons; however, low levels of GLUT4, the insulin-sensitive glucose transporter, have been reported. GLUT4 is highly expressed in peripheral tissues (heart, skeletal muscle, adipose) in which insulin and contraction induce acute increases in glucose transport by a recruitment of intracellular GLUT4 transporters to the plasma membrane. The purpose of this study was to examine GLUT4 expression by *in situ* hybridization, laser confocal microscopy (LCM), and Western blot analysis in brains of normal, genetically diabetic (*db/db*) and GLUT4 transgenic diabetic (hGLUT4*db/db*) mice. *In situ* hybridization studies localized GLUT4 mRNA expression primarily to 3 brain regions: cerebellum (CB), dentate gyrus of the hippocampus (DG) and olfactory bulb (OB). Expression is most pronounced in cerebellum where it is localized to the granule cells. Granule cell-specific expression appears true of DG and OB as well. LCM studies confirmed this localization for GLUT4 protein and demonstrated intracellular GLUT4, as is seen in peripheral tissues. Both *db/db* and hGLUT4*db/db* mice demonstrate enhanced GLUT4 expression in CB. The results of this study indicate that a subset of neurons, the granule cells, express the insulin-sensitive glucose transporter, GLUT4, in addition to the usual neuronal transporter, GLUT3, which may be integral to patterns of granule cell migration and maturation. The enhanced expression of GLUT4 in these cells may be a locus for the effects of diabetes on the brain. Supported by JDFI Grant # 194182 (SJV), NIDDK, Pfizer.

618.6

REGIONAL AND ULTRASTRUCTURAL LOCALIZATION OF THE GLUTAMATE TRANSPORTER EAAT4 IN HUMAN AND RAT BRAIN. M Dykes-Hoberg*, A Furuta, LJ Martin‡, G Lin, JD Rothstein. Johns Hopkins University, Dept. of Neurology and Pathology‡, Baltimore, MD 21287.

Glutamate transport is the primary mechanism for the synaptic inactivation of glutamate. EAAT4 is a novel glutamate transporter with properties of a ligand-gated chloride channel, that was recently cloned from human brain. The present study is an investigation of the protein expression and cellular localization of EAAT4 in human tissue, and the cloning, expression, cellular and ultrastructural localization of EAAT4 in rat brain (rEAAT4). Regional immunoblot analysis of EAAT4, using a monospecific oligopeptide (carboxy-terminal) affinity-purified polyclonal antibody, revealed that the protein is restricted to the central nervous system. The EAAT4 protein was largely expressed in cerebellum, with a much lower expression in hippocampus, cerebral cortex, striatum, and thalamus. Immunocytochemical study showed intense EAAT4-immunoreactivity in the human and rat cerebellar Purkinje cells, in a somatodendritic pattern. Other brain regions including cerebral cortex, hippocampus, striatum show faint neuropil staining of EAAT4. Ultrastructurally, rEAAT4 protein was localized in Purkinje cell dendrites and spines. In the hippocampus and neocortex, rEAAT4-immunoreactivity was found mainly in plasma membranes of distal dendrites, with enrichment at the post-synaptic density. Occasionally, rEAAT4 immunoreactivity was found in astrocytic cell processes. Thus, EAAT4 is primarily, although not exclusively, localized to cerebellar Purkinje cells, and to rarely in terminal dendrites and astrocytic processes of other brain regions. (Supported by NIH and MDA)

618.7

DIFFERENTIAL POSTNATAL DEVELOPMENT OF EXCITATORY AMINO ACID TRANSPORTER SUBTYPES IN RAT BRAIN [1,2]V.R. Roettger* and [1,2]S.G. Amara. [1]Howard Hughes Medical Institute and [2]Vollum Institute , Oregon Health Sciences University, Portland, OR

Maintenance of low extracellular glutamate (GLU), the major excitatory transmitter in mammalian brain, is due primarily to re-uptake via Na^+-dependent transporters. Previous studies measured ^{3}H-GLU uptake at 3 days post-birth (Blakely et al., J. Neurochem., 56:860, 1991; Christensen & Fonnum, Neurochem. Res., 17:457, 1992) but did not differentiate between transporter subtypes. We used polyclonal antibodies raised against excitatory amino acid transporter subtypes (EAAT1-4) and Western blotting to determine the protein developmental pattern in three rat brain regions: cerebellum, cortex, and hippocampus.

The developmental pattern of EAAT1 and EAAT2 protein expression was similar in all three regions; both proteins were present at very low levels beginning on post-natal (PN) day 1-4. Protein content continuously increased through the postnatal period leveling off at PN12 for EAAT1 and PN24 for EAAT2. In contrast, EAAT3 protein was present at adult concentrations beginning with PN1 in all three brain regions. Postnatal EAAT1-3 proteins showed no changes in molecular weight versus adult proteins. EAAT4 protein showed a different pattern. In cerebellum, a 68kD glycosylated protein species was observed beginning approximately PN14 and present through to adult. This species was not detectable at any timepoint in either cortex or hippocampus. In addition, a higher molecular weight species (app. 85kD) was present in all three regions on PN1; staining of this species was blocked when antibody was preincubated with GST-EAAT4 fusion protein but not with GST protein alone. The concentration of the second protein decreased during development becoming non-detectable after PN24. The identity of this second species has not been established; Northern blots and in situ hybridization studies are currently being conducted.

This differential pattern of expression of EAAT proteins raises the possibility that the transporters may play a role in regulating brain development by altering the level of extracellular glutamate. Funding Source: Howard Hughes Medical Institute.

618.8

DIFFERENTIAL REGULATION OF GLUTAMATE TRANSPORTER SUBTYPES IN DEVELOPING RAT BRAIN.

A Furuta*, LJ Martin‡, JD Rothstein. Johns Hopkins University, Dept. of Neurology and Pathology‡, Baltimore, MD 21287.

Glutamatergic systems participate in dendritic and axonal arborization, synaptogenesis and activity-dependent synaptic plasticity during development. Extracellular glutamate concentrations are regulated by transporter proteins localized in neuronal (EAAC1) and glial cell membranes (GLAST and GLT-1). Recently a novel neuronal glutamate transporter, EAAT4 was cloned in human brain. We have generated a monospecific polyclonal antibody for EAAT4, and the rat homolog, rEAAT4. The present study examined the protein expression and cellular localization of glutamate transporter subtypes in embryonic (E12, 15, 18) and postnatal (P1, 5, 10, 16, 24, 26, adult) rat brain, using immunoblotting and immunocytochemistry with anti-peptide antibodies for all four glutamate transporter subtypes. By immunoblotting, GLAST expression increased with age to reach adult levels by P26. However, the glycosylation of GLAST was differentially regulated- glycosylated GLAST increased with maturation. GLT-1 expression increased progressively to adult levels, which were achieved at about P26. EAAC1 expression increased with maturation and reached maximum levels around P5 to P16, then decreased to adult levels by P26. Strong EAAC1-immunoreactivity was seen as early as E15, and by E18, it was throughout forebrain regions including neocortex, striatum and hippocampus. In contrast, GLT-1 was expressed in rhinencephalon, hypothalamus and hippocampus at E18; but little immunoreactivity was seen in neocortex and striatum embryonically. rEAAT4 increased with maturation and was mainly localized to cerebellar Purkinje cells, although a small amount of protein was seen in the adult striatum and neocortex. Thus, the expression and localization of glutamate transporter immunoreactivity was subtype-specific during the developmental period. These findings indicate that glutamate transporters could participate in neuronal and glial maturation. (Supported by NIH and MDA)

618.9

IMPAIRMENT OF DNA REPAIR LEADS TO LOSS OF GLUTAMATE TRANSPORT *IN VITRO*.

L. Jin*, G. C. Lin, C. Coccia, I. Nagano and J.D. Rothstein. Department of Neurology, Johns Hopkins University, Baltimore, MD 21287.

A loss of high-affinity glutamate transport was identified in certain brain regions and spinal cord of patients with amyotrophic lateral sclerosis (ALS). Our laboratory previously developed an *in vitro* model of defective glutamate transport by chronically blocking the glutamate carriers in cultured organotypic spinal cord slices. This resulted in the slow degeneration of motor neurons (over several weeks), a pathogenic marker for ALS. In this study we applied this paradigm to pursue the potential mechanisms which could cause a loss of glutamate transport. We hypothesized that reduced DNA repair capacity may lead to accumulation of damage on the glutamate transporter gene, which consequently would result in a loss of glutamate transport. 1-beta-D-arabinofuranosylcytosine (araC), a DNA polymerase inhibitor, was used to inhibit DNA repair. Chronic inhibition of DNA repair synthesis with low concentrations of araC in spinal cord cultures initially produced a dose-dependent loss of glutamate transport, measured by [H^3]-D-aspartate binding assay. A loss of the motor neuron marker choline acetyltransferase activity (ChAT) occurred approximately 7 days later. This motor neuron toxicity was prevented by NBQX, a non-NMDA antagonist, or by t-BPN, an anti-oxidant. In addition, the toxicity was synergistically enhanced by concomitant treatment with threohydroxyaspartate (THA), a glutamate transport blocker. These results suggest that impairment of DNA repair may be relevant to altered glutamate transport function and could potentiate glutamate toxicity.
(Supported by NIH and MDA)

618.10

Modulation of astrocytic glutamate uptake by nitric oxide

Z-C. Ye* and H. Sontheimer. Neurobiology Research Center and Dept. of Physiology and Biophysics, Univ. of Alabama at Birmingham, Birmingham. AL 35294.

Glutamate is maintained at low micromolar concentrations in the extracellular space. This task, which in part serves to limit potential glutamate-mediated neurotoxicity, is believed to be primarily accomplished by the high affinity, Na^+-dependent uptake into astrocytes. Astrocytes are also a primary target for neuronally released nitric oxide (NO). We have used measurements of ^{3}H-glutamate/aspartate uptake in a hippocampal culture system to evaluate the modulation of glial glutamate uptake by NO. Exposure of astrocytes to several nitric oxide donors consistently inhibited astrocytic glutamate uptake in a dose and time dependent manner. According to the degree and time-course of their effectiveness, NO donors could be divided into two groups: 1.) SIN-1 and SNAP, in the presence of SOD/CAT for 5-30 minutes, inhibited uptake rates by 20-40%. These effects were abolished by treatment with the NO scavenger hemoglobin. Effects could be mimicked by activating astrocytic iNOS by coculture (24h) with lipopolysaccharides (LPS) or several cytokines including TNF-α, IL-1β, and IFN-γ. Their effects were abolished by the NOS inhibitors LNA, L-NMMA, and LNAME suggesting that inhibition was mediated by NO. 2.) Longer incubations (90 min) with SNP (0.1 mM) induced a dramatic release of glutamate by astrocytes with relatively little effect on uptake. These effects were not reversed by hemoglobin, but instead could be blocked by inhibition of guanylyl cyclase using methylene blue (20 μM, 30 min). SNP-induced release of glutamate from astrocytes may provide a alternative explanation for the frequently observed neurotoxic effect of SNP. Taken together our data provided further evidence that astrocytic glutamate uptake is dynamically regulated and sensitive to numerous factors released by neurons or non-neuronal cells in the brain. These modulatory influences may be important for normal synaptic transmission and could account for some of the uncontrolled glutamate accumulations observed with some CNS diseases. (Supported by R01-NS-31234 and P50-HD-32901)

618.11

PHARMACOLOGICAL CHARACTERIZATION OF NOVEL GLUTAMATE TRANSPORT IN RAT CORTICAL NEURONS. G. J. Wang*, K. Pratt, J. Schnuer, P. A. Rosenberg. Dept. of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115.

Glutamate transport in cortical synaptosomes is potently inhibited by dihydrokainate (DHK) but not L-α-aminoadipate (L-α-AA) [Robinson et al. (1993) J. Neurochem. 60 167-179]. In order to pursue the possibility of a novel neuronal transporter, we sought to characterize glutamate transport in cultures of cortical neurons (astrocytes < 0.5%). Uptake studies using [^{3}H]-L-glutamate showed Na^+-dependent high affinity glutamate transport with a K_m of 9.6 ± 2.3 μM (n=7). The V_{max} was 2.8 ± 0.3 nmol/mg protein/min. DHK, at 1 mM, inhibited uptake of radioactivity by 88 ± 3%, and had a K_i of 65 ± 12 μM. L-α-AA, at 1 mM, inhibited uptake by only 25 ± 4%. Five other excitatory amino acid analogs were examined for their ability to inhibit [^{3}H]-L-glutamate uptake in these cultures. Of the analogs tested, L-trans-pyrrolidine-2,4-dicarboxylate, L-serine-O-sulfate, and kainate potently inhibited transport activity with K_i values of 5 ± 0, 56 ± 6 and 102 ± 9 μM, respectively (n=3). α-Methyl-DL-glutamate and β-N-oxaly-L-α,β-diaminopropionic acid at 1 mM did not significantly inhibit transport. Autoradiography with [^{3}H]-D-aspartate labeled some but not all neuronal cell bodies and processes as well as astrocytes. DHK at 500 μM greatly reduced labeling on neuronal cell bodies and processes, but did not block labeling of astrocytes. In contrast, the labeling of both astrocytes and neurons was almost completely blocked by 1 mM PDC. Thus, the dominant glutamate transport activity in cortical neuronal cultures is similar to that of cortical synaptosomes.

This work was funded by grants from the NINDS (NS31353) and from the United Cerebral Palsy Foundation.

618.12

NEUROTOXICITY OF DIHYDROKAINATE TO RAT CORTICAL NEURONS IN CULTURE. R.C. Blitzblau, G.J. Wang, M. Yonezawa*, E. Aizenman[1], and P.A. Rosenberg. Dept. of Neurol., Children's Hospital & Harvard. Med. Sch., Boston MA 02115 and Dept. of Neurobiol. Univ. Pittsburgh. Sch. Med., Pittsburgh, PA 15261.

Glutamate transport into cortical synaptosomes is potently inhibited by dihydrokainate (DHK), but not by L-α-amino-adipate. This pharmacological signature is replicated in nearly pure (less than 0.5% astrocytes) cultures of rat cortical neurons, but not in mixed cultures of astrocytes and neurons. If glutamate transport is important in protecting neurons against excitotoxicity, then in neuronal cultures (but not in mixed cultures) DHK would be expected to be neurotoxic. In mixed cortical cultures, a 20-24 hour exposure to 1 mM DHK had no significant effect on neuronal survival (94 ± 17% of control; n = 4). In contrast, in neuronal cultures, survival in the presence of 1 mM DHK was 33 ± 22% of control ($p < 0.001$; n = 3). This neurotoxicity of DHK was blocked by 10 μM MK-801 plus 10 μM GYKI 52466. In order to clarify the mechanism of toxicity of DHK, we investigated its effect on [^{3}H]-MK-801 binding to rat brain membranes, ^{45}Ca influx into cortical cultures, as well as on membrane currents in whole-cell recordings from cortical neurons. The results of these studies were consistent with a weak antagonist action of DHK at the NMDA receptor. In addition, a small current was observed with 1 mM DHK (10% of the current produced by 30 μM NMDA) that was blocked by 100 μM 7-chlorokynurenate but not by 5 μM NBQX. It remains to be determined whether this current is due to a direct or an indirect action of DHK. Taken together, these data suggest that the toxicity of DHK that occurs in neuronal cultures is due to its ability to block glutamate transport in these cultures.

This work was funded by grants from the NINDS (NS31353) and from the United Cerebral Palsy Foundation.

618.13

ENDOTHELINS INDUCE REVERSED UPTAKE OF GLUTAMATE FROM RAT CULTURED ASTROCYTES. Y. Sasaki*, M. Takimoto, K. Oda, T. Früh, M. Takai, T. Okada, and S. Hori. International Research Laboratories, Ciba-Geigy Japan Ltd., Takarazuka 665, Japan.

Release of glutamate (Glu) from glial cells via reversal transport is supposed to be one of the most critical events for the neurodegeneration resulting from cerebral ischemia. To investigate the reversal transport of Glu in glial cells, we measured Glu efflux from rat cultured astrocytes preloaded with [^{3}H]Glu. Either 60 mM KCl or Na^+-free medium, but not 10 μM ionomycin, induced Glu efflux. This result indicate that the efflux was due to reversed uptake of Glu via Na^+/K^+-dependent Glu transporter. We investigated the effect of various neuropeptides and neurotransmitters on this response in astrocytes and found that only endothelins (ETs) induced the reversed uptake. ET-1, ET-3 and an ET_B-specific agonist, IRL 1620 increased the reversed uptake of Glu with similar potencies (EC_{50} = 0.6-2 nM). An ET_B-selective antagonist, IRL 2500, partially inhibited the reversed uptake induced by 1 nM ET-1 in a concentration-dependently manner, causing a maximum inhibition of 60% at 1 μM. However, 1 μM BQ-123, an ET_A-selective antagonist, did not cause significant inhibition. Combination of both antagonists completely inhibited the ET-1-induced reversed uptake. These results indicate that both receptor subtypes are involved in the reversed uptake with a major contribution of ET_B. Our findings suggest that ETs, which are known to be released in ischemia, exacerbate neuronal damage by stimulating the reversed uptake of Glu.

618.14

MECHANISMS INVOLVED IN RAPID STIMULATION OF THE EAAC1 GLUTAMATE TRANSPORTER BY PROTEIN KINASE C. L.A.Dowd*[1], C.Lamothe[1], J.D.Rothstein[2], and M.B.Robinson[1]. [1]Children's Seashore House, Depts. of Pediatrics and Pharmacology, Univ. of Pennsylvania, Philadelphia, PA 19104 and [2]Dept. of Neurology, Johns Hopkins Univ., Baltimore, MD 21287.

Four Na^+-dependent glutamate (Glu) transporter cDNAs have been isolated (EAAC1, GLAST, GLT1, & EAAT4). We have recently demonstrated that C6 glioma endogenously express EAAC1 but not GLAST or GLT1 protein. We also showed that the PKC activator, PMA, rapidly increases the V_{max} for L-[^{3}H]-Glu transport activity in C6 glioma (2- to 3-fold) but does not affect the K_m. This increase appears to be independent of protein synthesis. We are in the process of trying to define the mechanisms responsible for this regulation. To define which PKC isoform might be involved, we used the reported observation that, of the three isoforms expressed in C6 glioma (α, δ, and θ), the δ isoform is selectively down-regulated by 30 to 100 nM PMA (24h) (Chen & Wu, 1995). We found that a comparable pre-incubation blocked the effects of acute PMA treatment suggesting that the δ isoform of PKC is responsible for the stimulation of EAAC1 transport. To determine if the increase in activity is due to the movement of EAAC1 protein from intracellular vesicles to plasma membrane, as is observed with glucose transporters, C6 glioma cells were pretreated with PMA or vehicle, and subcellular fractionation was used to separate vesicular EAAC1 from plasma membrane EAAC1. Western analysis demonstrated that EAAC1 was present in both membrane fractions and PMA did not cause the movement of EAAC1 from vesicles to plasma membrane. We are in the process of determining whether direct phosphorylation of EAAC1 by PKC is occurring. (NS29868)

618.15

COS7 ENDOGENOUSLY EXPRESS THE GLAST SUBTYPE OF Na^+-DEPENDENT GLUTAMATE TRANSPORTER. M.K. Perez[1], P.A. Johansen*[1], L.A. Dowd[1], J.R. Vondrasek[1], J.D. Rothstein[2], and M.B. Robinson[1]. [1]Children's Seashore House, Depts. of Pediatrics and Pharmacology, Univ. of Pennsylvania, Philadelphia, PA 19104 and [2]Dept. of Neurology, Johns Hopkins Univ., Baltimore, MD 21287.

C6 glioma endogenously express the EAAC1 subtype of Glu transporter and are being used as a model system to study regulation (See Dowd *et al.*, adjacent poster). In a search of cell lines, we found that COS7 cells endogenously express GLAST-like immunoreactivity but not EAAC1- or GLT1-like immunoreactivity. We characterized the properties of this endogenous Na^+-dependent Glu transport in COS7 cells. Substitution of equimolar choline chloride for NaCl reduced the accumulation of both L-[^{3}H]-Glu and D-[^{3}H]-Asp to less than 15% of controls. Under conditions of initial velocity the Na^+-dependent transport of both L-[^{3}H]-Glu and D-[^{3}H]-Asp were consistent with a single site with K_m values of 22 μM and 45 μM, respectively. The V_{max} values were 442 and 145 pmol/mg protein per min, respectively. The pharmacology of endogenous transport in COS7 cells was characterized using EAA analogs which have been shown to selectively inhibit subtypes of Glu transport in brain tissue. Na^+-dependent L-[^{3}H]-Glu transport was inhibited by L-*trans*-pyrrolidine-2,4-dicarboxylate, L-α-aminoadipate, DL-threo-ß-hydroxyaspartate with IC_{50} values of 60 μM, 500 μM, and 18 μM, respectively. Na^+-dependent L-[^{3}H]-Glu transport was almost completely insensitive to dihydrokainate. This pharmacological profile is similar to that previously reported for primary astrocyte-enriched cultures. (NS29868)

618.16

EXPRESSION OF Na^+-DEPENDENT L-GLUTAMATE TRANSPORTER GLT1 IN PRIMARY CULTURES. J.R.Vondrasek[1], L.A.Dowd[1], J.D.Rothstein[2], and M.B. Robinson*[1]. [1]Children's Seashore House, Depts. of Pediatrics and Pharmacology, Univ. of Pennsylvania, Philadelphia, PA 19104 and [2]Dept. of Neurology, Johns Hopkins Univ., Baltimore, MD 21287.

Extracellular levels of the excitatory amino acid glutamate (Glu) are primarily regulated by a family of Na^+-dependent high affinity (SDHA) L-Glu transporters. The subtypes GLT1 and GLAST are expressed by glia *in vivo*. We previously characterized the pharmacology of SDHA transport in astrocyte-enriched cultures (14-17 days *in vitro*). This pharmacology differed from that observed for GLT1-mediated transport but was similar to that reported for GLAST-mediated transport. In the present study, the identification of transporter subtypes expressed by glial cultures and potential regulatory mechanisms were investigated. Unlike the cellular distribution of these subtypes *in vivo*, Western analyses indicated that astrocytes express GLAST but not GLT1. Two approaches were used to induce expression of GLT1. First, inclusion of L-Glu (1mM) or the mGluR agonist 1S, 3R ACPD (500μM) in the culture media did not induce GLT1. However, the addition of the cAMP analogs, dibutryl cAMP (250μM) or 8-bromo cAMP (250μM) to the culture media induced expression of GLT1. In addition, mixed cultures of neurons and glia also express GLT1. These data suggest that expression of the SDHA L-Glu transporter GLT1 is regulated through a cAMP response element (CRE) and potentially by the presence of neurons. (NS29868)

618.17

HYPOXIA RELEASES GLUTAMATE BY REVERSAL OF α-AMINOADIPATE-SELECTIVE TRANSPORTERS IN RAT HIPPOCAMPAL SLICES. J.E. Madl* and M.N. Hyden. Department of Anatomy & Neurobiology, CSU, Ft. Collins, CO 80523.

Hypoxia and other metabolic insults release toxic amounts of glutamate (Glu) and other excitatory amino acids. This release may be due to reversal of transporters. To identify the transporters primarily involved in pathologic release, we determined the effects of transportable Glu analogs on hypoxia-induced Glu release from rat hippocampal slices. Cloned members of the XAG^- transporter family have much lower affinities for α-Aminoadipate (AAA) and quisqualate than D,L-threo-β-Hydroxyaspartate (TBH) or D-Aspartate. Differences in the ability of Glu analogs to inhibit the uptake of TBH and AAA suggest these analogs are taken up primarily via different transporters. The effects of low pH and ion substitution also suggests transport of TBH and AAA occurs by different systems. Hypoxia released more preloaded AAA than TBH, suggesting that AAA cotransporters may be more easily reversed than TBH transporters. Azide-induced release of endogenous Glu was inhibited by extracellular AAA and quisqualate, suggesting that pathologic Glu release occurs primarily via nonXAG^- transporters. However, TBH and other analogs selective for XAG^- transporters potentiated release, suggesting XAG^- transporters continue to take up Glu during hypoxia. The apparent greater vulnerability of AAA transporters to reversal during metabolic insults and the ability of AAA to block ATP depletion-induced release of endogenous Glu strongly suggest that the initial pathologic release of Glu is primarily mediated by nonXAG^- transporters. Funding was provided by PHS grant NS28824.

618.18

REDOX SENSING PROPERTIES OF A HUMAN NEURONAL GLUTAMATE TRANSPORTER. D. Trotti* and M.A. Hediger. Brigham and Women's Hospital, Harvard Medical School, 75 Francis St., Boston, MA 02115.

In the CNS, control of the extrasynaptic concentration of L-glutamate is critical because of its role in neurotransmission and excitotoxicity. Mechanisms that modulate glutamate transporters are therefore relevant to the regulation of synaptic transmission. Using the two-electrode voltage clamp technique and *Xenopus* oocytes expressing the human neuronal glutamate transporter EAAC1, we performed a molecular characterization of the redox sensing site of EAAC1 by studying the effect of the sulfhydryl specific reagents DTT (3mM) and DTNB (500 μM) on the glutamate uptake current. Up and down changes in response to DTT (+35%) and DTNB (-20%) were observed in oocytes clamped at -60 mV. Blocking of free thiol groups with pCMPS (100 μM) prevented the effect of DTNB. The redox interconversion of cysteines by DTT/DTNB influenced the V_{max} of glutamate transport but the apparent affinity for glutamate was not affected. Redox modulation occurred at any membrane potential tested (-150 to +50mV). Endogenous oxidants such as peroxynitrite (100 μM) and H_2O_2 (5 mM) also affected the EAAC1 mediated uptake current (-35%, at -60mV) through a DTT reversible mechanism, suggesting that the redox site is targeted by oxidants. In addition to Na^+-coupled electrogenic glutamate transport, the current mediated by human EAAC1 is also affected by a glutamate gated anion influx. The net uptake current therefore reflects the inward current from electrogenic glutamate transport and the current arising from the glutamate-activated Cl^- conductance. Substitution of Cl^- with gluconate in the uptake medium did not abolish DTT-induced potentiation of uptake and had the same amplitude as observed in a Cl^- buffer, suggesting that redox modulation affects only the glutamate transport component of the current. Thus, our data provide new insights into the mechanism underlying the redox modulation displayed by the human glutamate transporter EAAC1. *Supported by NIH NS32001 and the ALS Association.*

618.19

TWO ELECTROGENIC GLUTAMATE UPTAKE TRANSPORTERS WERE FOUND IN SECONDARY CULTURES OF RAT ASTROCYTES EXPOSED TO INJURY. Y.L. Lee, P.G. Lal, and D.L. Eng*. VA Medical Center, Palo Alto CA and Stanford University, Stanford CA 94304.

Primary and secondary cortical astrocytes were tested for the expression of 3 different electrogenic glutamate uptake transporters. Astrocyte cultures were prepared from 1 day old rat pups and grown to confluence which is specified as time Day 0. The cultures were divided into 4 groups: Group A--untreated; Group B--astrocytes injured using a scratch wound model; Group C--astrocytes treated with dbcAMP, to induced morphological and cytoskeletal differentiation; Group D--astrocytes treated with dbcAMP and scratch wound injury. Astrocytes from each group were harvested, and total RNA was prepared from each group at the specified time points of Day 0, Day 1, Day 2, Day 4, and Day 7, after confluence. The total mRNA was reversed transcribed using AMV reverse transcriptase. Reverse transcriptase reaction was performed in duplicate for each Group A-D at each sample time point. cDNA samples were tested for 2 glial electrogenic glutamate uptake transporters GLT-1 and GLAST, and 1 neuronal form, EAAC-1, using PCR. PCR primers were designed so that they would specifically amplify GLT-1, GLAST, and EAAC-1. The 3 sequences were obtained from the Genbank and aligned using the program "pileup" and "Bestfit." The regions of the aligned sequences with no homology to each other were chosen to designed primers. The primers were designed using the program "Prime" to yield ~ 1.5kB products. The PCR reaction products were analysed using electrophoresis on 1.5% agarose gels. All samples from Groups A-D expressed mRNA for the 2 glial forms of the transporters, while none of the cultures expressed the neuronal glutamate uptake transporter. Of interest is the observation that all of these astrocyte cultures seemed to have expressed GLAST in greater proportion than GLT-1, whereas in vivo the GLT-1 is predominantly found in the astrocytes of the cortical hemispheres. By day 7, Group B and D, the scratch wound model, displayed an increase in the expression of glutamate transporter mRNA compared to untreated controls (Group A and C), however further quantitive analysis is being performed to confirm this finding. Supported by The Council for Tobacco Research and the Department of Veterans Affairs.

618.20

GLUTAMATE TRANSPORTER EXPRESSION IN RAT CORTICAL CULTURES. B.S. Stein*, J.W. Miller, K. Farrell, M.C. Longuemare, J.D. Rothstein, and R.A. Swanson
Dept. of Neurology, Univ. of California and VAMC, San Francisco, CA 94121.

GLT-1, GLAST, and EAAC are high affinity, Na^+-dependent glutamate transporters identified in rat forebrain. This study characterizes the expression of these transporters in rat cortical cultures by immunostaining and Western blots. Three types of cultures were studied: astrocytes with or without dBcAMP-induced differentiation, and mixed astrocyte neuronal cultures. Results are summarized as follows:

	GLT-1	GLAST	EAAC
Astrocyte cultures (- dBcAMP)	--	+	--
Astrocyte cultures (+ dBcAMP)	+++	++	--
Mixed cultures, Astrocytes	++	++	--
Mixed cultures, Neurons	--	--	++

Rare microglia were identified by OX42 immunostaining, and these cells were positive only for the GLT-1 transporter. Glutamate uptake in the astrocytes treated +/- dBcAMP differed only in V_{max}; the apparent K_M was unchanged. These studies show that transporter expression in the mixed astrocyte-neuronal cortical cultures exhibits the same pattern as seen *in vivo*, and that GLT-1 expression can be induced in astrocyte cultures by dBcAMP or by co-culture with neurons.

Supported by NIH RO1 NS31914

GLUTAMATE TRANSPORTERS II

619.1

SPECIFIC DOWN-REGULATION OF THE GLUTAMATE TRANSPORTER GluT-1 IN PURKINJE CELL-ASSOCIATED ASTROCYTES OF THE REELER AND WEAVER MUTANT MICE. M. WATANABE*, T. SHIBATA, K. YAMADA, K. TANAKA[1], K. WADA[1], Y. INOUE. Dept. of Anat., Hokkaido Univ. Sch. of Med., Sapporo 060, and Dept. of Degenerative Neurological Diseases, National Institute of Neurosci., NCNP, Kodaira 187, Japan.

The GluT-1 (GLAST or EAAT1) is one of the four glutamate transporter subtypes, with its high expression in the Bergmann astrocytes. In the present investigation, we employed expression analyses in neurological mutant mice having impaired Purkinje cell synapse formation. By *in situ* hybridization with ^{33}P-labeled antisense oligonucleotide probes, remarkable down-regulation of the GluT-1 mRNA was specifically detected in the *reeler* and weaver cerebella. In the *reeler*, the reduction was particularly remarkable in deep cerebellar regions, where most Purkinje cells lack synaptic contacts with the parallel fibers. A digoxigenin-labeled antisense cRNA probe revealed the presence of numerous GluT-1 mRNA-positive cells in both mutant cerebella, but they were labeled at much lower levels than those in the wild-type cerebellum. The labeled cells were all small in size and GFAP-immunopositive. In addition, these labeled cells were located in the vicinity of cell bodies of the Purkinje cells. Furthermore, specific antibody raised against the C-terminal peptide demonstrated that GluT-1-immunopositive structures were closely associated with Purkinje cell dendrites and soma. Thus, the present study clarified that the GluT-1 is down-regulated in Purkinje cell-associated astrocytes of neurological mutants with impaired Purkinje cell synapse formation. This, together with our previous result on the striking up-regulation in synaptogenesis, suggests that the gene expression of the GluT-1 may be controlled dynamically, in accordance with structural and functional state of synapses.

619.2

RECOMBINANT ADENOVIRUS MEDIATED TRANSDUCTION AND KNOCK-DOWN OF GLUTAMATE TRANSPORTER. J. Yang*, R. Sanga, and R. Roginski. Dept. Anesth. Pain Mgmt, UT Southwestern Med. Center., Dallas, TX and Dept. Anesth., UMDNJ Robert Wood Johnson Sch. Med., New Brunswick, NJ.

The Na-dependent glutamate transporter removes glutamate from the extracellular space. The ability to up or down-regulate the glutamate transporter will help elucidate the role of this transporter in a variety of CNS processes including synaptic transmission, excitotoxicity, and neurodegenerative diseases.

Replication deficient human adenovirus containing a RSV promoter driven expression cassette with the glutamate transporter cDNA inserted in the sense (ad(GluT1β-S)) or antisense (ad(GluT1β-AS)) orientations were constructed by homologous recombination in H293 cells. The GluT1β transporter is a variant of the glia specific Glt-1 transporter with a truncated amino terminal (Nsci. Abstr. 20: 628, 94). Transduction of functional glutatmate transporter was examined by infecting the MRC-5 (lung epithelial cell line) with ad(GluT1β-S), and the knock-down of constitutively present transporter activity by ad(GluT1β-AS) was evaluated by infecting the RCGU (rat glial cell line). Both the MRC-5 and the RCGU cells were infected by recombinant adenovirus at high efficiency. Control cultures exposed to ad(lacZ) at a titre of 1×10^8 pfu/ml showed that > 90% of the RCGU and 60-70% of the MRC-5 cells were infected. The MRC-5 cells lack Na-dependent ^{3}[H]-glutamate uptake, however, upon infection with ad(GluT1β-S) (2×10^8 pfu/ml, 48 hours incubation), Na- and [glutamate]-dependent uptake was demonstrated. Immunohistochemical staining with anti-GluT1β antibody confirms successful transduction of the transporter protein. RCGU cells constitutively express dihydrokainate blockable Na-dependent transporter activity. This native transporter activity was reduced by ad(GluT1β-AS) infection. We demonstrate the feasibility of adenovirus mediated transduction or knock-down of the glutamate transporter and should be useful for examining the role of this transporter in various CNS processes.

619.3

INHIBITION OF GLUTAMATE UPTAKE BY PEROXYNITRITE IN TRANSFORMED L CELLS EXPRESSING HUMAN GLUTAMATE TRANSPORTER SUBTYPES. I. Nagano*, G. C. Lin, L. A. Bristol and J. D. Rothstein. Department of Neurology, Johns Hopkins University, Baltimore, MD 21287.

Glutamate transport is essential to keep its extracellular levels below neurotoxic values. Altered transport function has been reported in CNS tissue of ALS patients. Recent studies have shown oxidative damage to proteins in ALS CNS tissue. In this study we demonstrate that a potent oxidant, peroxynitrite inhibits glutamate uptake mediated by two human glutamate transporter subtypes, excitatory amino acid transporter (EAAT) 2 (glial) and EAAT3 (neuronal). Stably transformed L cells expressing EAAT2 or EAAT3 were obtained by transfection with pcDNA3-EAAT constructs. After exposure to various concentrations of peroxynitrite, Na-dependent high-affinity glutamate uptake was measured in Krebs buffer containing [^{3}H]glutamate. In EAAT2 expressing L cells, 300 μM peroxynitrite inhibited glutamate uptake by ~30%, while the same concentration of peroxynitrite induced ~60% reduction in EAAT3 expressing cells. Application of DTT, a disulfide-reducing agent, partially restored transport function, suggesting that oxidation of protein sulfhydryls may be partially related to glutamate uptake inhibition. In addition, chronic exposure to a peroxynitrite releasing agent, SIN-1, also induced glutamate uptake inhibition. These results suggest the possibility that peroxynitrite locally produced in the CNS could inhibit glutamate uptake and might be implicated in neuronal cell death through excitotoxicity. (Supported by NIH and MDA).

619.4

DEVELOPMENT OF MONOCLONAL ANTIBODIES FOR HUMAN NEURON-SPECIFIC GLUTAMATE TRANSPORTER. P. Shashidharan[1]*, G.W.Huntley[2], J. Murray[1], M. J. Walsh[1], T. Moran[3]. [1]Dept. of Neurology, [2]Fishberg Research Center for Neurobiology and [3]Dept. of Microbiology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York NY 10029.

Glutamate is a major excitatory amino acid neurotransmitter in the mammalian central nervous system. The synaptic action of glutmate is thought to be terminated by a high-affinity uptake system, which is dependent on Na^+ and K^+. Here we report production and characterization of a monoclonal antibody for human neuron-specific glutamate transporter.

A monoclonal antibody was raised against a synthetic peptide (QYKTKREE-VKPPSDPEM) that corresponds to amino acid residues 161-177 of the human neuron-specific glutamate transporter cDNA cloned in our laboratory. The hybridomas were screened against the free peptide by ELISA initially and the clones were selected on the basis of their immunoreactivity. Western blotting of human and rat brain synaptosomal preparations from frontal cortex revealed a immunoreactive band at ~70 kD. The antibody was further characterized by immunoprecipitation and deglycosylation using N-glycosidase F. Deglycosylation of the immunoprecipitated material yielded a protein with a lower molecular weight (~65 kD) on western blots. These results are consistent with the molecular weight predicted by the cloned cDNA. Immunocytochemistry of cortex, hippocampus and substantia nigra of rat brain revealed strong immunoreactivity of neuronal cell bodies. In the cortical layers the large pyramidal neurons showed intense immunoreactivity, although there were other smaller neurons throughout the cortex showing lesser immunoreactivity. In the hippocampus neuronal somata and dendrites of the sub-fields CA1 to CA3 and the dentate gyrus were intensely immunoreactive. In substantia nigra in addition to neuronal somata with proximal dendritic labeling, the antibody stained fine caliber fibers and numerous puncta. Supported in part by Lowenstein Foundation for Parkinson's Disease Research.

619.5

CONFORMATIONALLY CONSTRAINED INHIBITORS OF THE HUMAN GLUTAMATE TRANSPORTERS: DIFFERENTIATION OF SUBSTRATES AND NON-TRANSPORTABLE BLOCKERS R.J. Bridges*, M.P. Kavanaugh°, N. Zerangue°, H. Koch, S. Esslinger, J. Humphrey°, S.G. Amara°, and A.R. Chamberlin°. Pharm. Sci., U.M., Missoula, MT 59812, °Vollum Inst., OHSU, Portland, OR 97201 and °Chem., UCI., Irvine, CA 92717.

High affinity uptake is recognized for its role in regulating extracellular glutamate levels in the CNS. We have begun using conformationally constrained glutamate analogues to delineate the specificity of three transporter subtypes cloned from human motor cortex: EAAT1, EAAT2, EAAT3 and EAAT4. A series of pyrrolidine dicarboxylates (PDCs) were prepared using L-*trans*-2,4-PDC as a prototype. In the present work transport activity was determined by measuring analogue-induced currents in voltage clamped (-60mV) oocytes expressing individual subtypes. Non-transportable inhibitors were evaluated using Schild analysis. L-*trans*-2,4-PDC was found to be a substrate of all three subtypes: $K_m \approx 10\mu M$ at EAAT2, $\approx 50\mu M$ at EAAT1 and EAAT3. Estimated I_{max} values were ≈30-80% that of L-glutamate. The bicyclic analogue, L-*anti-endo*-3,4-methano-PDC, was identified as a non-transportable inhibitor of EAAT2, $K_i \approx 2\mu M$. This inhibitor exhibited $K_i \approx 30\mu M$ and $40\mu M$ at EAAT1 and EAAT3, respectively, and I_{max} values 20% and 35% that of L-glutamate. L-*trans*-2,3-PDC was identified as a non-transportable inhibitor of EAAT2 ($K_i \approx 10\mu M$) that exhibited negligible activity at EAAT1 or EAAT3. Another bicyclic analogue, *meso*-methano-PDC, was unusual in that it exhibited a $K_m \approx 45\ \mu M$ at EAAT2, but an I_{max} of 115% that of L-glutamate. The analogues were also tested as uptake inhibitors and substrates in rat forebrain synaptosomes. Non-transportable inhibitors were differentiated from substrates by the inability of non-substrates to heteroexchange with ^{3}H-D-aspartate pre-loaded into synaptosomes. Comparisons demonstrated the closest correspondence was found between the synaptosomal preparation and EAAT2. This work was supported by NIH NS30570, NS33270, NS27600 and NS33273.

619.6

A POTENT GLUTAMATE UPTAKE INHIBITOR: L-*trans*-PYRROLIDINE-4-SULFONO-2-CARBOXYATE (PSC) H.P. Koch*, C.S. Esslinger, A.R. Chamberlin[†], and R.J. Bridges, Dept. of Pharm. Sci., U. of Montana, Missoula, MT 59812, Dept. of Chemistry[†], U. of California, Irvine, CA 92717.

High-affinity, sodium-dependent uptake of L-glutamate is postulated to participate in excitatory signal termination, transmitter recycling, and maintenance of sub-pathological levels of glutamate. In previous studies we identified L-*trans* -2,4-pyrrolidine dicarboxylate (L-*trans*-2,4-PDC) and L-*anti-endo* -3,4-methanopyrrolidine dicarboxylate (L-*anti-endo*-3,4-MPDC) as conformationally constrained analogues of glutamate that potently block the uptake of ^{3}H-D-aspartate, a non-metabolized substrate, into rat forebrain synaptosomes. These two inhibitors can be further differentiated with respect to their ability to serve as substrates, i.e. be translocated into the synaptosome. In the present work we have prepared a novel series of analogues in which the 4-COOH group of L-2,4-PDC has been replaced with a sulfonate moiety. When tested for their ability to inhibit the uptake of ^{3}H-D-aspartate, only the *trans* isomer was identified as active. Kinetic analyses of the inhibition yielded a K_i value of ≈ 9 μM for L-*trans*-2,4-PSC and ≈ 5 μM for L-glutamate. The activity of the sulfonate analogue as a substrate was evaluated by quantifying its ability to heteroexchange with ^{3}H-D-aspartate previously loaded into synaptosomes. The observed rank order of substrate activity was L-*trans*-2,4-PDC>L-*trans*-2,4-PSC>L-*anti-endo*-MPDC. These studies identify L-*trans*-2,4-PSC as a useful compound in delineating the binding site pharmacophore of the transport protein as well as an analogue with which to study the biochemistry of sulfonate-containing acidic amino acids. This work was supported in part by NIH NS30570 NS27600.

619.7

NOVEL COMPETITIVE INHIBITORS OF ^{3}H-L-GLUTAMATE TRANSPORT INTO SYNAPTIC VESICLES. R.D. Bartlett*, B.E. Garnett, A.R. Chamberlin°, and R.J. Bridges, Pharm. Sci., Univ. of Montana, Missoula, MT 59812 and °Chemistry, Univ. of California, Irvine CA 92717.

A number of biochemical parameters distinguish the glutamate transporter present in synaptic vesicles from the cellular high-affinity, Na-dependent uptake systems found on neurons and astrocytes. The vesicular transporter is Na-independent, coupled to a Mg-ATPase-generated proton gradient, and selective for L-glutamate (Km ≈ 1-3 mM) but not D-aspartate. Unfortunately, the development of selective competitive inhibitors of the vesicular system has lagged behind progress on the Na-dependent transporters. The recent identification of kynurenate (KYN) and *trans*-ACPD as two EAA analogues that exhibit some cross reactivity with the vesicular transporter has, however, begun to provide some insight into the pharmacological specificity of this system. In the present work we have assayed a series of conformationally constrained EAA analogues with the expectation of identifying more selective blockers. The compounds were tested for their ability to inhibit the uptake of ^{3}H-L-glutamate into synaptic vesicles prepared from the forebrains of male Sprague-Dawley rats. Two additional analogues of KYN were identified as potent competitive inhibitors: 7-Cl-KYN and xanthurenate. The latter compound is particularly interesting because previous reports suggest it exhibits little cross reactivity with EAA receptors. Among the pyrrolidine dicarboxylate (PDC) analogues tested, a previously identified inhibitor of Na-dependent synaptosomal uptake, L-*anti-endo*-3,4-methano-PDC, was found to also potently block vesicular transport. Surprisingly, this cross reactivity did not extend to the structurally similar analogue, L-*trans*-2,4-PDC. These newly identified inhibitors will be particularly valuable in modeling the binding site pharmacophore of the vesicular transporter. This work was supported in part by NIH NS30570 and NS27600.

619.8

TRANSMEMBRANE TOPOLOGY OF THE GAT-1 GABA TRANSPORTER. J. A. Clark.* Laboratory of Cell Biology, NIMH, Bethesda, MD 20892-4090.

While the predicted two-dimensional model of the Na^+/Cl^--dependent transporters derived by hydropathy analyses is commonly accepted, this transporter model has yet to be tested experimentally. The transmembrane topology of the GAT-1 GABA transporter has been studied as a representative member of this family. A FLAG epitope has been fused to GAT-1 at either the N- or C-terminus. When transiently expressed in mammalian cells, the membrane orientation of the FLAG epitope was determined using immunofluorescence under permeabilizing and non-permeabilizing conditions. To examine whether putative TM segments span the membrane, a series of C-terminal truncations were generated to which a prolactin epitope was fused. These truncated transporter constructs were expressed in *Xenopus laevis* oocytes and tested for protease sensitivity of the prolactin tag, as a determinant of transmembrane orientation. Results thus far support a model in which the N- and C-termini are intracellular, the large loop between TMs 3 and 4 is extracellular and glycosylated, and the first six hydrophobic segments span the membrane. Data from protease protection assays coupled with glycosidase treatment of these truncated transporters, reveal the presence of topogenic sequence(s) in TM5 responsible for correct orientation of the large extracellular loop. Study of the C-terminal six putative transmembrane segments is in progress. Knowledge of the transmembrane topology of these transporters is essential for interpretation of structure/function and chimeric analyses, and will contribute significantly to revealing their tertiary structure. Supported by an NIMH post doctoral IRT Award.

619.9

RESIDUES INVOLVED IN SUBSTRATE INTERACTIONS WITH A SODIUM-DEPENDENT GLUTAMATE TRANSPORTER IDENTIFIED USING CYSTEINE SCANNING MUTAGENESIS. [1,2]R.P. Seal*, and [2,3]S.G. Amara
[1]Program in Neuroscience, [2]Vollum Institute and [3]Howard Hughes Medical Institute, Oregon Health Sciences University, Portland OR 97201.

The Substituted Cysteine Accessibility Method (SCAM) was used to examine the contribution of residues in the Na^+-dependent glutamate transporter subtype, EAAT1 to the binding of substrates and inhibitors and to the permeation of chloride ions. A highly functional cysteine-less EAAT1 carrier has been generated and characterized by measuring substrate-elicited currents in the Xenopus oocyte expression system. In the cysteine-less mutant, the transport affinities and current-voltage relationships observed for L-glutamate, L-aspartate and D-aspartate are nearly identical to the wild type carrier. Twenty-four consecutive residues (Pro392-Gln415) have been individually mutated to cysteine using the cysteine-less transporter as the template. We focused on this domain of the transporter because of its high sequence conservation among glutamate carriers and because previous studies of EAAT chimeras and mutants have suggested a role for the region in substrate and inhibitor binding. The effects of three sulfhydryl-reactive methanthiosulfonate derivatives, MTSEA, MTSES and MTSET on the uptake of tritiated substrate and substrate-elicited steady-state currents for each of the mutants was evaluated. None of these derivatives exhibited an affect on the cysteine-less transporter. Significant inhibition of L-glutamate transport was observed after application of 5mM MTSEA to oocytes expressing the E406C transporter mutant. In addition, co-incubation of MTSEA with a saturating dose of either glutamate or kainate, a competitive inhibitor of the carrier, completely blocked the inhibition of uptake observed with MTSEA alone. These results suggest that Glu-406 resides within an aqueous environment that is accessible from the extracellular side of the membrane and it further implies a direct interaction between glutamate or kainate and this residue. By determining the accessibility of residues in the carrier and defining their possible interactions with substrates and ions we hope to provide insight into the structural and topological features required for transport. This work was supported by NS33273 and the Howard Hughes Medical Institute.

619.10

NEURONAL AND GLIAL GLUTAMATE TRANSPORTERS CONTAIN AN SH-BASED SITE RESPONSIBLE FOR REDOX MODULATION AND OXIDANT VULNERABILITY A. Volterra*, D. Trotti, B. Lodi Rizzini, D. Rossi, P. Bezzi, N.C. Danbolt+, G. Racagni Ctr. Neuropharmacol., Institute Pharmacol. Sci., Univ. Milan, 20133 Italy & + Dept. Anatomy, Univ. Oslo, N-0317 Norway

Glutamate (Glu) uptake controls the extracellular Glu level in the brain and prevents excitotoxicity. Our group has found that Glu uptake is long-lastingly inhibited by a number of endogenous oxidants, including oxygen radicals, hydrogen peroxide (H_2O_2 Volterra et al, J.Neurosci. 14:2924 (1994) and peroxynitrite ($ONOO^-$, Trotti et al, J.Biol.Chem. 271:5976 (1996). All of these agents affect uptake by direct interaction with the Glu transporters at unidentified "oxidant-vulnerable" site(s). We report here that 3 recombinant rat transporter subtypes, i.e. GLT1 and GLAST (glial) and rEAAC1 (neuronal), expressed in HeLa cells and reconstituted in liposomes, respond to thiol-specific oxidants (DTNB, 500 μM) and disulfide-reductants (DTT, 3 mM) with loss and gain of function respectively. The 3 transporters show almost identical sensitivity to SH-redox reagents ($\pm$20-30%). Sequential applications of DTT and DTNB to whole-cell clamped rat cortical astrocytes, cause dynamic up- (+80%) and down-modulation (-40%) of the Glu uptake current. In the same paradigm, H_2O_2 (1 mM) and $ONOO^-$ (nominally 250 μM), like DTNB, antagonize DTT potentiation, bringing the current level below control; subsequently, DTT partially reverses oxidant inhibition ($\approx$70% for H_2O_2). Therefore: (a) reversible interchange between free SH groups and disulfides regulates transport function; (b) GLT1, GLAST and rEAAC1 contain a similar SH-based redox site, probably formed by conserved cysteine residues; (c) this site most likely identifies with the "oxidant-vulnerable" site. Our findings may help to understand Glu uptake alterations in pathologies involving oxidative stress (e.g. ischemia and amyotrophic lateral sclerosis).
Supported by Telethon-Italy (grant 754) & EC Biomed 2 (BMH4-CT95-571) to A.V.

619.11

A MICRODIALYSIS STUDY OF BRAIN ADRENERGIC MODULATION OF GLUTAMATE REUPTAKE.
G.M. Alexander*, J.R. Grothusen, S.W. Gordon and R.J. Schwartzman.
Department of Neurology, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19102.

The central nervous system has high affinity uptake systems for the clearance of amino acid transmitters. These systems are found in both neurons and astrocytes. Previous studies have shown that the uptake of amino acid transmitters by astrocytes in culture can be modulated by adrenergic agents. The objective of this study was to determine whether amino acid uptake in the intact CNS is under adrenergic regulation. Male Sprague-Dawley rats weighing 250-450 grams were used in this study. A guide cannula was placed under anesthesia in the anterior cortex. The following day the microdialysis probe was inserted and sampling started 3 hours later in the awake, behaving animal. The extraction fraction of ^{3}H-L-glutamate using ^{14}C-mannitol as a reference was performed by the method of Bruhn T, *et al*, (J Neurosci Meth, 59:169-174, 1995). The cortical extraction fraction of ^{3}H-L-glutamate (an index of reuptake) was abolished by co-administration of the glutamate uptake blocker DL-threo-β-hydroxyaspartate. In agreement with tissue culture studies, the extraction fraction of ^{3}H-L-glutamate was increased by the α_1 adrenergic agonist phenylephrine and decreased by the α adrenergic antagonist phentolamine in a dose dependent manner. This study was supported by the Department of Neurology, MCPHU.

DOPAMINE TRANSPORTERS

620.1

DOPAMINE TRANSPORTER OVEREXPRESSION ALTERS PSYCHOSTIMULANT AND NEUROTOXIN RESPONSES. D.M. Donovan*#, L.L. Miner#, S. Prezedborski@, V. Jackson-Lewis@, S. Izenwasser$, R. Rothman+, C. Dersch+, R.M. Philpot%, C.L. Kirstein%, & G.R. Uhl!#. Molec. Neurobiol.#, +Clin. Pharm., $Preclin. Pharm. Branches, IRP/NIDA; @Col.-Pres. Univ.; %Univ. of S. Fla.; !Depts. Neurosci. and Neurobiol. JHUSM, Balto., MD 21224.

The dopamine transporter (DAT) is a critical site for cocaine reward, and plays a central role in terminating dopamine neurotransmission. Transgenic mice regionally overexpressing a variant DAT, via the 4.8 kb tyrosine hydroxylase promoter, reveal modest overexpression in several catecholaminergic cell groups as assessed by Northern blots, immunohistochemistry, ligand binding and dopamine uptake. DAT transgenic animals demonstrate reduced striatal dopamine and serotonin levels, display altered responses to both cocaine and morphine conditioned place preferences, and variable alterations in microdialysis studies. Their dopamine neurons show enhanced sensitivity to MPTP. These animals provide a valuable model for the striking influences that modest alterations in expression of DAT can yield.

620.2

DOPAMINE DYNAMICS AND PSYCHOSTIMULANT EFFECTS IN DOPAMINE TRANSPORTER KNOCKOUT MICE S.R. Jones*, M. Jaber, B. Giros, R.M. Wightman and M.G. Caron. HHMI Labs, Dept. Cell Biol., DUMC, Durham and Dept. Chem., Curr. Neurobiol., UNC-CH, Chapel Hill, NC

The knockout of the dopamine (DA) transporter gene results in a strain of mice (DAT-ko) which exhibits spontaneous locomotor hyperactivity and no response to cocaine or amphetamine (AMPH). This phenotype is surprising given the marked biochemical adaptive changes such as decreases in dopamine receptor levels and in dopamine itself as measured by HPLC. In order to determine the physiological basis of this hyperdopaminergic phenotype, cyclic voltammetry was used to measure stimulated DA release and uptake in striatal slices in real time. Release of DA in the DAT-ko mice is about one-fourth of control. The rate of clearance of released DA is 300-times slower in the DAT-ko mice than in wild-type controls (K = 0.05 vs. 16). There is no DA uptake in the DAT-ko mice, and inhibitors of other monoamine transporters or DA degradation enzymes have no effect on DA clearance. Cocaine and AMPH have a similar lack of effect. DA diffusion from the area is the only surviving mechanism for DA clearance. Thus, the hyperdopaminergic phenotype is best explained by the protracted period of time DA spends in the synapse. At the presynaptic level, D2 receptors are markedly down-regulated and their functional sensitivity is greatly diminished. AMPH has complex effects on DA neurons; it promotes DA release, inhibits uptake and disrupts vesicular stores. Whether these actions are strictly DA transporter dependent has remained controversial. We have used the DAT ko mice to further explore the mechaisms of AMPH action. In wild type mice, AMPH causes a 3-10 μM efflux of DA over a 30 min period while it has no DA releasing action in DAT-ko mice, even though AMPH enters terminals and destroys vesicular DA stores in both specimens. These results point to the DA transporter as a crucial determinant of DA tone and an obligatory mediator of the releasing action of AMPH. *Supported in part by NIH grants #T32 AG00029 (SRJ) and NS19576 (MGC).*

620.3

DRAMATIC REGULATION OF TYROSINE HYDROXYLASE IN THE BASAL GANGLIA OF MICE LACKING THE DOPAMINE TRANSPORTER. M. Jaber*, S. R. Jones, R. Bosse, B. Giros and M. G. Caron. HHMI labs, Dept of Cell Biology, Duke University Medical Center, Durham NC 27710.

Disruption of the mouse dopamine transporter gene results in spontaneous hyperlocomotion despite major adaptive changes, such as decreases in dopamine receptor levels. This phenotype is a direct consequence of the extended length of time that dopamine spends in the extracellular space following release. We have investigated the regulation of gene expression of tyrosine hydroxylase, the rate limiting enzyme of dopamine synthesis. While tyrosine hydroxylase mRNA levels were not modified, as evidenced by in situ hybridization, protein levels were dramatically decreased (>90%) in the ventral midbrain of homozygote mice, evidenced by western blotting. This decrease is correlated with a decrease in dopamine levels (>80%) in the striatum as measured by HPLC electrochemical detection. To determine whether this decrease is due to a down-regulation of the tyrosine hydroxylase protein expression levels or to the destruction of dopamine neurons, we have performed immunohistochemistry with a tyrosine hydroxylase polyclonal antibody and found no difference in the number of dopamine neurons of the ventral midbrain in homozygote compared to wild type mice. Moreover, we found that the decrease in tyrosine hydroxylase levels is less marked in the accumbens and olfactory tubercle than the striatum where dopamine transporter levels are normally higher. The dramatic extend of the down regulation documented here is only achieved in other animal models by destruction of dopamine neurons, which ultimately induce Parkinson-like syndromes. That mice lacking the dopamine transporter show a spontaneous increase in their locomotor activity despite the marked down regulation of tyrosine hydroxylase and dopamine levels strongly suggests that blockade of the dopamine transporter with highly selective antagonists could be beneficial in alleviating symptoms of Parkinson disease in humans.

This work was supported in part by NIH grant # NS 19576

620.4

KNOCK-OUT OF THE MOUSE DOPAMINE TRANSPORTER GENE LEADS TO PROFOUND MODIFICATIONS OF THE HYPOTHALAMO-PITUITARY AXIS. R.Bossé, F.Fumagalli, C.Missale, M.Jaber, B.Giros, and M.G. Caron*. HHMI Labs, Cell Biology, Duke University Medical Center, Durham, NC 27710

Despite the presence of both dopamine transporter (DAT) mRNA and protein in numerous nuclei of the hypothalamus, the physiological significance of dopamine uptake has remained enigmatic in this area of the brain. A strain of mice in which the dopamine transporter gene was disrupted by homologuous recombination (DAT-ko) has been generated. DAT-ko homozygous mice show significant deficit in weight gain compared to their littermates. DAT-ko mice mate and are fertile but females have an impaired maternal behavior and a markedly decreased ability to lactate their offspring. As these observations suggest significant hypothalamo-pituitary axis dysfunction, several parameters of this axis were investigated to gain insights into this phenotype. HPLC measurements show increased hypothalamus dopaminergic turnover as quantified by DOPAC/DA and HVA/DA ratios. Dopamine is known to inhibit prolactin secretion via its interaction with D2 receptors located on the lactotroph cells. It has also been shown to modulate the activity of the Pit-1 (GHF-1) gene *in vitro*. The Pit-1 gene modulates both somatotroph and lactotroph cell ontogeny and controls PRL and GH secretion by regulating the expression of their respective genes. Pituitaries of DAT-ko mice are about half the size of wild type glands. There is a marked reduction of both the anterior and intermediate lobe sizes while the posterior lobe remains unchanged. The D2 receptors density is significantly decreased in both the anterior and intermediate lobes. We observed an 85% decrease of the prolactin (PRL) content of the female pituitaries while the pituitary growth hormone (GH) content of both male and female DAT-ko mice is reduced by 70%. These data show that a hypothalamic hyperdopaminergia, consequent to gene targeted disruption of the dopamine transporter, leads to drastic alterations of the hypothalamo-pituitary axis of DAT-ko mice. Taken together, these observations represent the first ***in vivo*** demonstration that (1) hypothalamic dopamine uptake is physiologically relevant and (2) dopamine exerts a crucial control on pituitary gland development. *RB is a fellow of MRC of Canada. Supported by NIH grant # NS19756 and unrestricted Bristol-Myers Squibb Neuroscience Award.*

620.5

METHANETHIOSULFONATE (MTS) INHIBITS DOPAMINE (DA) TRANSPORTER FUNCTION IN RAT STRIATUM. L.P. Dwoskin*, S.E. Moore, W. Shaw and D.A. Butterfield. College of Pharmacy, Department of Chemistry and Center of Membrane Sciences, University of Kentucky, Lexington, KY 40536.

The cloned DA transporter contains 13 cysteine moieties distributed across the protein in its intracellular N-terminal and C-terminal tails, in the 1st and 3rd intracellular and 1st, 2nd and 3rd extracellular loops, and in putative transmembrane (TM) domains 4, 6, 9 and 11. Primarily based on reports that treatment with the thio-selective reagent N-ethylmaleimide (NEM) inhibits both DA transport and radioligand binding to the transporter and that DA transport inhibitors and substrates afford protection from effects of NEM, it appears that at least some of these cysteine moieties play an essential role for binding to and/or function of the DA transporter (Schweri, 1990; Johnson et al., 1992; Richfield, 1993; Saadouni et al., 1994). In the present study, DA transporter function was assessed following treatment with (1-oxyl-2,2,5,5,-tetramethyl-pyrroline-3-methyl)-methanethiosulfonate (MTS), a thiol-specific spin labeling agent. MTS inhibited [^{3}H]DA uptake into striatal synaptosomes with an IC50 of 15μM, indicative of sulfhydryl involvement in DA transport. Similarly, ascorbate-reduced MTS also inhibited [^{3}H]DA uptake with an IC50 of 28μM, indicating that the free radical moiety of MTS is not involved in the inhibition. Pretreatment with amphetamine (1μM - 1mM), a DA releasing agent and substrate for transport, or with DA uptake blockers (cocaine, GBR 12935 or BTCP, 0.1 nM-100 μM) which are not transport substrates, were not able to protect against the MTS-induced inhibition of [^{3}H]DA uptake. In contrast, dithiothreitol (10-100μM) provided complete protection from MTS-induced inhibition of [^{3}H]DA uptake. Thus, MTS may interact with a specific sulfhydryl group essential for transport, but not involved in binding of ligands to the transporter. Thereby, MTS may differentiate the transport site from uptake inhibitor binding sites. Supported by DA07219, DA05312, AG10836 and AG05119.

620.6

ANTISENSE OLIGONUCLEOTIDES AGAINST THE DOPAMINE TRANSPORTER (DAT) GENE.

C. Chatellard-Causse[1]*, M. Manier[1], N. Cristina[1], V. Leviel[2] and C. Feuerstein[1]
1. LAPSEN-INSERM U318 - Université Joseph Fourier, CHU, BP217, 38043 Grenoble cedex 9; 2. UMR CNRS 105, 69372 Lyon, France.

To evaluate the influence of dopaminergic (DA) phenotype on the susceptibility of rat mesencephalic DA neurons to degenerescence (as observed in Parkinson's disease) and/or its participation in their development and maturation, antisense strategy has been developed to selectively decrease the expression of a target protein. In our study, we focused on DAT, which acts to terminate DA transmission by rapid uptake of dopamine into these neurons. To decrease the expression of DAT, we used antisense oligonucleotides, because of their specificity, thereby allowing selective blockade of the production and the action of only DAT, thus leaving other DA markers (TH for instance) unaltered.

On the basis of the sequence of DAT, we designed and synthezised phosphodiester oligonucleotides: a 18 mer (ASDAT13) antisense to a region surrounding the initiation codon (-11 to +7) and a 20 mer (ASDATSIM) antisense to a coding region (396 to 415). Sense oligonucleotides were used as controls.

Beside test experiments performed *in vitro*, antisense oligonucleotides were administered intracerebrally in rats. Continuous infusions were made using ALZET® osmotic minipumps connected to a cannula implanted unilaterally dorsally to the substantia nigra. Time of treatment and concentration of antisense were tested. The level of expression of DAT was studied on coronal sections by quantitative autoradiography using (^{3}H)GBR 12935 as a ligand. In light of the interesting preliminary results observed, the efficacy of this antisense strategy will be presented.

(Supported by INSERM U318, UJF and Région Rhône-Alpes)

620.7

EXPRESSION OF NOREPINEPHRINE TRANSPORTER (NET) mRNA IS NOT REGULATED BY OVARIAN STEROIDS IN THE NON-HUMAN PRIMATE LOCUS CERULEUS (LC). W.E. Schutzer, S.R. Ojeda* and C.L. Bethea. Division of Reproductive Sciences & Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006

Dysfunction of the noradrenergic neurons of the LC has been implicated in depressive illness. Many effective antidepressants block NET re-uptake of NE, thereby increasing the concentration of NE in the synapse. Depression is twice as frequent in women than men and has been correlated with physiological withdrawal of the ovarian steroids, estrogen (E) and progesterone (P), which occurs in pre-menstrual syndrome, post-partum, menopause and hysterectomy. To determine whether E or P alters function of LC neurons, the expression of NET mRNA was examined in ovariectomized-control (spay), E treated (28 d) and E+P treated (28 d E + last 14 d P) monkeys (n=4/group) using *in situ* hybridization. Serum steroid concentrations mimicked follicular phase for E treatment (205±61 pg/mL) and luteal phase for P treatment (6.2±0.9 ng/mL). Perfusion fixed hindbrain sections (10 μ or 20 μ) which contained LC neurons were hybridized with a 730 bp ^{35}S-antisense monkey specific NET probe at 50°C, then stringently washed in 0.1X SSC at 60°C. Sections were exposed to β-MAX film for 5 days and densitometry was performed on the autoradiographs. Analysis showed that NET mRNA was expressed along the entire extent of the dorsolateral pontine tegmentum area and confined to the noradrenergic cells of the LC. Mean optical density (OD) throughout the entire LC averaged 93.08 ± 16.84, 82.05 ± 13.4 and 87.54 ± 12.80 (arbitrary units) for spay, E and E+P groups respectively. There was no significant difference ($p > 0.05$) in mean OD between treatment groups at any level of the LC. These results suggest that ovarian steroids, at these concentrations, do not regulate NET mRNA expression in the non-human primate LC. Nonetheless, lack of regulation of mRNA does not exclude E and P as potential effectors of noradrenergic transmission. For example, ovarian steroids may act at different concentrations, post-transcriptionally, on enzymatic activity or on receptor expression. Supported by HD17269, HD18185, R00163.

620.8

SIGMA RECEPTOR REGULATION OF AMPHETAMINE-STIMULATED (TRANSPORTER-MEDIATED) [^{3}H]DOPAMINE (DA) RELEASE. S. Izenwasser, D. Thompson-Montgomery, J. Weatherspoon, W. Hong, and L.L. Werling*. Psychobiology Section, NIDA Division of Intramural Research, Baltimore, MD; Department of Pharmacology and Neuroscience Program, George Washington University School of Medicine, Washington, DC.

Some sigma ligands have been shown to bind with low affinity to the DA transporter and to inhibit [^{3}H]DA uptake. However, it is difficult to be certain that the apparent inhibition of [^{3}H]DA uptake is not in fact due to an increased release of [^{3}H]DA via the transporter. To further examine the nature of the interaction between sigma ligands and the DA transporter, the effects of sigma ligands on amphetamine-stimulated [^{3}H]DA release were examined in rat caudate putamen. In the absence of exogenous calcium, (+)pentazocine, rimcazole, and ifenprodil each potentiated amphetamine-stimulated [^{3}H]DA release at concentrations well below those necessary for binding to the DA transporter, but consistent with their affinities for sigma receptors. This potentiation was blocked by the sigma receptor antagonist BD 1008. Neither the sigma agonists nor the antagonist alone had any effect on basal [^{3}H]DA release under these conditions. When amphetamine-stimulated DA release was measured in the presence of EGTA, to remove endogenous calcium, the potentiation by (+)pentazocine was absent. Thus, although the enhancement of amphetamine-stimulated [^{3}H]DA release by (+)pentazocine does not appear to be exocytotic in nature, since it occurs in the absence of added calcium, it does appear to rely on an internal calcium pool. Further, these effects appear to be unrelated to the uptake of amphetamine into the cell, since rimcazole and ifenprodil, but not pentazocine, inhibit DA uptake. These data suggest the possibility that the sigma ligands potentiate the effects of amphetamine at the vesicular transporter via an intracellular pool of calcium. (Supported by NIDA).

620.9

FOOD DEPRIVATION INDUCES UP-REGULATION OF NOREPINEHRINE UPTAKE IN THE NUCLEUS TRACTUS SOLITARIUS (NTS), BUT NOT IN THE LOCUS COERULEUS (LC) OF RATS. B. H. Hwang* and J. Guntz. Dept. of Anatomy, Indiana Univ., Sch. of Med., Indianapolis, IN 46202.

Food deprivation can sensitize animals to the reinforcing properties for food, self-stimulation and drugs of abuse. Many systems including the norepinephrine (NE) are associated with such a neuroadaptation. This study was designed to study how food deprivation may affect NE uptake capacity in the LC and NTS where NE-producing neurons are located. Adult male Sprague-Dawley rats (250-300 gm) were used for a 4-day food deprivation study for which water was *ad libitum*. The control rats had free access to both water and food. At the end of the experiment, the rats were decapitated. The brains were removed, frozen with dry ice powder and stored in a deep freezer. The NTS and LC were cut into 14-µm sections and processed for NE uptake study using 0.2 nM [^{3}H]-tomoxetine in 50 mM Tris-HCl buffer system. For the nonspecific control, 1 µM desipramine was added. After rinsing, tissue sections were processed for quantitative autoradiography. The quantitative analysis showed that (i) there was a similar high density (>290 x1000 cpm/mg protein) of NE uptake sites in the LC of both the control and deprived rats; (ii) however, in the NTS, the deprived rats (24.8 ± 2.4) had an up-regulation of NE uptake sites when compared with control rats (10.9 ± 0.7). Since food deprivation may induce stress, the present study suggests that norepinephrine uptake in the NTS could be an important parameter for studying stress related mechanisms. Supported in part by PHS NS25087.

620.10

REPEATED COCAINE AND STRESS INCREASE THE MAXIMAL VELOCITY OF DOPAMINE DISAPPEARANCE IN THE MEDIAL PREFRONTAL CORTEX, S. M. Meiergerd[1], J. O. Schenk[1] and B.A. Sorg*[2]. [1]Dept. Chemistry and [2]Dept. VCAPP, Washington State University, Pullman, WA 99164.

The effect of repeated footshock stress or cocaine on the kinetics of dopamine (DA) disappearance in the medial prefrontal cortex (mPFC) was measured by rotating disk electrode voltammetry (RDEV). Five groups of male Sprague-Dawley rats were used: naive rats, animals administered once-daily injections of either saline or cocaine (15 mg/kg, ip), and those given five daily 20-min sessions of either sham shock or footshock (0.05 mA/200 msec/sec). Rats were sacrificed one week later, and the mPFC was dissected, homogenized and washed with physiological buffer seven times. Km and Vmax values were determined by Eadie-Hofstee analysis. No difference in Km values was present among the treatment groups, with the average Km value approximately 0.3 µM for all groups. However, Vmax values were greater in daily sham shock, footshock and cocaine pretreated animals compared to daily saline and naive rats. Vmax values were approximately 40% higher in these three groups compared to naive and saline groups, which were not different from each other (Vmax, in pmol/s/g wet weight, = 28 ± 2 for pooled naive and saline groups). The increased ability to remove DA in the mPFC in daily sham shock, footshock and cocaine pretreated rats suggests that altered DA uptake may serve an adaptive mechanism in the mPFC, since a mild stressor such as daily handling (sham shock) produced changes in Vmax similar to animals treated with daily cocaine. Supported by USPHS Grants DA-07384 and DA-08212.

620.11

STRESS INCREASES DOPAMINE UPTAKE IN THE STRIATUM OF MICE. B.J. Copeland*, N.H. Neff and M. Hadjiconstantinou. Department of Psychiatry and Pharmacology and The Neuroscience Program, The Ohio State University College of Medicine, Columbus, Ohio, 43210.

Exposure of mice to restraint stress increased the uptake of dopamine into striatal synaptsomes in a biphasic manner. Uptake activity increased early during the stress, normalized, and rose again after the removal of the stressor. Similar changes were observed in olfactory tubercle. Kinetic analysis showed an increase in the apparent V_{max} without change in the apparent K_m for the substrate dopamine. Mazindol binding autoradiography revealed increased binding in accumbens, olfactory bulb, caudate/putamen and the region of A8, A9 and A10 dopaminergic nuclei. Other physiological stressors, also, enhanced the uptake of dopamine in the striatum and olfactory tubercle. Tolerance to the stressor, as well as cross-tolerance to novel stressors was observed.

620.12

ALTERED DOPAMINE UPTAKE FOLLOWING 7-DAY WITHDRAWAL FROM CONTINUOUS VS. INTERMITTENT COCAINE TREATMENT. T.H. Lee, K.R. Gee, E.H. Ellinwood and F.J. Seidler. Dept. of Psychiatry and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710 and Molecular Probes, Inc., Eugene, OR 97402.

Using fast scan cyclic voltammetry, we measured endogenous dopamine (DA) uptake and its inhibition by cocaine in the accumbens slices after 7-day withdrawal from chronic cocaine treatment. Rats were treated for 14 days with: (1) saline injections (1 ml/kg, s.c., qd); (2) cocaine injections (40 mg/kg, s.c., qd); or (3) continuous cocaine infusion (40 mg/kg/day, s.c., osmotic minipumps). Single-pulse, biphasic electrical stimulation was used to evoke endogenous DA release and extracellular DA concentrations measured in "real-time" (10 Hz) with nafion-coated carbon microelectrodes. Data were analyzed using an equation based on Michaelis-Menten kinetics (Jones et al., JPET 274, 396; $d[DA]/dt = [DA]_o - V_{max}/\{K_m/[DA]+1\}$).

While endogenous DA release and uptake kinetics were unaltered following either treatment regimen, the cocaine potency (1 - 10 µM) for inhibiting DA uptake was selectively enhanced in the injection group; no changes were observed in the infusion group. This enhanced cocaine potency on day 7 of withdrawal is consistent with the residual sensitization to cocaine-induced locomotion following daily injections. The differential effects of the two cocaine treatment regimens on the accumbens DA uptake kinetics following 7-day withdrawal will be further examined by measuring: (1) clearance of exogenous DA applied by photolysis of caged-DA (Lee et al., J. Neurosci. Meth., in press) and (2) uptake kinetics for [^{3}H] DA.

Supported by R01 DA-06519 to THL.

620.13

DOPAMINE CLEARANCE RATE IN RAT STRIATUM: EFFECTS OF UPTAKE INHIBITORS AND DOPAMINE CONCENTRATION. N.R. Zahniser, G.A. Larson and G.A. Gerhardt*. Depts. of Pharmacol. and Psychiat. and Neurosci. Prog., Univ. Co. Hlth. Sci. Ctr., Denver, CO 80262.

Changes in the *in vivo* activity of the dopamine transporter (DAT) can be determined using electrochemical recording to analyze the disappearance of locally-applied DA. In general, DAT inhibitors have been reported to alter one or more of the following: maximal DA signal amplitude, clearance time (T_{80}) and clearance rate (slope of the initial signal decline). We observed that administration (doses i.p. in mg/kg) of d-amphetamine (1-10), cocaine (20-30) and CFT (3-10) produced minimal changes in amplitude, prolonged T_{80} by as much as 250% and decreased clearance rate in medial dorsal striatum of urethane-anesthetized rats (N = 3-5). In contrast, nomifensine (10), benztropine (10), GBR 12909 (10) and mazindol (3) predominantly increased amplitude with minimal changes in T_{80} and thereby, paradoxically, increased clearance rate. These results suggest that DAT inhibitors with either high or low abuse potential alter DAT activity via somewhat different mechanisms. To investigate the relationship between extracellular DA concentrations and DA clearance, signals were measured in response to pressure-ejecting increasing amounts of DA (5-20 pmol; N = 4) at 5-min intervals at a single location. Amplitude increased nonlinearly from 0.37 to 10 µM, T_{80} increased linearly from 32 to 42 sec and clearance rate increased from 0.02 to 0.32 µM/sec. These results suggest that in striatum DA clearance rate increases over this DA concentration range and may explain why DAT inhibitors that preferentially increase DA signal amplitude appear to increase DA clearance rate. (Support: DA04216, DA00174 & NS09199)

620.14

EFFECT OF DSP-4 TREATMENT ON DOPAMINE UPTAKE IN RAT FRONTAL CORTEX

Y. Takahashi*, I. Kusumi and T. Koyama, Dept. of Psychiatry, Hokkaido Univ. Sch. of Med., Sapporo 060, Japan

Recent microdialysis studies reported that noradrenaline (NA) uptake inhibitors such as desipramine (DMI) increased dopamine (DA) concentrations in the medial prefrontal cortex (mPFC) probably by inhibiting DA uptake through NA transporter (Carboni et al., 1990). We previously reported that most antidepressants excluding selective serotonin reuptake inhibitors (SSRI) increase extracellular DA in the mPFC in freely moving rats by using brain microdialysis (Koyama et al., 1994). The purpose of the present study was to confirm the mechanism for crossed DA uptake through NA transporter. DSP-4, a neurotoxin that selectively destroys noradrenergic neurons, was administered to male Wistar rats (250-280 g) i.p. at the dose of 50 mg/kg. Seven days later the rats were sacrificed by decapitation and the dissected frontal cortices and striata were used for [^{3}H]NA or [^{3}H]DA uptake studies. Uptake assays were performed as a modification of the methods of Richelson and Pfenning (1984). In the frontal cortex, [^{3}H]NA uptake was reduced by DSP-4 treatment to approximately 30% of the control value. On the other hand, DSP-4 treatment significant decrease ($p < 0.001$) of [^{3}H]DA uptake to approximately 68% of the control value in the frontal cortex, although it didn't affect [^{3}H]DA uptake in the striatum. These findings suggested that DA was taken up by not only DA transporter but also NA transporter into NA terminals in the frontal cortex. The results support the postulated relationship between antidepressant effect and the preferential dopaminergic activation by antidepressant drugs in mPFC. This work was supported in part by Grant-in-Aid for Scientific Research (No. 05454308 T.K.) from the Ministry of Education, Science and Culture, Japan.

620.15

FASTING DECREASES THE INITIAL UPTAKE RATE OF DOPAMINE IN RAT STRIATUM, WHICH IS REVERSED BY 1 nM INSULIN *IN VITRO*. T.A. Patterson*[1], A. Zavosh[1], J.O. Schenk[2], D. Figlewicz Lattemann[1,3], [1]Dept. of Psychology, Univ. of Washington, Seattle, WA 98195, [2]Dept. of Chemistry, Washington State Univ., Pullman, WA, 99164, [3]V.A. Medical Center, Seattle WA, 98108

Previous behavioral and molecular data support the hypothesis that fasting - a condition of low endogenous insulin - modulates CNS dopaminergic function. The timing of uptake of exogenously applied dopamine (DA, 500 pmol) by individual striatum from male Wistar rats was determined using rotating disk electrode voltammetry (RDE). Rats were fasted for 24 hours or fed *ad libitum*. Striatum was dissected, minced and incubated with vehicle (170 nM HCl) or insulin (1 nM) for 30 min. at 37°C. The initial velocity (v) was determined from the DA uptake profile. In fasted animals, the observed v was significantly ($p < 0.02$) reduced vs. fed animals [91.6 ± 5.4 (avg ± SEM, n = 10) and 118.2 ± 8.1 (n = 7) pmoles/sec/g, respectively]. Addition of insulin to striatum from fasted animals reversed this change (111.8 ± 6.4 pmoles/sec/g, n = 5, $p < 0.05$ vs. fasted controls).

The observed decrease in v would result in DA being present in the synaptic cleft for a longer period of time in a fasted state, thereby resulting in increased synaptic action. The results also support the conclusion that the suppression of DA uptake rates observed in fasting animals is mediated via a lack of insulin action. Supported by NIH DK 40963 and VA Merit Review funds.

620.16

THE STRIATAL DOPAMINE RESPONSE TO REUPTAKE BLOCKERS AS MEASURED BY MICRODIALYSIS DECLINES OVER TIME POST-PROBE IMPLANTATION. B. Gough*, R.A. Gazzara, J.F. Bowyer, P. Clausing and R.R. Holson. National Center for Toxicological Research, Jefferson, AR 72079.

We have previously shown that amphetamine-stimulated dopamine (DA) release declines rapidly after microdialysis probe implantation. In this experiment 100 μM bupropion (BUP), a DA reuptake inhibitor, was infused for 10 min into rat striatum via the microdialysis probe three times at two hr intervals, with the first 10-min infusion beginning two hrs after probe implantation. The DA peak response to these three infusions declined rapidly (the response to the third infusion was only one third as great as that to the first infusion). This rapid decline in the striatal DA response to BUP was a function of time after probe implantation, since a separate set of rats given a single infusion at the same time as the third infusion in the above animals (i.e., six hours after probe implantation) showed identical peak DA levels. We conclude that neural tissue shows a time-dependent response to probe implantation which quickly reduces the response to both DA releasers and reuptake inhibitors. Supported by NCTR/FDA/DHHS.

620.17

TRANSIENT UP-REGULATION OF THE DOPAMINE TRANSPORTER GENE EXPRESSION

M.P. Martres*, B. Demeneix+, M. Hamon and B. Giros, INSERM U288, Faculté de Médecine Pitié-Salpétrière, 75013 Paris; + CNRS URA90, Museum National d'Histoire Naturelle, 75005 Paris, FRANCE.

In order to investigate the exact function of the brain dopamine transporter (DAT) in the central mechanisms of the development of dependence and tolerance to cocaine, methods are now available to selectively alter the gene expression of this membrane-bound protein and examine the induced changes in the dopamine transmission and the drug-associated behavior. As an alternative to the generation of a mouse line which does not express DAT ("knock-out" strategy, Giros et al., Nature 1996, 379,606), we have chosen to direct local expression through the intra-cerebral administration of cDNA plasmids. This transfer method should allow a long lasting and more efficient effect than that of an anti-sense oligo-nucleotide strategy (time and expression being a key feature related to delayed development of addiction). Thus, a plasmid construct containing the strong CMV promoter and the DAT sense cDNA was unilaterally injected in the rat together with the cationic polymer polyethylenimine (PEI, at a ratio of 3 charge equivalents) at the level of the ventral tegmental area and of the pars compacta of the substantia nigra, two cerebral structures rich in DA cell bodies. Three days after administration of 0.5 μg of the recombinant plasmid, the overexpression of the DAT protein was evidenced by a significant 20-40% increase of the immuno-reactive DAT like material as well as in the dopaminergic cell bodies than in the projection areas like the striatum, the nucleus accumbens and the olfactory tubercles, as compared with regions of rat administered with a non recombinant plasmid. This overexpression of the DAT is probably functional since the uptake of dopamine by striatal synaptosomes is increased by 35% and the mRNA of tyrosine hydroxylase decreased by 32%. This technic is now applied to DAT down-regulation and to other protein gene expression.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS III

621.1

EXPRESSION OF mRNA FOR SEROTONIN (5-HT) RECEPTORS IN HUMAN CORONARY ARTERIES. L. Bouchelet*, B. Case[1] and E. Hamel. Montreal Neurological Institute and [1]Royal Victoria Hospital, McGill University, Montréal, QC, Canada, H3A 2B4.

Treatment of acute migraine headache has improved considerably in recent years due to the availability of sumatriptan (Imitrex), a synthetic 5-HT1 receptor agonist. Sumatriptan exhibits high affinity at the 5-HT1Dα, 5-HT1Dβ and 5-HT1F receptor subtypes. Its efficacy in aborting migraine attack has been associated to its ability to interact with 5-HT1D receptors on cranial blood vessels and/or trigeminovascular afferents. Nevertheless, adverse side-effects such as coronary vasospasm have precluded its use in patients with a history of coronary artery disease. To better understand how sumatriptan can elicit such effects, we examined the expression of sumatriptan-sensitive 5-HT1 receptor messages in human coronary arteries using reverse transcriptase polymerase chain reaction (RT-PCR) and specific primers for the 5-HT1Dα, 5-HT1Dβ and 5-HT1F receptors. Fragments of the expected size for the 5-HT1Dβ and 5-HT1F receptors were evidenced in coronary arteries. In contrast, the 5-HT1Dα receptor message could not be detected in any coronary preparation. These results suggest that 5-HT receptors with high affinity for sumatriptan could exert vasocontractile actions on the cardiovascular system. Together with previous data in human brain vessels, they further point to the 5-HT1Dβ and 5-HT1F receptors being associated with cerebral and peripheral blood vessels. Moreover, they emphasize the importance of designing new drugs devoid of 5-HT1Dβ properties. Selective agonists at 5-HT1Dα receptors could be a primary choice to specifically target neural as opposed to vascular mechanisms involved in migraine manifestation. A vascular effect of 5-HT1F receptors needs to be investigated in order to clarify their possible contribution to the therapeutic and/or adverse effects of sumatriptan. Supported by the Heart and Stroke Foundation of Québec.

621.2

LATERAL *(l-)* VERSUS MEDIAL *(m-)* PREFRONTAL CORTEX (PFC): DIFFERENCE IN BASAL AND AMPHETAMINE (AMP)-INDUCED RELEASE OF DOPAMINE (DA) AND SEROTONIN (5-HT). T. Kuroki*, J. Ichikawa and H.Y. Meltzer. Dept. Psychiatry, Case Western Reserve Univ. Sch. Med., Cleveland, OH 44106.

Dopaminergic activity in the *l*-PFC has been suggested to play an important role in the pathophysiology of schizophrenia. However, less is known about the *l*-PFC DA neurons in animal studies. We examined the difference in basal and AMP-induced release of DA and 5-HT between the *l*-PFC (coordinates: A +3.2, L +3.0, V -5.0 mm) and the *m*-PFC (A +3.2, L +0.8, V -5.5 mm), using in vivo microdialysis in awake, free-moving rats. Basal extracellular levels (fmol/30 min) in the *l*-PFC were 0.71±0.04 for DA, obtained from six of 21 rats examined due to the lower DA levels in 15 rats than the detection limit (0.4 fmol/sample), and 0.63±0.07 (N=9) for 5-HT. Basal levels in the *m*-PFC were 1.49±0.20 (N=6) for DA and 0.67 ±0.09 (N=5) for 5-HT. AMP (1.0 mg/kg, s.c.) produced an increase in extracellular DA levels in the *l*-PFC (15.58±1.89 fmol/180 min, N=4) to the similar extent to that in the *m*-PFC (14.79±2.00, N=5). AMP-induced increases in extracellular 5-HT levels were 2.20±0.19 (fmol/180 min, N=4) in the *l*-PFC and 2.76±0.87 (N=5) in the *m*-PFC. These results suggest that the lower basal levels and comparable AMP-induced increases in DA release in the *l*-PFC, compared to those in the *m*-PFC, may reflect upon facilitated DA transmission in the *l*-PFC under AMP-stimulated conditions. Supported by NARSAD.

621.3

IMMUNODECTION OF AN 85 kDa PROTEIN IN BRAIN REGIONS WITH AN IMIDAZOLINE RECEPTOR ANTIBODY. T. R. Ivanov*, H. Zhu, and J. E. Piletz. Dept. of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS 39216.

The imidazoline receptor was first proposed to explain the nonadrenergic, hypotensive effects of clonidine following injection into cat brainstem nuclei. At least two subtypes of this receptor have been identified based on their pharmacology and subcellular distribution. Recently, we have characterized an I_1 subtype on platelet plasma membranes based on competition affinities for ^{125}I-p-iodoclonidine binding. A polyclonal antibody has been raised to a bovine adrenal chromaffin cell, affinity-purified, imidazoline binding protein (receptor)(Wang et al. 1993, Mol. Pharmacol 43, 509). We have used this antibody to investigate the size and distribution of the imidazoline receptor in rat brain regions. Western blot analysis of purified membranes revealed a predominant 85 kDa immunoreactive band across brain regions including hippocampus, brainstem, cortex, and cerebellum. This immuoreactive band is larger than MAOs (59-61 kDa) or α_2-adrenoceptors (approx. 62-64 kDa). The detection of the 85 kDa band was dependent on the inclusion of at least 8 protease inhibitors during membrane preparation. Smaller sized bands (i.e., 45 kDa) were found with fewer protease inhibitors. The brain immunoreactivy was subcellularly enriched in a plasma membrane fraction characteristic of the I_1 receptor subtype. In platelets, the immunodensity of the major immunoreactive band was correlated ($p=0.0036$) with I_1 receptor density (Bmax values) suggesting that this antibody recognizes the I_1 receptor subtype. Supported by NIMH grants MH42859 and MH49248

621.4

THE MU-OPIOID RECEPTOR IS DISTRIBUTED IN REGIONS OF THE RAT ROSTRAL VENTRAL MEDULLA CONTAINING LOCUS COERULEUS-PROJECTING NEURONS. E.J. Van Bockstaele*, M.E. Page#, and E.E.O. Colago. Dept. of Neurol. and Neurosci., Cornell Univ. Med. Coll., NY, NY. 10021; #Dept. Neurosci., Rutgers Univ., Newark, NJ, 07102.

The nucleus paragigantocellularis (PGi) in the rostral ventral medulla provides a strong opioid projection and potent excitatory amino acid input to the locus coeruleus (LC) in the rostral dorsal pons. Several lines of evidence indicate that this pathway is critical for mediating certain physical signs observed in the opiate withdrawal syndrome. Specifically, the increase in discharge of LC neurons observed during opiate withdrawal has been reported to be caused by excitatory amino acid (EAA) release most likely deriving from the PGi. Activation of EAA-containing neurons in the PGi may depend on changes in the occupancy of opioid receptive sites located on LC-projecting neurons in the PGi which subsequently effect EAA release in the LC. To determine whether the morphine-like mu-opioid receptor (μOR) is localized to plasmalemmal sites of LC-projecting neurons in the PGi, we combined retrograde transport of either Fluoro-Gold or the protein-gold tracer, wheat germ agglutinin-conjugated to inactive horseradish peroxidase, from the LC with immunocytochemical detection of μOR in the same section of tissue throughout the rostral ventral medulla. Sections were examined using both light and electron microscopy. Neurons containing either the retrograde tracer or immunolabeling for μOR were numerous throughout the ventral medulla. LC-projecting perikarya and dendrites also contained immunolabeling for μOR. These results indicate that μOR is distributed along plasmalemmal sites of neurons in the rostral ventral medulla which project to the LC. Elucidation of the anatomical substrates in the medullo-coerulear circuit may provide a model for our understanding of the neural circuits and neurochemicals associated with certain components of the opiate withdrawal syndrome. Supp. by R29 DA09082, NARSAD and AHA to EJVB, and NIMH 18974.

621.5

EXPRESSION OF THE CENTRAL CANNABINOID RECEPTOR mRNA DURING THE EMBRYONIC DEVELOPMENT OF THE RAT. **Nancy E. Buckley*, Stefan Hansson[1] and Eva Mezey. National Institute of Neurological Diseases and Stroke and [1]National Institute of Mental Health, Bethesda, MD 20892**

We mapped the distribution of the central cannabinoid receptor (CB1) mRNA in rat embryonic tissues to gain insight into how cannabinoids may affect development. *In situ* hybridization histochemistry studies were done using an antisense probe specific for the CB1 mRNA. We found that, at early embryological stages (i.e., at 11 days of gestation (E11)), CB1 mRNA is expressed within some cells of the neural tube and, in later embryological stages (from E15 to birth), it is found throughout the CNS (cortex, hippocampus, septum, subventricular zone, thalamus, hypothalamus, pons, medulla, in the differentiating cerebellum and in the spinal cord). In addition, high levels of CB1 mRNA were also found in the sympathetic ganglia, in the ganglionic cells within the retina and in the enteric ganglia of the gastrointestinal tract. High levels of CB1 mRNA were also present in two endocrine organs, the thyroid gland and the adrenal cortex. The high level of CB1 mRNA expression throughout the CNS and in endocrine organs may indicate that this gene has a very important regulatory role during embryonic development.

NIH Intramural Program

621.6

ISOLATION AND MEASUREMENT OF THE CANNABINOID RECEPTOR AGONIST, ANANDAMIDE, IN BRAIN AND PERIPHERAL TISSUE OF HUMAN AND RAT

C. C. Felder, M. Palkovits, †F. Karoum, ††J. M. Richardson, ††R. M. Riggin, J. Axelrod*, ††G. W. Becker

The Laboratory of Cell Biology, †Neurophychiatry Branch, National Institute of Mental Health, Bethesda, MD 20892, ††Lilly Research Labs, Eli Lilly and Co., Indianapolis, IN 46285

Anandamide (arachidonylethanolamide), first isolated from porcine brain, has been shown to be a functional agonist for the CB1 and CB2 cannabinoid receptors found predominantly in the CNS and immune system respectively. Studies were undertaken to measure anandamide by LC/MS/MS and GC/MS in the CNS and periphery of human and rat. In human brain, anandamide levels were highest in the hippocampus, striatum, parahippocampal cortex, thalamus and cerebellum, ranging from 25 to 148 pmol/gm wet weight of tissue. Levels of anandamide in rat brain where highest in striatum, cerebellum, and hypothalamus and ranged from 18 to 29 pmol/gm wet weight of tissue.. Anandamide was also found in human and rat spleen which expresses high levels of the CB2 cannabinoid receptor. Small amounts of anandamide were also detected in human heart and rat skin. Only trace quantities were detected in pooled human serum, plasma, and CSF. The distribution of anandamide in human brain and spleen supports its potential role as an endogenous agonist in both of these tissues. The presence of anandamide in brain regions expressing little if any CB1 receptor suggests that additional physiologic functions may exist for this compound.

621.7

IMMUNOREACTIVITY FOR RETINOID X RECEPTOR ISOFORMS IN SPECIFIC BRAIN SITES OF THE RAT. M.C. Langub Jr.*, J.P. Herman, H.H. Malluche and N.J. Koszewski. Departments of Internal Medicine and Anatomy/Neurobiology, Univ. KY. Med. Ctr., Lexington, KY 40536-0084.

The retinoid X receptor isoforms (i.e., RXRα, RXRβ, and RXRγ), are members of the steroid/thyroid hormone receptor superfamily. These ligand-dependent transcription factors are known to mediate a wide variety of physiological processes as critical heterodimer partners with vitamin D, retinoic acid, thyroid hormone and several orphan receptors. Altogether, these factors may play important roles in the pathogenesis of age-related cell death and AD. Characterization of RXR mRNA isoforms in embryonic and adult brain indicated that all three are present at both developmental stages. However, precise neuroanatomical localization of the proteins has yet to be established.

Therefore, immunocytochemistry and Western blot analysis were employed to detect RXR isoforms in specific brain cell populations. The results demonstrate distinct patterns of localization of positive cells in many regions implicated in aging and AD. The basal forebrain, basal ganglia, hippocampus, hypothalamic, thalamic and brainstem nuclei and neocortex contained labeled neurons. Staining was localized in neuronal nuclei, cytoplasm and distal processes. Cytoplasmic staining may reflect either untranslocated receptors or receptors which exert primary physiological action outside the cell nucleus. Labeling in some astrocytes was also observed. Interestingly, of the three isoforms, the RXRα alone was observed in varicosities, suggesting its localization in synaptic terminals. Western blot results confirmed the presence of RXRα and RXRγ proteins in the neocortex, hippocampus and hypothalamus. Altogether, central nervous system localization of the RXRs suggests that these isoforms are integral factors and may play specific roles in age-related disease. Forthcoming studies examine the emerging importance of these steroid receptors in brain function. {Supported by DK 47883 (NJK) and MH49698 (JPH).

621.8

THE erbB4 RECEPTOR IS EXPRESSED IN CELLS WHICH EXPRESS THE NEUROTRANSMITTER GABA. L. Weber, L. Ledesma, E. Battenberg, M. Morales, F.E. Bloom, G. Lemke+, and C. Lai*. The Scripps Research Institute and The Salk Institute+, La Jolla, CA 92037

The role of receptor protein-tyrosine kinases (PTKs) in the mature nervous system is not well understood. Here we report the observation that the receptor PTK, erbB4, is expressed in many of the cells that also express the neurotransmitter GABA. We have cloned the murine erbB4, the fourth member of the epidermal growth factor receptor subfamily of PTKs. The assembled cDNA encodes a protein having 97% identity with the human receptor. The predicted protein lacks 16 amino acids present in the human sequence suggesting that alternative splicing gives rise to at least 2 receptor forms. Both forms are detected in adult brain.

We provide evidence for the expression of erbB4 in GABA$^+$ cells using a double-labeling approach where erbB4 mRNA is detected by hybridization and GABA is detected immunocytochemically. Co-expression is most readily scored in hippocampus and cortex where GABA$^+$ cells are dispersed. Many hippocampal erbB4$^+$ cells also express NGF. In rat embryos, we detect the presence of both erbB4 and GAD (glutamate decarboxylate) mRNA as of E12, the earliest time point sampled. This finding suggests a possible role for erbB4 in the differentiation of these cells. We are currently assessing if cells bearing the erbB4$^+$/GABA$^+$ phenotype correlate with identified subpopulations of interneurons. Supported by NIH R01 NS32367.

621.9

HISTOLOGICAL AND ULTRASTRUCTURAL STUDY OF FROG (*Rana pipiens*) GASTRIC *MUSCULARIS EXTERNA*. M. Rubio-Dávila, R. Lugo-López, C. Hernández, R.E. Blanco and G. Escalona de Motta*. Inst. of Neurobiology, Univ. of Puerto Rico, Medical Sciences Campus, San Juan, P.R. 00901.

The frog gastric *muscularis externa* was used as a model preparation for pharmacological and toxicological studies showing two types of acetylcholine (ACh)-activated contractions: a tonic, sustained one that increased in amplitude with increasing ACh doses, and a spontaneous activity that increased in amplitude and frequency after ACh application. Four functional regions were observed along the vertical axis in response to ACh and muscarinc antagonists. In the upper, cardias region the distinct spontaneous activity was inhibited by M_3 blockers suggesting that this activity was initiated by endogenous ACh released from terminals in the myenteric plexus. We localized these terminals in histological and electron microscopic experiments. Staining with methylene-blue azure and fucshin red revealed the myenteric plexus between the two muscle layers. Sections of this area, stained with osmium tetroxide and observed under transmission electron microscope, showed nerve terminals with three types of vesicles: clear ones resembling cholinergic vesicles, dense-core ones similar to catecholaminergic vesicles, and a different and plentiful one that contained both clear and medium density core vesicles which were similar to granule vesicles found in neurosecretory neurons. Results of pharmacological experiments confirmed a sensitivity in the *muscularis externa* to the following potential neurotransmitters: muscarinic cholinergic, alpha and beta adrenergic, and delta, kappa and mu opiate suggesting a complex synaptic interaction in the myenteric plexus. (Supported by the NIH grants GM08102 and MH48190).

621.10

DISTRIBUTION OF DOPAMINE D_2 RECEPTOR IN THE RAT BRAINSTEM USING ANTI-PEPTIDE ANTIBODIES. *H. van Dijken, J. Dijk,[1] J.C. Holstege, and P. Voorn,[2]*. Dept. of Anatomy, Erasmus University Medical School, PO Box 1738, 3000 DR Rotterdam, [1]Sylvius Laboratory, Leiden, and [2]Dept. of Anatomy and Embryology, Free University, Amsterdam, The Netherlands.

We have investigated the distribution of the dopamine D_2 receptor in rat brainstem using anti-peptide antibodies described in our previous study on the D_2 receptor in rat spinal cord (*Eur. J. Neurosci.*, **8**: 621-628, 1996).

Adult, male Wistar rats were perfused transcardially with 4% paraformaldehyde in phosphate buffer. Brain and spinal cords were cut in 40 µm frozen sections, incubated as free floating sections with partially purified D_2 receptor antibody (24-48 hours, 4 °C) and processed with the ABC technique, in some cases followed by tyramide signal amplification (NEN Dupont). As a negative control adjacent sections were incubated with pre-immune serum instead of antiserum.

In all positive-staining regions, the antibody was highly localized to neuronal cell bodies and their proximal dendrites, while the controls resulted in no contrast staining. Within the mesencephalon, at levels caudal from the substantia nigra, strongest labelling was found in neurons located in parts of the periaqueductal gray and the superior and inferior colliculi. In the pons the most prominent labelling was found in the locus coeruleus and parabrachial nuclei. In the medulla oblongata, labelled neurons were present in the nucleus of the solitary tract, dorsal motor nucleus of the vagus, and the area immediately above the inferior olive. Furthermore, all raphe nuclei contained labelled neurons. Weakly labelled neurons were observed in several other areas, but the labelling was sometimes difficult to distinguish from background staining. Therefore we are currently increasing the purity of the antiserum.

The results provide anatomical support for specific effects of dopamine via its D_2 receptor on several areas of the brainstem, including serotonergic and noradrenergic cell groups. (Funding: FGG-EUR-ANA-11-02-01)

621.11

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE D4 DOPAMINE RECEPTOR IN THE MOUSE BRAIN.

C. Mauger*, M. Brockhaus‡, B. Sivan§, S. Fuchs§, O. Civelli, F. Monsma. PRPN, ‡PRPG F. Hoffmann-La Roche AG, Basel, Switzerland; §Weizmann Institute of Science, Rehovot, Israel.

In order to investigate the distribution and the properties of the dopamine D4 receptor, we have raised polyclonal antibodies (Ab) against the N-terminal part and the third intracellular loop of the mouse D4 receptor. Synthetic peptides D4-2 (composed of amino acid residues 2-11) and D4-266 (residues 266-278) were coupled to keyhole limpet hemocyanin and used to immunize rabbits. Ten weeks after immunization, the anti-peptide Abs were detectable by enzyme-linked immunosorbent assay at a dilution of at least $5x10^{-4}$ and were able to immunoprecipitate a 32 kDa mouse D4 receptor generated by *in vitro* translation. The antisera were affinity purified using peptide coupled to sulfolink gel. The purified Abs were successfully used in immunofluorescence (IF) experiments to detect the mouse D4 receptor in transiently transfected CHO cells, while no IF was detectable in untransfected or in cells transfected with rat D2 or human D4 receptors. Immunohistochemical studies with the anti D4-2 and anti D4-266 Abs demonstrated the presence of the D4 receptor immunoreactivity (D4-IR) in several regions of the mouse brain and showed the same immunoreactive pattern. D4-IR was abolished with peptide-blocked immune serum. D4-IR was present in the olfactory bulb, in the frontal, cingulate and entorhinal cortices, in the hippocampus and dentate gyrus, as well as in the lateral septal nucleus. Little or no D4-IR was seen in striatum. These anti-peptide Abs will help to further characterize the tissue distribution of the D4 dopamine receptor in the mouse brain and lead to new insights regarding the function of the D4 receptor.

[Supported by Roche]

621.12

mRNAs ENCODING D1, D2, D3, D4 AND D5 DOPAMINE RECEPTORS HAVE SIMILAR LAMINAR DISTRIBUTIONS IN THE PRIMATE PREFRONTAL CORTEX

F. Wang*, Yang Cao, and M.S. Lidow *Section of Neurobiology, Yale University, School of Medicine, New Haven, CT 06510*

In situ hybridization histochemistry was used to compare striatal and cortical levels of D1, D2, D3, D4 and D5 dopamine receptor mRNAs and to determine the laminar distribution of these messages in the primate prefrontal cortex. All five subtypes of dopamine receptor mRNA were present in both the monkey striatum and the cerebral cortex. Analysis of the autoradiograms also showed a noticeably stronger labeling of D1 and D2 mRNAs in the striatum than in the cortex. In contrast, D4 and D5 expression was clearly higher in the cortex. The levels of D3 transcripts were nearly equivalent in the striatum and the cortex. A major finding in the prefrontal cortex is that mRNAs encoding all dopamine receptor subtypes have very similar laminar distributions with their highest level of expression in layer V. The Northern blot analysis of the prefrontal cortical tissue showed the D1 receptor mRNA to be the most abundant in this cortical region. The level of D5 transcript was nearly five times lower than that of D1, with the levels of D2, D3 and D4 receptor mRNAs being lower by factors of 7, 33 and 10, respectively. The relatively high levels of expression of several dopamine receptor subtypes, including D4 receptor, in the primate cerebral cortex underscores the importance of the cortical dopaminergic system for cognitive function and for the therapeutic actions of antipsychotic drugs. Further, the prominence of all dopamine receptor mRNAs in layer V of the prefrontal cortex, which contains the cortico-striatal and cortico-tectal projection neurons, provides a neural basis for pharmacologic regulation of motor control systems.

This work was supported by the NIMH Center Grant P50-MH44866.

621.13

ANALYSIS OF D_1 AND D_2 DOPAMINE RECEPTOR mRNA DISTRIBUTIONS AND RELATIVE LEVELS IN THE PREFRONTAL CORTEX. A. Y. Deutch[1*], S. J. Watson, Jr.[2], and A. Mansour[2] [1]Depts. of Pharmacol. and Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508 and VA Medical Center, West Haven, CT 06516 and [2]Dept. of Psychiatry and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

The distribution of striatal dopamine (DA) receptors has been well characterized, but little is known of the distribution and relative expression of the D_1 and D_2 mRNAs in the prefrontal cortex (PFC). We examined the distributions and relative levels of D_1 and D_2 mRNAs in the PFC of rats by *in situ* hybridization. D_1 mRNA-containing cells were present in a distinct bilaminar pattern in the PFC, being present in layers II/III and in layer VI and adjacent deep V. In contrast, D_2-expressing neurons were almost exclusively confined to layer V. The characteristic laminar patterns of expression of both transcripts were seen in the infralimbic and prelimbic parts of the medial PFC; somewhat fewer DA receptor-expressing neurons were present in the medial precentral cortex. The laminar patterns of expression of the DA receptor transcripts were seen in low density in an arc surrounding the external capsule, and many DA receptor mRNA-containing cells were observed in the suprarhinal portion of the frontal cortex. Examination of the relative expression of D_2 receptor mRNA suggested the presence of two populations of PFC cells, including a smaller population with relatively high expression (as revealed by the percent area of the cell soma covered by silver grains). There was no significant difference between the infralimbic and prelimbic cortices in relative expression of the D_2 transcript. Preliminary assessments do not suggest significant differences in D_1 mRNA levels between cells in the superficial and deep layers of the medial PFC, but suggest that the expression of the D_1 receptor gene is slightly higher in the infralimbic than prelimbic cortex. Supported in part by MH-45124, the VA Center for Schizophrenia, and the NPF.

621.14

DOPAMINE D_4 RECEPTOR DISTRIBUTION IN NORMAL HUMAN POSTMORTEM BRAIN SECTIONS: AUTORADIOGRAPHIC STUDIES WITH [^{3}H]-NGD-94-1 R.A.Lahti*#, E.V. Cochrane#, R.J. Primus*, D.W. Gallager*, R.C. Roberts# and C.A. Tamminga#. #Maryland Psychiatric Research Center, Univ. of Maryland School of Medicine, Baltimore, MD 21228; *Neurogen Corporation, Branford, CN 06405

The dopamine D_2-family receptors are the D_2, D_3 and D_4 (Civelli, 1991). The D_2 receptor is primarily located in the caudate-putamen-accumbens, D_3 in the nuc. accumbens, and by indirect determination the D_4 receptors are located in the cortex and hippocampus (Lahti, 1995). Most neuroleptics are D_2 selective yet have high affinity for the D_4 receptor; clozapine, an atypical neuroleptic is selective for the D_4 receptor. Increases in D_4 receptor density were reported (Seeman, 1992; Murray, 1995) in caudate/putamen of schizophrenics vs normal postmortem tissue but others did not confirm these findings (Reynolds, 1994, 1995, Lahti, 1996).

In this study the distribution of the D_4 receptor was determined using the D_4 selective ligand [^{3}H]-NGD-94-1, autoradiography, and sections from normal human (n=5) postmortem tissue. Numerous brain structures were analyzed for the density of the D_4 receptor with the following findings: D_4 receptors were most dense in the hippocampus, especially in CA1, dentate gyrus and subiculum; significant levels were observed in entorhinal, perirhinal, and cingulate cortices, and parahippocampal gyrus. The receptor density was low in caudate, putamen and nuc. accumbens.

621.15

DOPAMINE (DA) D3 AND D2 RECEPTOR mRNA EXPRESSING NEURONS IN THE HUMAN MEDIAL TEMPORAL LOBE. J.N.Joyce* and E.V.Gurevich. Sun Health Research Institute, Sun City, AZ 85372.

The DA D2 and D3 receptor proteins is compartmentally organized in the hippocmapus and parahippocampus of the human. To identify the mechanisms responsible for this the istribution of DA D3 and D2 receptor mRNA bearing neurons was studied with non-radioactive in situ hybridization histochemistry (ISHH). Granular cell in the dentate gyrus exhibit intense staining for D2 mRNA. Pyramidal cells of CA3 field, hilus (sometimes referred to as CA4 field), as well as CA1 and CA2 fields, exhibit intense staining for D2 mRNA. Pyramidal cells of all fields of the subicular complex are stained for D2 mRNA. The D2 mRNA-expressing cells form a distinct layer of islands in the presubiculum and continuous layer II of the stained cells in the parasubiculum. In the entorhinal cortex D2 mRNA is found in the layer II comprised of large modified pyramidal cells. Deep pyramidal layers III and IV of the entorhinal cortex also contain cells stained for D2 mRNA. This is ony the region with a mismatch for receptor protein. In the hippocampal formation the level of D3 mRNA expression is quite low. D3 mRNA is barely detectable in the granular layer of the dentate gyrus. It is rather visible, however, in the pyramidal cells of the CA3, especially in the hilus. Many relatively densely stained neurons can be observed in this area. Pyramidal cells of all hippocampal fields as well as that of subicular formation express D3 mRNA. It is also possible to detect D3 mRNA in the layer II of pre- and parasubiculum, though the staining is weak, and the cells are sparse. The modified pyramids of the layer II of the entorhinal cortex are densely stained for D3 mRNA. Double labeling experiments showed that these cells also express D2 mRNA. Pyramids of the layers III and IV also appear to express D3 mRNa, though the staining is less intense than in the layer II. Double labeling experiments showed that the pyramids in the hippocampus and subiculum that stain for D3 mRNA also stain for D2, but there are more D2-positive that D3-positive neurons. D2 receptor protein detected by autoradiography overlaps with D2 mRNA distribution. Funded by MH 43852, MH55144.

621.16

DISTRIBUTION OF DOPAMINE (DA) D3 RECEPTOR mRNA and D2 RECEPTOR mRNA IN THE HUMAN FOREBRAIN. E.V.Gurevich* and J.N.Joyce. Sun Health Research Institute, Sun City, AZ 85372.

Studies of the DA D3 receptor mRNA in rats demonstrated that it was much less abundant and its distribution in the brain was more restricted that of the D2 receptor. Here we report on the distribution of D3 receptor mRNA and D2 receptor mRNA in the subcortical areas of the human brain studied using non-radioactive *in situ* hybridization histochemistry (ISHH). D3 mRNA can be detected in the basal ganglia, thought it is expressed at a lower level and by fewer cells than for D2. D3 expressing cells are relatively abundant in the nucleus accumbens and ventral portion of the putamen. D3-expressing cells are detected in both external and internal segments of the globus pallidus. In nucleus of diagonal band and nucleus basalis a considerable proportion of D3 and D2 positive neurons are cholinergic, as shown by ISHH followed by ChAT immunohistochemistry. Unlike the rat, D3 mRNA is co-localized to many D2 mRNA neurons. In the thalamus the highest density of the D3 expressing neurons was observed in the anteroventral, parafascicular, and medial part of the mediodorsal nuclei. In the hypothalamus neurons that stain for D3 message can be found in all major neuronal groups. The most intensely stained cells were observed in the tuberomammillary and supramammilary nuclei, and mammilary body. Neurons expressing D2 mRNA were more abundant that the neurons expressing D3 mRNA in all areas. Neurons stained for D3 message also stained for D2 mRNA, but many D2 expressing neurons did not contain D3 mRNA. In the midbrain D2 positive neurons are found in all subdivisions of the substantia nigra, as well as in ventral tegmental and reprorubral areas. Majority of D2 mRNA containing neurons stain are dopaminergic. D3 positive neurons are rare in the substrantia nigra, and only single neurons can be identified in the ventral tegmental and reprorubral areas. The segregation of D3 mRNA and D3/D2 neurons from D2 mRNA only neurons is also observed at the level of protein expression. Thus, they are differentially expressed in neuronal circuits. Funded by MH 43852, MH55144

621.17

DOPAMINE TRANSPORTER AND RECEPTOR DENSITY IN THE HUMAN CAUDATE-PUTAMEN AND PREFRONTAL CORTEX (B9). T. Hussain, S. Dimopoulos, G. Pavey, K. Opeskin, C. Hill F.A.O. Mendelsohn* B. Dean Rebecca L. Cooper Research Laboratories, The Mental Health Research Institute of Victoria, Vic. 3052, Australia. Department of Medicine, University of Melbourne, Austin and Repatriation Center, Austin Campus. Heidelberg 3064, Australia.

The importance of dopaminergic systems in human pathologies has led research to focus on the study of dopamine receptors and the transporters.[1] Using quantitative autoradiography, this study compares the density of dopamine receptors in the human caudate-putamen and prefrontal cortex (Brodmman's Area 9(BA9)). Post-mortem brain tissue was obtained from 5 subjects (Age: 61.4 ±10 yrs mean±SEM; 3 males and 2 females). The density of D1-like and D2-like receptor binding was determined using [^{3}H]SCH-23390 (3nM) displaced by 1μm cis-Flupentixol; and [^{3}H]YM 09151-2 (4nM) displaced by 1μm (+) butaclamol respectively. The dopamine transporter was measured using [^{3}H]mazindol (15nM) displaced with mazindol (1μm) all in the presence of desipramine (0.3μm). The density of D1-like and D2-like receptors in BA9 was 68 ±11% and 39 ±4% of that in the caudate-putamen. The level of dopamine transporter in BA9 was much lower (7 ±3%) compared to the caudate- putamen. These findings most probably reflect the low numbers of dopaminergic neurons in the frontal cortex and provide basic information for further studies of the dopaminergic system within these two regions of the human brain.

Supported by NH & MRC grant 957473

1. Strange P.G. (1992) Brain Biochemistry and Brain Disorders. Oxford University Press.

621.18

Differential Distribution of Dopamine D_2 Receptors in GABAergic Neurons in the Nucleus Accumbens Shell and Dorsolateral Caudate-Putamen. K.T. DelleDonne*[1], S.R. Sesack[2] and V.M. Pickel[1]. [1]Department of Neurology and Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021 [2]Departments of Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Dopaminergic modulation of GABAergic neurons through D_2 receptors has been implicated in motivational and motor functions processed through the nucleus accumbens shell (NAS) and caudate-putamen (CPn). To determine the cellular basis for this modulation, we examined the dual ultrastructural localization of the D_2 receptor with respect to GABAergic neurons in these regions in the rat. In both the NAS and CPn, peroxidase reaction product and gold-silver deposits indicating immunoreactivity (IR) for the D_2 receptor and GABA, respectively, were co-localized within axon terminals and dendrites, and in perikarya having features of spiny and aspiny neurons. The degree of co-distribution in terminals and dendrites was found to differ regionally. D_2-IR was detected most frequently in axons and terminals in the NAS, and a comparatively larger population of GABAergic terminals also contained D_2-IR in this region. Conversely, D_2-labeled dendrites and spines were more abundant in the CPn, where more GABAergic dendrites co-localized D_2-IR compared with the NAS. Furthermore, dendrites containing only D_2-IR more frequently received input from GABA-labeled terminals in the CPn. These results suggest a more prominent role for D_2 receptors in the presynaptic modulation of both GABA and non-GABA transmitter release in the NAS, whereas D_2 receptors may be relatively more important in mediating postsynaptic effects of dopamine on GABAergic neurons in the CPn.

(Supported by NIMH: MH00078 and 40342, and NARSAD).

621.19

EXPRESSION OF DOPAMINE D_1 AND D_2 RECEPTOR mRNAs IN THE HUMAN PLACENTA Wan Sung Choi*, Bong Hee Lee, Kyung Jae Cho, Myeong Ok Kim, Sook Kyeong Hwang, Jin Hyun Kim and Sang Ho Baik[1]. Dept. of Anatomy, College of Medicine, Gyeongsang Nat'l Univ, Chinju, 660-280, Korea and Dept. of Anatomy, College of Medicine, Seoul Nat'l Univ[1], Seoul, 131-742, Korea.

The presence of dopamine(DA) in the human placenta had already been described in the late 1970's. Recently, it was reported that the major effect of DA in the human placenta was the regulation of human placental lactogens(hPLs) release from trophoblastic cells and this effect was mediated at least by cAMP through DA D_2 receptors. However, the role of DA in the control of PL release is still conflicting. The purpose of this study was to determine the distribution of dopamine receptor mRNAs and to search the correlation between the DA receptors and hPLs in the human placenta. Expression of DA D_1 and D_2 receptor mRNAs in the human placenta was studied by *in situ* hybridization histochemistry, using radiolabeled cRNA probes. DA D_1 and D_2 receptor mRNAs were colocalized in the cytotrophoblastic cells and some stroma cells in the human placentas obtained at 7-38 weeks of gestation. Cytological localization of hPLs and prolactin like proteins(PLPs) in developing human placenta was analyzed by immunohistochemistry. hPLs were detected mainly in the syncytiotrophoblasts. However, PLPs were identified in the cytotrophoblast, and some stroma cells in the placental villi. Expression sites of DA receptor were well coincided with the cells which expressed the PLPs, but not with the PL-secreting cells. Our results suggest that DA might have a role in the release of PLPs through DA D_1 and D_2 receptor in the human placenta.

621.20

NEUROTENSIN RECEPTOR-IMMUNOREACTIVITY WITHIN DOPAMINERGIC NEURONS IN THE SUBSTANTIA NIGRA. Y. Delville*, J.C. Klick, C.F. Ferris, L. Lifshitz, S.P. Mitra and R.E. Carraway. Behavioral Neuroscience Program, Psychiatry Department, and Physiology Department, University of Massachusetts Medical Center, Worcester, MA 01655.

Synthetic peptides corresponding to portions of the rat sequence for neurotensin receptors (NTR) were used to raise polyclonal antibodies. Western blotting indicated staining of several bands (45-55 kDa) in cell lines producing NTR (amino acid content: 47 kDa). Two antisera towards the immediate C-terminus of NTR specifically labeled neuronal elements in rat brains. In the substantia nigra, NTR-immunoreactivity (NTR-IR) visualized by wide-field digital microscopy was partly associated with dopaminergic neurons labeled with a monoclonal antibody to tyrosine hydroxylase (TH). At a cellular level, NTR-IR was found within the soma and associated processes in the compact part of the substantia nigra. NTR-IR was limited to processes within the reticular part of the area. These results support the hypothesis that neurotensin can act directly on dopaminergic neurons in the substantia nigra.

Supported by IBN 9419100 from the NSF

622.1

ANTAGONISM OF THE RATE-DECREASING EFFECTS OF OPIOID PARTIAL AGONISTS. E.A. Walker*, L.A. Dykstra, M.J. Picker, Department of Psychology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

Opioid partial agonists may exert behavioral effects through a number of different opioid receptors. The hypothesis that the rate-decreasing effects of opioid partial agonists dezocine, *l*-α-acetylmethadol (LAAM), and *d*-propoxyphene are mediated through the μ opioid receptor was tested using naltrexone and β-funaltrexamine. Sprague-Dawley rats were trained to respond under a fixed ratio 20 schedule of food presentation. Cumulative doses of dezocine (0.1-10 mg/kg, s.c.) and *d*-propoxyphene (1.0-100 mg/kg, s.c.) dose-dependently decreased response rates. Pretreatments of naltrexone (0.01-1.0 mg/kg, s.c.) shifted the dose-response curves for dezocine and *d*-propoxyphene to the right in a dose-dependent manner. Apparent pA_2 analyses indicated that naltrexone was equi-potent as an antagonist of both agonists. Furthermore, naltrexone apparent pA_2 values were similar to those obtained with other μ opioid agonists. Twenty-four hr pretreatments of β-funaltrexamine (5 and 10 mg/kg, s.c.) also shifted the dezocine dose-response curve in a dose-dependent manner. These effects of β-funaltrexamine dissipated after 4 days. Cumulative doses of LAAM (0.032-10 mg/kg, s.c.) only produced a slight decrease in response rates. However, response rates for the following 2-3 days were depressed consistent with this compounds slow onset and long duration of action. No further antagonism tests were attempted with LAAM. These data support the hypothesis that the rate-decreasing effects of at least dezocine and *d*-propoxyphene are mediated through the μ opioid receptor. (Supported by DA07327, DA02749, DA00033)

622.2

SENSITIVITY OF DIFFERENT RAT STRAINS TO THE DISCRIMINATIVE STIMULUS PROPERTIES OF NICOTINE, J.R. James*, H. Covington III, and J.A. Rosecrans. Dept. Of Pharmacology and Toxicology, Virginia Commonwealth Univ., Richmond, VA 23298.

Male Sprague-Dawley (SD), Long-Evans (LE), and Fischer-344 (F-344) rats were trained in an operant procedure to discriminate between nicotine and saline. Rats (SD and LE) were trained at 400 ug/kg nicotine (free base, sc.) and F-344 at 900 ug/kg nicotine (free base, sc.). F-344 rats were unable to meet criteria at doses lower than 900 ug/kg. Dose response curves produced ED50 values of 140 ug/kg SD, 70 ug/kg LE, and 320 ug/kg F-344. Acute tolerance testing was performed using a cumulative dosing paradigm at time intervals of 0, 90, 180, and 270 min. Rats from all three strains exhibited unique acute tolerance (desensitization) 'fingerprints' similar to previously reported results by our laboratory using a drug discrimination paradigm. The variability of these 'fingerprints' suggests that each rat responds to the effects of nicotine in an individual manner. The underlying physiological mechanism determining the ability of a rat to either exhibit or fail to exhibit acute tolerance is possibly related to individual differences in distribution, sensitivity or quantities of specific nicotinic receptor subtypes. Cannulae were bilaterally implanted in the ventral tegmentum. Bolus injections of nicotine were used to determine the site specificity and sensitivity of this dopaminergic rich nucleus.
Funding provided by Philip Morris, Switzerland.

622.3

NALOXONE AS A DISCRIMINATIVE STIMULUS: AN ASSESSMENT OF MU, KAPPA AND DELTA OPIOID MEDIATION. A. L. Riley* and W. Klymczak, Psychopharmacology Laboratory, The American University, Washington, D.C. 20016.

Previously, we have demonstrated that animals trained to discriminate naloxone from its vehicle within the taste aversion baseline of drug discrimination learning generalize naloxone control to naltrexone (see Smurthwaite et al., Pharmacol. Biochem. Behav. 41:43-47; 1992), suggesting that the mu antagonist properties of naloxone mediate its discriminative effects. Naloxone, however, binds to and has antagonist activity at a number of opioid receptor subtypes. It is not known if and to what extent activity at these other subtypes underlies naloxone's stimulus effects. Accordingly, the present experiment assessed whether naloxone's activity at kappa and delta receptor subtypes also establish discriminative control. Specifically, rats were trained to discriminate naloxone (1 mg/kg) from its vehicle and then given selective mu (naltrexone), delta (naltrindole) and kappa (norBNI) antagonists to assess their ability to substitute for naloxone. Under these training conditions, naloxone generalized only to naltrexone. Naloxone-trained subjects responded similar to control subjects when administered naltrindole or norBNI. From these data, we suggest that when naloxone is the training stimulus only its mu antagonist properties establish discriminative control. It remains unknown whether selective delta or kappa antagonists would serve such a function if used as the training stimulus.

Supported by a grant from the Mellon Foundation

622.4

NALORPHINE'S OPIATE AGONIST/ANTAGONIST PROPERTIES WITHIN THE TASTE AVERSION BASELINE OF DRUG DISCRIMINATION LEARNING, S.D. Ziervogel* and A.L. Riley. Psychopharmacology Laboratory, Department of Psychology, The American University, Washington, DC 20016.

Recently, Melton and Riley (Pharmacol. Biochem. Behav. 46:237-242; 1993) reported that the stimulus properties of cholecystokinin (CCK) were blocked by the opioid agonist morphine and potentiated by the opioid antagonist naloxone. Given these opposite effects of agonists and antagonists on drug discrimination learning, this procedure may be useful in the general classification of the relative efficacy of partial opioid agonists, i.e., compounds with intermediate degrees of mu agonist effects. To that end, in the present experiment animals were trained within the taste aversion baseline of drug discrimination learning to discriminate between CCK and its vehicle. They were then given the partial *mu* agonist nalorphine either alone or in combination with CCK to assess its ability to block (agonist) or potentiate (antagonist) CCK's stimulus properties. The effects of nalorphine were like those of naloxone or morphine in individual subjects. In some subjects, nalorphine substituted for CCK and when given in combination with CCK potentiated its stimulus effects. In others, nalorphine did not substitute for CCK and when given in combination with CCK blocked its stimulus effects. Nalorphine appears to lie between naloxone and morphine in its relative efficacy at the opiate receptor, with the degree of similarity to naloxone and morphine dependent upon the specific subject examined.

Supported by a grant from the Mellon Foundation

622.5

INTRACEREBRAL MICROINJECTION OF THE ENDOGENOUS CANNABINOID ANANDAMIDE ALTERS RAT EXPLORATORY BEHAVIOR BUT DOES NOT AFFECT EITHER SPATIAL MEMORY OR LTP.
N. Meiri *[1], C. Collin[1], D. Tomsic[1], H. Shah[1], J. Axelrod[2], D. L. Alkon[1].
[1]LAS, NINDS/NIH, [2]LCB, NIMH/NIH, Bethesda MD, 20892

Ananandamide, an endogenous ligand for the brain cannabinoid receptors has been implicated in behavioral and neuronal plasticity. Here we describe the effect of in vivo intracerebral administration of anandamide and synthetic cannabinoid agonists. Bilateral intraventricular microinjection of anandamide 20 min prior to behavioral testing significantly altered the exploratory behavior of rats in an open field (ANOVA $P<0.01$; n=20). This reduction of movement was probably not caused by a motor deficit since rats that were similarly treated swam the same distance in the water maze as vehicle-injected controls. The intracerebral administration of anandamide did not affect the latency time to find the island in the water maze (n=40). Anandamide is rapidly processed in the rat brain. In preliminary experiments we found similar results after injection of THC which is metabolized here slowly. In order to evaluate the effects of cannabinoid administration in vivo on synaptic physiology, the injected rats were sacrificed immediately after performing the water maze, and hippocampal slices (300μm thick) were prepared. In an interface chamber extracellular field responses (fEPSP) recorded from the CA1 stratum radiatum were elicited by Schaffer collateral stimulation (100 μsec every 1 min). Non-associative long term potentiation (LTP) was induced by a single tetanus (100Hz, 1 sec). Both the magnitudes of the fEPSP and LTP were not affected in the cannabinoid treated animals. In addition, in vitro perfusion of naive slices with Anandamide (10uM in BSA) also failed to produce changes in the fEPSP and LTP. Anadamide perfusion did, however, cause a complete elimination of associatively induced LTP elicited by paired stimulation of distal and proximal CA1 dendrites.
Supported by NINDS/NIH

622.6

EFFECTS OF NICOTINE ON SEXUALLY DIMORPHIC PREFERENCES FOR DIFFERENT COGNITIVE STRATEGIES IN RATS DOES NOT INVOLVE DOPAMINE DA2 RECEPTORS. L. Kanit[1,2], D. Taskiran[1,2], R. McDonald[3], B. Kulali[1], J.J. Furedy[3] and S. Pogun[1,2] * [1]Ege Un. Center for Brain Research, Dept. of Physiology and [2]TUBITAK Basic Neuroscience Research Unit, Turkey; [3]Un. of Toronto, Dept. of Psychology, Canada

Nicotine exerts its central actions by impinging on neuronal nicotinic acetlycholine receptors. These actions exhibit sexual dimorphism: in rats, males and females show differences in receptor densities and regulation by chronic nicotine treatment (Pogun et al., 1995),and in the behavioral test of active avoidance(Yilmaz et al., 1995). Dimorphism in different strategies for problem solving have been shown only in humans (Kimura, 1992). Nicotine regulates dopaminergic activity in the CNS and some of the observed effects may be via dopamine DA2 receptors (DA2R) since the dopaminergic system is also differentially regulated in males and females. A modified version of the Morris Water Maze (MWM) developed by McDonald and White (1994) using visual vs. spatial cues, could discriminate between independent memory systems in rats. The present study employed the MWM to screen cognitive strategies in rats that may show sex differences, which cannot be captured by learning and memory tests like active avoidance. Rats received 0. 4 mg/kg nicotine, or saline, s.c., for 13 days prior to testing. The platform was in the same location for the first 12 days, either visible or hidden. On the 13th day, the visible platform was moved to a new location, leaving the animals with a choice. At the termination of behavioral testing, brain DA2R binding was determined in the frontal cortex, c. striatum, hippocampus, and amygdala. For the 12 days, nicotine improved performance, i.e. time to reach the platform, in both sexes. On the 13th day however, female rats that received only saline went directly to the visible platform whereas males and the nicotine treated females used their spatial cues and searched for the platform in the quadrant it had been for the past 12 days. Even though there were regional variations and sex differences in DA2R binding, no interaction with nicotine treatment was observed. The results showed clearly that males tended to use spatial cues, whereas females used visual cues to solve the problem. This difference, however, was eliminated by the administration of nicotine and the observed effects did not involve the DA2 Rs.

622.7

QUANTITATIVE GENETIC STUDY OF COCAINE INDUCED STEREOTYPY. C.A. Ballas, G.T. Golden, T.N. Ferraro, G.S. Smith, G.C. Brainard*, W.H Berrettini. Thomas Jefferson University, Phila., PA, DVAMC, Coatesville, PA.

Psychomotor (PM) stimulation is movement motivated by cognitive processes and is characteristic of many addictive substances, including cocaine. Mice treated chronically with high doses of stimulants such as cocaine or amphetamine exhibit intense PM stimulation manifested as stereotypy, an increase in small, repetitive, purposeless movements.

Mature DBA/2J (D2) mice exhibit marked stereotypy to repeated cocaine injections while C57BL/6J (B6) mice do not. We hypothesize that stereotypy is the result of differential neuronal adaptation originating in various genetic loci. We have studied the induction of stereotypy in B6, D2, F1 and F2 intercross populations of mice. Mice (8-10 weeks old) were given a seven day regimen of cocaine injections (40mg/kg IP); after the last injection, mice were observed and rated for stereotypy every five minutes for fifty minutes using a scale adapted from Tolliver and Carney (Pharm Biochem Behav 48:169, 1994) (0-4, with 4 being extreme stereotypy). For the test period, cumulative scores ranged from 0 to 40. Means $\pm$ SD for the one hour test period were: B6 (n=24) 11.10 $\pm$ 2.35; D2 (n=26) 28.33 $\pm$ 4.05; F1 (n=22) 20.13 $\pm$ 2.70; F2 (n=216) 17.76 $\pm$ 4.91. Genetic variance can be calculated by subtracting the F1 variance from the F2 variance, revealing that approximately 70% of the F2 variance is mediated by genetic factors. Wright's formula (Lande R., Genetics 99:541, 1981) shows that the minimum number of genetic factors (QTLs) is 2.2. Screening of the murine genome will begin shortly to determine correlations between DNA markers and locomotor activity and cocaine-induced stereotypy. Defining the location of genetic loci underlying this quantitative behavior may lead to identification of genes important in influencing behavior related to cocaine addiction in humans.

622.8

EFFECT OF INTRAPERITONEAL COCAINE PREEXPOSURE ON COCAINE-INDUCED TASTE AVERSIONS. H.F. Diamond* and A.L. Riley. Psychopharmacology Laboratory, Department of Psychology, The American University, Washington, DC 20016.

In the present experiments, the effect of preexposure to cocaine on cocaine-induced aversions was assessed under conditions that typically sensitize animals to its rewarding effects, i.e., following massed (vs. spaced) IP injections. Specifically, rats were administered cocaine (32 mg/kg) or vehicle daily (i.e., massed) or every fourth day (i.e., spaced) for five exposures. They were then given saccharin to drink and injected with cocaine (32 mg/kg, SC) or vehicle. Animals preexposed to the vehicle and then injected with cocaine acquired a taste aversion to cocaine. Animals given spaced preexposure to cocaine and injected with cocaine during conditioning failed to acquire an aversion (similar to that reported following spaced and massed SC cocaine exposure; see Diamond & Riley Soc. Neurosci. Abs. 21:1953; 1995). Animals receiving massed preexposure to cocaine and then injected with cocaine did not display attenuated aversions, instead acquiring aversions comparable to nonpreexposed subjects. These results are consistent with the position that massed preexposure either prevents the development of tolerance to cocaine or results in sensitization to both its rewarding and aversive effects, functionally resulting in the normal acquisition of aversions.

Supported by a grant from the Mellon Foundation.

622.9

RESPONSE ALLOCATION IN A DRUG DISCRIMINATION TASK WHEN ONE RESPONSE OPTION IS REMOVED. J. R. Stadler*, W. F. Caul, R. J. Barrett. Department of Psychology and V. A. Medical Center, Vanderbilt University, Nashville, TN 37240

It is important to know how intermediate cue states are represented by responding in two- and three-choice discrimination tasks. Using animals trained on a three-choice task, response allocation was evaluated when all three or only two levers were present.

Rats were trained using a VI schedule to discriminate among 0.5 mg/kg amphetamine (AM), distilled water (DW), and 0.03 mg/kg haloperidol (HA). Two groups were tested after being injected with DW. For one group, all three levers were present, whereas for the other group, the DW lever was removed. Animals given a choice of all levers allocated responses predominantly on the DW lever, whereas animals given a choice between the AM and HA levers allocated responses about equally between the two levers.

In a second study, animals were injected with 1.0 mg/kg HAL 24 h prior to the test session. Animals given a choice of all levers responded predominantly on the DW lever, although increased AM lever responding was observed relative to pretreatment levels. Animals given the choice between AM and HA levers showed post-HA rebound responding by allocating their responses predominantly on the AM lever. Supported by NIDA grant DA 08202

622.10

EFFECT OF INTERDOSE INTERVAL ON AMPHETAMINE-INDUCED REBOUND RESPONDING. W. F. Caul*, R. J. Barrett, and J. R. Stadler. Department of Psychology and V. A. Medical Center, Vanderbilt University, Nashville, TN 37240

Our previous research, using a three-lever amphetamine-vehicle-haloperidol drug discrimination task, has shown that time-dependent, rebound changes in choice responding occur following administration of a single dose of drug. The present study evaluated rebound responding following 5 doses of amphetamine as a function of interdose interval.

Rats were trained using a VI schedule of food reinforcement to discriminate among 0.35 mg/kg amphetamine (AM), distilled water (DW), and 0.033 mg/kg haloperidol (HA). Orderly dose-response functions were obtained for both AM and HA. Discrimination performance was used to form 3 matched groups. The group given DW on each of the 9 days of the treatment period responded predominantly on the DW lever when tested 24 h after the final injection. The group given AM (0.75 mg/kg) on days 5 through 9 responded predominantly on the HA lever while an intermediate level of rebound HA-like responding was observed for the group given the same dose of AM 5 times but on alternate days during the treatment period.

Supported by NIDA grant DA 08202

622.11

STIMULUS EFFECTS OF COCAINE GIVEN CENTRALLY AND SYSTEMICALLY. W. B. West, S. Livreri, B. Simon, M. Goding, J. Buggy, and J. B. Appel*. Behav. Pharmacol. Lab., Dept. of Psych., Univ. S. Carolina, Columbia, SC 29208.

This study compared the discriminative stimulus effects of cocaine given systemically (i.p.) and directly into the nucleus accumbens (NAc), a probable site of action of many of the behavioral effects of CNS stimulants and related drugs. Spraque-Dawley rats were trained to discriminate i.p. injections of cocaine (10 mg/kg) from saline in a standard, water-reinforced, two-lever drug discrimination paradigm. Cannulae for intra-cranial (i.c.) injections were implanted bilaterally into the NAc (coordinates: 2.2 mm anterior to bregma, 1.5 mm lateral, and 6.0 mm ventral).

Centrally administered cocaine (1 μl per side) substituted for i.p. cocaine in a dose-related manner with complete substitution at a dose of 20 μg. Systemic injections of the D1/D2 receptor antagonist cis-flupenthixol dose-dependently blocked the stimulus properties of i.c. cocaine, with complete antagonism occurring at 0.4 mg/kg. When given i.c., cis-flupenthixol blocked the i.p. cocaine cue only partially. Immunohistochemistry, using c-Fos antibodies (1:5000) demonstrated that i.c. administration of cocaine was confined to an area of 2000 μ surrounding the injection site.

Substitution of i.c. cocaine for i.p. cocaine supports the hypothesis that the NAc plays a role in the stimulus effects of cocaine. Antagonism by cis-flupenthixol suggests that DA receptors mediate the cocaine cue. However, the failure of i.c. cis-flupenthixol to antagonize i.p. cocaine completely indicates that sites other than the NAc may also be involved.

Supported by USPHS Research Grant 5 R37 DA02543, from the National Institute on Drug Abuse.

622.12

WITHDRAWAL FROM COCAINE "BINGES" in RATS: ULTRASOUNDS AS INDICES OF AFFECTIVE DISTRESS. Nicole H. Mutschler*, Jack Sutherland II, and Klaus A. Miczek. Dept. of Psychology, Psychiatry, and Pharmacology Tufts Univ., Medford MA 02155.

Human cocaine abusers report that they experience intense anxiety during withdrawal from chronic use or "bingeing". Because the symptoms of cocaine withdrawal are not easily observed, it has been difficult to develop an adequate animal model for cocaine withdrawal that detects anxiety-like behavior. The objective of the present study was to examine the effects of continuous access to intravenously self-administered cocaine on distress vocalizations induced by startling tactile stimuli as a possible animal model for cocaine withdrawal. Five days after implantation of a jugular catheter rats were placed into self-administration chambers with access to cocaine (0.25mg/inf). Once the animal had a stable response rate over 3 days, on FR5, they were given free access to cocaine (0.25mg/inf) for 48 or 12 hours. Subsequently, animals were exposed to 18 air puffs (20 or 10psi) at 6, 24, 72 hours, 7 and 14 days after the "binge". Rats that "binged" on cocaine for 48 hours and were subsequently startled with 20psi stimuli increased the number of automatically recorded ultrasonic distress calls when compared to handled controls at 6 and 24 hours after the last cocaine infusion. After a 48 hour "binge", animals showed an increase in amplitude of the startle response up to 7 days after withdrawal in response to 20psi tactile stimuli but not to 10psi. Animals that "binged" for 12 hours also showed an increase in ultrasonic distress calls when compared to handled controls when the USVs were induced by 10psi tactile stimuli. Rats that experienced repeated 12 hour "binges" showed no evidence for habituation or sensitization due to multiple "bingeing". USVs during cocaine withdrawal may be interpreted to reflect affective distress during the first 72 hours after the last cocaine infusion of a 12 or 48 hour "binge". (PHS grant RO1 DA02632)

622.13

BRIEF SOCIAL STRESS SENSITIZES COCAINE EFFECTS ON MOTOR ACTIVATION AND OPERANT BEHAVIOR. J. Sutherland II*, G. J. Carter, N. H. Mutschler and K. A. Miczek. Dept. Psychology, Psychiatry and Pharmacology, Tufts Univ. Medford MA 02155.

Brief confrontations with an aggressive opponent comprise behavioral and physiological stress responses. This form of "social stress" interacts with psychomotor stimulants in a long-lasting sensitizing manner. The present study investigated how cocaine's effects on motor acts and positively reinforced responding behavior were sensitized by "social stress" episodes. Male Long-Evans rats lever pressed and male CFW mice nose poked, reinforced by milk presentation, according to joint FR5-FI180sec (rats) or joint FR5-FI300sec (mice) schedules. After response rates stabilized, subjects were exposed to social stress. For rats, social stress is defined as a threat of attack by an aggressive conspecific while protected in a cage for 15 or 60 minutes, and in mice, it is defined as attacks by an aggressive conspecific (20 bites) followed by 30 minutes inside a protective cage. Experimental subjects were randomly assigned to a dose of 3,6,10,17, or 30 mg/kg cocaine, receiving the same dose of cocaine 7,14, 21 and 28 days after social stress. Compared to pre-stress rates, cocaine (6.0 and 10.0 mg/kg) challenge increased response rates on days 7,14,21 and 28. Peak effects were observed on day 14 for rats and day 21 for mice. After a single social defeat by an aggressive resident, cocaine challenges increased locomotor and stereotyped behaviors. These data suggest that brief social stress produces sensitization to the stimulant effects of cocaine on conditioned and unconditioned motor acts at different times (Supported by PHS grant R01 DA 02632.)

622.14

CUMULATIVE LOCOMOTOR ACTIVITY CURVES FOR HABITUATION STUDIES WITH RODENTS. F.A. Barrios, M. Giordano and W. Freed*. Centro de Neurobiologia, UNAM, México D.F. 04510 and NIMH, Neuroscience Center. Washington, D.C. 20032.

Locomotor behavior is commonly used in pharmacological and behavioral studies to assess the effects of pharmacological and experimental manipulations. Many of these studies rely on some means of automated measurement of locomotor behavior. We propose a novel approach for studying locomotor activity data. Ordinarily, activity is monitored by measuring total amounts of activity for defined time intervals. Alternatively, cumulative locomotor activity data might be analyzed by fitting the data to a mathematical model. This approach could provide information not only about the quantity of locomotion, but also about the properties of locomotion over time. Locomotor activity data collected from several types of apparatus were fitted to mathematical models with the assumption that cumulative activity reaches a stable asymptote. By using the chi-square test for goodness of fit the best model was selected. The best model is based on the saturation function $Ac(t) = At/(B+t)$, where Ac is the cumulative activity, t is time, and A and B are parameters. From the first derivative $dAc(t)/dt = AB/(B+t)^2$ at t=0, it can be seen that A/B represents the initial rate of activity. In addition the rate of activity will have diminished to A/4B at time t=B, and as t approaches infinity Ac(t) approaches A (which represents the asymptotic level of activity). Using this model we observed that stimulants (e.g. amphetamine) induced a ten-fold increase in the initial rate of activity. Analysis of locomotor activity by fitting the data to a mathematical model has several potential advantages. First, it may be possible to predict overall activity curves from a relatively short period of testing. Secondly, this information could provide hypotheses regarding a drug's mode of action by comparing its parameters those of drugs for which the mode of action is known. Support provided by NIMH-Intramural Research Program and UNAM.

BEHAVIORAL PHARMACOLOGY: SEROTONIN

623.1

PSYCHOSOCIAL CONFLICT IN TREE SHREWS: NEURO-BEHAVIORAL EFFECTS OF CLOMIPRAMINE TREATMENT. E. Fuchs*, E. Nonn, M. Kramer, G. Flügge, C. Hiemke[a]. German Primate Center, D-37077 Göttingen and [a]Department of Psychiatry, University of Mainz, D-55131 Mainz.

Male tree shrews (*Tupaia belangeri*) provide a suitable model to study the neuro-behavioral effects of chronic psychosocial conflict. Since the overall pattern of the behavioral and physiological changes in subordinate male tree shrews resemble a depression-like symptomatology, we analyzed the effect of a chronic treatment with the antidepressant clomipramine in these individuals. The drug was administered orally (50mg/kg/day) for 28 days, while the psychosocial conflict continued during the whole treatment period. A clear and time-dependent improvement of locomotor activity, marking and grooming behavior, risk assessment, and the activity of both the HPA-axis and the sympathetic nervous system was observed. In 17 brain regions and the pituitary, numbers of binding sites for serotonin ($5HT_{1A}$), noradrenaline ($alpha_2$-adrenoceptors, $beta_1$-adrenoceptors, $beta_2$-adrenoceptors), and corticotropin-releasing factor were quantified. The antidepressant treatment affected these receptor systems in a region and treatment specific pattern. Since the clomipramine treatment counteracts behavioral and endocrine effects of the chronic psychosocial conflict, the present data reflect distinct neurochemical processes that may underly the therapeutic actions of antidepressants. Supported by the DFG Fu 174/9-1 and Hi 399/3-1.

623.2

THE EFFECTS OF LESIONS OF BRAIN SEROTONIN AND NOREPINEPHRINE ON ANXIETY-LIKE BEHAVIOR. P.A. Ardayfio, A.J. Barron, G.U. Ikeri and R.L. Commissaris*. Pharmaceutical Sciences, College of Pharmacy & AHP, Wayne State University, Detroit, MI 48202

The present studies examined the hypothesis that the neurotransmitters serotonin (5-hydroxytryptamine; 5HT) and norepinephrine (NE) are redundant systems in the modulation of anxiety-like behavior. Anxiety-like (conflict) behavior in female rats was investigated before and after treatment with vehicle, the 5HT neurotoxin 5,7-DHT, the NE neurotoxin 6-OHDA, or the combination of the two neurotoxins. When the absolute number of shocks accepted in conflict sessions was used as the measure of "anxiety", there was no apparent effect of any neurotoxin treatment on conflict behavior. Treatment with vehicle, 5,7-DHT or 6-OHDA alone resulted in a very modest reduction in water intake (unpunished responding). In contrast, subjects receiving the combination lesion treatment exhibited a dramatic reduction in water intake, perhaps the result of a generalized behavioral disruption produced by the combination lesion. To normalize for the differences in water intake across the various treatments, conflict (anxiety-like) behavior during the first week following lesion treatment also was evaluated in terms of a "suppression ratio" (i.e., shocks accepted/water consumed). This analysis again revealed that the vehicle and single lesion groups did not differ in their conflict behavior; in contrast, the subjects receiving the combination lesion exhibited a dramatic anticonflict effect (reduced "anxiety"). These findings with the suppression ratio measure are consistent with the hypothesis that 5HT and NE are redundant neurotransmitters in the modulation of anxiety-like conflict behavior. (Supported in part by GM08167).

623.3

EFFECTS OF DULOXETINE, A DUAL SEROTONIN AND NOREPINEPHRINE REUPTAKE INHIBITOR, ON THE STIMULUS PROPERTIES OF COCAINE AND LSD. B. Simon* and J. B. Appel, Beh. Pharm. Lab., Dept. of Psych., Univ. of S. Carolina, Columbia, SC, 29208.

Duloxetine, a newly developed antidepressant, inhibits the reuptake of both 5-HT and NE. Both of these neurotransmitters have been implicated in certain effects of cocaine and LSD. Although cocaine acts primarily by inhibiting the reuptake of DA, it also increases NE concentration in the amygdala and 5-HT throughout the brain. Studies of the stimulus properties of cocaine indicate that both NE and 5-HT uptake inhibitors substitute at least partially for this drug or potentiate the effects of relatively low doses. LSD acts primarily as an agonist at 5-HT, but some of its behavioral effects (*e.g.*, neophobia, acoustic startle response) depend on NE projections from the locus coeruleus or involve NE activation in general. While there is considerable evidence that the stimulus properties of LSD are mediated by 5-HT receptors, drugs such as yohimbine, which acts at α_2 NE as well as 5-HT_{1A} and 5-HT_{1D} receptors, substitute for LSD. For these reasons, doloxetine was tested in rats trained to discriminate cocaine (10 mg/kg) or LSD (0.08 mg/kg) from saline.

Results indicate that duloxetine alone (2 - 16 mg/kg) does not substitute for either cocaine or LSD. However, two doses (8 and 16 mg/kg) enhanced the stimulus effects of a low dose of cocaine (2.5 mg/kg). Duloxetine (4 mg/kg) also potentiated the effects of a low dose of LSD (0.02 mg/kg).

These findings indicate that the stimulus properties of duloxetine do not resemble those of either cocaine or LSD. However, the fact that this drug enhanced the effects of both drugs suggests that 5-HT and NE reuptake plays some role in the actions of both cocaine and, to a lesser extent, LSD.

Supported by USPHS Research Grant 5 R37 DA02543, from the National Institute on Drug Abuse.

623.4

AN ETHOGRAPHIC STUDY OF REVERSAL OF FIGHTING BEHAVIOR IN ISOLATED MICE BY SEROTONERGIC AGENTS USING CORRESPONDENCE ANALYSIS J. K. Chamberlain*. Terre Haute Center for Medical Education, Indiana University School of Medicine, Terre Haute, IN 47809

Reversal of fighting behavior in mice is an animal model used for screening potential anxiolytic agents. Putative anxiolytic activity is inferred when an agent inhibits the aggressive response of an isolated mouse toward a group-housed intruder stimulus mouse. Ethological observation of resident-intruder encounters allows analysis of a broad spectrum of behaviors in assessing drug action. In the present study, correspondence analysis is used to evaluate ethological data. CD-1 mice weighing 18-22g were isolated for three weeks. Mice were then trained to a criteria of a biting attack on a stimulus mouse in 10 seconds or less on three consecutive training sessions. The mice were injected with various serotonergic agents at a previously determined ED_{50} dose for inhibition of fighting behavior. Isolated mice were scored on the frequency of several behaviors ranging from a biting attack to flight for a three minute period. A Fisher's exact test and a Monte Carlo simulation revealed a significant difference between drug treatments. Two-dimensional graphical representation reveals several associations. Between set comparisons of drug treatments with behaviors show 8OH-DPAT associates with defensive posture, and the arylpiperazine axiolytics, buspirone and ipsapirone, group together and associate with aggressive unrest. Propranolol and penbutolol, two ß-adrenergic antagonists with high affinity for 5-HT_{1A} receptors, group together and associate with alert posture. These data demonstrate that differently acting serotonergic agents with anti-aggressive activity, can be distinguished by ethological observation using multivariate correspondence analysis.

623.5

NOVEL MURINE AVOIDANCE ASSAY SELECTIVELY SENSITIVE TO ANXIOLYTICS. T.W. Seale*, Y. Zhang and K.M. Garrett. Depts. of Pediatrics and Physiology, Univ. Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK 73190

Well characterized behavioral assays responding selectively to anxiolytic agents are vital for drug discovery, for *in vivo* pharmacological studies and for pharmacogenetic analyses of anxiety-related behaviors. We have developed a new behavioral assay, the mirrored chamber aversion assay, which is based on innate approach-avoidance of mice and most other vertebrate species to mirrors placed in their environment. The assay has several attractive attributes: 1) selectivity of its pharmacological response profile; 2) its simplicity, rapidity and non-punishing nature; 3) its quantitative nature involving two endpoints, latency to enter and duration spent in the avoided environment; 4) the large magnitude of the observed behavioral changes; 5) the ability to respond to both anxiolytic and anxiogenic agents. Untreated or vehicle treated male and female mice (e.g. BALB/cByJ, C57BL/6J) exhibit high avoidance (mean latencies $\geq$ 280 sec, mean duration $\leq$ 3 sec) during a 300 sec test period. Mean latency is reduced to a nadir of 60 sec and duration increased to 60 sec in a dosage dependent manner following diazepam administration.+ Anxiolytic-like responses also are observed following several 5-HT_{1A} agonists, 5-HT_3 antagonists, the glucocorticoid dexamethasone, nicotine and ethanol. Propranolol, clonidine and yohimbine, haloperidol, fluoxetine and tricyclic antidepressants, nisoxetine, caffeine, methylphenidate and phencyclidine had no effect up to motor depressant doses. Behavior of habituated mice resembles that of anxiolytic-treated mice but these respond to anxiogenic agents like DMCM and high dosage caffeine. Several situational variables have been identified which impact this behavioral assay: recent litter change, intra-cage aggression, diurnal rhythm, recurrent noise or vibration. Genotype has a major impact on both innate and drug-induced responsiveness in this assay. This new method may have value for the identification and characterization of novel anxiolytics. (Supported by NIMH grant MH49791).

623.6

MICROINJECTIONS OF 5-HT_{1A} AND 5-HT_{1B} AGONISTS INTO THE LS AND MPO OF MALE MICE: EFFECTS ON OFFENSIVE AGGRESSION. A. Cologer-Clifford, S. Smoluk, J. Nyby*, and N. Simon. Department of Biological Sciences, Lehigh University, Bethlehem, PA 18015.

Testosterone and its metabolites facilitate the expression of offensive intermale aggression, while serotonin inhibits this behavior. Previous findings demonstrated that androgens and estrogens differentially effected the ability of 5-HT receptor agonists to attenuate aggression. The present study examined whether steroidal modulation of agonist function occurred in the lateral septum (LS) or medial preoptic area (MPO), brain regions which have been linked to the expression of aggression and exhibit androgen, estrogen, 5-HT_{1A}, and 5-HT_{1B} receptors. Castrated CF-1 male mice implanted with either the estrogen DES or the nonaromatizable androgen DHT were microinjected bilaterally into the LS or MPO with either 8-OH-DPAT (a 5-HT_{1A} agonist), CGS12066B (a 5-HT_{1B} agonist), or DPAT+CGS and tested for aggression and motor behavior. All procedures complied with Federal guidelines for animal care. In the LS, CGS decreased aggression in androgen-treated, but not estrogen-treated males. DPAT injections had no effect. In the MPO, agonist treatments reduced aggression in both androgen- and estrogen-treated animals. The only exception was DPAT+CGS in androgen-treated males where the effects were marginal ($p<0.054$). The findings demonstrate regional variation in endocrine modulation of 5-HT_{1A} and 5-HT_{1B} agonist effects on aggression.

Supported by A.P.S. Dissertation Award (A.C.), a Howard Hughes Science Education grant (S.S.), and grants to N.S. from the Guggenheim Foundation, NIH (1R15HD31696), and NSF (IBN-9512015).

623.7

DISCRIMINATION BETWEEN THE 5-HT_1 RECEPTOR AGONISTS FLESINOXAN AND ELTOPRAZINE. J. Gommans, T.H. Hijzen, J.C. Compaan* and B. Olivier. Dept. of Psychopharmacology, Utrecht Univ., Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands.

In previous drug discrimination studies flesinoxan (F) and eltoprazine (E), having cues mediated by 5-HT_{1A} and 5-$HT_{1A/1B}$ receptors, resp., partially cross-generalized to each other. In a two lever FR10 operant procedure, rats learned to discriminate F (1.0 mg/kg, p.o.) from E (1.5 mg/kg, p.o.) readily. Saline administration resulted in approximately 50% of the responses being made on either lever. Different doses of the training drugs gave dose dependent increases in drug appropriate lever responding and in response rate, and a decrease in latency to the first response and lever selection. The training doses in the dose response curves, differed between the drugs, i.e. E was located more to the right than F. This might explain the following results: when the training doses of F and E were administered together this resulted in predominantly E lever responses. Lower doses of E together with F reduced E lever presses and, concomitantly, increased F lever responses. A second saline test resulted, again, in approximately 50% responses on either lever. We have no explanation why the point on the generalization curve representing the E training dose is located more to the right than that of F, while at the same time saline administration results in 50% responding on both levers. The full 5-HT_{1A} receptor agonist 8-OH-DPAT (0.01-0.1 mg/kg, p.o.) and the 5-HT_{1B} receptor agonist anpirtoline (0.063-0.25 mg/kg, p.o.) substituted completely for F and E, resp., showing that the discrimination is based on the effects of 5-HT_{1A} versus 5-HT_{1B} receptor activation. Mianserin (1.0-12.0 mg/kg, p.o.) did not substitute for either F or E. Buspirone (1.0-30.0 mg/kg, p.o.) partially substituted for F, probably due to its partial 5-HT_{1A} receptor agonistic character.

This research was sponsered by Solvay-Duphar B.V., Weesp, The Netherlands.

623.8

ACUTE EFFECTS OF DEXFENFLURAMINE ON RHESUS MONKEY PERFORMANCE IN AN OPERANT TEST BATTERY. M.G. Paule, M.P. Gillam, J. Gossett and D.L. Frederick*. Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079.

The acute effects of dexfenfluramine (d-FEN), an anorectic drug believed to facilitate serotonergic neurotransmission, were assessed in rhesus macaques using behavior in several complex food-reinforced tasks designed to model aspects of time estimation, short-term memory, motivation, learning, and color and position discrimination. The endpoints monitored included measures of response rate and accuracy (or breakpoint for the motivation task). The acute effects of d-FEN (0.0-1.75 mg/kg; i.m.) manifest as dose-dependent disruptions in all tasks, but disruptions in the motivation and time estimation tasks occurred at a dose (1.0 mg/kg) below those affecting performance in all other tasks. Learning task performance appeared to be the least sensitive to d-fen, while the short-term memory and color and position discrimination tasks were intermediate in sensitivity. In both the learning and color and position discrimination tasks, d-FEN decreased response rate at a dose or doses that did not decrease accuracy, suggesting that these functions remained intact even at doses that affected the motoric components of task performance. These observations support the contention that d-FEN is an effective anorectic agent in the monkey (i.e., the task assessing motivation to work for food reinforcers is one of the most sensitive).

Supported by FDA/NCTR

623.9

THE ANTIEMETIC EFFECT OF THE R- AND S-ENANTIOMERS OF ONDANSETRON AGAINST CISPLATIN AND MORPHINE-INDUCED EMESIS IN THE FERRET. R.H. Wong*, A. Carter, G. Haynes, P.D. Thut, R.L. Wynn, J.R. McCullough, and T.P. Jerussi. Department of Family Medicine, School of Medicine, and Department of Pharmacology, Dental School, University of Maryland, Baltimore, MD 21201, and Sepracor, Inc., Marlborough, MA 01752.

Ondansetron possesses an asymmetric carbon center which imparts chirality to the molecule. Previous studies with other antiemetics have shown differences in the pharmacological properties between the racemate and the constituent enantiomeric chemical pairs. This study examined the antiemetic effect of the R- and S-enantiomers of ondansetron against cisplatin and morphine-induced emesis. Ferrets were injected i.v. with cisplatin 10 mg/kg. After 30 min, the animals were injected i.v. with a test compound (racemic ondansetron or the R- or S-enantiomer; n=5/drug). For morphine-induced emesis, ferrets were injected i.v. with a test compound 5 min prior to 0.3 mg/kg morphine s.c. The results indicated that R-ondansetron was approximately 9-times more potent than the S-enantiomer against cisplatin-induced emesis. Moreover, R-ondansetron was more potent and showed greater efficacy against morphine-induced emesis than the S-form which was lethal at higher doses. These results indicate that the enantiomers of ondansetron inhibit cisplatin and morphine-induced emesis in a graded dose response manner, with the R-isomer being the most potent and most effective antiemetic drug. Support provided by Sepracor, Incorporated.

623.10

FLESINOXAN'S ANTIDEPRESSANT PROPERTIES IN VARIOUS FORCED SWIM TEST PARADIGMS. J.A.M. van der Heyden*, J. Mos and B. Olivier. Solvay Duphar b.v., Dept of Pharmacology, P.O. Box 900, 1380 DA Weesp, The Netherlands

The 5-HT_{1A} receptor has been found an important locus for the action of antidepressant treatment. In putative animal depression models 5-HT_{1A} agonists are highly active. Here we describe the effect of flesinoxan in the forced swim test, using several protocols.

Flesinoxan potently reduces the immobility in the forced swim test under standard conditions (i.e. swim period followed by two drug administrations and a final swim test session). The full agonists (flesinoxan., 8-OH-DPAT) reduced immobility over the entire 6 minute test period, whereas the partial agonists (buspirone, ipsapirone), like classic antidepressants, only reduced the immobility in the first three minute period. This reduction of immobility in the entire 6 minute period cannot be attributed to stimulation of motor activity. Flesinoxan has a good duration of action. The ED_{8}- value (dose needed to reduce immobility by 8 seconds) calculated 4 hrs after the second drug administration only decreased a factor 3 over a four hour period. Tolerance for the effect of flesinoxan in the forced swim test did not occur, since a subchronic 1 week pretreatment with flesinoxan (1 mg/kg po once daily) did not affect the efficacy of the drug in the swim test after this period. Flesinoxan and imipramine were also tested in a deviant protocol of the forced swim test in which the compound was administered only once before the two consecutive swim sessions. Flesinoxan was active 24 hours after a single administration in contrast to the classic antidepressant imipramine. Similar effects were found using this deviant swim protocol if the animals were treated with the drugs for 4 consecutive days.

In conclusion, flesinoxan shows a clearcut dose-dependent and long-lasting effect in the forced swim test in rats, on basis of which it is predicted to possess antidepressant activity in man. Its profile is clearly different from the classic tricyclic antidepressants.

Solvay Duphar

623.11

VARYING THE TRAINING DOSE OF 8-OH-DPAT FACILITATES CHARACTERIZATION OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF 5-HT_{1A} SELECTIVE COMPOUNDS WITH MIXED AGONIST/ANTAGONIST ACTIVITY. M. C.Wolff and J. D. Leander*. Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285.

Pigeons were trained to discriminate either 0.16 mg/kg (low dose) or 0.64 mg/kg of a selective 5-HT_{1A} agonist, 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT), from saline. In the low dose condition, the dose response curve for the training drug was shifted to the left (ED_{50} = 0.04 mg/kg) with respect to the high dose condition (ED_{50} = 0.14 mg/kg). A number of 5-HT agonists, partial agonists and putative antagonists were tested for stimulus generalization. Compounds, such as buspirone, which engendered only partial discrimination in the high dose condition, produced full discrimination in the low dose condition. Some compounds, such as (-)pindolol, which completely antagonized the 8-OH-DPAT cue in pigeons trained with the higher dose, produced full generalization in pigeons trained to recognize the lower training dose. Other compounds, such as WAY100635 and LY206130, completely antagonized the 8-OH-DPAT cue in both groups without producing 8-OH-DPAT-like responding in either group. Thus the training dose of 8-OH-DPAT determines whether some compounds will appear to discriminate as full agonists, partial agonists, or antagonists of 8-OH-DPAT. Using a relatively low training dose of 8-OH-DPAT may facilitate detection of the agonist properties of mixed 5-HT_{1A} agonists/antagonists.

623.12

EFFECTS OF 5-HT RECEPTOR ANTAGONISTS GR127935T (5-$HT_{1B/1D}$) AND MDL 100,907(5-HT_{2A} ON LEARNING. A. Meneses* & E. Hong. Terapéutica Experimental, Depto. Farmacología y Toxicología, CINVESTAV-IPN. Ap. Postal 22026, México, D.F., 14000. MEXICO.

It has been previously reported that TFMPP, mCPP, 1-NP and mesulergine impaired the consolidation of learning, whereas, DOI or ketanserin improved it. In this work, we tested the effects of GR127935T (GR1) and MDL 100,907 (MDL) plus the afore-mentioned drugs in the autoshaping learning task. The results showed that the post-training injection of the 5-$HT_{1A/1B/1D}$ receptor agonist GR46611X (GR4) (1-10 mg/Kg) decreased learning, whereas GR1 (1-10 mg/Kg) increased it, the latter being reversed by PCA pretreatment. GR1 eliminated the decrement induced by GR4, TFMPP and mCPP. Moreover, MDL (0.1-3.0 mg/kg) had effect by itself and eliminated the effect of DOI, ketanserin, TFMPP, mCPP, 1-NP and mesulergine but did not modify the increase in learning induced by 8-OH-DPAT Therefore, it seems that presynaptic stimulation of 5-$HT_{1B/1D}$ receptors impaired the consolidation of learning, whereas their blockade improved it. Additionally, 5-HT_{2A} receptors might be also involved in the consolidation of learning. Supported by CONACYT grant 4367-M9406.

623.13

5-HT_{1A} RECEPTOR AGONISTS AND SILENT ANTAGONISTS REVERSED THE DECREMENTS IN LEARNING INDUCED BY DIZOCILPINE AND SCOPOLAMINE E. Hong*, & A. Meneses Terapéutica Experimental, Depto. Farmacología y Toxicología, CINVESTAV-IPN. Ap. Postal 22026, México, D.F., 14000. MEXICO.

We have previously reported that the 5-HT_{1A} receptor agonist 8-OHDPAT (DPAT) enhanced consolidation of learning, whereas the silent 5-HT_{1A} receptor antagonists WAY 100635 (WAY) or S-UH-301(S-UH) had no effect by themselves. In this work, the effect of post-training injection of DPAT, WAY and S-UH plus dizolcipine (DIZ) or scopolamine (SCO) was determined in autoshaping learning task, which consisted of illumination of a retractable lever for 8 sec (conditioned stimulus, CS) followed by the delivery of a food pellet (US) every 60 sec. If the animal pressed the lever (conditioned response, CR) the trial was shortened and the lever was retracted, the light was turned off and the US was delivered. The results showed that both 5-HT_{1A} stimulation (by DPAT) and blockade (by S-UH) reversed the learning deficit induced by DIZ and SCO, whereas WAY reversed only the effect of SCO, but not that of DIZ. These data confirm the role of 5-HT_{1A} receptors in learning and might further suggest a possible interaction of cholinergic systems with 5-HT_{1A} receptor function. Supported by CONACYT grant 4367-M9406

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION IV

624.1

ESTROGEN EFFECTS ON GALANIN IMMUNOREACTIVITY IN THE GNRH NEURONS IN FEMALE MICE. G. V. Rajendren* and M. J. Gibson. Div. Endocrinology, The Mount Sinai Medical Center, New York, NY 10029.

Galanin is reported to be present in a significant proportion of GnRH neurons in the rat. Expression of galanin immunoreactivity in GnRH neuronal cell bodies reaches its peak in female rats when circulating levels of estrogen are high. The present experiments were undertaken to investigate the role of estrogen in the expression of galanin immunoreactivity in GnRH neurons in female mice. The females were ovariectomized (OVX) and either untreated, or given subcutaneous implants of 5 μg estradiol (E_2) to induce a strong feedback inhibition on LH secretion. A group of cycling females in proestrus were also included in the studies. Two representative coronal sections were selected from each mouse, one passing through the anterior septum and the other containing the organum vasculosum of lamina terminalis (OVLT), for analysis using a confocal laser scanning microscope. The GnRH cell bodies in these sections coexpressing galanin immunoreactivity, and those which were in close contact with galanin immunoreactive fibers were counted and compared. In the presence of strong steroid inhibition (OVX-E_2) less than 7% of GnRH neurons colocalized galanin, while in the other groups 30-37% of the GnRH cells were also immunoreactive for galanin. In contrast, GnRH cells in all groups were closely associated with galanin immunoreactive structures, presumably synapses, with no apparent differences among the groups. GnRH neurons receiving synaptic contacts from galanin immunoreactive fibers were particularly abundant (74-92%) at the level of the OVLT. Thus, galanin immunoreactivity in GnRH neurons during E_2 inhibition of LH release was suppressed, in contrast to facilitation of galanin immunoreactivity in GnRH neurons during a period of enhanced LH release (proestrus or OVX). This suggests that galanin may be involved in the steroid modulation of the GnRH system. Supported by HD 19077.

624.2

BASIC FGF IS A MAJOR NEUROTROPHIC SIGNALING AGENT DURING LHRH NEURON-ASTROGLIA INTERACTIONS:bFGF PRIMING SENSITIZES LHRH NEURONS TO GROWTH FACTOR NEUROTROPHIC EFFECTS. F. Gallo[1]*, [2]R. Avola, A. Beaudet[3], and B. Marchetti[1,4]. Depts. of [1]Pharmacol. and [2]Biochem, Catania Univ., [3]Montreal Neurological Institute, Montreal, Quebec, Canada, and [4]OASI Institute for Research and Care (IRCCS) Mental Retardation and Brain Aging Troina (EN), Italy

Luteinizing hormone-releasing hormone (LHRH) neurons play a pivotal role in the neuroendocrine control of mammalian reproduction. We have recently established an in vitro model system to study LHRH neuron-astroglial interactions and shown the presence of a bidirectional informative network between astroglial cells in primary culture and the immortalized hypothalamic LHRH neuronal cell line (GT_{1-1} subclone), that displays highly differentiated properties of LHRH neurons (Endocrine 3: 863, 1995). In the present work we have characterized at a morphological and biochemical levels the neurotrophic effects exerted by different growth factors during neuronal differentiation, with the aim to elucidate a. whether certain growth factors are capable to directly exert neurotrophic and functional effect without glia intermediacy; b. whether there is a synergism/cooperation between growth factors in the induction of neuronal differentiation; and c. whether a specific growth factor participates in LHRH neuron-astroglia crosstalk. Treatment of GT_{1-1} neurons for 24 hrs with bFGF exerted the most potent neurotrophic effects compared to epidermal growth factor (EGF), insulin (Ins), and insulin-like growth factor (IGF-I). Specificity of bFGF was underscored by a. the ability of a submaximal priming dose of bFGF to markedly potentiate EGF, IGF-I and Ins neurotrophic effects and b. the almost complete inhibition of astroglia neurotrophic effects after neutralization of endogenous bFGF action during GT_{1-1} neuron-astroglia interactions. EGF, Ins and IGF-I significantly affected LHRH release, bFGF was inactive alone, but induced a marked down-regulation of GFs-induced spontaneous LHRH release upon priming. All together these informations point to bFGF as a candidate intercellular signaling agent coordinating neuron-astroglia activity and suggest that bFGF in partnership with LHRH participates in the regulation LHRH release.

624.3

MODULATION OF LUTEINIZING HORMONE BY ANGIOTENSIN II AND GABAERGIC SYSTEM IN THE PREOPTIC MEDIAL AREA. R.C.M.Dornelles; C.R.Franci*;Faculty of Medicine of Ribeirão Preto,University of São Paulo, Brazil,14049 900.

Luteinizing hormone (LH) secretion in female rats is controled by several factors, including γ aminobutiric acid (GABA) and angiotensin II (AII). Medial preoptic area (MPOA) contains LH-releasing hormone (LHRH) neurons which are stimulated by AII and receive gabaergic inputs. There are also estrogen-sensitive neurons in this area although is still unkown if this steroid can modulate AII and/or GABA effects on LH secretion. The aim of this study was investigate the interaction of AII, GABA and estrogen (E_2) on LH secretion. Adult ovariectomized rats with cannula into the MPOA received subcutaneous injection of estradiol benzoate (EB) or vehicle (V) during 3 days. Jugular vein was cannulated one day before the experiment. Blood sample was collected 10 minutes before microinjection of saline, GABA agonists (muscimol, 5 nmol or baclofen, 10 nmol) or GABA antagonists (bicuculline, 60 pmol or phaclofen, 120 pmol) in the MPOA. Ten minutes after this first microinjection, saline or AII (100 pmol) were microinjected in the same area and blood samples for LH radioimmunoassay were collected immediately before and 10,20,30 and 60 minutes after. GABA agonists and antagonists decrease by themselves the plasma LH in the control group (V) but they had no effect in the EB group.The increase of plasma LH induced by AII in the EB group was blocked by GABA agonist and $GABA_A$ antagonist (bicuculline) but not by the $GABA_B$ antagonist (phaclofen).Thus, the inhibition of LH secretion by MPOA-GABA system as well as the interaction of AII-GABA system only occur when E_2 is in the milieu.

Supported by CAPES and FAPESP.

624.4

CHANGES IN GLUTAMATE RECEPTOR SUBTYPE mRNA EXPRESSION IN GONADOTROPIN-RELEASING HORMONE (GnRH) NEURONS DURING THE SEXUAL MATURATION OF THE FEMALE RAT. Ozhan Eyigor* and Lothar Jennes. Dept. Anatomy & Neurobiology, Univ. Kentucky, Coll. Medicine, Lexington, KY 40536.

Excitatory amino acids, particularly glutamate, have been associated with the development of puberty in the rat. We have previously shown that, in the adult female rat, GnRH neurons express preferentially the KA_2 receptor subtype. The aim of the present study was to determine if the onset or rate of KA_2 receptor expression in GnRH neurons can be correlated with the onset of puberty. Dual *in situ* hybridization using digoxigenin labeled GnRH cRNA probes and ^{35}S labeled KA_2 receptor probes, followed by the autoradiography and image analysis was used to measure KA_2 receptor mRNA content in 15-50 days old female rats which were sacrificed at 0800 or 1700 hours. The results show that: a) GnRH neurons of 40 days old animals have the highest KA_2 mRNA content, b) in 20-40 days old animals more KA_2 mRNA is present in GnRH neurons at 0800 when compared to 1700 hrs, while in 45 and 50 days old animals more KA_2 mRNA is present in GnRH neurons during the afternoon. Since the timing of highest KA_2 receptor mRNA content in GnRH neurons as well as the reversal of diurnal rhythmicity in KA_2 receptor mRNA content coincide with vaginal opening, it is suggested that glutamate, acting through KA_2 receptors on GnRH neurons is an important factor in the development of puberty. Supported by NIH HD 24697.

624.5

DENDRITIC REMODELING OF ARCUATE NEUROENDOCRINE NEURONS FOLLOWING ORCHIDECTOMY. S.C. Danzer*, N.T. McMullen and N.E. Rance. Depts. of Cell Biology and Anatomy, and Pathology, U. of Arizona, College of Medicine, Tucson, AZ 85724.

A combination of retrograde labeling with systemically injected Fluorogold and intracellular injection of neurons in a fixed slice preparation was used to examine the morphology of neuroendocrine neurons in the rat arcuate nucleus. The same preparation was used to study the effects of orchidectomy on arcuate neuronal morphology. Intracellulary-filled neuroendocrine neurons (8-21 neurons per brain) from intact (N=5) and orchidectomized (N=5) animals were reconstructed in three dimensions using computer assisted microscopy. The morphology of arcuate neuroendocrine neurons in intact animals was relatively simple, with the majority of neurons (79%) having only two primary dendrites and a moderate number of dendritic spines. In comparison to intact controls, arcuate neuroendocrine neurons in the orchidectomized group had significantly larger somatic profile areas and exhibited significant increases in dendrite length, dendrite volume, terminal branch number, and spines per unit length of dendrite. The majority of the increase in terminal branch number in orchidectomized animals was due to the appearance of short (less than 50 μm) branches, giving a striking, claw-like appearance to many of the dendrites. These results provide the first evidence for hormonal regulation of dendritic morphology of arcuate neuroendocrine neurons in adult mammals. (Supported by NIH Grant AG09214 and the Flinn Foundation of Arizona)

624.6

HORMONE REPLACEMENT SUPPRESSES NEUROKININ B (NKB) GENE EXPRESSION IN THE ARCUATE NUCLEUS OF OVARIECTOMIZED MONKEYS. T.W. Abel, M.L. Voytko and N.E. Rance* Department of Pathology, University of Arizona College of Medicine, Tucson, AZ 85724 and Department of Comparative Medicine, Bowman Gray School of Medicine, Winston-Salem, NC, 27157.

Human menopause is associated with increased tachykinin gene expression in the arcuate nucleus (Rance and Young, *Endocrinology* 128:2239, 1991). We have hypothesized that these changes are secondary to ovarian failure and not due to age per se. To test this hypothesis, we determined the effects of hormone replacement upon NKB neurons in the arcuate nucleus of young ovariectomized cynomolgus monkeys. Arcuate proopiomelanocortin (POMC) gene expression was also assessed. Twenty-two months after ovariectomy, monkeys were treated with either continuous physiologic levels of estrogen (E_2, n = 8), estrogen plus progesterone (E_2P, n = 8) or left untreated (n = 8). The animals were sacrificed 30 months later. Every 20th section was hybridized to synthetic radiolabeled 48-base cDNA probes complementary to either NKB or POMC mRNA. Two sections were chosen from each animal for cell counts. In the untreated group, there were 18.3 ± 5.0 (mean ± SEM) neurons expressing NKB mRNA per arcuate section. Hormone replacement of either E_2 or E_2 P resulted in undetectable levels of NKB gene transcripts. In contrast, the number of POMC neurons was unchanged among treatment groups (untreated: 101.2 ± 7.8; E_2: 103.8 ± 21.3; E_2P: 95.6 ± 18.1 neurons/arcuate section). These results indicate that steroid hormones modulate levels of NKB mRNA in the primate hypothalamus and support the hypothesis that the increased tachykinin gene expression in postmenopausal women is due to steroid withdrawal. (supported by NIH AG-09214)

624.7

SEXUAL DIFFERENCES AND THE EFFECTS OF GONADECTOMY ON EXPRESSION OF FOS PROTEIN IN TYROSINE HYDROXYLASE IMMUNOREACTIVE (TH-IR) NEURONS LOCATED IN SUBDIVISIONS OF THE RAT HYPOTHALAMIC ARCUATE NUCLEUS. S. Cheung*, K.E.Moore and K.J.Lookingland. Dept. of Pharm & Toxicol., Michigan State University, East Lansing, MI 48824

Sexual differences in the activity of tuberoinfundibular dopamine (TIDA) neurons are due to circulating gonadal steroids; i.e. estrogen stimulates, whereas testosterone inhibits neurochemical estimates of the activity of TIDA neurons terminating in the median eminence. Expression of the immediate early gene product FOS also reflects TIDA neuronal activity and permits the determination of the responsiveness of subpopulations of these neurons located in subdivisions of the arcuate nucleus (ARC). In the present study sexual differences and effects of gonadectomy were examined on FOS protein expression in TH-IR neurons in the dorsomedial (DM) and ventrolateral (VL) ARC using dual immunohistochemistry. Frontal sections through the middle ARC were chosen macroscopically, and the number of TH-IR cells and TH-IR + FOS-IR double labeled cells were counted bilaterally. There were no gender differences in the number of TH-IR cells in either the DM- or VL-ARC, but in the DM-ARC there were more double-labeled cells in females vs. males. Ovariectomy decreased the number of TH-IR cells containing FOS-IR in the DM-ARC, but not in the VL-ARC. Orchidectomy increased the number of TH-IR cells containing FOS-IR in both the DM-ARC and VL-ARC. These results reveal that sexual differences in FOS protein expression in subpopulations of TIDA neurons in the DM- and VL-ARC are due to circulating gonadal hormones. (Supported by NIH Grant MH42802)

624.8

ESTROUS EWE INTRODUCTION INCREASES FOS EXPRESSION IN A SUBSET OF GONADOTROPIN-RELEASING HORMONE-CONTAINING NEURONS IN THE MEDIAL BASAL HYPOTHALAMUS OF THE MALE SHEEP. R. Boukhliq, R.L. Goodman*, and B.A. Adrian. Physiology Department, West Virginia University Morgantown WV 26506-9229

Previous work, using the early immediate gene product, Fos, demonstrated that a subset of gonadotropin-releasing hormone (GnRH) neurons in the ovine medial basal hypothalamus (MBH) play a role in the increase in pulsatile LH secretion that occurs in response to an opioid antagonist. In this study, we examined the physiological significance of this pharmacological model, by testing the hypothesis that a physiological stimulus that increases pulsatile LH secretion will also induce Fos in MBH GnRH neurons. The specific physiological stimulus used was exposure of intact rams to estrous ewes, which is known to induce pulsatile LH release in the males. Seven rams were preconditioned by isolation from ewes for several weeks during the non-breeding season. Five of the rams were exposed to estrous ewes and 2 controls were exposed to another intact male. Rams were bled every 12 min for 4 hr before and 2 hr after introduction of the ewes (or ram) and then killed with an overdose of pentobarbital. After fixation, brains were removed, sectioned, and dual immunostained for GnRH (LR-1 antibody) and Fos (c-Fos K-25 antibody from Santa Cruz, Inc). Exposure of rams to estrous ewes induced a rapid increase in pulsatile LH secretion that lasted at least 2 hr and Fos was found in GnRH neurons in the MBH of all 5 rams. Although the percentage of Fos-containing GnRH neurons was low (5.9 ± 1.5 %), more than 80% of these double-labeled cells were found in the rostral MBH, just posterior to the optic chiasm. No GnRH neurons in this area contained Fos in 2 control rams and almost no GnRH neurons in more rostral areas contained Fos in any of the sections examined from all rams (3 out 2,605 GnRH neurons). These data suggest that a subset of GnRH neurons in the rostral MBH are involved in stimulation of episodic LH secretion following exposure of rams to estrous ewes.(NIH HD17864)

624.9

SEXUAL DIFFERENTIATION OF SYNAPTIC INPUT TO GNRH NEURONS IN SHEEP: ROLE OF PRENATAL TESTOSTERONE. S-J. Kim[1], D.L. Foster[2]*, R.J. Wood[1]. [1]Dept of Ob/Gyn, Yale University, New Haven, CT 06520; [2]Depts of Ob/Gyn and Biology, Univ. Michigan, Ann Arbor, MI 48109.

In sheep, the control of tonic and surge GnRH secretion is sexually differentiated by testosterone during prenatal development. However, despite marked sex differences in the control of GnRH release, there are no differences between male and female lambs in the number, distribution or gross morphology of GnRH neurons. Therefore, this study tested the hypothesis that prenatal steroids influence the synaptic input to GnRH neurons. The approach was to compare the number of synapses on GnRH neurons from male, female and androgenized female lambs (n=5 each). Androgenized female lambs were exposed to testosterone prenatally from 30-90 days of gestation (145 days is term). Gonadectomized, postpubertal lambs were perfused at one year of age with 4% paraformaldehyde containing 0.2% glutaraldehyde. GnRH neurons were visualized using the LR-1 antibody (gift of R. Benoit). Individual neurons were punched from the rostral preoptic area at the level of the organum vasculosum of the lamina terminalis for observation at the EM level. Five GnRH neurons were selected from each animal. The number of afferent synapses on each neuron was counted in a single section passing through the plane of the nucleus. GnRH neurons from female lambs received approximately twice as many contacts (2.75±0.3 synapses per neuron) as those of male lambs (1.56±0.2), similar to a previous study performed in rats (*Endocrinology*, 126: 695-702, 1990). In addition, the number of synapses on GnRH neurons from androgenized female lambs resembled that of male lambs, suggesting that prenatal steroids give rise to sex differences in synaptic input to GnRH neurons. (Supported by USDA CSRS 92-02629).

624.10

PHOTOPERIOD ALTERS THE NUMBER OF ESTROGEN RECEPTOR (ER), NEUROPEPTIDE Y (NPY) AND ß-ENDORPHIN (ßE)-IMMUNOREACTIVE NEURONS IN THE EWE HYPOTHALAMUS. D.C. Skinner, R.G. Dyer*, and A.E. Herbison. Dept. Neurobiology, Babraham Institute, Cambridge, UK.

The reproductive activity of ewes fluctuates markedly with season in response to photoperiodic cues. The neural mechanisms driving the reproductive axis during the breeding season and in anestrus are not well established but appear to involve changes in both gonadal steroid-dependent and -independent components. As gonadotrophin -releasing hormone (GnRH) neurons do not to express nuclear ERs, estrogen (E) may act indirectly to influence their activity. The present study has used immunocytochemistry to examine whether differences exist between breeding and anestrous ewes in the number of cells containing ERs and/or the neurons synthesizing neurotransmitters implicated in the regulation of GnRH neurons. The breeding season was induced by short day (SD; 8L:16D) and anestrus by long day (LD; 16L:8D) artificial photoperiods. Reproductive status was confirmed by progesterone analysis of bi-weekly blood samples and ewes were killed and perfused in SD/LD pairs (n=6). SD ewes were taken in the mid-luteal phase. Perfusion fixed brains were sectioned and immunostained for ER, tyrosine hydroxylase (TH), NPY and ßE. The cellular density of ER-expressing cells was decreased by approx. 20% ($P<0.05$) in the preoptic area of LD ewes compared with SD animals but did not change in the ventromedial and arcuate nuclei (ARN). No differences were detected in the number of TH neurons within the A_{12}, A_{14} or A_{15} cell groups or in the percentage of TH cells containing ERs (A_{12}:13-17%; A_{14}:15-20%; A_{15}:0%). The number of ßE neurons within the ARN was reduced by 35% ($P<0.05$) in SD compared with LD animals while a 3-fold increase ($P<0.05$) in the number of NPY immunoreactive neurons was evident within the median eminence (ME), but not ARN, of LD ewes. Around 3 and 7% of NPY cells express ERs in the ME and ARN respectively, and this did not change with season. These results demonstrate that the number of ER-expressing cells within the preoptic area is increased during anestrus while reduced ßE, and elevated NPY, -immunoreactive cell numbers within the ARN and ME are associated with reproductive activity in the breeding season.

624.11

MODULATION OF NEUROPEPTIDE Y Y1 RECEPTOR (NPY1R) GENE EXPRESSION IN THE MALE RAT ANTERIOR PITUITARY BY GONADAL STEROIDS. J.H. Urban*, D.M. Gelinas, J.E. Levine. Dept. Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

We have shown that NPY increases luteinizing hormone releasing hormone (LHRH) release from hypothalamic explants in a steroid-dependent manner. Other reports have shown that the actions of NPY on the stimulation of LH secretion from the pituitary is dependent upon steroid levels as well. Ineffectiveness of NPY on LHRH or LH release in tissue from castrated rats is likely due to changes in NPY receptor levels or signalling systems. The present studies were designed to examine whether the expression of the NPY1r gene in the pituitary of male rats is regulated by gonadal factors, namely testosterone (T). Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to quantify NPY1r gene expression in RNA extracted from the anterior pituitaries of males that were either castrated (14 d), sham-operated or sham-operated and received physiological levels of T. NPY1r gene expression was quantified as a ratio of NPY1r/tubulin for 4 different dilutions of the RNA, and then expressed as fold change from the sham-operated controls. After castration, there was a significant decrease in the levels of NPY1r gene expression in the anterior pituitary (intacts: 1.00 relative units; castrates: 0.339 $\pm$ 0.170 relative units, $p < 0.05$). This decrease was prevented by treatment with physiological levels of T (0.845 $\pm$ 0.090 relative units). These studies demonstrate that in the male rat, the expression of the NPY1r gene is regulated by T. Work is continuing to map the distribution of NPY1r in the pituitary, and to further understand how the transcriptional regulation of this receptor contributes to alterations in physiological responsiveness. (Supported by AHA-MC Grant-in-Aid; NIH HD20677, HD219121, HD28048)

624.12

HUMAN LUTEINIZING HORMONE-RELEASING HORMONE-SYNTHESIZING NEURONS RECEIVE NEUROPEPTIDE-Y IMMUNOREACTIVE INNERVATION. **B. Dudás[1], E. Dobó[1], I. Merchenthaler[2] and Zs. Liposits[1]*. [1]Dept. of Anatomy, Albert Szent-Györgyi Medical University, Szeged, Hungary, and [2]Women's Health Research Institute, Wyeth-Ayerst Research, Philadelphia, USA.**

The luteinizing hormone response to luteinizing hormone-releasing hormone (LHRH) is potentiated by neuropeptide-Y. In the present study, we provided morphological evidence for this putative communication in the human prosencephalon by means of double labeling immunocytochemistry utilizing diaminobenzidine (DAB) and silver-intensified DAB chromogens.

This study first evaluated the distribution of the LHRH and NPY-immunoreactive (IR) neuronal elements and then analized the interrelationship of the two peptidergic systems in overlapping areas. The LHRH-IR perikarya were located mainly in the preoptico-septal, diagonal band of Broca, lamina terminalis, medial preoptic and infundibular areas of the brain. NPY-containing perikarya and fibers were widely distributed in the prosencephalon with high densities in the preoptico-septal, periventricular and infundibular regions. The overlap between the NPY and LHRH systems was apparent in the medial preoptic and infundibular regions. In this loci, brown DAB-labeled LHRH-IR cells received NPY-IR axon terminals marked by black silver deposits. The putative innervation of LHRH neurons by NPY-IR fibers was also confirmed in 1 µm thick plastic sections by demonstrating juxtapositions.

The present findings suggest synaptic regulation of hypophysiotropic LHRH-synthesizing neurons by the NPY system in the human prosencephalon.

624.13

TIME COURSE OF THE EFFECT OF OLFACTORY BULBECTOMY TO INCREASE SERUM FSH LEVELS IN MALE GOLDEN HAMSTERS David R. Pieper* and Catherine Lobocki Providence Hospital, Department of Physiology, Southfield, MI, 48037

Bilateral removal of the olfactory bulbs (BX) increases tonic reproductive hormone secretion in male golden hamsters and prevents the testicular regression associated with short photoperiod. The objective of the present study was to study the time course of the facilitatory effect of BX on FSH secretion of hamsters on long photoperiod.

In Experiment I, 12 BX and 12 sham operated (SH) adult male golden hamsters maintained on long (LD 14:10) photoperiod were implanted with atrial catheters and bled at 4, 8, 24, 48hr and at 1 and 2 weeks after surgery. In Experiment II, 12 BX and 12 SH adult male hamsters were bled weekly from the orbital sinus for 11 weeks.

In Experiment I, serum FSH was similar in BX and SH groups at all time points examined. In Experiment II, SH and BX hamsters also had similar FSH levels for the first 3 weeks following surgery, but thereafter serum FSH was much higher in BX than SH animals at all time points ($p < 0.001$).

In summary, the increase in serum FSH secretion following removal of the olfactory bulbs takes over 3 weeks to become evident. There are various interpretations to these results. The delayed effect of BX may be related to neuronal degeneration of axons to or from the bulbs, the delay may be related to the trauma of the surgery, or the tonic modulatory influence of the olfactory bulbs on reproductive hormone secretion may simply take some time to become evident following removal of the bulbs.

(This research was supported by the Providence Hospital Dept of Philanthropy)

624.14

EFFECTS OF CASTRATION AND TESTOSTERONE REPLACEMENT ON THE COLOCALIZATION OF ESTROGEN RECEPTORS AND AROMATASE IN THE QUAIL BRAIN. A. Foidart*, M. Houbart, N. Harada and J. Balthazart. Lab. Biochemistry, Univ. Liège, Belgium & Molecular Genetics, Toyoake, Japan

In Japanese quail, castration decreases (50-80%) and testosterone (T) treatment restores aromatase activity (AA) in the preoptic area. These effects are paralleled by variations in the number and size of the aromatase-immunoreactive (ARO-ir) cells in the medial preoptic nucleus (POM) but the medial and lateral portions of the nucleus differentially react to the treatments. T-induced changes in AA and ARO-ir cells are ultimately caused, at the cellular level, by estrogens produced by aromatization (positive feedback of the product on its enzyme). This is namely demonstrated by the fact that T no longer affects aromatase in the presence of aromatase inhibitors but, surprisingly, immunoreactive estrogen receptors (ER) are present in only a small fraction (<20%) of ARO-ir cells in the POM . We therefore wondered whether the few ARO-ir cells that are still observed in castrates are those that also contain ER (ARO+ER-ir). Effects of castration (CX) associated or not with T replacement (CX+T) on the number of ARO-ir cells colocalizing with ER were studied in the POM and in the ventromedial nucleus (VMN) of the hypothalamus selected as a control region where the colocalization is more frequent. Because anatomical heterogeneity had been detected in the reaction to endocrine changes of the POM ARO-ir cells, the distribution in space of the ARO+ER-ir cells was also analyzed. This study first confirmed the low degree of colocalization of ARO and ER in the POM (<20% of the ARO-ir cells) compared to the VMN (30-70%). Castration markedly decreased the number of ARO-ir cells in both nuclei. A proportional decrease in the number of ARO+ER-ir cells was observed in the POM but this decrease was more limited in the VMN so that castration did not affect the percentage of colocalization in the POM but increased it in the VMN. In the POM of gonadally intact and CX+T males, colocalized cells were mainly observed in the dorsolateral part of the nucleus. By contrast, this peak of frequency was seen in the medial POM after castration. All effects of castration were reversed by T treatment. These data indicate that T (or its metabolite E2) differentially affect ARO-ir and ARO+ER-ir cells in the VMN but not in the POM. In the POM, however, ARO+ER-ir cells are preferentially maintained in the medial part of the nucleus after castration which indicates a differential control by steroids.

Supported by NIMH 50388, FRFC 2.9003.91, AC 93/98-171, EC-CT94-0472.

624.15

INTRACRANIAL IMPLANTS OF THE ANTI-ESTROGEN ICI 182,780 ON VASOPRESSIN EXPRESSION IN THE BED NUCLEUS OF THE STRIA TERMINALIS. W.C.J. Chung, C.J. Auger, G.J. De Vries*. Neuroscience and Behavior program, University of Massachusetts, Amherst, MA 01003-7710.

Vasopressin-immunoreactive (AVP-ir) neurons in the bed nucleus of stria terminalis (BST) and medial amygdaloid nucleus are very responsive to gonadal hormones. After gonadectomy, these neurons lose their AVP immunoreactivity. Testosterone treatment reverses these changes. Estrogen receptor-mediated mechanisms appear to be crucial for this effect, because AVP expression in castrated rats can be stimulated by peripheral administration of estradiol but not by dihydrotestosterone alone. Here we test the idea that estrogen acts directly on estrogen receptors within the BST by implanting the antiestrogen, ICI 182,780 (ICI), into the brain. Intact males were implanted bilaterally with 30-gauge cannulae aimed at the BST. The cannula on one side of the brain was filled with ICI, the other was filled with cholesterol or kept empty. Four weeks after implantation, the animals were perfused and their brains were processed for AVP immunocytochemistry. The number of AVP-immunoreactive cells in the BST was significantly lower on the ICI side than at the cholesterol or blank side ($p<0.005$). There were no differences in the number of cells between animals with blanks or cholesterol implants. ICI implants eliminated virtually all AVP-immunoreactivity of cells that were within a radius of about 300 μm of the cannula tip, but had a much smaller impact on cells outside that radius. Intracerebral implants of ICI may therefore be useful to study local effects of testosterone on brain mechanisms.

This study was funded by NSF grant, IBN 9421658 to GJD.

624.16

CHARACTERIZATION OF BRAINSTEM ESTROGEN-RECEPTIVE NEURONS PROJECTING TO THE VICINITY OF THE GONADOTROPHIN-RELEASING HORMONE (GnRH) CELL BODIES IN THE RAT. A.E. Herbison* and S.X. Simonian. Laboratory of Neuroendocrinology, The Babraham Institute, Cambridge CB2 4AT, UK.

Brainstem noradrenergic (NA) neurons exert an important estrogen-dependent permissive influence on the electrical and synthetic activity of GnRH neurons. The present study has used double- and triple-labelling immunocytochemistry combined with retrograde labelling techniques to examine the relationship of estrogen receptor (ER), NA and neuropeptide Y (NPY) expression amongst brainstem cells and determine which of these neurons project to the rostral preoptic area. **Exp.1** Brainstem sections from ovariectomized rats (n=6) immunostained for the ER (monoclonal ID5) and dopamine-b-hydroxylase (DBH) revealed that ER-expressing NA neurons were located exclusively in the caudal-most aspects of medulla where 30% of A1 and 60% of A2 neurons were immunoreactive for the ER. **Exp.2** Sections from colchicine-treated, ovariectomized rats (n=5) immunostained for NPY, tyrosine hydroxylase (TH) and the ER showed a rostrocaudal pattern of NPY expression by NA cells opposite to that of the ER with 100% and 50% TH and NPY co-expression in the rostral A1/C1 and A2/C2, respectively, and less than 10% in the caudal A1 and A2. Of all cells examined, only 2 were triple labelled for TH+NPY+ER. Double-labelling for ER and NPY showed the same almost complete absence of ER expression by brainstem NPY neurons. **Exp.3** Fluorescein-labelled latex microspheres (20nl) were injected into the rostral preoptic area of intact female rats (n=3) and brainstem sections immunoreacted one week later for DBH and ER. Five to 7% of NA neurons within the A1 and A2 cell groups were retrogradely labelled and approximately one third of these A2 neurons were found to express ERs. No triple labelled cells were found in the A1. **Summary** These findings provide evidence for the differential expression of ER and NPY by brainstem NA neurons and indicate that the NA cells of the A2, and not the A1, are likely to play a role in transmitting estrogen-dependent information to the GnRH cell bodies.

Supported by The Lister Institute and Medical Research Council (UK).

624.17

ANDROGEN AND OESTROGEN REGULATION OF CALCITONIN GENE-RELATED PEPTIDE mRNA IN MEDIAL PREOPTIC AREA OF THE MALE RAT. D.P. Spratt and A.E. Herbison. [SPON: Brain Research Association] Laboratory of Neuroendocrinology, The Babraham Institute, Cambridge, CB2 4AT, UK

Previous immunocytochemical studies in this laboratory have identified a substantial female-predominant sex difference in the number of cells expressing calcitonin gene-related peptide (CGRP) in the rat preoptic area (POA). We have found that 27±6% of CGRP neurones in the male POA possess oestrogen receptors (ERs), and that the observed sexual dimorphism results from an inhibitory action of testosterone (T) exerted both perinatally and in adulthood in the male. The current study was undertaken to determine the mechanisms through which testosterone exerts its suppressive influence on CGRP expression. **Experiment 1:** Double-labelling immunocytochemistry was carried out on adult male rats (n=6) using antibodies directed against α-CGRP and the androgen receptor (AR; PG-21). We observed that 9±4% of CGRP neurones in the male medial preoptic nucleus (MPN), also possessed ARs. **Experiment 2:** *In situ* hybridization was used to examine the effects in the adult male rat of gonadectomy and oestrogen (E), T or dihydrotestosterone (DHT) replacement on CGRP mRNA expression in the MPN. The number of cells expressing CGRP mRNA in the male MPN doubled following castration ($p<0.03$), and was returned almost exactly to intact levels by E, T or DHT replacement. Likewise, CGRP mRNA expression per cell increased by approximately 30% after gonadectomy ($p<0.03$), with intact levels being similarly restored by any one of the three hormone treatments. Distribution analysis revealed that the CGRP population responded to the steroid treatments in an homogeneous manner. These findings suggest that the suppressive action of testosterone on preoptic CGRP neurones in the male results at least in part through alterations in CGRP mRNA content and that testosterone could achieve this through either AR- or ER-mediated pathways. The low occurrence of nuclear AR and ER expression by preoptic CGRP neurones suggests the involvement of trans-synaptic or paracrine factors. Supported by The Lister Institute and The Medical Research Council (UK)

624.18

COEXISTENCE OF NEUROTENSIN/NEUROMEDIN N mRNA AND PROGESTIN RECEPTOR mRNA IN HYPOTHALAMIC NEURONS OF THE FEMALE RAT. M.J. Alexander*, E. Bartolák-Suki, and S.E. Leeman. Department of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118.

In the female rat, estrogen stimulates synthesis of neurotensin (NT) in several hypothalamic cell groups important in reproductive neuroendocrine regulation, including neurosecretory cells in the arcuate nucleus as well as neurons in the anteroventral periventricular nucleus (AVPv) and medial preoptic nucleus (MPN). Since these regions also display estrogen-stimulated synthesis of progestin receptor (PR), we sought to determine whether NT neurons are capable of synthesizing PR and therefore subject to direct regulation by progesterone. Sections were prepared from brains of estradiol-treated ovariectomized rats, and cRNA probes labeled with digoxigenin or ^{35}S were used for simultaneous detection of neurotensin/neuromedin N (NT/N) mRNA and PR mRNA in individual cells by double-label *in situ* hybridization histochemistry. The 548-base probe used to detect PR mRNA corresponds to the steroid-binding domain of the receptor and therefore detects both A and B isoforms. Double-labeled cells were numerous in the dorsomedial division of the rostral arcuate nucleus, where as many as 90% of NT/N mRNA-labeled cells also displayed labeling for PR mRNA. Double-labeled cells were also observed in the AVPv and medial division of the MPN. These results imply that multiple populations of hypothalamic neurons, including neurosecretory cells, synthesize both NT and PR in response to stimulation by estrogen and further suggest that NT-synthesizing neurons mediate effects of progesterone on reproductive neuroendocrine systems. Supported by NIH Grants NS 32391 and DK 29876.

624.19

COLOCALIZATION OF NEUROPEPTIDE Y (NPY) Y_1 RECEPTOR mRNA IN ANTERIOR PITUITARY GONADOTROPES. J.M. Lloyd* Dept. of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Neuropeptide Y (NPY) has been implicated in the generation of preovulatory luteinizing hormone (LH) surges through actions at both the hypothalamus and anterior pituitary gland. NPY facilitates gonadotropin-releasing hormone (GnRH)-induced LH release directly at the anterior pituitary by activation of the NPY Y_1 receptor. Recently, it was demonstrated that anterior pituitary NPY Y_1 receptor expression is regulated during the estrous cycle. The present study was designed to assess whether anterior pituitary gonadotropes express the NPY Y_1 receptor mRNA using a combined *in situ* hybridization/ immunocytochemical technique. Regularly cycling female rats were sacrificed at 1000 h on proestrus. Anterior pituitary cells were dispersed enzymatically, plated on glass coverslips and processed for dual labelling of a biotinylated NPY-Y_1 receptor oligonucleotide probe and immunocytochemical localization of LHβ antigen. Expression of the biotinylated NPY-Y_1 receptor probe was observed in 80% of LHβ-labelled cells which constituted 13% of the total pituitary cell population. These results demonstrate that anterior pituitary gonadotropes do express NPY receptor mRNA. Further studies are in progress to assess whether the number of gonadotropes expressing the NPY-Y_1 receptor changes during the estrous cycle. Supported by the Oklahoma Center for the Advancement of Science and Technology, HN3-017.

624.20

THE EFFECTS OF AGING AND STEROIDS ON THE EXPRESSION OF SYNAPSE MARKERS IN THE RAT HIPPOCAMPUS

Helen M. Chao*[1], Robert L. Spencer[2], Randall R. Sakai[3] and Bruce S. McEwen[1], [1]Rockefeller University, Laboratory of Neuroendocrinology, 1230 York Avenue, New York, NY 10021, [2]Univ. of Colorado, Dept. of Psychology, Boulder, CO 80303, [3]Univ. of Pennsylvania, Dept. of Animal Biology, Philadelphia, PA 19104

The neurons of the hippocampus are vulnerable to the damaging effects of aging and neurodegenerative disease. Adrenal steroids have been shown to influence the morphology and survival of neurons in the hippocampus, and have been implicated in the neuronal loss associated with aging. As a way of investigating the consequences of these changes on synapses in the hippocampus, we have employed molecular probes for the synaptic markers synaptophysin, synaptoporin and synapsin II, to determine whether their expression is altered by age or adrenal steroid manipulation. We have found that there is an age-related decrease in the level of synaptophysin mRNA in the hippocampus, which appears to be independent of adrenal steroid regulation.

While in the mammalian brain the actions of ovarian steroids have primarily focused on the hypothalamus, these steroids have also been shown to affect the neurons of the hippocampus. Low levels of estrogen, as a result of ovariectomy or during the estrous cycle, have been shown to result in a decrease in the dendritic spine density of hippocampal pyramidal cells and the changes in spine density are correlated with changes in synaptic density. We have monitored synapse marker expression following estrogen treatment and our results indicate that in the hippocampus, the level of synaptophysin mRNA appears to be responsive to ovarian steroid.

(Supported by NS07080 and MH41256.)

625.1

LOCAL GLUTAMATERGIC NEURONS IN THE HYPOTHALAMIC ARCUATE NUCLEUS. A.B. Belousov* and A.N. van den Pol. Dept. Biological Sci., Stanford Univ., Stanford, CA 94305 and Sect. Neurosurgery, Yale Univ. Med. Sch., New Haven, CT 06520.

The hypothalamic arcuate nucleus (ARC) contains neuroendocrine neurons that regulate hormone secretion from the anterior pituitary. Many neuroactive substances have been identified in the ARC, but whether or not excitatory neurons exist in this nucleus, and the identity of an excitatory transmitter, have not been investigated physiologically. In the present experiments using whole cell current- and voltage- clamp recording of neurons from cultures and slices of the ARC, we demonstrate that many of the neurons in the ARC secrete glutamate as their transmitter. By stimulating presynaptic neurons in ARC slices electrically or with microdrops of glutamate (n=12) we found that excitatory axons from these neurons make local synaptic contact with other neurons in the ARC, and all evoked excitatory postsynaptic potentials (EPSP) could be blocked by the selective ionotropic glutamate receptor antagonists CNQX (10 μM) and AP5 (100 μM). In ARC cultures (n=63), spontaneous EPSPs were virtually eliminated by glutamate receptor antagonists AP5 and CNQX, underlining the functional importance of glutamate within this part of the neuroendocrine brain. GABA was secreted by another group of ARC neurons. The $GABA_A$ receptor antagonist bicuculline (50 μM) released glutamatergic neurons from chronic inhibition mediated by synaptically released GABA, resulting in further depolarization and an increase in the amplitude and frequency of glutamate-mediated excitatory postsynaptic potentials.

625.2

INHIBITION OF HYPOTHALAMIC CHICKEN GONADOTROPIN RELEASING HORMONE (cGnRH-I) BY OPIOID PEPTIDES IN VITRO. Y. Fan* and M.A. Ottinger. Dept. of Poultry Science, Univ. of Maryland, College Park, MD 20742.

In a series of *in vitro* experiments, we studied the effects of the opioid peptides, met-enkephalin (ENK), endorphin (END) and dynorphin (DYN) on the release of hypothalamic cGnRH-I. Longitudinal hypothalamic slices spanning the preoptic-lateral septal region to the median eminence (POA-MBH) were taken from adult, reproductive male Japanese quail (*Coturnix coturnix japonica*). Slices from a single brain were placed into a 2.5 ml chamber in a perifusion system (Endotronics, Inc.) and perifused (Medium 199, Sigma) at a rate of 12ml/hr. Samples were collected at 5 min intervals and analyzed for cGnRH-I by EIA. All experiments had the same overall design. Tissue was equilibrated and baseline release was monitored for 2 hrs followed by a 15 min norepinephrine (NE; 10^{-8}M) challenge and 1 hr recovery period. One of the opioid peptides (10^{-7}M) was then added to the medium for the remainder of the experiment. A second NE challenge was conducted; n=6 for each opioid peptide. Results showed that ENK, END, or DYN exposure all significantly ($p<0.05$) reduced both baseline cGnRH-I release as well as inhibited NE stimulated cGnRH-I release. Decreased cGnRH-I release was reflected in reduced pulse amplitude with no change in pulse frequency. These results confirm opioid peptide inhibition of the cGnRH-I system in an avian model and suggest direct effects on the cGnRH-I neuron as well as inhibition of NE neuronal input.

Supported in part by USDA/NRI 92-37023-7742 (MAO)

625.3

MECHANISM OF PROGESTERONE-EVOKED RELEASE OF GONADOTROPIN RELEASING HORMONE (GnRH) FROM GT1-1 NEURONS. M. El-Etr*, K. Shazand, Y. Akwa, P. Robel and E.E. Baulieu. INSERM U.33, 94276 Bicêtre, France.

Progesterone (PROG; 0.5-10 μM) stimulates the release of GnRH from hypothalamic GT1-1 cells (Soc.Neurosci., 1995, 21, 547.10). Although PROG receptor mRNA is present in these neurons, GnRH release occurs too rapidly for a genomic mechanism and is also evoked by PROG-3-O-carboxymethyloxime:BSA (PROG-BSA), which does not enter the cells, thus suggesting a membrane site of action. Additionally, the PROG antagonist RU 486, not only inhibits PROG- but also PROG-BSA-induced GnRH release. In spite of a membrane-like effect of PROG, binding experiments performed with ^{3}H-PROG or the synthetic progestin ^{3}H-ORG-2058 (20-50 nM) on GT1-1 cell membranes were unsuccessful. This indicates that the putative membrane PROG receptor might be distinct from the intracellular one. Contrary to its metabolite TH-PROG (3α-OH-5α-pregnan-20-one), PROG does not potentiate the release of GnRH evoked by muscimol, and the $GABA_A$ receptor antagonists bicuculline and picrotoxin do not counteract PROG effect. The release of GnRH induced by the $5HT_{1A}$ receptor agonist 8-OH-DPAT is enhanced by PROG, however the selective antagonist WAY 100635 (0.1-5 μM) does not abolish the effect of PROG alone. Finally, the release of GnRH induced by PROG is blocked by selective antagonists of calcium channels, particularly of L-type. The coupling of these PROG-sensitive calcium channels to G-proteins is under investigation.

625.4

OLDER MALES SECRETE LUTEINIZING HORMONE (LH) AND TESTOSTERONE MORE IRREGULARLY, AND JOINTLY MORE ASYNCHRONOUSLY, THAN YOUNGER MALES. S.M. Pincus, T. Mulligan, A. Iranmanesh*, S. Gheorghiu, M. Godschalk, J.D. Veldhuis. VAMC Salem, VA 24153, Guilford, CT 06437, VAMC Richmond VA 23249, and University of VA, Charlottesville, VA 22908.

New statistical perspectives on the secretory patterns of both LH and testosterone may prove useful in further understanding the aging process, and possibly ultimately for improved diagnosis and treatment of spermatogenetic failure and loss of sexual interest. We examined secretory time-series for LH and testosterone in 14 young (21-34 yr) and 11 aged (62-74 yr) healthy men. For each subject, blood samples were obtained at 2.5 min intervals during an overnight sleep period, average sampling duration 7 h. For each of LH and testosterone, we utilized the model-independent statistic Approximate Entropy (ApEn) to quantify irregularity. To quantify joint LH-testosterone secretory asynchrony, we employed the recently introduced cross-ApEn. Although mean (and SD) LH and testosterone concentrations were indistinguishable in the 2 age groups ($P > 0.25$), for LH aged subjects had greater ApEn values (1.525 +/- 0.221) than younger subjects (1.207 +/- 0.252), $P < 0.003$, indicating more irregular LH secretion in the aged cohort. For testosterone, aged subjects also had greater ApEn values (1.622 +/- 0.120) than younger subjects (1.384 +/- 0.228), $P < 0.004$. Aged subjects had greater cross-ApEn values (1.961 +/- 0.121) than younger subjects (1.574 +/- 0.249), $P < 10^{-4}$, with nearly 100% sensitivity and specificity, indicating greater LH-testosterone asynchrony in the older group. In conjunction with previous findings of greater irregularity of insulin and GH release with increasing age, we propose that increased secretory irregularity with advancing age may be a widespread hormonal phenomenon reflecting attenuation of coordinate feed-forward and feedback regulation. Finally, theoretically, we clarify the need for quantifications such as ApEn and cross-ApEn via a study of a "variable lag" pulsatile process, and empirically note the potential wide applicability of cross-ApEn to quantify asynchrony in settings in which network characteristics might be of interest, beyond the study of variables individually.

NIH, Veterans Affair Administration

625.5

EXTRAHYPOTHALAMIC CORTICOTROPIN RELEASING FACTOR (CRF) NEURONS CONTAIN ANDROGEN RECEPTOR (AR). G. Wishbach, E.M. Wilson and M.Sar*.Departments of Pediatrics (M. S., E.M.W.) and Biochemistry and Biophysics (E..M.W.), School of Medicine, University of North Carolina, Chapel Hill, NC 27599

The influence of gonadal steroids on the regulation of hypothalamic CRF secretion was studied. Although the effects of estrogen on the regulation of CRF in the hypothalamus have been reported, the effects of androgen on hypothalamic CRF remain controversial. Since androgen action is mediated through receptor mechanisms, we investigated whether CRF neurons in brain contain the AR. Colocalization of CRF and AR in rat brain was performed by dual immunocytochemistry using antipeptide antibody CRF and AR32. Adult male Sprague-Dawley rats (n=8) were each treated with testosterone propionate and received colchicine intraventricularly. Twenty four hours later, the rats were perfused with Zamboni's fixative (pH 7.2) and processed for immunostaining. Sections were stained first with AR32 antibody using diaminobenzidine followed by immunostaining with CRF antibody using 4-chloro-1-naphthol. Nuclear localization of AR and cytoplasmic localization of CRF in neurons were observed in several areas of the brain. CRF neurons that contain AR include the cortex, amygdala and bed nucleus of the stria terminalis but not the paraventricular nucleus. AR was localized in 30 - 35 % of CRF cells in the bed nucleus, particularly the lateral part and 25 -30% of CRF cells in the central nucleus of the amygdala. In the cortex, AR was localized in CRF cells of layers 4 and 5. The results demonstrate that a certain population of CRF neurons in extrahypothalamic but not hypothalamic areas contain AR and raise the possibility of androgen regulated events in extrahypothalamic CRF neurons. (Supported by NIH Grant NS - 17479).

625.6

THE AP-1 AND CREB TRANSCRIPTION FACTORS MEDIATE GLUCOCORTICOID REGULATION OF TRH GENE EXPRESSION, LU-GUANG LUO*, EMILY SU, IVOR M.D. JACKSON, DIVISION OF ENDOCRINOLOGY, RHODE ISLAND HOSPITAL, BROWN UNIVERSITY SCHOOL OF MEDICINE, PROVIDENCE, RI 02903.

Glucocorticoids stimulate TRH gene regulation in rat hypothalamic and anterior pituitary cells, but the underlying mechanism has not been elucidated. Accordingly, the purpose of this study was to examine the role of the protooncogenes *cfos/cjun* (AP-1 complex) and CREB in this process. TRH content was measured by radio-immunoassay (RIA) in fetal hypothalamic neurons exposed to Dexamethasone(Dex) at 10-8M and treated with chemical agents which affect protein formation. N-ethyl-maleimide (NEM), an alkylating agent, decreased TRH content in a dose-dependent manner (from 87.45 ± 6.05 to 35.20 ± 9.35 fmol/well; $p<0.05$), while the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) increased the TRH content, also in a dose-dependent manner (from 50.45 ± 3.85 to 188.02 ± 12.62 fmol/well; $p<0.05$). In addition, TPA(10-8M) enhanced the Dex (10-8 M) stimulation of TRH expression (374.00 ± 28.60 *vs* 180.95 ± 13.75 fmol/well; $p<0.05$). In contrast NEM (10-4 M) reduced the effect of Dex stimulation (89.10 ± 9.35 *vs* 180.95 ± 13.75 fmol/well; $p<0.05$). Western blot analysis of AtT20 cells (a pituitary tumor cell line transfected with a cDNA for proTRH) was performed in order to directly determine relative levels of AP-1 and CREB. The findings were consistent with the RIA data. Both Dex and TPA increased the levels of AP-1 and CREB while NEM inhibited the formation of these proteins. As anticipated, the glucocorticoid stimulatory effect was reduced by the addition of NEM. Anterior pituitary monolayer cells, undergoing electrophoretic mobility shift assays (EMSA), were used to determine DNA binding ability to both the protooncogene protein AP-1 and CREB. The data showed that Dex increases the DNA binding ability of both AP-1 and CREB to the TRH gene. In conclusion, these findings suggest that glucocorticoid stimulation of TRH gene expression is regulated, at least in part, by the protooncogene AP-1 complex and the CREB pathway, associated with enhanced DNA binding.

625.7

CHARACTERIZATION OF A PITUITARY CELL LINE PRODUCING THE PEPTIDE GALANIN J.F. Hyde*, A. Cai and J.P. Moore, Jr., Dept. Anatomy & Neurobiology, Univ. Kentucky College of Medicine, Lexington, KY 40536.

We previously reported that the GH- and PRL-producing transplantable pituitary tumor MtTW-10 also synthesizes and secretes the peptide galanin in copious amounts. The MtTW-10 tumor is estrogen responsive and must be maintained subcutaneously in Wistar-Furth rats. During the past several years, we have examined a variety of pituitary cell lines (including GH3, GH4C1 and MMQ) and found that only αTSH cells were found to produce minute amounts of galanin. Harrison and co-workers established a pituitary tumor cell line (RC-4B/C) derived from a male rat in 1977, and they showed that this cell line contains all pituitary cell types as determined by immunocytochemical analysis. We have studied the RC-4B/C cell line to determine if they produce the peptide hormone galanin. Northern blot analysis showed that the cell line contains galanin mRNA, identical in size with the normal pituitary galanin mRNA transcript (~0.9 kb). *In situ* hybridization using an ^{35}S-UTP-labeled cRNA galanin probe revealed that only a subpopulation of the RC-4B/C cells contain galanin mRNA. Immunofluorescent cytochemistry using a specific rat galanin antiserum showed that the RC-4B/C cells produce galanin peptide, and confirmed that only a subpopulation of the cells synthesize galanin. It is not known at present, which pituitary cells type(s) in this pituitary tumor cell line produces galanin mRNA/peptide. This cell line may be useful for studying galanin peptide processing and secretion as well as for studying the regulatory sequences controlling galanin gene transcription. (Supported by DK-45981 and HD-07436).

625.8

BASAL PROENKEPHALIN (PENK) GENE EXPRESSION IN THE HAMSTER IS INDEPENDENT OF GLUCOCORTICOIDS Rosa Jimenez and S.O. Franklin*, Dept. of Pharmacology, Cornell U. Med. College, N.Y., N.Y. 10021.

Proenkephalin (Penk) gene expression is low In the rat adrenal medulla and high in the rat striatum, and glucocorticoids play a major role in regulating that gene's expression. In contrast to the rat, preproenkephalin (PPenk) mRNA levels in the adult hamster adrenal are high (90 times rat levels) and comparable to striatal PPenk mRNA levels (Franklin et al, 1991). To determine the role that glucocorticoids play in regulating Penk gene expression in the hamster, male hamsters were either hypophysectomized 14 days prior to sacrifice (HYPOX), HYPOX and treated with the glucocorticoid receptor antagonist RU486 (30 mg/kg/day, sc beginning on day 8) or subjected to treatments known to reduced adrenal cortical glucocorticoid biosynthesis (150 mg/kg/day metyrapone, ip, or 75 mg/kg/day mitotane, sc, bid for 6-7 days). All treatments reduced plasma glucocorticoid levels. HYPOX + RU486 treatments reduced plasma glucocorticoid levels by as much as 67% and phenylethanolamine-N-methyltransferase (PNMT) mRNA levels by 57% in the hamster adrenal. PNMT, which converts norepinephrine to epinephrine, is influenced by glucocorticoids in the rat. Yet, all applied treatments did not effect basal enkephalin containing peptide levels nor basal PPenk mRNA levels in either the hamster adrenal or striatum. These results suggest that basal Penk gene expression in the hamster is independent of glucocorticoids, despite divergent observations in other species and the presence of glucocorticoid response elements (GRE's) in the hamster Penk gene. (Supported by NIDA GRANTS DA09901 and DA05130.)

625.9

IMMUNOHISTOCHEMICAL LOCALIZATION OF SNAP-25, A SYNAPTIC PROTEIN, IN THE RAT PITUITARY. L.C. Saland* and V. Hernandez, Dept. of Anatomy, Univ. of New Mexico Sch. Medicine, Albuquerque, NM 87131.

SNAP-25 is a neuronal membrane-associated protein which may be involved in docking of synaptic vesicles. We have previously found other proteins, including synaptophysin (SN), in nerve fibers terminating in the rat pituitary neural (NL) and intermediate (IL) lobes (Saland et al, '95, Soc. Neurosci. Abst. 21:1993). Here, adult male Sprague-Dawley rats were halothane anesthetized, perfused intracardially with buffered saline followed by paraformaldehyde, and tissues prepared for paraffin sectioning. Sections were immunostained for SNAP-25 using a monoclonal antibody from Chemicon, dilution 1:50, followed by secondary antibody, Vector ABC reagent, then a peroxidase reaction. A fine network of SNAP-25-positive fibers extended throughout the NL, and among some areas of the IL. Relatively sparse staining contrasts with greater intensity for SN as well as for GAP-43 seen in earlier studies (Saland et al,'93, Mol. Cell. Neurosci. 4:576; Saland et al,'96, Neurosci. Lett., in press). SNAP-25 may be another component of innervation which regulates secretion from the pituitary. Support: NIH GM-08139.

625.10

A PROGESTERONE EFFECT UPON NEOCORTICAL CYCLIC AMP THAT DEPENDS UPON THE $GABA_B$ AGONIST BACLOFEN. M.I.Al-Dahan*,M.H.Jalilian Tehrani , and R. H. Thalmann, Baylor Coll. of Med. , Houston,TX 77030.

Progesterone, without the necessity of estrogen priming, is associated with increases in $GABA_B$ receptor density in neocortex (Al-Dahan and Thalmann, Brain Research, in press). We have begun to examine the interaction of progesterone with second messenger systems and with $GABA_B$ receptors. Progesterone or vehicle alone was injected subcutaneously in sesame oil, and the brains were removed four hours later. Neocortical slices were incubated in a Krebs bicarbonate buffer with baclofen (10uM) followed 15 minutes later by forskolin (3uM). Progesterone had little effect upon basal cyclic AMP and produced an insignificant effect upon forskolin-stimulated cyclic AMP (10 mins forskolin exposure). However, when 10uM baclofen was introduced 15 minutes prior to the addition of forskolin, cyclic AMP levels were lower in the presence than in the absence of progesterone (N=4/4 experiments). This evidence for increased $GABA_B$ dependent suppression of cyclic AMP after exposure to progesterone may reflect the functional effect of the increased density $GABA_B$ receptors that are detected after a similar exposure to the hormone. (See also Thalmann et al, this meeting). Supported by National Institutes of Health grant NS21713.

625.11

BIOLOGICAL AND IMMUNOLOGICAL CHARACTERIZATION OF A GONADOTROPIN-RELEASING HORMONE (GnRH)-LIKE SUBSTANCE IN A MOLLUSK, *APLYSIA CALIFORNICA*. P.-S. Tsai*[1], N.M. Sherwood[2], and N.L. Wayne[1]. [1]Department of Physiology, UCLA School of Medicine, Los Angeles, CA 90095-1751 and [2]Department of Biology, University of Victoria, Victoria BC, V8W 2Y2.

GnRH is a neuropeptide central to the initiation and maintenance of reproduction in vertebrates. Members of the GnRH family share almost 100% sequence identity at both amino and carboxy terminals, suggesting remarkable structural conservation over the course of evolution. In this study, we investigate if a GnRH-like molecule exists outside Phylum Chordata in an invertebrate, *Aplysia californica*. Immunocytochemical studies using antisera against dogfish and tunicate I (tI) GnRHs revealed the presence of GnRH immunoreactivities in neuronal cell bodies, fibers, and acellular regions of the CNS connective sheath (possible site of neurohormone release) in *Aplysia*. A specific radioimmunoassay for tI-GnRH also demonstrated tI-GnRH immunoreactivity in acid/acetone extracts of the CNS (28.9 ± 10.6 pg/g tissue) and hemolymph (68 ± 7.8 pg/ml hemolymph). However, the immunoreactive *Aplysia* GnRH is biochemically distinct from other known forms of GnRH as it cannot be successfully retained by the C18 reverse-phase column. *In vitro* administration of 100 nM of chicken II GnRH to the neuroendocrine bag cell clusters (during the nonbreeding season) significantly suppressed the duration of electrical afterdischarge (AD) and the number of action potentials fired during an AD ($P \leq 0.02$, n=5), but the secretion of egg-laying hormone from bag cells was not significantly affected. These findings demonstrate the presence of GnRH immunoreactivity and bioactivity in a protostomic invertebrate and suggest that the structural and functional conservation of GnRH may extend beyond phylum Chordata to other members of invertebrate phyla. (Supported by NIH Grants HD-28336 and HD 07228-15)

625.12

CYTOSOLIC Ca^{2+} OSCILLATIONS EVOKED BY PACAP IN MELANOTROPHS OF THE PITUITARY PARS INTERMEDIA OF RATS. K. Tanaka, I. Shibuya, N. Harayama, Y. Ueta*, M. Nomura & H. Yamashita Dept. of Physiol., Univ. Occup. & Environ. Health, Kitakyushu 807, Japan

Melanotrophs of the pituitary pars intermedia (PI) exhibit spontaneous secretion at a high rate, which is maintained by spontaneous Ca^{2+} entry. This spontaneous secretion is thought to be regulated mainly by the secreto-inhibitory neurotransmitters, dopamine and GABA. We found remarkably high density of mRNA of the type-I receptor of pituitary adenylate cyclase activating polypeptide (PACAP) in PI, which is in a direct contact with the pars nervosa where PACAP is released. To examine the function of PACAP in PI, we measured $[Ca^{2+}]_i$ and ionic currents in dissociated melanotrophs of rats. PACAP (10^{-10}-10^{-7} M) raised $[Ca^{2+}]_i$ and often initiated phasic $[Ca^{2+}]_i$ elevations (Ca^{2+} oscillations), which were suppressed when extracellular Ca^{2+} was removed or the Ca-channel blocker, nicardipine was added. Moreover, PACAP augmented voltage-dependent Ba^{2+} currents under voltage-clamp mode of the whole-cell patch-clamp technique. These results suggest that PACAP potentiates Ca^{2+} entry mechanisms of melanotrophs to initiate cytosolic Ca^{2+} oscillations and that PACAP may serve as a physiological secretagogue of rat melanotrophs.

625.13

DIMORPHIC EXPRESSION OF CALRETININ IN THE MEDIAL BASAL HYPOTHALAMUS FROM PERINATAL MALE AND FEMALE RATS. E. D. LEPHART*, D. R. LADLE AND N. A. JACOBSON. Dept. Zoology - Cell Biol. Div., Brigham Young University, Provo, UT 84602.

Calcium-binding proteins play an important role in mediating cell proliferation, programmed cell death and neurotoxicity. Conversely, evidence exists that hypothalamic and limbic regions display sexually dimorphic structures which are regulated by sex hormones during perinatal development. Calcium-binding proteins such as Calretinin (CALRET) within certain brain areas may regulate these brain regions in concert with the hormonal action of sex steroids during perinatal development. In the present study, we examined the developmental pattern of CALRET expression (via Western analysis) in MBH and AMY tissue homogenates from perinatal male and female rats. Tissue samples were collected on gestational days (GD) 17, 19, 21 and postnatal days (PND) 1 and 3. The CALRET antibody recognized a single protein band with an apparent molecular weight of 29,000 in brain. Notably, males displayed significantly higher CALRET levels compared to females in the MBH (but not the AMY) brain region from GD 19 through PND 1. We have previously shown a dimorphic expression of Calbindin-D_{28K} in the MBH (but not the AMY) from perinatal male and female rats and the present data for CALRET are similar to these earlier studies. The notable sex difference in MBH CALRET (and calbindin) levels suggest that androgens or other hormonal factors may regulate the dimorphic expression of CALRET during perinatal development. Finally, the calcium-binding proteins potentially play an important and critical role in neuronal programmed cell death during perinatal development where females, presumably, exhibit a greater cell loss than males due to the lower abundance of calcium-binding proteins. Supported, in part, by a grant from NSF.

625.14

SEROTONERGIC REPRESSION OF CALCITONIN GENE-RELATED PEPTIDE GENE EXPRESSION. P.L. Durham, N.J. Pantazis* and A.F. Russo. Dept. of Physiology and Biophysics, Univ. of Iowa. Iowa City, IA 52242.

We investigated the regulatory effect of serotonergic agents on transcriptional activity of the calcitonin/calcitonin gene-related peptide (CT/CGRP) gene in rat CA77 medullary thyroid carcinoma C-cells. CGRP, a potent vasodilator, plays an important role in cardiovascular homeostasis. High levels of CGRP are associated with vascular headaches, such as migraines. Administration of sumatriptan, a serotonin type-1 (5-HT_1) receptor agonist, causes plasma CGRP levels to return to normal, coincident with alleviation of migraine symptoms. Sumatriptan is belived to act through the human 5-HT_{1D} receptor (rat 5-HT_{1B}) to inhibit adenylate cyclase activity via its coupling to a G_i protein. To test whether sumatriptan and serotonergic agents could directly inhibit the expression of CGRP in neuronal cells, we utilized CA77 cells which exhibit a neuronal phenotype and possess characteristics of serotonergic neurons. Treatment of CA77 cells with the 5-HT_{1B}-specific receptor agonist, CGS 12066B maleate (CGS), caused a 2.5-fold decrease in the steady-state level of CGRP mRNA as determined by northern blot analysis. Utilizing luciferase reporter genes containing the rat CT/CGRP promoter and 1.3 kb of the 5' flanking sequence in transient transfection assays, we determined that sumatriptan or CGS inhibit forskolin-mediated stimulation of CGRP promoter activity. Data from our studies are suggestive that 5-HT receptor agonists downregulate CGRP gene expression via a cAMP-dependent pathway. Furthermore, the CA77 cells will provide a useful neuronal/serotonergic model system to investigate the mechanism by which sumatriptan and other anti-migraine drugs regulate the expression of the vasoregulatory neuropeptide CGRP. This work was supported by NIH grant HD25969.

625.15

ULTRA-SHORT LOOP FEEDBACK OF GnRH IN THE EWE: DOES THIS INVOLVE GAMMA AMINOBUTYRIC ACID (GABA) NEURONS IN THE PREOPTIC AREA? J.E. Robinson*, N.P. Evans, and J.A. Taylor. Laboratory of Neuroendocrinology, The Babraham Institute, Cambridge CB2 4AT, UK.

There is evidence for an ultra-short feedback loop regulating GnRH release in several species including sheep. Whether this effect involves the direct actions of the decapeptide on GnRH neurons themselves or a self-regulatory action via another neurotransmitter system is unknown. We have explored the latter possibility by using microdialysis to measure the local release of GABA following the infusion of either GnRH or a receptor antagonist, Antide, directly into the preoptic area (POA). Microdialysis probes (5mm membrane; CMA/10) were placed in the POA of 7 ovariectomised Clun Forest ewes via preimplanted guide cannulae and artificial cerebrospinal fluid (aCSF) pumped through the probes at a flow rate of 2-3 µl/min. Beginning 180 min later, samples of dialysate were collected at 20 min intervals for 260 min. Animals received 2 GnRH infusions (10^{-6}M in aCSF for 20 min), one 60 min after sample collection began and the second 80 min later. Antide (10^{-3}M) was infused for 20 min before the second GnRH infusion. GABA concentrations were variable among the 7 ewes and values were log-transformed for statistical analysis. A significant ($p<0.02$) increase in POA GABA levels was observed in all animals in the 20 min following the first GnRH administration (20 min before, 1061±339 vs 20 min after, 4971±2453 nM). Infusion of the GnRH antagonist, Antide, rapidly depressed local GABA concentrations ($p<0.01$: before, 926±499 vs after, 419±236 nM). However, this concentration of antagonist did not prevent the second GnRH infusion from stimulating GABA ($p<0.01$; before, 419±236 vs after, 3070±1279 nM). These data suggest that endogenous GnRH exerts a tonic stimulatory action on GABA release in the POA that is receptor mediated. As GABA can alter GnRH release, then preoptic GnRH and GABA neurons may form the neural substrate for an ultra-short feedback loop by which GnRH is able to modulate its own release. Supported by BBSRC.

625.16

RAT SPERM IMMOBILIZATION BY ORAL INGESTION OF RUTA CHALEPENSIS AQUEOUS EXTRACT. E. Gijón*, L. Cartas, M. Lorenzana-Jímenez, V. Anaya, and X. García. Dept. of Physiol., Dept. of Pharmacol., and Division of Postgraduate Studies and Research, Sch. of Medicine. UNAM. México D. F. 04510, México.

Ruta chalepensis extract is used in traditional medicine as a vaginal wash before sexual intercourse as it has been suggested to have an anticonceptive action. As a more likely target for the compounds is the sperm if any of the compounds interfered with sperm activation or motility, we showed that Ruta chalepensis extract affects motility of rat and frog sperm **in vitro**, until complete immobilization of spermatozoa, in a dose dependent form. The aim of this study was to find out if the substitution of drinking water to male rats for aqueous extract of Ruta chalepensis **in vivo**, might induced any change in sperm motility. After 4 days of this treatment we found under light microscopy and videomicroscopy complete immobilization of spermatozoa obtained from rat testicle while control rat testicle rendered sperm with clear visible motility. As **in vitro** observations sperm from treated animals showed morphological changes in the tail and head of the spermatozoa. This results support the postulated contraceptive action on male side of Ruta chalepensis extract. Work in progress in our laboratory is looking also for morphological changes on rat testicle or spermatogenesis.

625.17

INDOMETHACIN (INDO) PREVENTS THE RISE IN PLASMA OXYTOCIN (OT) AFTER INTRACEREBROVENTRICULAR (ICV) INJECTION OF L-NAME BUT NOT NALOXONE. J. Summy-Long*, V. Bui, S. Gestl and M. Kadekaro[1]. Dept. of Pharmacology, Penn State Univ., M.S. Hershey Med. Ctr, Hershey, PA 17033, [1]Div. Neurosurg., Univ. Texas, UTMB, Galveston, TX 77555-0517.

Both nitric oxide (NO) and endogenous opioid peptides inhibit OT release from the magnocellular system without affecting glucose utilization. To determine if they share a similar mechanism involving prostaglandins, conscious female control rats or those water deprived (24h, WD) were injected icv with 5µl of Na_2CO_3 or Indo (100µg) 15 min before CSF or NAME (250µg). Other WD rats were treated similarly except Indo or 0.9% NaCl was given before 0.9% NaCl or naloxone (50 µg) icv. Rats were decapitated 5 min later. Plasma OT was quantified by RIA and changes determined by 2 way AOV and Newman Keuls t. Inhibiting NO synthase with NAME increased (CSF vs NAME; $p<0.01$) plasma OT (pg/ml ± SEM; n=7-10) in control (10±2 vs 48±8) and WD (20±2 vs 72±7) animals, responses prevented ($p<0.01$) by Indo (Control: Na_2CO_3+CSF < Indo+CSF = Indo+NAME < Na_2CO_3+NAME; WD: Na_2CO_3+CSF = Indo+CSF = Indo+NAME < Na_2CO_3+NAME). However, the rise in plasma OT in WD rats given naloxone (19±2 vs 39±3) was unchanged by Indo (41±4). Thus, NO inhibits the excitatory effect of a prostaglandin(s) on OT release whereas opioid peptides do not. Funded by RO1-25498 and 2RO1-23055.

625.18

INVOLVEMENT OF BETA RECEPTORS IN COMPENSATORY GROWTH OF THE RAT ADRENAL GLAND: AN UNEXPECTED CHANGE IN RECEPTOR FUNCTION AND cAMP SIGNALING. Z. Lacković*, V Trkulja A. Horvat, D. Zdilar and H. Sečić. Lab. Molecular Neuropharmacology, School of Medicine, Zagreb University, Zagreb, Croatia.

Catecholaminergic innervation influences compensatory growth of the adrenal gland remnant after unilateral adrenalectomy. The aim of the present study was to identify the receptors involved. Mature, female Wistar rats were used in 10-day experiments. Several parameters of adrenal growth were determined - wet weight, protein content, and total RNA and DNA. Both splanchnecotomy and chemical sympathectomy (guanethidine or reserpine) prevented the compensatory adrenal growth induced by contralateral adrenalectomy. While ß-adrenergic agonist isoproterenol had a stimulatory effect and reversed the effect of guanethidine, ß-blocking agents (propranolol, sotalol, atenolol, but butoxamine, too) prevented the compensatory adrenal growth. In contrast, neither the cold stress-induced adrenal enlargement nor the normal adrenal growth followed throughout a 45-day period, were modified by ß-blocking agents. ^{3}H-Alprenolol binding did not indicate alterations in ß-receptor density or affinity. While isoproterenolol stimulated cAMP formation in the control adrenal gland, in the adrenal gland remnant after unilateral adrenalectomy cAMP formation was inhibited by isoproterenol. This inhibitory effect was clearly antagonized by butoxamine but not by atenolol. These observations suggest that ß-receptors stimulate the adrenal growth induced either by adrenalectomy or by denervation of the contralateral gland, changing thereat their physiological function and intracellular signaling mechanism.

Supported by grant from the Ministry of Science, Republic of Croatia.

626.1

FURTHER EVIDENCE THAT OSCP, A SUBUNIT OF MITOCHONDRIAL ATP SYNTHASE, IS A STEROID BINDING PROTEIN. J. Zheng* & V. D. Ramirez. Neurosci. Program & Dept. Mol. & Integrat. Physiol., Univ. of Illinois, Urbana, IL 61801

17β-Estradiol linked to bovine serum albumin at C-6-position (17β-E-6-BSA) has been used as a ligand in our laboratory to demonstrate membrane estrogen binding sites and to purify membrane estrogen binding proteins from affinity columns. One membrane estrogen binding protein has been identified as oligomycin sensitivity-conferring protein (OSCP), a subunit of mitochondrial ATP synthase/ATPase. In order to further clarify the issue that OSCP is an estrogen binding protein, we have used recombinant bovine OSCP (kindly provided by Drs. Y. Hatefi and A. Matsuno-Yagi of the Scripps Research Institute). Ligand blotting and radioligand binding assay revealed that recombinant OSCP bound iodinated 17β-E-6-BSA (17β-E-6-[^{125}I]BSA). The binding was completely displaced by 17β-E-6-BSA at 0.5 μM with affinity in the range of 0.2 nM, partially by 17α-E-6-BSA at 0.5 μM with affinity in the range of 50 nM, and not by 10 μM BSA. Both 17β-estradiol linked to BSA at C-17 position (17β-E-17-BSA) and testosterone linked to BSA at C-3 position (T-3-BSA) showed lower affinity (about five times less) than 17β-E-6-BSA. The results are consistent with our early studies of affinity column-purified rat brain OSCP. In contrast, it was found that 17β-E-6-BSA and 17α-E-6-BSA displaced the [^{3}H]17β-estradiol binding to nuclear estrogen receptors in immature rat uterine cytosol with Ki of 1.5 and 3.0 nM, respectively, whereas 17β-E-17-BSA showed very high affinity (Ki of 0.024 nM). T-3-BSA and BSA were without any effect at the concentration of up to 1 μM. The results support the hypothesis that the estrogen binding groups for the OSCP are different from those for the nuclear estrogen receptor. In conclusion, 17β-E-6-[^{125}I]BSA is a useful ligand in identifying membrane steroid binding proteins both in radioligand binding assay and ligand blotting. The findings using recombinant OSCP provide further evidence that OSCP is a binding protein for estrogen, and probably other steroids. Work is under way to develop a method to demonstrate binding affinity of different steroids to the recombinant OSCP using [^{3}H]17β-estradiol.

This work was supported by a NSF grant to VDR.

626.2

TIME COURSE AND SPECIFICITY OF ESTROGEN NEUROPROTECTION AGAINST GLUTAMATE TOXICITY IN PRIMARY CORTICAL NEURONS. C.A. Singer,*, R.H. Batchelor and D.M. Dorsa. Departments of Pharmacology and Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195

We have previously reported neuroprotective effects of estrogen in rat primary cortical neurons following a toxic glutamate exposure. In these experiments, a twenty-four hour pretreatment with 15 and 50 nM 17ß-estradiol significantly reduced cellular lactate dehydrogenase (LDH) release by 23% when compared to vehicle treated cells. This effect is specific to estradiol and is not mimicked by pretreatment with related steroids such as progesterone, dihydrotestosterone, dexamethasone or cholesterol. Neuroprotection is elicited, however, by the potent estrogen diethylstilbestrol (DES) at doses of 10^{-10} M. The anti-estrogen tamoxifen blocked the protective effects of 17ß-estradiol suggesting that a classical steroid hormone receptor may be involved and the presence of estrogen receptor in these cultures has been verified by immunoblot. An examination of the time course for this neuroprotective effect has demonstrated that estrogen neuroprotection can occur with a five minute pretreatment of 10^{-9} M 17ß-estradiol. The ability of estrogen to protect against toxicity mediated by different types of glutamate receptors is being evaluated by exposing cultures to various glutamatergic agonists. Preliminary experiments indicate that 10^{-8} M 17ß-estradiol will protect cultures from cell death induced by a five minute exposure to NMDA, suggesting that estrogen neuroprotection during glutamate toxicity may involve, in part, the NMDA receptor component of glutamate toxicity. This work is supported by a Molecular Neurobiology Training Grant to C.A.S. and ADRC Grant AG05136 to D.M.D.

626.3

TRANSCRIPTIONAL EFFECTS OF ESTROGEN ON THE NEUROTENSIN GENE INVOLVE NON-CLASSICAL SIGNALLING MECHANISMS J. J. Watters*[a], P. R. Dobner[b], and D. M. Dorsa[a,c]

Departments of Pharmacology [a] and Psychiatry [c], Univ. of WA, Seattle, WA 98195 and Dept. Mol. Genetics and Micro., Univ. Mass., Worcester, MA[b]

Estrogen is known to regulate the expression of neuropeptide genes whose promoters do not contain classical estrogen response elements (EREs). Neurotensin (NT) gene expression is regulated by estrogen in a sexually dimorphic nucleus of the rat brain, the medial preoptic nucleus (MPO). Although the NT promoter does not include an ERE it does contain several other transcription factor binding sites, including a cyclic AMP response element (CRE). It has been shown that estrogen can rapidly elevate intracellular levels of cAMP in various cell lines and tissues. In addition, we have previously reported that estrogen induces the phosphorylation of the cyclic AMP response element binding protein (CREB) in the MPO, indicating that estrogen could potentially employ this second messenger system to influence gene transcription. To test this possibility in the context of the NT gene, we have used a promoter reporter gene construct, and transient transfection techniques to evaluate estrogen's effects on transcription using an estrogen-responsive human neurblastoma cell line, SK-N-SH. In these cells, estrogen elicits a 2-3 fold increase in transcription at 8 hours, which is preceeded by an increase in intracellular cAMP that appears to be maximal about 90 minutes after estrogen exposure. Additionally, the effects of estrogen on the NT reporter gene construct are blocked by administration of the PKA inhibitor H-89, but not by Cam Kinase or PKC inhibitors. Overexpression of the human estrogen receptor in these cells dramatically enhanced transcription of an ERE reporter gene, but not of the NT construct. Treatment with the estrogen receptor antagonists tamoxifen or ICI 182,780 fails to antagonize, and may potentiate, the effect of estrogen. (Supported by NS20311, and Pharmacological Sciences Training Grant 5T3GM07750).

626.4

17 ß-ESTRADIOL MODULATION OF GLUCOSE TRANSPORTER 1 (GLUT 1) EXPRESSION IN BLOOD-BRAIN BARRIER J. Shi* and J. W. Simpkins Center for the Neurobiology of Aging and Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL 32610

The present study was designed to evaluate the 17 ß-estradiol (E_2) modulation of GLUT 1 protein and mRNA expression in BBB endothelium. Female rats were ovariectomized (OVX) for 12-14 days, then E_2 was injected *sc.* at doses of 1-100 μg/kg at 2-16 hours before sampling. Glucose transport was assessed using ^{14}C-2-deoxyglucose (^{14}C-2-DG) uptake and GLUT 1 protein and mRNA samples of brain microvessel endothelial cells were analyzed by Western and Northern blotting, respectively. E_2 treatment caused dose- and time-dependent increase in ^{14}C-2-DG uptake by microvessels and the whole microvessel endothelial cell GLUT 1 protein expression. The peak responses were induced by 10 μg/kg E_2 dose at the 4-hour sampling time (36.0% and 31.3% increase, $p<0.05$, respectively). GLUT 1 mRNA demonstrated a transient increase at 15-minute (55% increase, $p<0.05$), then decreased to basal level by 2 hours. This study shows that *in vivo* treatment with E_2 increases ^{14}C-2-DG uptake into the BBB endothelial cells and suggests this E_2 effect is due to its modulation of GLUT 1 mRNA and protein. (Supported by NIA grant AG 10485)

626.5

APOLIPOPROTEIN J AND E mRNA IN THE HYPOTHALAMUS AND HIPPOCAMPUS DURING THE RAT ESTROUS CYCLE DJ Stone, I Rozovsky, CP Anderson, TE Morgan, and CE Finch*. Andrus Gerontology Center and Department of Biology, University of Southern California, Los Angeles, CA 90089

The arcuate nucleus of the hypothalamus and the CA1 region of the hippocampus undergo rapid transient synaptic remodeling during the rat estrous cycle. Synapse number is highest when estradiol levels are high; decreases in estradiol are accompanied by decreases in synapse number. Because the glial apolipoproteins J and E are implicated in synaptic remodeling after injury, we investigated mRNA levels of apoJ and apoE by in situ hybridization in the brains of rats at 4 different points in the estrus cycle (diestrus, proestrus morning, proestrus afternoon, and estrus). During proestrus afternoon, when blood estradiol and synapse number are highest, we observed maximum levels of apoJ mRNA in the hippocampus and hypothalamus. Levels decrease significantly by the morning of estrus when synapse number and estradiol level are lowest. ApoE mRNA in the CA1 region of the hippocampus is highest on the morning of proestrus, and decreased by the morning of estrus. ApoE levels in the arcuate nucleus show a similar but non-significant trend.

These results implicate apoJ and apoE in synaptic remodeling during the estrous cycle in the rat brain. The temporal course of apoJ and apoE mRNA changes where apoJ levels are peaking while apoE levels are decreasing suggests that these mRNAs are differentially controlled in different glial subpopulations and may be involved in different aspect of the remodeling process. (Supported by grants AG-000-93 to Vern Bengston and AG13499 to CEF)

626.6

MOLECULAR MEMBRANE INTERACTIONS OF STEROIDS: IMPLICATIONS FOR A NON-GENOMIC MECHANISM OF STEROID ACTION. G.A. Golden*, R.T. Rubin, P.E. Mason and R.P. Mason. Neurosciences Research Center, Medical College of Pennsylvania and Hahnemann University, Allegheny Campus, Pittsburgh, PA 15212.

Recent experimental evidence points to rapid cellular effects that occur within seconds to minutes of steroid application, a time frame incompatible with a classical (genomic) mechanism of steroid action. In this study, we examined the membrane interactions of two representative steroid hormones, cortisol and 17-β-estradiol. Partition coefficients (Kp) were determined in phosphatidylcholine liposomes at cholesterol-to-phospholipid mole ratios (C:P) ranging from 0:1 to 1:1 in increments of 0.2. Equilibrium partitioning of both steroids was achieved at t<5 min. In liposomes of a physiologically relevant cholesterol content (0.4:1 C:P), the Kp values for cortisol and estradiol were 147 ± 55.0 and 1290 ± 265, respectively. For estradiol, increasing membrane cholesterol between 0.2:1 and 0.6:1 C:P correlated with a significant reduction in Kp; there were no further changes in Kp above 0.6:1 C:P. In contrast, the Kp for cortisol did not change significantly between 0.2:1 and 0.4:1 C:P; membrane partitioning was highly variable between 0.4:1 and 0.8:1 C:P, and significantly elevated at 1:1 C:P.

Electron density profiles generated from small angle x-ray diffraction of reconstituted human erythrocyte membranes demonstrated that the time-averaged location of cortisol was near the phospholipid headgroup region, while estradiol was located deep within the hydrocarbon core. These data indicate that cortisol and estradiol bind rapidly to energetically favorable locations in the membrane bilayer, and that the extent and location of binding are highly influenced by membrane lipid composition and steroid structure. This study supports a model for rapid steroid action at the cell membrane independent of slower genomic effects.

Supported by a Dean's Scholarship, Medical College of Pennsylvania and Hahnemann University, and NIH grant MH28380 (to RTR).

626.7

REGULATION OF AROMATASE EXPRESSION IN PRIMARY CELL CULTURES OF DEVELOPING ZEBRA FINCH TELENCEPHALON
F. Freking*, B. Ramachandran and B. Schlinger, Depts.of Physiological Science, Psychiatry and Lab of Neuroendocrinology of the BRI, UCLA, Los Angeles, CA 90095.

Aromatase (estrogen synthetase), is expressed in the vertebrate brain, with highest levels often in the hypothalamus-preoptic area (HPOA). In non-songbirds, testosterone (T), estradiol (E2) and 5α-dihydrotestosterone (DHT) can upregulate POA aromatase. This upregulation is a key property of steroid control of reproductive behavior in males. In songbirds, aromatase is expressed at unusually high levels in the telencephalon (Tel). Presumably, regulation of Tel aromatase is important in controlling steroid dependent telencephalic function. To test this idea, we examined factors that might regulate aromatase activity and mRNA expression in 1° cell cultures of developing zebra finch Tel. Cultures (+/- serum) were treated for 0 to 72 hrs with sex steroids or db-cAMP. Total RNA was extracted from cultures for Northern blot analyses or they were incubated with ^{3}H-androgen to measure aromatase activity. Northern blots were hybridized with a ^{32}P-labeled zebra finch aromatase cDNA probe. Compared to control cultures, 1μM E2 reduced aromatase activity on average 58% and mRNA levels by 56%. 1μM T, 5α-and 5β-DHT had no effects on aromatase activity. 400μM db-cAMP increased both aromatase activity and aromatase mRNA by 220%. These results suggest that E2 regulates its own synthesis in the songbird Tel by negative feedback on aromatase expression. Factors that regulate intracellular cAMP levels may also influence Tel aromatase expression. Supported by HD32050 & HD07228.

626.8

INTERACTIVE EFFECTS OF ESTROGEN AND MPP^{+} UPON CORPUS STRIATAL DOPAMINE RELEASE, K. A. Disshon* and D. E. Dluzen. Dept. of Anatomy, NEOUCOM, Rootstown, OH 44272.

The effects of estrogen upon MPTP-induced neurotoxicity were examined using *in vitro* superfusion. Striatal tissue from ovariectomized rats was infused with MPP^{+} (10uM), or a combination of MPP and estrogen (E) using .1, 1, or 100 ng/ml of estrogen. The infusion of MPP^{+} resulted in an increase in dopamine release (in pg/mg of tissue/min, 46.8 ± 7.6, N=20) which was significantly greater than the MPP^{+} + E-100ng/ml (18.6 ± 3.1, N=5) treated group. Neither the MPP^{+} + E-.1ng/ml (41.3 ± 5.5, N=9) or the MPP^{+} + E-1ng/ml (63.6 ± 3, N=10) groups were significantly different from the MPP^{+} group. These results demonstrate that the gonadal steroid hormone estrogen can modulate responses of striatal dopamine neurons to MPP^{+} by altering the immediate increase in dopamine release which occurs in response to this neurotoxin. This effect showed a dose dependency with only the 100ng/ml E dose significantly reducing the MPP^{+} response. Supported by an NIH grant to D.E.D.

626.9

THE ROLE OF ESTROGEN IN GLIAL GENE EXPRESSION AND GLIAL REACTIVITY IN VITRO.
I.Rozovsky*, D.J. Stone, T.E. Morgan, C.P. Anderson and C.E.Finch. Andrus Gerontology Center and Department of Biology, University of Southern California, Los Angeles, CA 90089.

Astrocyte hypotrophy in the response to local neuronal activities and/or damage and increased expression of GFAP were demonstrated during the estrus cycles. Recent studies from our lab showed that two astrocyte lipoproteins, ApoE and ApoJ, which are involved in synaptic plasticity, and modify Aß toxicity in vitro (Oda et al., Exp. Neurology, 1995) are also differentially expressed in hippocampus and arcuate nucleus of hypothalamus during the estrus cycles (Stone et al., Neuroscience Abst., 1996). Both ApoE and ApoJ are expressed and secreted by rat cultured astrocytes. However, cultured microglia express high prevalence ApoE mRNA, but no ApoJ mRNA. Treatment of mixed glial cultures with low physiological concentrations of 17ß-estradiol demonstrated 2-fold increase of ApoE mRNAs. In contrast, pure astrocyte cultures did not show the change in ApoE mRNA level. Heterotypic cell-cell interactions, as well as differential regulation of microglial ApoE by estrogen, might be responsible for the ApoE mRNA increase in mixed glial cultures.

The role of estrogen in controlling astrocyte reactivity in the response to neuronal damage using the in vitro model of "wounding in a dish" was also examined. GFAP was induced in the response to mechanical wounding of astrocyte-neuron co-cultures. Addition of 17ß-estradiol to these cultures prevented the "wound"-induced increase of GFAP. The role of estrogen response elements in rat GFAP promoter was also defined. (Supported by AG13499-01 to C.E.F.).

626.10

ESTROGEN RECEPTORS ARE EXPRESSED IN OLFACTORY AND VOMERONASAL RECEPTOR NEURONS
M.L. Getchell*[1,2], A. Kulkarni-Narla[3], R. Marcinek[1,3], and T.V. Getchell[1,2,3]. [1]Otolaryngol., Dept. of Surgery; [2]Sanders-Brown Center on Aging, [3]Dept. of Physiology, Univ. of Kentucky Coll. of Med., Lexington, KY 40536.

Estrogen receptors (ERs) are ligand-dependent nuclear transcription factors that regulate the tissue-specific expression of numerous genes. ERs have been localized in several central olfactory and vomeronasal pathways. Using molecular and cellular techniques, we demonstrate the localization of ERs in olfactory and vomeronasal receptor neurons (ORNs, VRNs, respectively). Northern blots of total and poly $(A)^{+}$ RNA extracted from nasal mucosa and uterus from 6- and 12-week-old rats probed with a cocktail of 3 ^{32}P-labeled oligonucleotides with unique sequences in the coding region of the ER gene showed a single 6.2 kb band that was more intense in the older rats. *In situ* hybridization with these probes labeled with ^{35}S and immunohistochemistry with 2 antibodies recognizing sequences in either the hormone- or DNA-binding ER domains demonstrated the localization of ER message and protein in ORNs and VRNs in female and male rats. No ER immunoreactivity was detected in the nasal cavity at embryonic (E) day 12; ER protein was first detected in both ORNs and VRNs at E14; staining intensity increased at E16 and was maintained at E19, P6, 11, 17, and 20. In contrast to the uniform staining pattern at E14-19, in postnatal animals regions of intensely stained ORNs were intermingled with regions of less intensely stained ORNs. ERs were also immunolocalized in ORNs of adult humans of both genders. Estrogen may influence development, neuronal turnover and plasticity, and reproduction-related chemosensation in these receptor neurons. Supported by NIH grants DC 01715 (MLG) and DC 00159 (TVG).

626.11

FAILURE TO FIND VARIATIONS IN PERFORMANCE ON GENDER-SPECIFIC COGNITIVE TASKS AS A FUNCTION OF MENSTRUAL CYCLE PHASE.
K. Epting, K. Barbour, W. Overman*. Psychology Department, Univ. North Carolina at Wilmington, Wilmington, N.C. 28401

Three putative female-sensitive cognitive tests (Purdue peg board, finger tap, spatial array) and three putative male-sensitive cognitive tests (mental rotation, rod-and-frame, water-level) were administered twice, at six-week intervals, to 27 college women and 20 men. Counterbalanced for order, women received the tests once during menstruation (low hormone condition) and once during mid luteal phase (high hormone condition). The mid-luteal phase was determined by projection from ovulation, as verified by commercially available ovulation detection kits and by follow up confirmation of subsequent menstruation.

Results showed a significant gender difference for 4 tasks in the expected male- or female- direction; however, for females there was no evidence that cognitive performance differed with menstrual cycle phase. Results are consistent with previous negative findings of effects of the menstrual cycle upon cognition (Sommer, B. in J. Richardson (Ed.), Cognition and the Menstrual Cycle, Springer-Verlag, 1992) and inconsistent with reports of cognitive variations during the menstrual cycle (Hampson, E., Kimura, D. 1988, Beh. Neurosci., 102). Supported by UNC-W Research Fellowship Award to K. Epting and NIMH grant RO1-MH50724-01 to W. Overman.

626.12

EFFECTS OF ESTROGEN ON BASAL FOREBRAIN CHOLINERGIC NEURONS VARY AS A FUNCTION OF DOSE AND DURATION OF TREATMENT.
R.B. Gibbs*. University of Pittsburgh School of Pharmacy, Pittsburgh, PA, 15261.

Immunohistochemical techniques were used to examine the effects of estrogen replacement on the number of choline acetyltransferase (ChAT) and p75NGFR expressing cells detected in the medial septum (MS) and nucleus basalis magnocellularis (NBM) of adult (~250g), ovariectomized, Sprague-Dawley rats.

Animals were maintained on a 12h:12h light:dark cycle. Estradiol benzoate (E; in 0.1 mL sesame oil, injected s.c.) was administered every other day at a dose of 2, 10, 25, or 100 μg/250g.b.w. for a period of 1, 2, or 4 weeks (n=5-7 animals/group). Controls received vehicle alone. Twenty-five micron sections through the basal forebrain were cut and stained for immunocytochemical detection of ChAT (AB144P; Chemicon, Inc.) or p75NGFR (mouse monoclonal AB#192). The number of immunoreactive (IR) cells in each of 3 matched sections/region/animal was counted and compared at each time-point using analysis of variance followed by the Dunnett's test.

Average serum levels of estradiol ranged from 14.0-26.3 pg/mL, 37.9-54.4 pg/mL, 105.4-170.4 pg/mL, and 439.8-888.3 pg/mL in animals treated with 2, 10, 25, and 100 μg E every other day for 1-4 weeks. Treatment with 2-25 μg E produced a dose-related increase in the number of ChAT-IR cells detected in the MS at 1 week, with the peak increase (30%) detected at the 25 μg dose. No significant increase was detected with the 100 μg dose or following longer periods of treatment. Treatment with 10 μg E for 1 week or with 2 μg E for 2 weeks produced a significant increase (21.6% & 23.3%) in the number of ChAT-IR cells detected in the NBM. In contrast, E administration produced a dose-related decrease in the number of p75NGFR-IR cells detected in the MS (but not the NBM) with the greatest effects detected after 4 weeks of treatment with the 25 μg (-29%) and 100 μg (-28.9%) doses.

These data demonstrate that the effects of estrogen on basal forebrain cholinergic neurons are dependent on both the dose and duration of estrogen treatment with maximal increases in ChAT-IR detected following short-term replacement with physiological levels of E, and decreases in p75NGFR detected following longer-term treatment with higher levels of E. Supported by NIH grant # RO1-NS28896.

627.1

DISTRIBUTION OF PROTEIN KINASE C (PKC) ISOFORMS IN CULTURED PREMOTOR RESPIRATORY NEURONS FROM NEONATAL RAT Miranda Jarnot* and Michael S. Dekin, Dept. of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903

An outward rectifying K^+ channel ($K_{(OR)}$) is activated by both serotonin type 1B ($5\text{-}HT_{1B}$) and γ-aminobutyric acid type B ($GABA_B$) receptors in cultured premotor respiratory neurons (Dekin and Wagner, *Soc. Neurosci. Abstr.*, this volume). While $K_{(OR)}$ channels activated by $5\text{-}HT_{1B}$ have a much smaller single channel conductance than those activated by $GABA_B$, both types of channel are inhibited by thyrotropin-releasing hormone (TRH) which is an activator of PKC. In this report, we studied the distribution of PKC isoforms in cultured premotor respiratory neurons and determined how they were modulated by TRH, $5\text{-}HT_{1B}$, and $GABA_B$. Indirect immunofluorescence was used to detect labeling by antibodies specific for the α, β, γ, ε, δ, and λ isoforms of PKC. PKC(α) was localized in the cytoplasmic membranes of unstimulated neurons suggesting that it was endogenously active. PKC(γ), PKC(δ), and PKC(λ) were diffusely distributed throughout the cytoplasm suggesting that they were inactive. No staining for PKC(β) or PKC(ε) was detected. Preincubation with TRH altered the distribution of PKC(δ) and PKC(λ). PKC(δ) translocated to the nuclear membrane while PKC(λ) translocated to the cytoplasmic membrane. Preincubation with CGS-12066B, a $5\text{-}HT_{1B}$ agonist, decreased PKC(α) in the cytoplasmic membrane. Exposure to baclofen, a $GABA_B$ agonist, did not alter the distribution of any PKC isoforms. These data suggest that translocation of PKC(λ) to the cytoplasmic membrane by TRH is involved in the inhibition of $K_{(OR)}$ channels. The decrease in endogenously active PKC(α) by $5\text{-}HT_{1B}$, but not $GABA_B$, further suggests that this PKC isoform may play a role in determining the single channel conductance of $K_{(OR)}$ channels. (Supported by NIH Grants HL54666, HL07467, and an AHA-NJ Affiliate Grant)

627.2

MULTIPLE NEUROTRANSMITTERS EMPLOY PROTEIN KINASE A (PKA) TO MODULATE OUTWARD RECTIFYING K^+ CHANNELS IN CULTURED PREMOTOR RESPIRATORY NEURONS. M.S. Dekin* and P.G. Wagner Dept. of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903

The serotonin type 1B ($5HT_{1B}$) receptor agonist CGS-12066B activates a Ba^{++}-insensitive outward rectifying K^+ channel ($K_{(OR)}$) in cultured premotor respiratory neurons (see Wagner and Dekin, *Soc. Neurosci. Abstr.*, this volume). $K_{(OR)}$ channels are also activated by γ-aminobutyric acid acting at its β receptor ($GABA_B$) and inhibited by thyrotropin-releasing hormone (TRH) (Wagner *et al., Soc. Neurosci. Abstr.*, 19(2):1194, 1994). In this study, we tested the hypothesis that all three neurotransmitters modulate $K_{(OR)}$ channels through PKA. Single channel recordings (cell-attached configuration) were made from cultured neurons obtained from neonatal rats. Baclofen, a selective $GABA_B$ agonist, activated $K_{(OR)}$ channels with a variable conductance (γ) ranging from 90-200 pS and a mean open time probability (nP_O) <30%. $5HT_{1B}$ activated channels had a lower γ and showed much less variability (30 to 40 pS). $5\text{-}HT_{1B}$ channels also had a higher nP_O (>50%). TRH decreased nP_O in both types of channel to <2% but had no effect on γ. Rp-cAMP, a competitive inhibitor of PKA, increased nP_O of the baclofen activated channel to over 50% and reduced γ to a value comparable to that of the $5\text{-}HT_{1B}$ channel (36.3 pS±2.1, n=10). Rp-cAMP had no effect on either nP_O or γ of the $5HT_{1B}$ activated channel. Rp-cAMP did, however, protect both channel types from inhibition by TRH. These data demonstrate that $GABA_B$, $5\text{-}HT_{1B}$, and TRH converge on PKA to modulate $K_{(OR)}$ channels. The differential effects of these neurotransmitters on nP_O and γ also indicates that PKA acts (directly or indirectly) at two distinct sites on the channel. (Supported by NIH Grant HL54666, an AHA-NJ Affiliate Grant, and the Parker B. Francis Foundation).

627.3

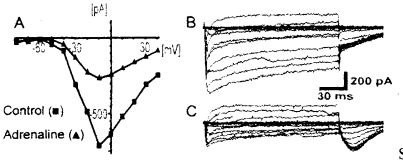
SEROTONIN 1B ($5HT_{1B}$) RECEPTORS ARE COUPLED TO A BA^{++} INSENSITIVE OUTWARD RECTIFYING K^+ CHANNEL IN CULTURED PREMOTOR RESPIRATORY NEURONS OF THE NEONATAL RAT. P.G. Wagner* and M.S. Dekin Dept. of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

Activation of $5\text{-}HT_{1B}$ receptors in the phrenic motor nucleus of the rat inhibits the release of L-glutamate from premotor respiratory neurons (Lindsay and Feldman, *J. Physiol.*, 461:213, 1993). We have previously demonstrated the existence of a second messenger operated Ba^{++} -insensitive outward rectifying K^+ channel ($K_{(OR)}$) in cultured premotor respiratory neurons from neonatal rats and suggested that this channel is involved in the regulation of neurotransmitter release (Wagner and Dekin, *J. Neurophysiol.*, 69:286, 1993). In this study, we tested the hypothesis that $K_{(OR)}$ channels can be activated via $5HT_{1B}$ receptors. Using the single channel recording technique from cell attached patches we observed that bath application of 0.5 μM CGS-12066B, a selective $5\text{-}HT_{1B}$ receptor agonist, activated $K_{(OR)}$ channels. Mean channel open time probability was typically > 50%. Current-Voltage (I-V) relationships were measured in 140 mM $[K]_O$ to zero the membrane potential (V_m). When V_m was more negative than 0 mV, the I-V relationship displayed pronounced outward rectification. Neither Ba^{++} nor Cs^+ had any affect on outward rectification. The single channel conductance, measured over the linear portion of the I-V relationship at V_m more positive than 0 mV, was 35 pS ± 2.3 (n= 8). The reversal potential (E_r) was 0 mV with 140 mM K^+ in the pipette. When the pipette K^+ concentration was altered, E_r changed in accordance with the Nernst equation prediction for a K^+ channel. These data support the hypothesis that $K_{(OR)}$ channels are involved in the modulation of L-glutamate release from premotor respiratory neurons. (Supported by NIH Grant HL54666 an AHA-NJ Affiliate Grant, and the Parker B. Francis Foundation).

627.4

CALCIUM-CURRENTS IN NEURONS OF THE ISOLATED RESPIRATORY SYSTEM OF MICE. F.P. Elsen*, P. Telgkamp, J.M. Ramirez, D.W. Richter. Department of Physiology, University of Göttingen, 37073 Göttingen, FRG.

The transverse medullary slice preparation of mice containing the pre-Bötzinger complex (pBC) generates spontaneous respiratory rhythmic activity at different postnatal stages (P0-P25). During this time, changes occur in the motor pattern, network organization, responsiveness to neuromodulators and hypoxia (Funk et al. 1994, J. Neurophys. 72: 2538; Ramirez et al. 1996, J. Physiol. 491: 799). To understand the underlying mechanisms, whole-cell patch clamp recordings were obtained from neurons within the pBC. Here, we concentrated on the characterization of calcium currents, isolated by intracellular CsCl [110mM] and TEA [30mM] and by extracellular TTX [1μM] (B). Different stimulation protocols and the application of specific toxins revealed the presence of T- and P-type, as well as high voltage-activated N-type calcium currents. The amplitude of the steady state currents decreased during postnatal development which was most obvious for high-voltage steps from -70 to -10mV, suggesting that these are developmental changes in the expression of high voltage activated calcium currents. These currents were modulated by the application of adrenaline [5μM] which led to a reduction in peak (A) and steady state currents (C). Adrenaline also evoked a delayed tail current (C), which was blocked by cobalt [2mM] and ω-conotoxin GVIA [5μM]. Currently, we examine the consequences of these changes for respiratory rhythm generation.



Supported by DFG

627.5

LONG-TERM SYNAPTIC DEPRESSION IN NUCLEUS TRACTUS SOLITARIUS OF NMDAR1 KNOCKOUT MICE. C.-S. Poon*, Z. Zhou, and J. Champagnat. Harvard-MIT Division of Health Sciences and Technology, M.I.T., Cambridge, MA 02139, USA and Institut Alfred Fessard, C.N.R.S., Gif-sur-Yvette, France.

Recent studies have shown that mutant mice whose NMDA receptors are genetically disrupted suffer from certain developmental abnormalities in the brainstem and die within the first day of life. We have previously shown that neonatal mice with genetic knockout of the NMDAR1 subunits exhibit severe respiratory depression, although their CO_2 chemosensitivity remain largely intact (Poon et al., 1994, 1995). To investigate whether the respiratory depression was caused by abnormalities in the respiratory controller, we used an in-vitro brainstem slice preparation to study synaptic transmissibility in the nucleus tractus solitarius (NTS) of normal and mutant mice at postnatal day 0. Synaptic strength of NTS neurons was assessed by measuring the postsynaptic excitatory responses using either whole-cell recordings or extracellular field potentials elicited by electrical impulse stimulation of afferent fibers in the tractus solitarius (TS). Inhibitory responses via $GABA_A$ receptors were eliminated by bath application of bicuculline (20 μM). Low-frequency stimulation (LFS; 5 Hz for 5 min) of TS resulted in long-term depression (LTD) of synaptic transmission in the mutant cells (7 out of 10 cells) lasting > 30 min, but had no effect on any of the normal cells (n = 7). Application of D-APV (50 μM) had no effect on the response of normal cells to LFS (n = 2). Thus, genetic disruption of NMDA receptor activity may trigger a form of LTD that is absent in normal cells even after acute blockade of NMDA activity. Such LTD in the NTS offers a possible central mechanism for the respiratory depression in NMDAR1 knockout mice. (Supported by ONR grant N00014-95-0414 and NIH grant HL50641)

627.6

GENERATION OF RESPIRATORY OSCILLATIONS AFTER DISRUPTION OF INHIBITORY NEUROTRANSMISSION DURING POSTNATAL DEVELOPMENT: *IN VITRO* AND *IN VIVO* STUDIES WITH MUTANT AND NORMAL MICE. J.C. Smith*, N. Koshiya, and E.S. Simon. Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892.

We have proposed that respiratory rhythm in mammals is generated by a hybrid pacemaker-network in which excitatory interneurons with oscillatory bursting properties in the pre-Bötzinger complex (pre-BötC) form the kernel of the oscillator (Smith et al. *Science 254*, 1991). This model predicts that rhythm generation can occur without network-based inhibitory synaptic interactions. This has been confirmed for neonatal rats (PO-P7) from our *in vitro* studies with medullary slice preparations that generate respiratory rhythm (*ibid.*), but has only been partially tested later in postnatal development (Ramirez et al. *J. Physiol. 491.3*, 1996). We extended earlier work by analyzing the role of synaptic inhibition *in vivo* in mice up to P23 and *in vitro* in medullary slices from mice up to P15. Perturbations of rhythm generation were analyzed *in vivo* in the mutant mouse *oscillator* (*Human Mol. Gen. 3: 2025*, 1994), which has a mutation of the adult isoform of the alpha subunit of the glycine receptor (GlyR) that causes death by P23. This mutation is reported to result in a loss of GlyR function by ~P15 when the developmental switch from a normal neonatal to the mutant adult alpha subunit is complete. These mutants generate a stable breathing rhythm and normal pattern of phrenic motor discharge until death while disturbances, including tremor and rigidity, develop in other motor systems after P15. In *in vitro* studies, rhythmic activity of respiratory (XII) motoneurons and pre-BötC inspiratory neurons was maintained in slice preparations after simultaneous blockade in the respiratory network of glycine, $GABA_A$ and $GABA_B$ receptors by bath-application of pharmacological antagonists (up to 50 μM strychnine, 100 μM bicuculline, 1 mM hydroxysaclofen). These results are consistent with the hybrid pacemaker-network model.

627.7

EXPIRATORY (E-)NEURONS MEDIATE EXPIRATORY LENGTHENING IN A LUNG ATTACHED *IN VITRO* BRAINSTEM-SPINAL CORD PREPARATION. N.M. Mellen*, D.R. McCrimmon & J.L. Feldman. Depts. of Physiol. Sci. and Neurobiology, UCLA, Los Angeles, CA, 90095-1527 & Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago IL, 60611-3008

Lung inflation during expiration activates slowly adapting pulmonary stretch receptors causing expiratory prolongation (Breuer-Hering reflex, B-H). We have used a preparation in which afferent vagal innervation is maintained between lungs and an otherwise isolated neonatal rat brainstem-spinal cord preparation (Murakoshi et al.; *Neurosci. Lett. 62:*63, '85), to investigate cellular and synaptic mechanisms mediating this reflex. Lung inflation-induced changes in firing patterns of ventral medullary respiratory neurons were studied using extracellular electrodes. We inflated lungs with mock CSF. Inflation was triggered by inspiratory onset monitored at ventral roots C1-C4. Baseline lung pressure ranged between 0-2 cm H_2O and inflation-induced pressure change was 2-5 cmH_2O. We classified cells according to Smith *et al.* (*J. Neurophys. 64:*1149, '90). Lung inflation led to a sustained increase in activity of phase-spanning E cells and a matching decrease in activity of late-E cells. This enhanced firing or inhibition persisted until lung deflation, triggered by the next inspiratory phase. E-tonic cells were transiently inhibited by lung inflation, and I cells were either inhibited or showed no change in firing pattern. Non-respiratory cells were transiently inhibited or excited by lung inflation. We suggest that activation of phase spanning E neurons and inhibition of late-E neurons provides the mechanism for the expiratory lengthening associated with the Breuer-Hering reflex. This work was supported by NIH grants HL09234 and HL37941.

627.8

LUNG INFLATION-INDUCED EXPIRATORY LENGTHENING REQUIRES $GABA_A$ RECEPTOR ACTIVATION IN A LUNG ATTACHED *IN VITRO* BRAINSTEM-SPINAL CORD PREPARATION. D.R. McCrimmon*, N.M. Mellen, & J.L. Feldman. Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago IL, 60611-3008 & Depts. of Physiological Science and Neurobiology, UCLA, Los Angeles, CA, 90095-1527

Using a modified neonate rat *in vitro* brainstem spinal cord preparation in which lungs and their vagal innervation are preserved, and in which the Breuer-Hering reflex (B-H) can be elicited, we compared the effect of lung inflation on expiratory duration and discharge patterns of ventral medullary respiratory neurons before and after bath application of the $GABA_A$ receptor antagonist, bicuculline (BIC, 5-20 μM). Lung inflation-induced changes in baseline firing patterns of these neurons are described in an accompanying abstract. Lungs were filled and inflated with mock CSF via a cannula inserted into the trachea. Inflation was delivered every 6-12 respiratory cycles, triggered by inspiratory onset monitored at a ventral root (C1-C4). Baseline lung pressure ranged between 0-2 cm H_2O and the change in pressure during inflation was 2-5 cm H_2O. Cells were recorded from extracellularly and classified according to Smith *et al.* (J. Neurophys. 64:1149, '90). BIC markedly and reversibly attenuated lung inflation-induced expiratory lengthening. Expiratory neurons excited by lung inflation under control conditions retained their response under BIC. In contrast, neurons inhibited by lung inflation during control cycles were no longer affected after BIC. These findings identify neurons affected by GABAergic inhibition which mediate the B-H reflex. This work was supported by NIH grants HL09234 and HL37941.

627.9

HYPOXIA ENHANCES PHRENIC NERVE ACTIVITY FOLLOWING DISINHIBITION OF NEURONS IN THE PRE-BÖTZINGER COMPLEX. I.C. Solomon*, N.H. Edelman, and J.A. Neubauer. Dept. of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ

Recently, we have demonstrated that local hypoxia of neurons located in the pre-Bötzinger (pre-Böt) complex produces a gasp-like pattern in the phrenic neurogram (PN), suggesting that neurons in this region are hypoxia chemosensitive. Further, we have shown that $GABA_A$-mediated inhibition of neurons in this region plays a role in the suppression of gasping during eupnea, and that prior disinhibition of these neurons unmasks central hypoxia chemosensitivity in response to a hypoxic challenge induced systemically. The combined effect of hypoxic stimulation of this central hypoxia chemosensitive region and the peripheral chemoreceptors on PN output is unknown. We, therefore, examined the effects of hypoxia before and after blockade of $GABA_A$ receptors in the pre-Böt complex in chloralose- or chloralose/urethane-anesthetized vagotomized, paralyzed, mechanically ventilated cats. Approximately 45-60 minutes after a control response to a hypoxic challenge (6-12% O_2 in N_2 for 3 min) was obtained, bicuculline methiodide (BIC; 4.5 mM; 42 nl) was microinjected, and 15-20 minutes later, the hypoxic challenge was repeated. Microinjection of BIC produced either a gasp-like (n=6) or augmented (n=1) pattern in the PN and decreased the frequency of phrenic activity. Before microinjection of BIC, hypoxia increased PN amplitude by 126% and elicited no change in the frequency of phrenic bursts. After microinjection of BIC, hypoxia similarly increased PN amplitude by 124%; however, hypoxia also increased the frequency of phrenic activity by 220%. Subsequent microinjection of sodium cyanide (1 mM; 21 nl) into 3 of these sites similarly increased the frequency of phrenic activity by 210%. These findings demonstrate that hypoxia stimulates both amplitude and frequency of phrenic activity following disinhibition of neurons located in the pre-Böt complex. Further, our findings suggest that the enhanced frequency results from hypoxic excitation of neurons in the pre-Böt complex. Supported by HL16022, AHA/NJ Affiliate.

627.10

$GABA_A$ NEUROTRANSMISSION AND RESPIRATORY MOTOR OUTPUT IN THE BRAINSTEM + SPINAL CORD OF NEWBORN MICE. J. P. Farber*. Univ. of Oklahoma HSC; Okla. City, OK 73190.

Results of blocking $GABA_A$ neurotransmission on respiration-like motor output among in-vitro preparations have been variable. Small changes in respiratory timing are observed in the brainstem + spinal cord of the rat (Pflügers Arch. 417:425, 1990) while the tilted sagittal medullary slice preparation from the mouse shows substantial increases of the interburst interval (J. Physiol. 484:505, 1995). The present studies utilized brainstem + spinal cord from neonatal mice to evaluate effects of the $GABA_A$ antagonist, bicuculline (bic), at 2-10 mM, on the motor output from either hypoglossal or C4 ventral roots. Preparations were superfused using carbogen-saturated ringers solution (K^+ = 5mM in most cases), with a flow of 4-5 ml/min, at 27-28°C. Newborns, at day 0, in a minority of instances showed relatively short interburst intervals (2-3 sec) which were approximately doubled by bic; motor burst duration was increased. More often, day 0 preparations were not rhythmic, but could be induced to burst with bic. In those instances, motor bursts were comparable to that observed in bic-treated animals which originally expressed a rapid rhythmic output. When treated with bic, preparations from older animals (day 2-3) having spontaneous rhythm showed decreases in interburst interval. Sometimes this response was transient. Changes in the motor bursts were generally minor. Both induction of rhythm and slowing of rhythm after bic could result from the relatively unrestrained influence of a pacemaker, but the original situations of rapid bursting as well as apnea suggest a substantial inhibitory input onto relevant neuronal circuitry. The relatively modest effects of bic occurring after day 0 may be a consequence of more impaired O_2 diffusion through a larger tissue mass. (Supported by HL37318).

627.11

PHARMACOLOGICAL IDENTIFICATION OF GABA RECEPTORS IN THE PHRENIC MOTONUCLEUS OF RAT. **H.N. Sapru* and V.C. Chitravanshi, Departments of Neurosurgery & Pharmacology, New Jersey Medical School, Newark, NJ 07103.**

GABAergic terminal arborizations have been reported to be present in the phrenic motonucleus (PMN). In this study phrenic nerve (PN) responses to activation of GABA receptors in the PMN were studied. Experiments were carried out in the adult male Wistar rats, anesthetized with urethane, bilaterally vagotomized, paralyzed and artificially ventilated. A pneumothorax was produced to eliminate movement artifact. All the dorsal and ventral rootlets from C_2-C_6, except those at C_3 segment, were sectioned so that the PN activity represented the output of C_3 phrenic motoneurons only. The PMN was identified by microinjecting (50 nl) of NMDA (5 mM) at C_3 segment; an increase in PN background discharge was observed. Muscimol (3 mM/50 nl) was microinjected into the same site. A decrease in the amplitude of spontaneous PN bursts was observed. Prior injections of bicuculline methiodide (2 mM/50 nl) into the PMN completely blocked the inhibitory effects of muscimol. The inhibitory effect of muscimol was also reversed by microinjections of bicuculline methiodide. Excitatory PN responses to peripheral chemoreceptor activation, by tracheal administration of 100% N_2 for 7 sec, were blocked by prior microinjections of muscimol into the PMN. These results suggest that activation of $GABA_A$ receptors in the PMN results in inhibition of spontaneous PN bursts which is prevented as well as reversed by microinjections of bicuculline.

Support: NIH: HL24347 and AHA (NJ).

627.12

CARBACHOL-INDUCED INHIBITION OF PHRENIC MOTONEURONS IS MEDIATED BY M_1 MUSCARINIC RECEPTORS. **V.C. Chitravanshi* and H.N. Sapru., Departments of Neurosurgery & Pharmacology, New Jersey Medical School, Newark, NJ 07103.**

Phrenic nerve (PN) responses to activation of cholinergic receptors in the phrenic motonucleus (PMN) were studied. Experiments were carried out in the adult male Wistar rats, anesthetized with urethane, bilaterally vagotomized, paralyzed and artificially ventilated. A pneumothorax was produced to eliminate movement artifact. All the dorsal and ventral rootlets from C_2-C_6, except those at C_3 segment, were sectioned so that only the output of C_3 phrenic motoneurons was represented in the PN activity. The PMN was identified by microinjecting (50 nl) of NMDA (5 mM) at C_3 segment; an increase in PN background discharge was observed. Carbachol in different doses (25 nM -5 mM/50 nl) was microinjected into the same site. A dose-dependent decrease in the amplitude of spontaneous PN bursts was observed. This inhibition was not due to some non-specific local actions of carbachol because microinjections of NMDA into the PMN continued to elicit excitatory PN responses. Prior injections of pirenzepine (1 mM/50 nl), but not bicuculline methiodide (6 mM/50 nl) or strychnine (2 mM/50 nl), into the PMN completely blocked the inhibitory effects of carbachol. These results suggest that: (1) muscarinic cholinergic agonists inhibit phrenic motoneurons; this effect was unexpected considering that acetylcholine has been implicated as an excitatory transmitter in the central regulation of respiratory function, (2) the inhibitory effect of carbachol is mediated via M_1 muscarinic receptors, and (3) activation of GABA or glycine receptors does not contribute to the inhibitory effect of carbachol.

Support: NIH: HL24347 and AHA (NJ).

627.13

CORRELATION OF SYNAPTIC VESICLE MORPHOLOGY WITH AMINO ACID CONTENT OF INPUTS TO RAT PHRENIC MOTONEURONS Susan M. Murphy*[1], Paul M. Pilowsky and Ida J. Llewellyn-Smith, Departments of Anatomy and Histology[1], and Medicine, Flinders Medical Centre, Bedford Park SA 5042, AUSTRALIA.

The amino acids glutamate (GLU) and γ-aminobutyric acid (GABA), are neurotransmitters in excitatory and inhibitory pathways, respectively, from bulbospinal respiratory neurons to phrenic motoneurons. Another amino acid, glycine (GLY), has not yet been shown to play a role in transmission to phrenic motoneurons, although GLY is involved in inhibitory control of other groups of spinal motoneurons. We retrogradely labelled phrenic motoneurons by injecting cholera toxin B subunit conjugated to HRP (CT-HRP) into the diaphragm, and used post-embedding immunogold labelling to identify nerve terminals containing GLU, GABA or GLY, that made synapses or direct contacts with CT-HRP-labelled phrenic motoneuron cell bodies or dendrites. We classified nerve terminals into two groups on the basis of the morphology of their synaptic vesicles. S terminals contained round synaptic vesicles. F terminals, contained mixtures of round and flattened synaptic vesicles. S terminals had higher levels of GLU ($t=5.0$, $p<0.001$: Wilcoxon two-sample test) and lower levels of GABA ($t=4.7$, $p<0.001$) than F terminals. GLY labelling was higher in F terminals than in S terminals ($\chi^2=15.8$, $p<0.001$: median test). These data demonstrate a strong correlation between synaptic vesicle morphology and neurotransmitter content in the inputs to phrenic motoneurons, suggesting that S terminals are excitatory, and F terminals are inhibitory. Supported by grants from the NH&MRC, and the National SIDS Council of Australia.

627.14

ANATOMICAL LOCALIZATION OF THE VENTRAL RESPIRATORY GROUP AND NUCLEUS AMBIGUUS IN THE NEONATAL RAT. H.H. Ellenberger*. Department of Anatomy & Neurobiology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

In vitro preparations of the neonatal rat have provided fundamental information about the cellular mechanisms underlying respiratory network function. However, the anatomical location of the medullary respiratory neuron populations in the neonatal rat has not been determined. Fluorescent retrograde tracers were utilized to identify the ventral respiratory group (VRG) and nucleus ambiguus (AMB) in the neonatal rat. Sprague-Dawley rats (day zero) were anesthetized with 20µl of 5% Innovar-Vet (I.P.) and received injections of 2% (20-60nl) Fluoro-Gold (FG) into the C_4 spinal cord and/or applications of 1% Fast Blue (FB) to the cervical vagus nerve. After a 48 hr survival period, FG labelled bulbospinal neurons were identified within a densely packed column within the ventrolateral intermediate reticular nucleus from the level of the pyramidal decussation to the facial nucleus. This cell column corresponded closely to the location of the VRG of the adult rat. FG labelled neurons were also located in the medial reticular nuclei, raphe pallidus and obscurus, parapyramidal nucleus, and spinal vestibular nucleus. FB labelled vagal motoneurons were distributed throughout the dorsal motor nucleus of the vagus and AMB. Labelled VRG neurons overlapped with the external formation, and partially with the loose formation, but not with the semicompact or compact formation of AMB. These results indicate that the distribution and relative locations of bulbospinal VRG neurons and vagal motoneurons are similar in neonatal and adult rats.

This study was supported by MRC operating grant #MT-12212.

627.15

***IN VITRO* AND *IN VIVO* CHARACTERIZATION OF VENTROLATERAL PAG NEURONS PROJECTING TO RESPIRATORY NUCLEI.** R. Pásaro*, S.P. Gaytán, and J. Ribas. Dept. of Animal Physiology and Biology, and Dept. of Medical Physiology and Biophysics, University of Sevilla, 41012-Sevilla, Spain.

The Periaqueductal Gray (PAG) is a highly complex structure involved in several autonomic functions. In this report, the PAG link with the Ventral Respiratory Group (VRG) was elucidated by means of electrophysiological identification of the VRG and simultaneous injection of a mixture of Fast Blue (FB) and Fluoro Ruby (FR) fluorochromes. These results showed four different populations of FB-labelled neurons within the PAG and moderate FR-labelled fibers and varicosities. From all of these populations of PAG neurons projecting to the VRG, the ventrolateral one was intracellular recorded in slice preparation. The membrane resting potential of these neurons ranged from 60 to 75 mV and controlled the type of electro-physiological response induced by the depolarizing pulses. The lower membrane potentials were related to tonic firing and the higher ones to burst firing of action potentials, the latter lacked calcium conductance. Furthermore, the ventrolateral PAG neurons were unsensitive to opioid receptor agonists. Supported by a CICYT PB94-1443 grant.

627.16

IDENTIFICATION OF SEPARATE SITES RESPONSIBLE FOR GILL AND LUNG RESPIRATORY RHYTHMICITY IN THE *IN VITRO* BRAINSTEM PREPARATION OF THE BULLFROG TADPOLE, *RANA CATESBEIANA*. M.J. Gdovin*, C.S. Torgerson, and J.E. Remmers. Department of Medical Physiology, University of Calgary Faculty of Medicine, Calgary, Alberta, Canada, T2N 4N1.

We recently described an *in vitro* brainstem preparation of the bullfrog tadpole *Rana catesbeiana* in which we were able to distinguish between fictive gill and lung ventilatory activities in cranial nerve (CN) V, VII, and X neurograms (Torgerson *et al.*, 1996). In addition, this *in vitro* brainstem preparation increased both fictive gill and lung ventilation in response to elevations in superfusate PCO_2. The neural substrates responsible for central respiratory rhythm generation of gill and lung ventilation in the tadpole are unknown. We recorded efferent activities of CN V or VII, and X during changes in superfusate PCO_2 before and after transection of the *in vitro* brainstem between CN VIII and IX. After transection, robust gill ventilation was recorded from CN X but was absent in CN V and VII neurograms. By contrast, this transection eliminated fictive lung bursts in CN X, while they persisted in CN V and VII. Moreover, the frequency response of lung bursts to increases in superfusate PCO_2, observed before transection, persisted in CN V and VII after transection. We conclude that the neural substrate required for fictive gill and lung ventilation exist in anatomically separate regions such that the gill central pattern generator (CPG) is located in the caudal medulla at the level of CN X, and the lung CPG is located more rostrally in the medulla at the level of CN VII (supported by grant MA-9719 from the MRC of Canada).

RESPIRATORY REGULATION: CHEMORECEPTION AND HYPOXIC RESPONSES

628.1

DIFFERENTIAL EXPRESSION OF HEME OXYGENASE ISOFORMS IN THE ROSTRAL VENTROLATERAL MEDULLA OF THE RAT. E Mazza*, S Thakker-Varia, M Zingariello, CA Tozzi, JA Neubauer. Departments of Medicine and Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ.

Neurons in the rostral ventrolateral medulla (RVLM) are directly excited by hypoxia. However, the mechanism by which the RVLM senses changes in O_2 is unknown. Since heme oxygenase (HO) has been shown to be involved in O_2 sensing mechanisms in the carotid body and pulmonary artery, the goal of these studies was to determine if HO is present in the RVLM and whether the expression of HO is altered by chronic hypoxia. Two isoforms of HO have been described; HO-1 which is inducible and HO-2 which is constitutive. To determine whether HO-1 and HO-2 are expressed in the RVLM and whether this expression is altered by chronic hypoxia, RT-PCR was used to measure mRNA in RVLM, cerebellum and whole brain. Sprague-Dawley rats were exposed to hypoxia (10% O_2) for 10 days while corresponding age matched controls were maintained in normoxia (21% O_2). RT-PCR demonstrated expression of HO-2 mRNA in RVLM, cerebellum, and whole brain in both control and hypoxic samples. However, hypoxia induced expression of HO-1 mRNA in RVLM but not in cerebellum or whole brain. In addition, HO-2 was immunocytochemically localized in brain sections (40 µm). HO-2 immunoreactive staining was seen in RVLM, central gray, inferior olivary nucleus, cerebellum, and pontine nuclei. Together, these results indicate that HO-2 is present in the RVLM under control conditions and that HO-1 is induced in the RVLM during chronic hypoxia. We conclude that HO is expressed in the RVLM suggesting that it may be important for the O_2 sensing function of this medullary region. Supported by HL-16022, Medical Research Service of the VA, AHA/NJ Affiliate Grant-in-Aid and Predoctoral Fellowship.

628.2

THE CO_2 SENSITIVE RESPIRATORY CHEMORECEPTION REGION IN THE ROSTRAL VENTROLATERAL MEDULLA (VLM) IDENTIFIED BY A CO_2 DIFFUSION PIPETTE

Aihua Li and Eugene E. Nattie.* Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756-0001

In anesthetized rats and cats, work from this lab has demonstrated that central chemoreception is located in many brainstem sites. These include the region of the VLM, at previously identified central chemoreceptor areas, and the regions of the nucleus tractus solitarius, the locus ceruleus, the midline raphe, and the ventral respiratory group. This localization was achieved by one nl injections of the carbonic anhydrase inhibitor, acetazolamide, which result in a focal tissue acidosis limited to 350 µm from the injection center and a significant increase in integrated phrenic nerve amplitude (PNA). These responses last up to 90 min making it difficult to test mechanistic or integrative hypotheses. In this study, we examine the tissue pH and PNA responses following a focal acidosis in the rostral VLM produced by a CO_2 diffusion pipette. No tissue injection is made. Instead, a CO_2 enriched solution perfused through an inner barrel exits via an outside barrel. The $[CO_2]$ at the pipette tip (outside barrel) is constantly renewed allowing diffusion into the tissue. A second attached barrel measures pH at the tip and a separate electrode measures pH at some distance from the tip. In group one (CO_2 diffusion; N=19), 15 of 19 one min diffusion periods resulted in a significant increase in PNA and a significant decrease in tissue pH. The peak pH change at the pipette tip was at 1 min and peak PNA and distal pH change were at about 2 min. PNA and distal pH returned to baseline in about 5 min; pipette tip pH in about 15 min. In group two (control; N=5), use of a non-CO_2 solution in the diffusion pipette did not result in any significant change in PNA or pH. This type of diffusion pipette allows study of chemoreceptor induced responses to short periods of focal acidosis in vivo. (Supported by HL 28066).

628.3

Effect on breathing of ejecting excitatory amino acid (EAA) receptor agonists on the ventrolateral medullary (VLM) surface of awake goats. H.V. Forster*, T.F. Lowry, L.G. Pan, T. Feroah, A. Whaley, and M.M. Forster. Physiol., Med. Col. Wis., Phy. T. Marquette U., and Zablocki VA Med. Ctr. Milwaukee, WI 53226

In 5 adult goats, 3-4 guide tubes were chronically implanted bilaterally on the VLM about 2 mm caudal to CN VI where cooling under anesthesia caused apnea. While awake 3 to 8 days after surgery, N-methyl-D-aspartic acid (NMDA) or α-amino-3-hydroxy-5-Methyl-4-isoxazolepropionic acid (AMPA) were ejected on the VLM surface. In 4 goats, a single 1 or 10 ul ejection of 250 or 500 mM NMDA increased pulmonary ventilation 100-300% and decreased $PaCO_2$ 15 mmHg. In 3 goats, the onset, peak and duration of the responses were 2, 10, and 30 minutes after ejection respectively. In the 4th goat, the onset was 5 minutes after ejection and the hyperpnea was sustained for 60 minutes. For most goats, the response was elicited at only 1 guide tube. NMDA did not stimulate breathing when a NMDA antagonist was ejected 15 minutes before NMDA ejection. In a 5th goat, NMDA ejection did not change breathing but AMPA (40 uM) ejection elicited a transient hyperpnea and hypocapnia. AMPA elicited a hyperpnea in only one other goat, but it was delayed and progressive over an hour, eventually resulting in apneustic breathing and terminal apnea. These data suggest a rather discrete population of EAA receptors near this VLM surface which affect breathing and it appears there is a predominance of the NMDA receptor subtype. (Support: NIH25739 and Veterans Administration).

628.4

MEMBRANE PROPERTIES OF CHEMOSENSITIVE NEURONS IN THE SOLITARY COMPLEX (SC) IN YOUNG RATS. R.-Q. Huang* and J.B. Dean. Department of Physiology & Biophysics, Wright State University, Dayton, OH 45435.

The immature rat, postnatal day (P) ≤ 21, is used extensively in studies of central chemosensitivity. However, neural development during the first 3 weeks may change membrane properties, anatomical coupling and chemosensitivity of neurons in the SC (= n. tractus solitarius & dorsal motor n.). The present study employed the rapid perforated-patch recording technique in medullary slices (120-350μm) to determine the effects of age (P0-20) on: responses to hypercapnic acidosis (5 to 10-15% CO_2, 22-25°C), membrane properties, and coupling. Once the effects of age were identified, the relationship between chemosensitivity and membrane properties was determined. Neurons were visualized for recording using infrared-Hoffman modulation contrast video microscopy. There was no relationship between age (P0-20) *vs.*: action potential amplitude, resting membrane potential and input resistance. Likewise, coupling between CO_2/H^+-neurons in SC was unrelated to age (coupled, P8.9 *vs.* noncoupled, P8.3). During hypercapnic acidosis (n = 151), 35% of SC neurons were excited, 25% inhibited, and 40% insensitive. Type I repetitive firing properties (Dekin et al., 1987) occurred in younger rats (P6.2) compared to Type II (P9.0). Further, CO_2/H^+-inhibited neurons were more common in younger rats (P6.2) compared to excited (P8.3) and insensitive neurons (P8.7). CO_2/H^+-excited neurons were either Type I or II, whereas inhibited and insensitive neurons were Type I, II or III. No other differences in membrane properties were found among SC neurons based on their response to CO_2/H^+. Coupling did not occur in CO_2/H^+-inhibited neurons and only rarely in insensitive neurons. We conclude that CO_2/H^+ excitation, most membrane properties, and coupling do not correlate with development during P0-20 in SC neurons. However, CO_2/H^+-inhibited, Type I neurons are more common during the first week. Finally, CO_2/H^+-excited neurons in SC are either Type I or II, but not Type III. (supported by NIH HL 46308).

628.5

EFFECTS OF CARBONIC ANHYDRASE INHIBITION ON INTRACELLULAR pH IN NTS NEURONS. J.S. Erlichman* and J.B. Dean, Department of Physiology & Biophysics, Wright State University, Dayton, OH 45435.

Carbonic anhydrase (CA) catalyzes the reversible hydration of CO_2 and is noncompetitively inhibited by acetazolamide (AZ). It has been hypothesized that CA is involved in CO_2 chemoreception, specifically, in sensing rapid changes in CO_2. It is known that CA inhibition decreases both extracellular buffering power and pH (pHo), however its effects on intracellular pH (pHi) have not been studied. Here we describe the effects of AZ on pHi recorded from neurons located in the nucleus tractus solitarius (NTS), which has been identified as a site of CO_2/H^+-sensitivity and contains ~1/3 chemosensitive neurons. pHi was measured in 200 μm thick slices (P1-13 rat) loaded with the fluorescent pH-sensitive dye, 2'7'-bis-(2-carboxyethyl)-5,6-carboxyfluorescein (BCECF). AZ treatment (10 μM) had no effect on steady state pHi during normocapnia (5% CO_2), but resulted in a greater acidification of pHi (~0.1 pH unit) during hypercapnic acidosis (10% CO_2) compared to control conditions. In addition, the rate of acidification following exposure to hypercapnic acidosis in 65% of the cells was unaffected by AZ (control: -0.43 ± 0.02 pH unit/min, r^2 = 0.82 *vs.* AZ: -0.37 ± 0.02, r^2 = 0.71). However, 35% of the cells exhibited a decreased rate of acidification during exposure to AZ and hypercapnic acidosis (control: -0.43 ± 0.02 pH unit/min, r^2 = 0.87 *vs.* AZ: -0.24 ± 0.02, r^2 = 0.86). In contrast, the rate of increase in pHi following the return to normocapnia was similar in all cells (control: 0.30 ± 0.01 pH unit/min, r^2 = 0.73 *vs.* AZ: 0.30 ± 0.03, r^2 = 0.74). We conclude that inhibition of CA slows the initial rate of CO_2-induced intracellular acidification, suggesting that ~1/3 of the neurons in NTS contain CA. However, since AZ has been reported to decrease pHo, and pHi regulation in NTS neurons is inhibited by decreased pHo, the larger steady state acidification in the presence of AZ and 10% CO_2 may be due to inhibition of extracellular and/or glial CA. We are currently investigating the effects of AZ on pHo (NIH HL46308).

628.6

COMPARING INTRACELLULAR pH REGULATION OF NEURONS IN CHEMOSENSITIVE AND NON-CHEMOSENSITVE AREAS OF THE MEDULLA. **N.A. Ritucci, J.B. Dean* and R.W. Putnam**. Department of Physiology & Biophysics, Wright State University, Dayton, OH 45435.

Central chemoreceptors are thought to modulate respiration by monitoring the level of CO_2 in the blood and making synaptic connections with respiratory neurons that regulate respiration. The response of these chemoreceptors is believed to be mediated by CO_2-induced changes of intracellular pH (pH_i). If so, the manner in which they respond to changes in pH_i should be different than that of non-chemoreceptors. The present study tests this hypothesis by investigating whether neurons in a chemosensitive area (nucleus tractus solitarius; NTS) respond to pH_i changes differently than neurons in a non-chemosensitive area (inferior olive; IO) at 37°C. We found that pH_i recovery from hypercapnic acidosis at constant pH_o in NTS neurons is 96% inhibited by 100 μM 5-(N-ethyl-N-isopropyl)-amiloride (EIPA) but unaffected by 0.5 mM 4,4'-diisothiocyanato-stilbene-2,2'-disulfonic acid (DIDS). The rate of pH_i recovery from hypercapnic acidosis at constant $[HCO_3^-]_o$ in IO neurons, however, is inhibited 33% by 0.5 mM DIDS but only 67% by 100 μM EIPA. We also found that pH_i recovery from acidification with NH_4Cl prepulses in NTS neurons is completely inhibited by 100 μM EIPA but unaffected by 0.5 mM DIDS. Further, the rate of pH_i recovery from acidification with NH_4Cl prepulses in IO neurons is inhibited 24% by 0.5 mM DIDS but only 58% by 100 μM EIPA. The intrinsic buffering power of NTS and IO neurons were calculated to be 23 mM/pH unit and 7 mM/pH unit, respectively. These data indicate that pH_i recovery in response to an acidification in NTS neurons is mediated solely by Na^+/H^+ exchange but that pH_i recovery in response to an acidification in IO neurons is mediated by both Na^+/H^+ exchange and HCO_3^--dependent transport. [Supported by NIH Grant HL46308, an Ohio Research Challenge Grant, an NIH Small Instrumentation Program Grant and the Wright State University Biomedical Sciences Ph.D. Program.]

628.7

EFFECTS OF CYANIDE APPLIED TO THE CAUDAL VENTROLATERAL MEDULLARY SURFACE (CVMS) ON RESPIRATION AND BRAINSTEM C-FOS EXPRESSION.
Douglas, R.M.*, Trouth, C.O., James, S.D., Sexcius, L.M., Cheatham, J.G., Johnson, S.M., and Reynolds, M.
Department of Physiology and Biophysics, Howard University College of Medicine, Washington, D.C. 20059.

The caudal ventrolateral medullary surface (cVMS) has been implicated in respiratory and cardiovascular chemosensory control mechanisms. The unilateral, topical application of CN- (20mg/ml) to the cVMS induces a transient apnea that can be ameliorated by artificial ventilation. Perfusion of the CN- lesioned animal 2 hr post application reveals the immunocytochemical expression of the c-fos protein bilaterally along the VMS, in the nucleus tractus solitarius, in the A1/C1 areas bilaterally, the midline raphe pallidus, in the dorsolateral pons and other subcortical regions. These results suggest that the cVMS may be involved in the cardiorespiratory responses to hypoxia.
(Support: Grant # N00014-94-1-0523)

628.8

MORPHOLOGY OF THE RETROTRAPEZOID NUCLEUS RETROGRADELY LABELED BY HORSERADISH PEROXIDASE IN ADULT CATS. S. Sheng, G. Song [1], and L. Xin* [2]. Dept. of Pharmacology and Physiology [1], Pre-Clinical Medicine Division, Shandong Med. Univ., Jinan, Shandong 250012 P. R. China. [2] Dept. of Pharmacology, Temple Univ. Sch. of Med., Philadelphia, PA 19140.

The morphology of the retrotrapezoid nucleus (RTN) in the ventrolateral medulla in adult cats was investigated with retrograde cell labeling techniques. The experiment was performed on 12 cats weighing 2.3-2.8 kg. Following extracellular recording of the multiunits expiratory discharges in the Bötzinger complex (Böt.c) in the vicinity of the retrofacial nucleus, 30 nL of 5% wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) was injected into the center of the neuronal activity. The injected WGA-HRP successfully labeled a group of neurons in the RTN. On crosscut sections, three layers of neurons were identified with detailed observation. The near surface layer was about 20 μm below the ventral surface and two types of neurons were observed: vertically arranged spindle-shaped neurons (10x20 μm) with dendrite projecting toward and even touching the ventral surface; round or triangle neurons (15 μm) near or touching the ventral surface. The middle layer was filled with large amount of horizontally arranged small ovoid and spindle-shaped neurons (15x30 μm). About 80% of the neurons in the RTN clustered in this layer. The inner layer was composed of large multipolar neurons (20-40 μm). The total thickness of the three layers was around 800 μm. The morphological characteristics of the RTN revealed in this study were consistent with those proposed for central chemoreceptor. This suggested that the RTN is involved in central chemoreception and respiratory control.

Source: National Foundation for Natural Sciences of China

628.9

INCREASED TEMPERATURE AUGMENTS CENTRAL CO_2-SENSITIVITY IN TURTLE BRAINSTEM IN VITRO. S.M. Johnson*, R.A. Johnson, and G.S. Mitchell. Dept. of Comparative Biosciences, School of Veterinary Medicine, Univ. of Wisconsin, Madison, WI 53706.

In reptiles, increased temperature augments the ventilatory response to changes in arterial CO_2 (Q_{10} ~ 2), but the relative effects of temperature on central *versus* peripheral CO_2-sensitivity are not known. Thus, we tested the effects of CO_2 on respiratory rhythm at two levels of temperature in an isolated turtle brainstem *in vitro*. Adult turtles *(Chrysemys picta)* were anesthetized with halothane and cardioperfused with oxygenated ice-cold HEPES and bicarbonate buffer. The brainstem between spinal segment C1 and the optic lobes was isolated and superfused with buffer; suction electrodes were attached to hypoglossal nerve roots. Respiratory-related discharge was highly variable, occurring in single bursts or episodes of two or more bursts. To test for acute effects of increased temperature, bath temperature was increased from 22.8±0.8°C (mean±S.D.) to 31.0±1.2°C at 5% CO_2 (n=3; pH increased from 7.39±0.03 to 7.58±0.04). Burst duration decreased ~35%, but there were variable effects on burst amplitude and frequency. In separate groups of turtles, CO_2 was varied between 1-9% at either low (21-23°C, n=7) or high temperature (30-32°C, n=10). At both temperatures, disharge was abolished at pH≥8.00. With decreasing pH between 7.40-8.00, episode frequency increased progressively to maxima of 0.45/min (slope=-0.85±0.42 episodes/min/pH unit) and 0.59/min (slope=-2.04±1.51, P≤0.05) at low and high temperature respectively. At pH<7.40, frequency was unchanged or decreased slightly. Amplitude was not greatly altered by changes in pH. These preliminary results indicate that: (i) acute increases in temperature alter respiratory pattern formation (ie. shorter burst duration), (ii) burst frequency in the *in vitro* turtle brainstem increases as pH decreases in the range of 7.40-8.00, and (iii) increased temperature increases this CO_2/pH sensitivity. Thus central chemoreceptors may contribute to the increased hypercapnic ventilatory response at increased temperature in reptiles, although it is not likely to account for the entire effect. (Supported by NIH HL53319 and the Parker B. Francis Foundation)

628.10

DEVELOPMENT OF CENTRAL CHEMORECEPTION IN AN *IN VITRO* BRAINSTEM PREPARATION OF LARVAL *RANA CATESBEIANA* DURING FICTIVE GILL AND LUNG VENTILATION. C.S. Torgerson, M.J. Gdovin, and J.E. Remmers*. Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

The *in vitro* brainstem of the bullfrog tadpole *Rana catesbeiana* displays "fictive" gill and lung ventilations. In decerebrate, spontaneously breathing tadpoles, coordinated rhythmic bursting activities in cranial nerves V, VII, and X are temporally linked to fluctuations in buccal and lung pressures and to EMG bursts in the buccal levator (H3a) (Gdovin *et al*, 1996). PO_2 depth profile measurements in completely isolated stage 19 (Taylor-Kollros) brainstems *in vitro*, superfused with control mock CSF (pH = 7.8, PO_2 = 598 Torr, PCO_2 = 17, at 23 °C), showed PO_2 gradients of about 25 Torr/100 μm below the ventral surface, with a minimum PO_2 of 217 Torr at 800 μm. The brainstem of stage III-IX tadpoles *in vitro* displayed fictive gill ventilation with infrequent lung bursts occurring at irregular intervals. Stages X-XIX maintained fictive gill ventilation, and the frequency of fictive lung ventilation gradually increased with maturation. Stages XX-XXV displayed a predominance of fictive lung burst activity. The amplitude of gill ventilation in all stage tadpoles increased with reductions in pH (7.8-7.4) (produced by raising the [CO_2] in the superfusate) and decreased with pH elevation (7.8-8.4), a response that was progressively augmented throughout development. Similarly, the frequency of fictive lung ventilation increased with pH reductions (7.8-7.4) in all stages, but this response was particular striking for stages XX-XXV. We conclude that the pH of the superfusate bathing the tadpole brainstem has a developmentally dependent influence on both fictive gill and lung ventilation, demonstrating the ontogeny of central respiratory chemoreceptive drive in the amphibian. (Supported by MRC grant MA-9719).

628.11

MECHANISMS OF RAT VENTROMEDIAL MEDULLARY NEURONS SENSITIVE TO ACIDOSIS. G.B. Richerson* and W. Wang. Neurology, and Cellular & Molecular Physiology, VAMC & Yale University, West Haven, CT, 06516.

Pacemaker neurons of the rat medullary raphe are highly sensitive to respiratory acidosis in brain slices (J. Neurophysiol., 73:933, 1995). We have used cultured neurons microdissected from this region to examine the mechanisms of this response. Cultures included the medullary raphe and immediately surrounding structures (VMM), and excluded neurons from the ventrolateral and dorsal medulla. Using perforated patch-clamp recordings, cultured neurons displayed baseline firing properties similar to those previously seen in brain slices. Changes in CO_2 between 3% and 9% with constant HCO_3^- (respiratory acidosis) resulted in large changes in firing rate in a subgroup of neurons in solutions blocking chemical synaptic transmission. In neurons sensitive to respiratory acidosis, changes in pH induced by changes in [HCO_3^-] at constant PCO_2 (5%) (metabolic acidosis) resulted in a similar response (n=5). In some neurons, whole-cell conductance (G_m) was measured in voltage clamp using -10 mV hyperpolarizing steps from a holding potential of -60 mV. Neurons from the hippocampus (n = 4) without a change in firing rate in response to acidosis had no change in (G_m). In most VMM neurons tested with a change in firing rate in response to acidosis, there was also no change in G_m in response to acidosis. However, in some neurons stimulated by acidosis, there was a large decrease in net outward current due to a decrease in G_m. We propose that in those neurons sensitive to acidosis without a change in G_m, the currents affected by pH are voltage- and/or time-dependent, and are inactive in the steady state at a holding potential of -60 mV. In order to more quantitatively characterize these currents it will be necessary to use protocols which isolate individual currents, rather than examining resting whole-cell conductance or using ramp protocols. These results also demonstrate unequivocally that the VMM contains all of the elements required for chemosensitivity at the cellular level. Neurons of the medullary raphe project to many functional systems known to be sensitive to acidosis. By responding to changes in pH, raphe neurons could in turn modulate these other systems in response to changes in blood CO_2. (Supported by VAMC, and NIH HL52539).

628.12

RAT VENTROMEDIAL MEDULLARY NEURONS SENSITIVE TO ACIDOSIS ARE BULBOSPINAL. W. Wang* and G.B. Richerson. Dept. of Neurology, VAMC & Yale University, West Haven, CT, 06516.

Pacemaker neurons from the rat medullary raphe are highly sensitive to respiratory acidosis in brain slices (J. Neurophysiol., 73:933, 1995). We have used nanoinjections of fluorescent latex microspheres centered in the phrenic motor nucleus (PMN) to determine which of these neurons were bulbospinal (Wagner & Dekin, J. Neurophysiol, 69:286, 1993). Neonatal rats (P0-P1) were anesthetized, and rhodamine beads were injected into the region of the phrenic motor nucleus using stereotactic technique. 1-2 days later the medullary raphe and immediately surrounding structures were microdissected from the ventrolateral and other portions of the medulla. Primary dissociated glial-neuronal cultures were prepared and maintained for 10-35 days. All injection sites were visualized to verify their location and size. Perforated patch-clamp recordings were made in Ringer solution with either blockers of GABA and glutamate receptors, or high magnesium (10-20 mM), and low calcium (0.5 mM). In both solutions, increases in CO_2 between 3% and 9% at constant [HCO_3^-] (respiratory acidosis) resulted in an increase in firing rate in a subgroup of neurons, a decrease in firing rate in another subgroup, and no change in a third subgroup. Neurons labelled with dye were more likely to be either stimulated or inhibited by respiratory acidosis (n = 16 of 18) than those which were not filled (n = 2 of 8), were from animals that were not injected (n=2 of 7), or were cultured from the hippocampus (n = 0 of 7). These results suggest that chemosensitivity is not a nonspecific response, but is a specialization of neuroanatomically specific subtypes of neurons. It has previously been shown by neuroanatomical and electrophysiologic techniques that there is a major projection by neurons of the medullary raphe to phrenic motor neurons. Our results strongly suggest that raphe neurons that project to the phrenic motor nucleus are chemosensitive. Since the phrenic nerve is the major output pathway for respiration, projections to the phrenic motor nucleus would enable chemosensitive raphe neurons to strongly modulate respiratory output in response to changes in blood CO_2. (Supported by VAMC, and NIH HL52539).

628.13

CHOLINERGIC FINDINGS PERTINENT TO CAROTID BODY (cb) NEURAL ACTIVITY. R.S. Fitzgerald, M. Shirahata*, Y. Ishizawa, B. Schofield, Department of Environmental Health Sciences, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

We have designed several sets of experiments to test the possibility that acetylcholine (ACh) is an excitatory neurotransmitter during the chemotransduction of hypoxia in the carotid body (cb) of the anesthetized cat. Experiments A measured the release of ACh from the in vitro cb with HPLC. Experiments B_1 altered the release of ACh from a postulated "presynaptic" component of the "synapse" (the glomus cell [GC]), and B_2 blocked cholinergic receptors postulated to be on the "postsynaptic" site (neuron apposed to the GC) during a selective perfuson of the cb in situ with pharmacological agents. Experiments C identified cholinergic receptors in the cb with immunocytochemcal techniques.

(A) Under control conditions the 2 cbs released 316 fmoles/4min; under 0-4, 4-8, 8-12 min of hypoxic/hypercapnic stimulation the 2 cbs released 831, 853, and 1029 fmoles of ACh respectively. (B_1) After 4 min of perfusion with hypoxic Krebs Ringer bicarbonate (KRB) containing 30μM hemicholinium (blocks uptake of choline by GC) cb neural activity was reduced to ~65% of the control response. (B_2) Both atropine and mecamylamine reduced the cb neural response to hypoxic KRB in a dose-related manner; and whereas pirenzepine (M1 muscarinic receptor blocker) decreased the response to hypoxic KRB, gallamine and AFDX116 (M2 muscarinic receptor blockers) augmented the response to hypoxic KRB. (C) The α4 subunit of the neuronal nicotinic receptor has been identified on GCs in culture and on tissue sections of the cb; cell bodies and nerve fibers in the petrosal ganglion also stain positive for the α4 subunit. These data support an excitatory role for ACh in the cb chemotransduction of hypoxia. Precise mechanisms and interplay of neurotransmitters remain to be identified. Supported by HL 50712.

628.14

CHANGES IN PROTEIN EXPRESSION OF CULTURED CAT CAROTID BODY CELLS. Y. Ishizawa*, M. Shirahata, R.S. Fitzgerald, B. Schofield, Department of Environmental Health Sciences, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205

Freshly dissociated or cultured carotid body (CB) cells have been frequently used to study cellular mechanisms of chemotransduction.

A frequently encountered problem in using primarily cultured cells is that the processes involved in dissociation and culturing, as well as culture conditions, may affect basic functions of the cells. The purpose of the present study was to examine the changes in the expression of several proteins in glomus and sheath cells in the CB during two weeks of culture. The expression of the same proteins was also examined in tissue sections of the CB.

We cultured the CB cells from adult cats in serum-free chemically defined medium. Standard immunocytochemical techniques were applied using monoclonal antibodies against the following proteins; tyrosine hydroxylase (TH), neuron specific enolase (NSE), glial fibrillary acidic protein (GFAP), and neurofilament 160kD (NF). TH, NSE, and GFAP have been observed throughout the two weeks of culture. However, staining for TH and NSE in glomus cells was weak on day 1 compared with the staining later in the period, suggesting dissociation and culturing processes may affect basic protein expression in glomus cells over the initial few days in culture. The stains for GFAP in sheath cells did not seem to change during the culture period. In tissue sections both TH and NSE were expressed in glomus cells and GFAP was in sheath cells. NF has not been detected in glomus cells in culture, or in tissue sections. Although the basic phenotypes for these proteins do not seem to change under these culture conditions, the present results suggest that some functional changes may occur in culture, and that glomus cells might be susceptible to the culturing procedures. Supported by HL47044 and HL50712.

628.15

CHEMORECEPTOR RESPONSES OF THE SWINE CAROTID BODY
Ayuko Igarashi*, Robert S. Fitzgerald, and Machiko Shirahata.
Department of Environmental Health Sciences, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

The swine has been used as an animal model for the sudden infant death syndrome and the sleep apnea. Although the carotid body seems to be involved in the development of these conditions, little is known about neurophysiology of the swine carotid body. The purpose of this study was to obtain basic information as to the effects of natural and pharmacological stimuli on carotid chemoreceptor neural activity (CCNA) of the swine. *In vitro studies:* Carotid bodies obtained from 3- to 4-month-old swine were perfused with modified Krebs solution at a rate of 6 ml/min at 35±0.5 °C. CCNA was recorded from whole carotid sinus nerve with bipolar silver-silver chloride electrodes. Flow interruption, hypoxia (PO_2=30torr), nicotine (1-10 μg), and cyanide (1-10 μg) increased CCNA significantly. Hypercapnia tended to increase CCNA. Elevation of PCO_2 augmented the hypoxic response. *In vivo studies:* CCNA and ventilation were recorded in the anesthetized 3-month-old swine. Responses of CCNA were similar to those observed *in vitro*. Under condition of poikilocapnic hypoxia, ventilation was initially stimulated, but subsequently depressed. The addition of CO_2 to the hypoxic inspirate attenuated the hypoxic depression of ventilation. Supported by HL52652, HL50712 and HL47044.

628.16

MODULATION OF CAROTID BODY CHEMORECEPTOR FUNCTION BY ENDOTHELIN.
L. He, J. Chen, B. Dinger and S. Fidone*. Dept. of Physiology, Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

Previous immunocytochemical studies in our laboratory showed that endothelin (ET) peptides are contained in chemoreceptor type I cells in the carotid body and that ET-immunostaining is elevated in carotid bodies from animals exposed for two weeks to hypobaric hypoxia (350 torr). We additionally demonstrated that ETs (ET-1 and ET-3) enhance carotid sinus nerve (CSN) activity evoked by hypoxia and also stimulate the production of inositol phosphate (IPn) and cyclic AMP (cAMP) second messengers in this chemosensory tissue. In the present study, we have examined 1), the effects of ETs on intracellular Ca^{2+} levels in type I cells and 2), the role of endogenous ETs in the generation of chemoreceptor activity.

Superfusion of cultured rabbit carotid body type I cells in ET-1 or ET-3 (100 nM) did not alter basal $[Ca^{2+}]_i$, whereas peak Ca^{2+}-responses evoked by hypoxia were elevated more than 2-fold. Low O_2-evoked Ca^{2+} responses were depressed by analogs of atrial natriuretic peptide (ANP; also contained in type I cells), and this effect was reversed in the presence of ETs (100 nM). RIA measurements showed that acute hypoxia (breathing 5% O_2, 40 min) reduces carotid body ET-immunoreactivity by 40% ($p<0.01$) and is consistent with the notion that hypoxia evokes the release of ET from type I cells. Superfusion of normal rabbit carotid bodies *in vitro* with the specific ET-A receptor antagonist BQ-123 (5 µM) did not alter basal CSN activity, and the drug caused only a slight depression of activity evoked by hypoxia. However, in carotid bodies from chronically (2 wks) hypoxic rabbits the antagonist depressed hypoxia-evoked CSN activity by more than 50%. Our data suggest that ETs modulate type I cell activity by altering cell Ca^{2+} responses and that endogenous ET participates in the physiological adjustments to chronic hypoxia. Supported by USPHS Grants NS12636 and NS07938.

628.17

RESPIRATORY AND BRAINSTEM NEUROCHEMICAL RESPONSES TO REPEATED *VS* ACUTE HYPOXIA IN PIGLETS.
I.R. Moss*, K.A. Waters, A. Laferrière, J. Paquette and C. Goodyer. Developmental Respiratory Laboratory and Endocrinology & Metabolism Division, Dept. Pediatrics, McGill University, Montreal, Québec, Canada H3H 1P3.

Some victims of Sudden Infant Death Syndrome (SIDS) appear to have been exposed to repeated episodes of hypoxia. This study aimed to assess whether repeated hypoxia might increase the risk for SIDS in infants by blunting respiratory responses, and whether such blunting is associated with altered, respiratory-related neuromodulators. Twenty to 33 day-old piglets were instrumented for diaphragmatic electromyogram (EMGdi) measures and for repeated *in vivo* microdialysis from the nucleus tractus solitarius (NTS) via a chronically implanted guide. Experiments were conducted on 5 sequential days. On days 1 and 5, 30 min microdialysis samples were collected during 2 h normoxia, 30 min 10% O_2/90% N_2, then 1 h normoxia. On days 2, 3 and 4, piglets underwent sessions with or without hypoxia, but with no microdialysis. Eight out of the 11 microdialysis probe placements were either within, or <200 μm from the NTS. For met-enkephalin (ME) and substance-P (SP) levels from the dialysates by RIA, the peptide containing fractions were collected after separation by HPLC. Thus far, ME and SP levels at control, 1 h after initial probe insertion, were similar on days 1 and 5. Whereas, on day 1, all piglets had similar EMGdi responses to hypoxia, on day 5, those exposed to the daily repeated hypoxia displayed lower respiratory responses than those subjected to hypoxia on days 1 and 5 only. The possible association of this blunting to changes in select peptide levels at the NTS will be revealed from the ongoing RIAs of the *in vivo* NTS microdialysates. (HL36939, HD29608; Glaxo/CLA; MCH-RI support)

628.18

THE RESPONSE TO HYPOXIA IS AFFECTED BY INCREASING THE ATP CONTENT IN THE CENTRAL RESPIRATORY NETWORK OF MAMMALS.
B. Wilken*, J.M. Ramirez, I. Probst, D.W. Richter, F. Hanefeld. Depts. Pediatric Neurology, Physiology and Biochemistry, University of Göttingen, 37073 Göttingen, FRG

Newborns are not capable of maintaining enhanced respiratory activity during prolonged hypoxia. This typical neonatal response is retained in the transverse brainstem slice preparation of mice and was used as a functional in vitro model to study the hypoxic response of the respiratory system (Ramirez et al. 1996, J. Physiol.429: 599). In this study we examined the possibility that the inability to maintain augmented hypoxic responses is due to a depletion of ATP, which could affect the hypoxic response by altering various components of ionic metabolism, such as the function of the Na^+/K^+ pump or ATP-dependent K^+ channels. In order to avoid ATP depletion brainstem slices were incubated for 3 h in creatine (200 µM), which via the production of phosphocreatine, significantly increased ATP concentrations (as measured with a bioluminescence assay). As shown in the table, creatine treatment enhanced respiratory activity during the initial period of hypoxia by increasing the amplitude and duration of rhythmic hypoglossal activity. The values obtained for two different age groups represent percentage change from control before hypoxia and during hypoxic augmentation for respiratory burst discharges (amplitude and duration of bursts) recorded from hypoglossal nerve.

	P0-5 (control)	P0-5 (Creatine)	P8-14 (control)	P8-14 (Creatine)
Amplitude	+ 14%	+ 58.5% ($p< 0.05$)	+ 11.6 %	+ 47.8% ($p<0.05$)
Duration	+ 3%	+ 17.9% ($p< 0.05$)	+ 14.4 %	+ 21 % (n. s.)

Thus, the response of newborns to hypoxia can be stabilized by increasing ATP levels with creatine, which may be beneficial in protecting the nervous system against acute anoxia.

628.19

INHIBITION OF VENTROLATERAL PONTINE NEURONAL ACTIVITY REVERSES POST-HYPOXIC FREQUENCY DECLINE IN VAGALLY INTACT RATS.
S.K. Coles* and T.E. Dick. Dept. of Medicine, Div. of Pulmonary and Critical Care Medicine, Case Western Reserve Univ., Cleveland, OH 44106

Post-hypoxic frequency decline (PHFD) is characterized by a prolongation in expiration (T_E) after hypoxic (8% O_2) exposure. PHFD may have a neurogenic component, because it is attenuated following inhibition (10 mM muscimol microinjections) of neuronal activity in the ventrolateral (vl) pons (Coles and Dick, 1994). However, the baseline timing of the breathing pattern is altered by inhibition of the vl pons in vagotomized rats. To determine if the apparent attenuation of PHFD after vl pontine inhibition results from baseline breathing pattern changes, we tested rats in which vagi remained intact to minimize these changes.

Adult, male Sprague-Dawley rats (360-492 g) were anesthetized with Equithesin. Rats (n=4) breathed spontaneously and were exposed to hypoxia (15 min of 15% O_2 in one and 20-60 sec of 8% O_2 in the remaining 3 rats). Diaphragmatic EMG was recorded, and T_E was measured before, during, and after hypoxia before and after inhibiting vl pontine neuronal activity. Small volumes (20 nl) of 10 mM muscimol were pressure injected into the vl pons using glass micropipettes. Blood pressure, air flow, pCO_2, and body temperature were monitored.

Baseline T_Es were similar before and after muscimol injections (0.48 s compared with 0.49 s), but post-hypoxic T_Es were significantly shorter after muscimol (0.94 s compared with 0.40 s).

We conclude that the attenuation of PHFD is due to intervention in the vl pons rather than changes in baseline phase durations.

Supported by HL-07288 and HL-42400 (TED).

628.20

IN VITRO RESPONSE OF VENTROLATERAL MEDULLARY NEURONS TO HYPOXIA FOLLOWING NORMOBARIC HYPOXIC ACCLIMATIZATION.
P.C. Nolan* and T.G. Waldrop. Depts. of Molecular & Integrative Physiology, Neuroscience Program and College of Medicine, Univ. of Illinois, Urbana, IL 61801.

Hypoxic acclimatization involves an initial rapid ventilatory response followed by a more gradual increase in ventilation over a period of 24 to 48 hours in both humans and rats. In addition, the acute ventilatory response to hypoxia is accentuated following hypoxic acclimatization. This laboratory has previously shown that neurons in the ventrolateral medulla (VLM), an area of cardiorespiratory integration, are excited by acute hypoxic exposures independent of synaptic activity. The purpose of the present investigation was to determine if hypoxic acclimatization augments the acute hypoxic response observed in VLM neurons *in vitro*. Brain slices (400-500 µm) containing the ventrolateral medulla were prepared from Sprague-Dawley rats acclimatized to hypoxia (10% O_2) for 4-5 days (n=4) and 9-10 days (n=4) and from rats maintained in a normoxic environment (n=4). *In vitro* extracellular recordings demonstrated that there were no significant differences in the basal pattern or discharge rate of VLM neurons from animals exposed to short (10.8±0.9 Hz, n=51), or long (10.1±1.1 Hz, n=59) periods of hypoxia compared to control neurons (10.8±1.1 Hz, n=52). The proportion of neurons stimulated (~70%), inhibited (~20%) and unaffected (~10%) by an acute bout of hypoxia (10% O_2, 90 sec) was also similar among groups. However, acute hypoxia elicited a greater increase in discharge frequency in neurons from rats exposed to the short period of hypoxia compared to the responses from neurons in the control and longer acclimatization groups. These observations suggest the early stages of hypoxic acclimatization result in altered hypoxic responsivity of VLM neurons which may contribute to the respiratory responses associated with acclimatization. (Supported by NIH32876, AHA-IL Affiliate and HD-07333).

628.21

EFFECT OF 6-NITRO-7-SULPHAMOYLBENZO[F]QUINOXALINE-2,3-DIONE (NBQX) ON THYROAREYTENOID (TA) MUSCLE ELECTROMYOGRAM (EMG) DURING HYPOXIA. J.M. Bissonnette,* A.R. Hohimer and S.J. Knopp. Oregon Health Sciences University, Portland, OR 97201.

In fetal sheep acute hypoxia results in a marked depression of respiratory output often to apnea (J Physiol 243:599, 1974). Using TA muscle activity in unanesthetized animals, as an index of early expiratory (post inspiratory) neuronal activity it has been shown that hypoxia induces a tonic activation which coincides with the arrest of inspiratory activity (The Physiologist, in press). Since neuronal populations rostral to TA motoneurons have been shown to be activated by hypoxia (e.g. Respiration Physiol 100:185, 1995) these studies were undertaken to determine if non-N-methyl-D-aspartate receptor blockade in the spinal cord-brain stem inhibited the tonic TA EMG activity induced by hypoxia. Electrodes were placed in the TA muscle and a catheter in the fourth cerebral ventricle in fetal sheep and experiments carried out 4-5 days later in unanesthetized animals. Hypoxia was induced by having the ewe breath a mixture of 10% O_2, 4% CO_2 in air for 10 minutes which lowers fetal Po_2 from ~21 torr to ~13 torr with no change in CO_2 tension. In experiments in which the hypoxic challenge was preceded by a 100 µg NBQX instillation in the cerebrospinal fluid of the fourth ventricle (a dose which inhibits respiratory activity for 60-90 minutes) in 12 of 18 challenges (8 animals) tonic activity in the TA EMG was seen. This 67% incidence was not different from the 69% observed without NBQX (9 of 13 trials). These results indicate that excitation by glutamate at non-NMDA receptors is not required for tonic output of expiratory neurons during hypoxia and suggests it may result from loss of inhibitory inputs. (HL 38039 from NIH)

628.22

DEVELOPMENT OF HYPOXIA-INDUCED FOS EXPRESSION IN THE CAUDAL HYPOTHALAMUS OF THE CONSCIOUS RAT. Eric M. Horn* and Tony G. Waldrop. Neuroscience Program, Department of Molecular & Integrative Physiology and College of Medicine, University of Illinois, Urbana, IL 61801.

The caudal hypothalamus is an important central nervous system site in the control of cardiorespiratory integration during systemic hypoxia. Previous findings from this laboratory have identified neurons in the caudal hypothalamus of anesthetized rats that are stimulated during hypoxia *in vivo* and in the *in vitro* brain slice preparation in the absence of synaptic connections; these electrophysiological responses increase during development. The purpose of the present study is to characterize the induction of Fos in response to hypoxia in caudal hypothalamic neurons throughout postnatal development in the conscious, freely moving rat. Immunocytochemistry was utilized to analyze the expression of the transcription factor Fos, which is induced after neuronal depolarization. Both postnatal and adult (3 days through 6-8 weeks) Sprague-Dawley rats from a regulated colony were placed in a normobaric chamber circulated with either 10% oxygen (hypoxic) or room air (normoxic) for a period of three hours. Following the hypoxic/normoxic exposure period, the animals were anesthetized and immediately transcardially perfused with 4% buffered paraformaldehyde. The brains were removed, post-fixed in 4% paraformaldehyde for 24 hours, sliced and analyzed for Fos immunoreactivity using a polyclonal antibody (Zeneca) and the avidin-biotin peroxidase reaction (Vector Labs). Cells were quantified using image analysis software (NIH Image) and reported as a number of neurons demonstrating Fos immunostaining per square millimeter. A significant increase in the number of neurons expressing Fos was observed in the caudal hypothalamus of hypoxic compared to normoxic adult (6-8 weeks) rats. A smaller increase in Fos induction occurred during hypoxia in the caudal hypothalamus of younger (6-12 days) rats. Based on these and previous findings, we believe that neurons in the caudal hypothalamus are sensitive to hypoxia; this sensitivity follows a developmental time course. (Supported by NIH 38726 and USPHS T32-GM07143)

628.23

PRENATAL CARBON MONOXIDE EXPOSURE AND ITS EFFECTS ON RESPIRATORY REGULATION AND BRAINSTEM DEVELOPMENT IN THE NEWBORN GUINEA PIG. H.P. McGregor[1], M. Tolcos[2], K. Westcott[1], D.W. Walker[1] & S. Rees[2]*
[1] Department of Physiology, Monash University, Clayton, Australia, 3168.
[2] Department of Anatomy & Cell Biology, University of Melbourne, Australia, 3152.

The effect of prenatal exposure to carbon monoxide (CO), a major component of cigarette smoke, on fetal & neonatal development is largely unknown. Therefore, we examined the ventilatory responses of newborn guinea pigs to specific respiratory challenges, and the neurochemical development of brainstem nuclei controlling ventilation, after exposing pregnant guinea pigs (n=4) to 200 ppm CO for 10 hours a day from day 25 of gestation until term (68 days). The control group consisted of 4 pregnant guinea pigs housed in an air filled chamber. On postnatal day 4, the ventilatory responses of 9 control and 10 CO exposed neonates were determined during progressive asphyxia and steady state hypercapnia. The brains were then fixed with 4% paraformaldehyde. Basal ventilation was not different between groups but CO exposed neonates had a significantly greater ventilatory response during progressive asphyxia and steady state hypercapnia. Immunohistochemical analysis of the brainstem (CO,n=6; control,n=6) revealed an increase in tyrosine hydroxylase (TH) immunoreactive (IR) fibres in the nucleus tractus solitarius (NTS) and medullary reticular formation, an increase in the number of TH-positive neurons in the caudal NTS, and an increase in Substance P-IR fibres in the NTS, ventrolateral medulla, and the spinal trigeminal nucleus. Proliferation of astrocytes, as determined by GFA-IR, was observed in the area postrema of CO exposed neonates. These results indicate that prenatal exposure to CO affects respiratory responses postnatally. This may be due to modification of chemosensitivity or cardiovascular control mechanisms, which may in part be due to the altered expression of neurochemicals in the brainstem nuclei involved in cardio-respiratory control.
This work was supported by the National SIDS Council of Australia.

RESPIRATORY REGULATION: INTEGRATIVE MECHANISMS

629.1

MICROINJECTION OF N-METHYLNALOXONIUM IODIDE (MN) INTO THE CAUDAL VENTROLATERAL MEDULLA (cVLM) REVERSES RESPIRATORY DEPRESSION INDUCED BY INTRAVENOUS (IV) ALFENTANIL (AL) IN THE CAT. MJ Kelly, RA Gillis, AM Taveira Da Silva* Pulmonary and Critical Care Medicine, Depts of Medicine and Pharmacology, Georgetown University School of Medicine, Washington, DC 20007.

The ventral medulla is an important area were opiates act to depress respiration (Taveira Da Silva, 1983). The caudal subretrofacial area (cSRFA) and the caudal ventrolateral medulla (cVLM) are areas which have been identified as important in the control of respiratory activity in the cat. We have demonstrated that the microinjection of MN into the cSRFA reverses the respiratory effects of continuous IV alfentanil (Kelly, 1996). The aim of this study was to determine if the cVLM is also an important site in opiate-induced respiratory depression. Studies were conducted in 9 choralose anesthetized spontaneously breathing cats while monitoring tidal volume (Vt), respiratory rate (RR), minute ventilation (Ve), and end-tidal CO2 (EtCO2). Respiratory depression was induced by a continuous infusion of AL which caused significant decreases in RR (-9.8±0.9 breath/min, p<0.05) and Ve (-266±22 mL/min, p<0.05); and increases in EtCO2 (+1.6±0.4%, p<0.05) and Vt (+18.4±6.8 mL, p<0.05). Microinjection of MN, 250 ng/side, into the cVLM reversed the effects of AL resulting in an immediate increase in RR (+7±1 breaths/min, p<0.05) and Ve (+228±40 mL/min, p<0.05), and returned Ve to 80±13% of its baseline value. Treatment with 100 and 1000 ng/side of MN returned Ve to 46±22% and 114±44% of its baseline values respectively. Microinjection of (+)-naloxone, a stereoisomer of naloxone devoid of opiate receptor activity, into the cVLM failed to reverse AL induced respiratory depression. We conclude that the cVLM is an important medullary site where systemically administered opioids act to influence respiratory activity.

629.2

NMDA OR AMPA RECEPTOR ACTIVATION IN THE ROSTRAL VENTROLATERAL MEDULLA PRODUCES BRONCHOCONSTRICTION P.J. Mueller, K.J. Dormer, E.J. Zuperku, J.B. Buckwalter, and P.S. Clifford*. Departments of Anesthesiology and Physiology, Medical College of Wisconsin and VA Medical Center, Milwaukee, WI 53295.

Recent evidence implicates glutamate as an important excitatory neurotransmitter in the control of airway tone by the rostral ventrolateral medulla (RVLM). The purpose of this study was to determine which glutamate receptor subtypes are involved in the bronchoconstrictor response observed after glutamate microinjection into the RVLM. Mongrel dogs of either sex (18-20 kg) were maintained under alpha chloralose and urethane anesthesia and mechanically ventilated with room air. Arterial blood gases were monitored and pH was maintained by bolus injections of sodium bicarbonate. Tracheal smooth muscle tone was assessed by pressure changes in the saline-filled cuff of an endotracheal tube placed at the level of the sternal notch. A multibarreled micropipette was advanced from the dorsal surface of the medulla and positioned in the RVLM (7-8 mm rostral to obex and 4-5 mm lateral to the midline) at the site where microinjections of glutamate (10 nmol) produced the greatest increases in tracheal tone (7.5-18.8 mmHg). Microinjections of NMDA (51-255 pmol) increased tracheal tone (3.1-21.9 mmHg). AMPA (3.6-15.2 pmol) also produced increases in tracheal tone (2.0-15.6 mmHg) when microinjected at this site. Rank order of potency was AMPA>NMDA>glutamate. These results demonstrate that both NMDA and non-NMDA receptors in the RVLM are capable of producing increases in airway tone.
(Supported by NHLBI and VA Medical Research Service).

629.3

ALTERED EXCITATORY AMINO ACID NEUROTRANSMISSION IN BRAINSTEM RESPIRATORY REGIONS OF PORTACAVAL SHUNTED RATS. C.A. Connelly* and S.D. Colquhoun. Depts. of Surgery, University of California-Davis, Sacramento, CA 95817, Liver and Pancreas Transplantation, Cedars-Sinai Medical Center, Los Angeles, CA 90048 and The Dumont-UCLA Transplant Center, Los Angeles, CA 90095.

A portacaval (PC) shunt diverts portal venous blood flow from the liver directly into the systemic circulation. Ammonia and other neuroactive substances then remain in the circulation, cross the blood-brain barrier and may differentially affect NMDA (N-methyl-D-aspartate) receptor function. This study examines the chronic influence of PC shunts on NMDA vs. non-NMDA mediated neurotransmission in brainstem respiratory regions. The non-NMDA (AMPA/kainate) receptor antagonist 6-Nitro-7-sulphamoylbenzo(f)quinoxaline-2,3-dione (NBQX, 40 mg/kg, i.v., pH= 7.45-7.49 in 5.5% glucose) was administered to chloral hydrate anesthetized, spontaneously breathing Sprague-Dawley rats 4 months (113-125 days) after creation of PC shunts (n=6). Diaphragm electromyogram activity, blood gases, pH and arterial pressure were monitored. Blood ammonia levels of the PC shunted rats were significantly elevated compared to unoperated (n=6) or sham-operated (n=6) rats. Following NBQX, respiratory frequency decreased and apnea occurred in 4 of 6 PC shunted rats, lasting 5s (n=1) to ≥ 1 hr (n=3). In 2 of 6 rats respiratory frequency decreased, but without apnea.

Combined activation of NMDA and non-NMDA receptors ordinarily underlies excitatory neurotransmission necessary for generation of the respiratory rhythm (Connelly, *Soc. Neurosci. Abs.* 21:1877, 1995). These results suggest that neuroactive factors not cleared by the liver after a PC shunt can impair NMDA receptor function, causing a shift toward predominantly non-NMDA mediated mechanisms to sustain excitatory neurotransmission in brainstem respiratory regions. *(Supported by The American Lung Association of California and Hibbard E. Williams Foundation).*

629.4

LONG TERM MODULATION OF VENTILATORY CONTROL IN EXERCISING HUMANS. Duncan L. Turner*, J.R. Greenwell, H. Lawrence, P. Lyons, M.R. Taylor and Z. M. Iqbal. Dept. Physiology, University of Leeds, Leeds LS2 9NQ, UK.

Repeated associations of exercise (conditioned stimulus) and increased respiratory dead space (ΔV_D; unconditioned stimulus) alter future blood gas and ventilatory responses to exercise alone in goats. These results were suggested to represent long term modulation (LTM) of the control system subserving the exercise ventilatory response (*i.e.* a form of associative learning; Martin & Mitchell, J. Physiol., **470**, 601, 1993). The present study was conducted to test the hypothesis that LTM also occurs in exercising humans. Ventilation (V_I, $L_{BTPS}\cdot min^{-1}$), carbon dioxide production (V_{CO_2}, $L_{STPD}\cdot min^{-1}$), heart rate (F_H, min^{-1}) and end-tidal P_{CO_2} (mmHg) were measured and arterial P_{CO_2} was estimated (P_aCO_2; Jones *et al*, J. Appl. Physiol., **47**, 954, 1979) at rest and during steady state cycling exercise (5-7 min; 80 watts; Δ = exercise - rest) in 9 male subjects. Resting CO_2 responsiveness ($S = \delta V_I/\delta P_aCO_2$; +$V_D$ = 1.8 L for 7 min; δ = +V_D - no added V_D) and the feedforward exercise stimulus ($G_{EX} = \Delta V_I/\Delta VCO_2 - [S(\Delta P_aCO_2/\Delta VCO_2)]$) were calculated (Mitchell, J. Appl. Physiol., **69**, 718, 1990). Duplicate measurements were made at least 24 hours before (PRE) and 1 hour after conditioning (POST) with ten, repeated associations of exercise (5-7 min; 80 watts) and added dead space (+V_D = 1.35 L). POST vs. PRE values (mean ± SE) were compared by Wilcoxon's Signed Rank Test (*, one-tailed $p<0.05$).

	$\Delta V_I/\Delta VCO_2$	$\Delta P_aCO_2/\Delta VCO_2$	S	$S(\Delta P_aCO_2/\Delta VCO_2)$	$\Delta F_H/\Delta VCO_2$
PRE	23.8 ± 0.9	3.3 ± 0.4	5.7 ± 1.7	19.2 ± 6.0	39.8 ± 4.3
POST	25.3 ± 0.6*	2.2 ± 0.8*	5.4 ± 1.7	5.6 ± 4.5*	41.5 ± 2.9

G_{EX} increased more after conditioning (4.6 ± 6.5 *vs* 19.7 ± 4.4*; PRE *vs* POST). The lesser increase in $\Delta P_aCO_2/\Delta VCO_2$ and greater increases in $\Delta V_I/\Delta VCO_2$ and G_{EX} after conditioning suggest that LTM also exists in humans. We propose that prior experience may influence the normal exercise ventilatory response (ADF315710).

629.5

INSPIRATORY RESISTIVE LOAD THRESHOLD FOR ELICITING THE EARLY PEAKS OF THE RESPIRATORY RELATED EVOKED POTENTIAL (RREP) IN CHILDREN. P.W. Davenport*. Department of Physiological Sciences, Univ. of Florida, Gainesville, FL 32610.

It has been previously demonstrated that the amplitude of the early exogenous peaks of the RREP, P_1 and N_f, increase with increasing inspiratory resistive load magnitude using supra-threshold, easily detected loads. The present study tests the hypothesis that the P_1 and N_f peaks will only be present with supra-threshold, detectable inspiratory resistive loads. EEG recordings were made from normal children, aged 11-16 years from scalp electrode sites: F_3, F_Z, F_4, C_3, C_Z and C_4 (referenced to the joined earlobes). Three magnitudes of resistive loads, R_1=1.6, R_2=3.2, R_3=12.0cmH_2O*L^{-1}*sec, and no-load were presented 100 times each by interruption of inspiration in randomized blocks with 2-4 unloaded breaths separating each loaded breath. The R_1 load was below the reported detection threshold (ΔR_{50}) for normal subjects, R_2 was slightly above threshold and R_3 was easily detectable. A minimum of 50, 350 msec EEG epochs free of EOG artifact for each load (and no-load) magnitude were signal averaged. The averaged traces were examined to determine the presence, latency and amplitude of the P_1 (latency range = 18-40 msec) and N_f (latency range = 30-60 msec) peaks. Both peaks were found with increasing incidence and peak amplitude (when present) as the load magnitude increased. These results demonstrate that inspiration against inspiratory resistive loads that are below the ΔR_{50} does not elicit the P_1 and N_f peaks of the RREP. The presence of the early peaks of the RREP may be related to the detectability of these loads. Supported by NIH-NHLBI grant HL48792.

629.6

CARDIOPULMONARY INTERACTIONS FOLLOWING REM SLEEP DEPRIVATION IN SPRAGUE - DAWLEY RATS. S. M. Trbovic, D. W. Carley, T. Thai and M. Radulovacki*. Department of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612

Changes in blood pressure (BP) and heart period (HP)during REM sleep have been ascribed to increased baroreflex sensitivity in humans and animal species including rats, but there have not been studies describing cardiopulmonary interactions following selective REM sleep deprivation (REMD).These studies are of interest because REMD has been suggested to impair baroreflexes in Wistar rats. We implanted ten rats with EEG and EMG electrodes for sleep/wake scoring and with a telemetry transmitter for monitoring BP and HP. Control recordings were done 1 week after surgery whereas experimental recordings were done following 48 h of REMD for which the "flower pot" method was used. For each rat, sleep , BP and respiration were polygraphically recorded for 6 hours. We found that BP was highest during waking (W), lower during non - REM sleep (NREM) and lowest during REM sleep and these state dependent decreases were found to be statistically significant in control and experimental conditions (p = .05 to .002). HP exhibited mirror image changes (p < .01). REMD was associated with lower BP and longer HP over all sleep/wake states, although this achieved statistical significance only during REM sleep during the first hour of recovery sleep. There was 15% - 25% decrease in respiratory rate (RR) during REM sleep in comparison to W in the control group. After REMD there was a further decrease in RR in REM sleep compared to control recordings in the same state (p = .039). Minute ventilation (MV) showed the same pattern as RR, i. e. it was lower during REM sleep than in W in the control group. In REM sleep after REMD, MV decreased 15% (p = .025) in comparison to REM sleep of control animals. We conclude that changes in BP and HP during REM sleep may reflect increased baroreflex sensitivity during REM in the Sprague-Dawley rat. The observed decreases in RR and MV suggest that baroreflex effects on HP and MV may be enhanced and attenuated in concert with changes in sleep/wake state. The amplification of REM-related effects on BP, RR and MV suggests a further augmentation of baroreflexes following REMD.

629.7

PERIPHERAL EFFECTS OF PENTOBARBITAL ON RESPIRATORY MUSCLE ACTIVATION. J.R.Romaniuk*, K.E.Kowalski, G.Supinski, A.F.DiMarco. MetroHealth Med. Ctr., CWRU, Cleveland, OH 44109

Pentobarbital (Pb) has differential effects on centrally generated expiratory and inspiratory motor activity. The potential effects of the Pb on peripheral muscle activation and contractility are, however, less clear. Electrical stimulation of the lower thoracic spinal roots (SRS) results in the generation of large positive airway pressures (P) consistent with the predominant activation of expiratory muscles. In contrast, upper thoracic SRS results in negative airway pressures and predominant inspiratory muscle activation. We evaluated the efficacy of SRS after administration of increasing doses of Pb in 5 dogs artificially hyperventilated to apnea. The efficacy of SRS was assessed by measurements of P during tracheal occlusion. Pb was administered at 2 mg/kg every 5 min. Administration of a cumulative total dose of 24 mg/kg caused P to fall from 44.8 cmH_2O ± 2.7 SE to 16.0 cmH_2O ± 3.6 SE ($P < 0.05$) during expiratory muscle activation and from -16.6 cmH_2O ± 1.6 SE to -15.9 cmH_2O ± 2.0 SE during inspiratory muscle activation (NS). We speculate that the differential depressive actions of Pb on inspiratory and expiratory pressure generation reflects fundamental differences in the physiological properties of the neuromuscular junction and/or muscle contractile apparatus of the inspiratory intercostal and abdominal muscles.
(HL 34143).

629.8

ROLE OF THE NUCLEUS TRACTUS SOLITARIUS IN CONTROL OF VOMITING. M. S. Siniaia*, K. Shiba and A. D. Miller. Neurophysiol., Rockefeller Univ., New York, NY 10021.

The neural pathways responsible for producing vomiting have not yet been completely elucidated. Lesion studies have shown that the essential coordinating circuitry for producing vomiting is located within the medulla of the brainstem. It is known that the nucleus tractus solitarius (NTS) participates in regulation of sympathetic and respiratory outflow and the triggering of vomiting. The importance of the NTS for vomiting was tested using the fictive vomiting model in adult cats that were decerebrated, paralyzed and artificially ventilated. Injections of the neurotoxin kainic acid into the NTS blocked vomiting induced by a combination of abdominal vagus nerve stimulation and the emetic drugs cisplatin, apomorphine, lobeline, protoveratrine, and naloxone. Preliminary studies indicate that vomiting can also be blocked by injections of the serotonin 1A receptor agonist 8-OH-DPAT into the NTS. Thus, the NTS may represent both a site of convergence of different emetic inputs and of the broad spectrum anti-emetic action of serotonin type 1A agonists.

Supported by NIH grant NS20585.

629.9

EFFECTS OF NATURAL VESTIBULAR STIMULATION ON RESPIRATORY EFFERENT NERVE ACTIVITY IN THE CAT. C.D. Rossiter*, N.L. Hayden, S.D. Stocker and B.J. Yates. Depts. of Otolaryngology and Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15213

Activity was recorded from inspiratory (phrenic and hypoglossal) and expiratory (abdominal) nerves during natural vestibular stimulation in decerebrate, paralyzed and artificially ventilated cats. Vestibular stimulation was produced by rotating the head of animals with C1-C3 dorsal root transection, carotid sinus and upper airway denervation and vagus nerve transection. Stimuli consisted of sinusoidal pitch, roll and yaw (15-20°) at 0.05 to 1.0 Hz and trapezoidal nose-up pitch (50°) and roll (40°). Phrenic nerve activity increased in 2 of 3 animals during 50° trapezoidal nose-up pitch but not during 40° ear-down roll. Modulation of phrenic nerve activity did not occur using sinusoidal 20° pitch, roll or yaw in 15 of 16 animals. Similarly, hypoglossal nerve activity increased during 50° trapezoidal nose-up pitch but was not effected by sinusoidal 20° pitch, roll or yaw (n=13). Abdominal nerve activity also increased during 50° trapezoidal nose-up tilt (not 40° ear-down roll) in 4 animals. In contrast to the inspiratory nerves, however, sinusoidal 20° pitch and sometimes roll were effective in modulating abdominal nerve activity (n=16). Typically, maximal modulation of abdominal nerve activity was elicited by head rotations within 45° of pitch with increased discharges during the nose-up rotation. The gains of the responses remained relatively consistant across stimulus frequencies and the phases were near stimulus position, consistent with otolith afferent influences. Lesions of the vestibular system eliminated modulation of respiratory nerves during stimulation. These data suggest that the vestibular system influences respiratory muscles as well as those muscles typically considered to have roles in antigravity, movement and posture. Supported by NIH grant DC02644.

629.10

BRAIN STEM NEURAL ASSEMBLY SYNCHRONY DURING INDUCTION OF LONG-TERM ENHANCEMENT OF RESPIRATORY ACTIVITY. K. F. Morris*, R. Shannon and B. G. Lindsey. Physiol. & Biophysics, Univ. South Florida Med. Ctr., Tampa, FL 33612.

Stimulation of carotid chemoreceptors results in an immediate increase, an after discharge (short term memory) and long term enhancement of respiratory drive (J. Physiol., 490.2:463). The amplitude of integrated phrenic nerve activity was significantly increased for >10 min. after multiple injections of 200 ml of CO_2 saturated saline solution via the external carotid arteries of 2, anesthetized, vagotomized, artificially ventilated cats. We analyzed spike trains with low or no respiratory firing rate modulation in raphe obscurus and the region of the ventral respiratory group with respiratory cycle-triggered histograms, spike triggered average histograms of phrenic multiunit activity, cross-correlograms and the gravity method. Spark plots were calculated from the results of gravity analysis (Lindsey, et al., these proceedings). We found momentary increases in the impulse synchrony of the monitored neurons associated with chemoreceptor stimulation. Some of these transiently configured assemblies contained neurons that did not change rate during stimulation. These preliminary results are consistent with the hypothesis that chemoreceptor modulation of breathing involves dynamic changes in synchrony not necessarily related to changes in rate of distributed brain stem neural network assemblies. Supported by N.I.H. grant NS19814.

RETINAL ANATOMY

630.1

AMACRINE CELLS TRACER-COUPLED TO PARASOL GANGLION CELLS CONTAIN CHOLECYSTOKININ. R. A. Jacoby* and D. W. Marshak. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical Scool, Houston, TX 77225.

Parasol ganglion cells are the large retinal cells in primates that project to the magnocellular layers of the LGN. The goal of this study was to identify the amacrine cells that make gap junctions with parasol cells. Parasol cells from peripheral macaque retina were injected with Neurobiotin, and the retinas were labeled with antibodies to the glycine-extended precursor to cholecystokinin (G6-gly). The first type of tracer-coupled amacrine cell is larger and has sparsely-branching dendrites that give rise to axon-like processes. The second has smaller diameter dendrites and no axons, confirming Dacey and Brace ('92). Processes of the larger amacrine cell were more intensely-labeled with Neurobiotin, but both were G6-gly-IR. The effect of the amacrine cells onto nearby ganglion cells might be excitatory, even though their chemical synapses are likely to be inhibitory. Axon-bearing amacrine cells like the large coupled type would be particularly well-suited for this if the gap junctions were located on the proximal dendrites and the output synapses on the axons. In that case, the ganglion cells that fell within the dendritic field would be excited, but those falling outside would be inhibited. A second possibility is that the gap junctions act to enhance the inhibition of the parasol cell. This would occur if the gap junctions were rectified so that they conduct depolarizing current preferentially from ganglion cells to amacrine cells. When the ganglion cells depolarize, the amacrine cells would also depolarize via the gap junctions and, after a brief synaptic delay, they would inhibit the ganglion cells. Our working hypothesis is that the large amacrine cells mediate the excitatory interactions and the small ones mediate the inhibitory interactions.

Supported by grants R01 EY06472, P30 EY10608 and T32 EY07024 from the NEI, F31 MH10957 from the NIMH, PD 92040 from Fight for Sight, Inc. and 011618027 from the Texas Higher Education Coordinating Board Advanced Research Program.

630.2

STAINING OF NEURONS OF DIFFERENT RETINAL LAYERS VISUALIZED BY INTERFERENCE CONTRAST MICROSCOPY ON AN INVERTED MICROSCOPE. R.Pflug, H.P.Groiss, H.Reitsamer, K.H.Huemer*. Dept.of Gen.and Comp.Physiology, University of Vienna, Schwarzspanierstr.17, A-1090 Vienna, Austria.

In an isolated superfused rabbit retina preparation we have stained living cells of different layers including horizontal cell networks with Lucifer Yellow. We have used a newly developed set-up that allows us to penetrate cells under visual control without previous labeling.

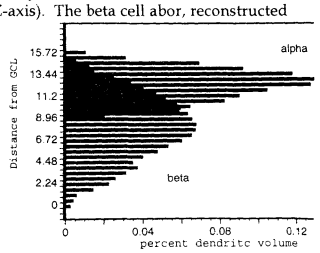
The retinal preparation is located on the stage of an inverted microscope. Cells are visualized by Nomarski interference contrast. A computer program developed in our department is used to calibrate the position of the electrode and to drive the electrode to any predefined position in the retina. Cells are penetrated by further advancing the electrode with a stepper motor. Dark adapted retinal preparations are observed with a CCD camera sensitive in the infrared range.

A connected amplifier allows electrophysiological recordings of intracellular potentials in response to light stimuli With this set-up pharmacological modulation of cell coupling as well as influences of light adaptation on gap junctional resistance can beinvestigated supported by FWF P09670-MED

630.3

VOLUMES OF BETA AND ALPHA CELL DENDRITIC ARBORS PEAK IN DIFFERENT STRATA. M. Luo, M. Pu, and P. Sterling*. Dept Neuroscience, U Penn, Phila, PA 19104

Retinal ganglion cells comprise about 20 types, and for each type the dendritic arbors "tile" the plane of the retina completely and efficiently. But the degree to which arbors of different types intermingle in the inner plexiform layer has not been quantified because conventional optical sections can be thicker than the dendritic strata to be resolved. Hence beta and alpha ganglion cells are assumed to occupy the same strata, the sparsely branched, planar, alpha dendrites insinuating among the densely branched, 3-D beta arbors. Confocal microscopy shows this to be an oversimplification.Adjacent ON beta and ON alpha ganglion cells were injected with Lucifer yellow + neurobiotin and visulized with CY3. Whole mounts were coverslipped in glycerol mounting medium, and the intensely fluorescent neuron arbors were optically sectioned with 100X, 1.4 NA objective (resolution ~ 0.6 um in Z-axis). The beta cell abor, reconstructed and rotated into vertical view was flat-topped, like a 'crew-cut', and 95% of its volume occupied a stratum from 1 µm to 13 µm above the ganglion cell layer. The alpha cell arbor was flatter: 95% of its volume occupied a stratum from 8 to 14 above the ganglion cell layer. Only the upper 30% of the beta cell's dendritic volume was overlapped by the alpha cell arbor. (EY00828)



630.4

DENDRITIC COVERAGE FACTOR OF CAT RETINAL GANGLION CELLS FROM NEUROBIOTIN FILLED RETINAS. X. J. Zhan and J. B. Troy*. Neuroscience Institute & BME Department, Northwestern University, Evanston, IL 60208

It is generally believed that the dendritic trees of a functionally and morphologically homogeneous class of retinal ganglion cell would cover the retina with a constant degree of overlap. However, direct measurement of the dendritic coverage factor (DCF) has proven difficult, especially for beta cells. A method for direct and systematic evaluation of ganglion cells' DCFs has been developed, and the beta cells' DCF measured directly, across the retina.

Complete dendritic fields together with all ganglion cells in selected retinal areas were revealed, using neurobiotin retrograde transport in an *in vitro* eye cup preparation. The DCF was evaluated directly from the cell density and the dendritic field size of the same type of ganglion cell in the same retinal location.

The newly developed method provided a direct means to measure DCFs. Direct DCF measurement was used to evaluate DCFs for both alpha and beta cells across the retina. The DCF for alpha cells was found to be in good agreement with previously published data, but the DCF for beta cells was found to be significantly smaller than estimated by other investigators using indirect methods.

Supported by NIH R01 EY06669 and Northwestern University.

630.5

THE NUMBER OF UNIDENTIFIED AMACRINE CELLS IN THE RABBIT'S RETINA. E. Strettoi and R. H. Masland*, Istituto di Neurofisiologia del C.N.R., Pisa and Howard Hughes Medical Institute, Boston, MA, 02114.

The three largest known populations of amacrine cells in the rabbit retina were stained using fluorescent probes in whole mounts. The cells were counted at a series of retinal eccentricities. The retinas were counterstained using a fluorescent DNA-binding molecule and the total number of nuclei in the inner nuclear layer were counted in confocal sections. From the total number of inner nuclear layer cells and the fraction occupied by amacrine cells (J. Neurosci., 1995) the fraction of amacrine cells made up by the stained populations could be calculated. Starburst cells made up 3%, indoleamine-accumulating cells 4%, and AII cells 11% of all amacrine cells. By referring four smaller populations of amacrine cells to the number of indoleamine-accumulating cells, they were estimated to comprise, taken together, 4% of all amacrine cells.

Thus, 78% of all amacrine cells in the rabbit's retina are known only anecdotally (mostly from Golgi catalogues) if at all. For comparison, horizontal cells make up 2% of all cells in the rabbit's inner nuclear layer. The number of *unidentified* amacrine cells is thus 14 times the total number of horizontal cells: amacrine cells represent the main laterally conducting pathway in the rabbit's retina. The proportion of unidentified amacrine cells in the retinas of the mouse, rat, cat and monkey is similar.
Supported by HHMI and EY01075.

630.6

ANTI-KLH ACTIVITY IN FROG AND TOAD RETINA. E. Kicliter[1]*, D. L. Meyer[2], A. Reichenbach[3] and N. Lugo[1]. Institute of Neurobiology and Department of Anatomy, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico 00901[1], Department of Neuroanatomy, School of Medicine, University of Göttingen, Germany[2] and Paul Flechsig Institute for Brain Research, University of Leipzig, Germany[3].

Keyhole limpet hemocyanin (KLH) is a large protein which functions as a respiratory pigment in this mollusc. Although KLH has been used as a carrier molecule to be conjugated with low molecular weight neuroactive peptides, antisera to KLH itself have been shown to react with neural elements, for example in the olfactory mucosa. While studying anti-KLH reactivity of olfactory mucosa in anuran amphibians, it was noticed that in some species strong anti-KLH reactivity was present in the outer plexiform layer (OPL) of the retina. This reactivity was observed in processes in the OPL and also in cell bodies. The somata exhibited label only in the cytoplasm. The locations of these labeled somata and processes is consistent with their identification as horizontal cells. Anti-KLH antibody from two sources (U.S. Biochemical and Organon Technika) and two immunochemical detection techniques (PAP and ABC) produced similar results. Labeling of the OPLwas not observed in all of the anuran species tested. Marked labeling was observed in bufonids, hylids, and leptodactylids, but not in pipids, microhylids, ranids or hyperoliids. In all species some activity was also noted in the ganglion cell layer. The strong anti-KLH reactivity of presumed horizontal cells in some species offers the possibility of identification of this cell type when the retina is dissociated. That OPL reactivity was observed only in members of the superfamily Bufonoidea suggests that the reactivity is with a protein whose structure varies between anurans. Differences in OPL reactivity may offer an indication of the closeness of phyletic relation. Supported, in part, by NIH Grants MH-48190 and NS-07464 and the German Science Foundation.

630.7

CALRETININ AND CALBINDIN-D28k DISTRIBUTION IN PHOTORECEPTIVE ORGANS OF THE LIZARD *IGUANA*. M. S. Grace*, A. Ziyada#, and M. Menaker, Dept. of Biology and NSF Center for Biological Timing, Univ. of Virginia, Charlottesville, VA 22903; #Dept. of Anatomy and Histology, Faculty of Medicine, Aleppo University, Syria.

Iguanid lizards have at least four different populations of photoreceptors (PRs), located in the lateral eyes, parietal eye, pineal organ, and brain. These sites also contain circadian clocks, one output of which is rhythmic melatonin production. Because calcium is involved in both photoreception and melatonin synthesis, we set out to further characterize known photoreceptive organs and putative "deep brain" photoreceptors (see Grace et al., 1996, J. Comp. Neurol., *367*:565) in *Iguana*, using immunocytochemistry for the calcium-binding proteins calretinin (CaR) and calbindin-D28k (CaB). Anti-CaB stained all PR inner and outer segments in the retina of the lateral eye. Confocal microscopy of retinal whole-mounts confirmed that both large and small single PRs as well as double PRs were immunolabeled. Anti-CaB also stained a subpopulation of bipolar cells in the outer nuclear layer. Anti-CaR did not stain PRs, but stained most if not all horizontal cells in the outer nuclear layer, as well as a subset of amacrine cells at the inner border of the inner nuclear layer. Immunostained fibers of amacrine cells formed five distinct bands in the inner plexiform layer. A subset of ganglion cells was weakly stained. In pineal, anti-CaR and anti-CaB immunoreactivities appeared restricted to different populations of pinealocytes. Long cylindrical cell bodies and their outer segments exhibited faint punctate CaB-like staining. Anti-CaR intensely stained ependymal pinealocytes, their outer segments, and their axons. Neither antiserum labeled the opsin- and vasoactive intestinal polypeptide-immunoreactive PR-like cells in the ependyma of the basal lateral ventricles. Thus, while retinal PRs may contain only CaB, one class of pinealocytes may contain CaR, another may contain CaB, but putative deep brain photoreceptors contain neither. We are currently investigating the distribution of CaB and CaR in the *Iguana* parietal eye.
Support: NIH T32 07646-03 (MSG), U.S. Air Force OSR F49620-94-1-0356 (MM)

630.8

HIGH PRESSURE FREEZING OF RETINAL SLICES FOR ULTRASTRUCTURAL EXAMINATION.
M. T. Wilson*[1], C. J. Karwoski[1], & M. A. Farmer[2]. [1] Vision Research Laboratory and [2] Center for Ultrastructural Research, University of Georgia, Athens, GA 30602.

High pressure freezing (HPF), followed by freeze substitution, can produce excellent ultrastructural preservation of tissues at depths over 10 times that obtained by other cryofixation techniques. However, in the case of neural tissue, the benefits of HPF have not been realized.

In the present study, isolated frog (*Rana Pipiens*) retina was sliced at a thickness of 150 or 350 μm, rapidly frozen in a Balzers HPM 010 high pressure freezer, and freeze substituted with 1% OsO_4 in acetone. Specially designed HPF chambers and specific freezing media (35% high MW dextran for 150 μm slices or 15% low MW dextran for 350 μm slices) were required for adequate freezing.

The quality of preservation throughout the retina was excellent in both the 150 and 350 μm slices, as compared to chemically fixed slices. Specifically, HPF resulted in excellent preservation of the cellular, mitochondrial and nuclear membranes in all retinal layers. Previous studies with other cryofixation techniques have not been able to adequately preserve these structures in many of these layers. In the nervous system, HPF should be useful for ultrastructural studies of dynamic events and cytochemical localization, as well as studies of extracellular and intracellular matrices. (Supported by NIH grant EY-03526)

630.9

REGULATION OF GENE EXPRESSION IN THE RETINAL PIGMENT EPITHELIUM OF THE EYE: ANALYSIS OF THE E61 PROMOTER
A. Nicoletti[1], P.A. Sieving[2]*, and D.A. Thompson[1,2]. Departments of Ophthalmology[2] and Biological Chemistry[1], University of Michigan Medical School, Ann Arbor, MI, 48105.

The retinal pigment epithelium (RPE) of the eye expresses an abundant 61 kDa protein (E61) whose expression is developmentally regulated and tissue-specific. E61 transcripts are present at high levels in native RPE, and are down regulated in both proliferative and differentiated RPE cells in culture. Although the function of E61 is unknown, these characteristics suggest involvement in the specialized functions of the RPE, as well as regulation by signals from the retina and native environment.

In order to identify the cis-acting elements which mediate the high levels of tissue-specific E61 gene expression, the promoter region of the E61 gene has been characterized. Genomic clones containing 7.0 kb of the 5'-flanking region of E61 have been isolated and 4.0 kb proximal to the transcription start site have been sequenced. Potential DNA binding sites for a number of transcription factors are present, including a site for the retina-specific factor, Ret1. In functional studies using luciferase reporter gene constructs, the most proximal 256 bp of 5'-flanking sequence is sufficient to drive expression when transfected into a human RPE cell line, but not when transfected into HeLa cells. Studies focused on the identification of the cis-acting elements in the 5'-flanking region which mediate this tissue-specific promoter activity are in progress.

Supported by grants from NIH (EY09193, EY07003), Michigan Eye Bank & Transplantation Center, National Center for Research Resources (RR00042).

630.10

NITRIC OXIDE SYNTHASE TYPE I AND TYPE II DISTRIBUTION IN NORMAL RAT RETINA. J.J. López-Costa*, J. Goldstein and J. Pecci Saavedra. Inst. de Biologia Celular y Neurociencia "Prof.E.DeRobertis", Fac. Medicina, UBA. Buenos Aires, 1121, ARGENTINA.

Two isoforms of Nitric Oxide Synthase (NOS) have been described: Type I (constitutive) and Type II (inducible). NOS activity has been reported in vertebrate retina in rod outer segments, rod and cone inner segments and in a subpopulation of amacrine cells. NO is known to activate a soluble guanylate cyclase in photoreceptors, gating a Ca^{+} channel with importance in visual transduction and dark adaptation. The aim of this work was to perform a detailed study about the distribution of both NOS isoforms in the rat retina using immunocytochemistry. Adult Wistar rats were anaesthetized with sodium pentobarbital (60 mg/kg) and perfused transcardially with a solution containing 4 % paraformaldehyde in 0.1 M phosphate buffer. Eyes were removed, postfixed in the same solution for 2 hours, cryoprotected and cut on a Leitz cryostat. The sections were immunocytochemically stained employing either a polyclonal rabbit antibody to neuronal NOS (nNOS), type I, (generously provided by Dr. Julia Polak; dil. 1: 2000) or a polyclonal rabbit antibody to macrophagic NOS (mNOS), type II, (Trasduction Lab.; dil. 1:400).

Sections stained using nNOS antibody showed an immunoreactive band corresponding to inner photoreceptor segments, immunolabeled amacrine cells and nNOS containing fibres in inner and outer plexiform layers. On the other hand mNOS antibody demonstrated immunoreactivity in cells localized in inner nuclear layer and in slender Müller cell processes along inner plexiform and ganglion cell layers. In addition mNOS immunoreactivity was also found in a band corresponding to inner photoreceptors segments showing a punctate immunostaining pattern. The results show a differential distribution of type I and type II NOS in rat retina, the only exception being inner photoreceptor segments where both types of NOS are colocalized.

631.1

A COMMON PATTERN FOR A THIRD VISUAL CHANNEL IN THE PRIMATE LGN. S. H. C. Hendry* and V. A. Casagrande. Krieger Mind/Brain Institute, Johns Hopkins Univ., Baltimore, MD 21218 and Department of Cell Biology, Vanderbilt Univ., Nashville, TN 37232

Two populations of neurons, comprising magnocellular (M) and parvocellular (P) layers of the lateral geniculate nucleus (LGN), are usually thought of as the source of geniculocortical input to primary visual cortex (V1) in primates. Yet a third population of small neurons making up the koniocellular (K) layers has been described repeatedly in the LGN. Using the selective expression by K neurons of the 28kD calcium binding protein, calbindin, we examined this third visual channel across a series of primate species, including prosimians (Galago crassicaudatus), Tarsiers, Old World monkeys (Macaca mulatta), New World monkeys (Cebus apella, Samiri sciureus and Aotus trivirgatus), orangutans (Pongo pygmaeus) and humans. A common pattern is evident in which neurons immunoreactive for calbindin occupy four distinct layers, each of which is intercalated between principal layers or between M layer 1 and the optic tract. Consistent also is the large number of K neurons inserted between two P layers, a pattern most obvious in Galago, and between the ventral P and dorsal M layers, seen most clearly in Tarsier. Two specializations in monkeys, orangutans and humans are the expanded K layer ventral to M layer 1 and, where P layers are divided into leaflets (in macaques, orangutans and some humans), the insertion of two additional K layers of variable thickness. In the context of this pattern, only Cebus apella stands out as anomalous, with the more dorsal calbindin-immunoreactive neurons distributed not in K layers but in P layers. As do calbindin cells in other primates, however, those in Cebus monkeys send axons to V1, specifically to layer I and to the CO-rich puffs of layer III. Thus common to the primate LGN is a robust, neurochemically distinct K channel, in which variations among species likely represent selective adaptations to visual environment. Supported by EY06432 (SH) and EY1778 (VC).

631.2

WHAT VISUAL STIMULUS PARAMETERS CONTROL THE AMPLITUDE AND SPATIOTEMPORAL PHASE PATTERNING OF OSCILLATIONS IN THE SUBCORTICAL VISUAL SYSTEM?

Rowshanak Hashemiyoon* and John K. Chapin, Department of Physiology and Biophysics, Hahnemann University, Philadelphia, Pa. 19102

Using simultaneous multielectrode recordings, we have determined that retinally derived subcortical visual system oscillations (SVSOs; 10-40Hz) are ubiquitous throughout the direct retinal receiving zones of the pretectal area, superior colliculus (SC) and lateral geniculate nucleus (LGN) in rats. To elucidate the functional significance of these phenomena, we investigated the effects of various parameters of visual stimulation on the amplitude, frequency, and spatiotemporal phase patterning of these SVSOs via recordings through electrode arrays in the target nuclei. Whereas static light stimulation over the whole visual field markedly decreased the amplitude of oscillatory bursts, it had minimal effect on frequency and did not change spatiotemporal phase patterning. On the other hand, several types of dynamic visual stimulation produced marked changes in both amplitude and spatiotemporal phase patterning. Light flashes produced transient strong oscillatory episodes with phase resetting. The magnitudes of these effects were correlated with the flash frequency, as well as the size and brightness of the flashed area. Small localised flashes produced complex spatial phase patterning consistent with travelling waves. Light/dark edge stimuli moving across the visual field also produced increased oscillatory amplitude and phase resetting, with magnitudes dependent on the movement velocity, size and contrast of the edge. These results suggest that such oscillatory dynamics could play a significant role in the processing of flashing or moving visual stimuli. In particular, the propagation of spatiotemporally complex phase information across the retina may constitute a dynamic-distributed representation of visual information.

Supported by grants NS23722 and ONR N00014-95-1-0246 to JKC.

631.3

PRINCIPAL COMPONENTS ANALYSIS OF THE TRANSFER OF VISUAL INFORMATION THROUGH THE LGN. T. Ozaki[1,3], R. Everson[2], E. Kaplan[3] & K. Purpura[3]*. Biophysics Lab., The Rockefeller University, NY, NY 10021; Applied Math Lab., Ophthalmology, Mt. Sinai Medical Sch., NY, NY 10029; Neurol. & Neurosci. Dept., Cornell Univ. Med. Col., NY, NY 10021

The lateral geniculate nucleus (LGN) is strategically placed along the central visual pathway to filter the flow of visual information from the retina to the visual cortex. While the LGN receives its main excitatory input from the retina, it also receives connections from the perigeniculate nucleus, brainstem and layer VI of the visual cortex. These non-retinal connections are thought to influence the transfer of information to higher visual areas. We previously reported that the transfer ratios (LGN/Retina) of two cells recorded simultaneously in the LGN were strongly correlated regardless of receptive field proximity, suggesting a global control in the LGN. We seek to better understand the mechanism of this control.

We applied an extension of the Karhunen-Loève (K-L) principal component analysis to recordings of retinal input (recorded as synaptic (S) potentials) and LGN spike output in anesthetized and paralyzed cats. The cats viewed either a blank CRT or various visual stimuli. The K-L analysis yielded a single component which reflected a change of activity in the LGN output, but not in the retinal input. Furthermore, the time course of this component's strength closely resembled the transfer ratio. The K-L component provides additional information on the nature of the high and low transfer ratio epochs. Results from thalamocortical relay neuron simulations suggest that the cells' resting membrane potential (RMP) is important in the control of transfer ratio. Thus, controlling the RMP provides a convenient mechanism for non-retinal inputs to influence LGN signal transmission. The fact that a single K-L component (which is necessarily uncorrelated with all other components) accounts for the variations in transfer ratio suggests that a single conduit is employed for control. However, the possibility that more than one non-retinal input may exert control via this conduit cannot be excluded.

Support: EY 4888, EY 01867, EY 06476, MH 50166, RPB, ONR N0014-93-12079, NRSA GM07524-19. NS.1677(KP)

631.4

SYNCHRONOUS OSCILLATIONS IN THE LGN REFLECT STIMULUS-DEPENDENT LONG-RANGE INTERACTIONS IN THE RETINA. S. Neuenschwander*, S. Herculano and W. Singer Max-Planck-Institut für Hirnforschung, Deutschordenstraße 46, 60528 - Frankfurt a. M., Germany.

Perceptual binding of stimulus features has been proposed to be coordinated by synchronization of neuronal activity. So far, most of the experimental work has focused on cortico-cortical interactions, and only a few studies have examined sub-cortical structures. Recently we demonstrated that synchronous oscillatory responses in the retina are transmitted reliably by the lateral geniculate nucleus (LGN). Here we investigate with multi-electrode recordings the stimulus-dependency of synchronization of oscillatory responses from retinal ganglion cells in anesthetized cats.

Oscillatory responses were evoked best by large stationary stimuli or moving gratings, and also by global changes in luminance, suggesting the involvement of center-surround interactions in the generation of rhythmic activity. An important requisite for synchronization over large distances was that cells be activated with a continuous stimulus. Sliding window analysis reveals that oscillatory activity builds up quickly, accompanied by a decrease in oscillation frequency over time. This pattern does not depend on the spiking activity of individual cells, suggesting that synchronous oscillations emerge from population dynamics. The oscillation frequency is in general lower for OFF- (mean 69.8 ± 12.2 Hz) than for ON-responses (89.6 ± 11.5 Hz). Interestingly, synchronization is not restricted to cells of similar center type, as ON-center responses of an ON-cell and ON-surround responses of OFF-cells can synchronize.

Our results suggest that depending on its spatial properties, a visual stimulus is capable of organizing large cell populations in the retina into ensembles that cooperate over long distances. Stimulus-dependent long-range synchronization of responses in the retina could signal elementary spatial relations such as size and continuity of the stimuli. Since the temporal patterning of retinal responses is transmitted reliably by LGN neurons, information about basic spatial relations could then be exploited in perceptual grouping at higher levels of visual processing.

Supported by the Max-Planck-Gesellschaft.

631.5

CORRELATED CHANGES IN THE VISUAL ACTIVITY OF TOPOGRAPHICALLY MATCHED PGN AND LGN CELLS. K. Funke* and U.T. Eysel. Inst. of Physiology, Dept. of Neurophysiology, Ruhr-Universität Bochum, D-44780 Bochum, Germany.

Relay cells of the cat lateral geniculate nucleus (LGN) are reciprocally connected with the GABAergic cells of the associated perigeniculate nucleus (PGN), thereby forming a negative feedback loop. Anatomical and physiological studies of the past, demonstrating the diversity of afferent inputs to LGN and PGN, have indicated a couple of possible functions for this feedback loop (binocular and long-range lateral inhibition, arousal effects, oculomotor interactions). In an attempt to find evidence for these functions, we performed double-recordings from single, topographically matched LGN and PGN cells in anesthetized (N_2O/O_2 70:30, halothane 0.4 %) and paralysed (alcuronium chloride 0.15 mg/kgh) cats during the presentation of visual stimuli, and combined with a reversible, local inactivation of PGN activity by micro-iontophoretical application of GABA or ACh. In most cases, PGN activity was like a mirror-image of LGN activity: increasing tonic activity in the PGN was directly followed (<5sec) by a decrease in LGN activity and vice versa. Visually induced activity varied oppositely within milliseconds. Very prominent opposite changes in LGN and PGN activity were found during changes in EEG activity and with visual stimulation by a large uniform stimulus or a moving grating. The latter caused a strong and sustained increase in PGN activity accompanied by a reduced tonic activity in the LGN, but with an enhanced signal to noise ratio for the transient responses elicited by the contrast borders. Preliminary results obtained with reversible inactivation of PGN areas reveal a slight reduction of inhibitory responses in the corresponding LGN regions (e.g. binocular and long range lateral inhibition). The moderate changes obtained with local PGN inactivation indicate that the contribution of single PGN cells to LGN responses may be small, although the correlated changes in PGN and LGN activity are strong at the single cell level.

Supported by the Deutsche Forschungsgemeinschaft (SFB 509/A2).

631.6

FEEDBACK IMPROVES THE TEMPORAL ACCURACY OF NEURONAL EVENTS IN THE VISUAL PATHWAY. E. Nelle, K. Funke, B. Li and F. Wörgötter*. Inst. of Physiology, Dept. of Neurophysiology, Ruhr-Universität Bochum, D-44780 Bochum, Germany.

The temporal accuracy of the signal transmission in the visual pathway is expected to deteriorate with increasing distance from the retina due to noise and other sources of activity uncorrelated to a driving retinal input. In a set of extracellular recording experiments in anesthetized (N_2O/O_2 70:30, halothane 0.4%) and paralysed (alcuronium chloride 0.15mg/kgh) cat, using flashed spot stimuli, we find on the contrary that the temporal precision of spike events is in many geniculate relay cells higher than in the corresponding retinal afferents: this is reflected in a reduction of the width of the peaks in the inter-spike interval histograms of many LGN cells by about 20% as compared to the retinal pre-potentials. A mechanism to explain the increased accuracy is proposed and implemented in a biophysically computer model for the dynamics of the membrane potential in the thalamo-cortical network: The action of excitatory cortico-geniculate feedback allows a temporal fine-tuning of the spike-pattern in geniculate cells. The temporal correction introduced by the cortex is mainly determined by an averaging process among those LGN cells projecting to one cortical cell. The improved temporal accuracy is accompanied by an increase in the mean firing rate of the geniculate cells. We were able to confirm the theoretical results with experimental data, using reversible inactivation of the visual cortex (cooling or GABA micro-iontophoresis) to switch off the excitatory corticofugal feedback. A disfacilatory effect induced by cortex inactivation was found in about 65% of the LGN cells. Consistently this effect was accompanied by a broadening of the interval distributions. Therefore, it could be speculated that one aspect of feedback loops might be to reduce the temporal dispersion of spike trains.

Supported by the Deutsche Forschungsgemeinschaft (Wo 388/4-3, SFB 509/A4).

631.7

LOCALIZATION OF METABOTROPIC GLUTAMATE RECEPTORS IN MONKEY LGN AND VPL. M. Xiong*, S.C. Van Horn, D.W. Godwin, A. Erişir, and S. M. Sherman. Dept. Neurobiology, SUNY, Stony Brook, NY 11794-5230.

We have recently shown in the cat LGN that different metabotropic glutamate receptors (mGluRs) have different distributions (Van Horn et al., *Soc. Neurosci. Abstr.* **21**:658, 1995; Godwin et al., *Soc. Neurosci. Abstr.* **21**:659, 1995). Labeling for mGluR1α (a splice variant of mGluR1) mostly labels fibers, which are relay cell distal dendrites at sites of cortical inputs. The mGluR5 antibody mostly labels puncta, which are F2 (dendritic) terminals of interneurons. Labeling for mGluR1α and mGluR5 is also seen in the thalamic reticular nucleus (TRN). However, mGluR2/3 labeling is very heavy in the TRN but light in LGN. We extended these observations to the macaque monkey, using light microscopic immunocytochemistry to reveal staining for the various mGluRs. In the monkey LGN, the mGluR1α antibody labeled a meshwork of fibers and was dense throughout, although magnocellular (M) laminae appeared more densely stained than parvocellular (P). Laminar borders could not be seen, because the interlaminar zones were also stained. This was similar to the pattern in the cat LGN, where the staining in interlaminar zones is due to the fact that many distal dendrites of relay cells cross laminar boundaries. Staining for mGluR5 was mostly punctate and also darker for M than P laminae, but laminar borders were quite distinct, because staining was much lighter in interlaminar zones. This, too, is similar to the pattern in the cat and is explained there by the limitation of all interneuron processes, including all F2 terminals, to the lamina in which the interneuron's soma is located. Thus the staining in the monkey LGN is consistent with the notion that mGluR1α labels distal dendrites of relay cells and mGluR5 chiefly labels the dendritic terminals of interneurons, as in the cat. We also observed both mGluR1α and mGluR5 labeling in the monkey's TRN. The labeling for mGluR2/3 was also similar to that in cat: dense in TRN but very light in the LGN. Finally, to extend the observations from LGN to other thalamic nuclei, we investigated the staining pattern seen in monkey VPL. Label for both mGluR1α and mGluR5 was dense in VPL, but that for mGluR1α chiefly labeled fibers and that for mGluR5 chiefly labeled puncta. Labeling for mGluR2/3 was much lighter in VPL. (We thank Carl Romano for providing the mGluR5 antibody. Supported by EY03038.)

631.8

COMPARISON OF CORTICAL AND CHOLINERGIC BRAINSTEM PROJECTIONS TO THE LGN IN CATS: AN EM STUDY. A.Erişir*, S.C. Van Horn, and S.M. Sherman. Dept. Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Visual cortex and brainstem provide the major nonretinal extrinsic inputs to LGN relay cells and modulate the relay of retinal information. We used the EM to compare the distribution of synaptic terminals from these inputs onto relay cells in the cat's LGN A-laminae. We identified corticogeniculate (CG) terminals via orthograde transport of biocytin into cortical areas 17, 18, or 19; we identified brainstem terminals via immunocytochemical labeling for ChAT or BNOS (all ChAT positive terminals also labeled for BNOS), since >90% of brainstem inputs to LGN derive from ACh & NO containing cells of the parabrachial region (PBR). Most CG and PBR terminals share the same basic RSD morphology (*r*ound vesicles, *s*mall terminals, *d*ark mitochondria) and make asymmetric synapses. However, despite a size overlap, PBR terminals were significantly larger. PBR terminals contacted thick, proximal dendrites amongst retinal terminals both inside and outside of glomeruli; this is the *retinal recipient zone*. CG terminals contacted thin, distal dendrites, where no retinal terminals were found, in the *cortical recipient zone*. We saw no overlap on relay cell dendrites between the zones of CG and PBR inputs. Of all LGN terminals, 55% are RSD, and despite the lack of supporting data, it has been generally assumed that >95% of RSD terminals are CG and very few are PBR. From our data, we used 3 different means of determining the ratio of CG and PBR inputs: 1) a comparison of interneuron (identified by postembedding anti-GABA immunocytochemistry) and relay cell target preferences of unlabeled RSDs and labeled CG terminals; 2) a re-evaluation of the distributions of unlabeled RSD terminals found in the retinal and cortical recipient zones of relay cell dendrites; and 3) an algorithm based on size distributions of unlabeled RSD, labeled CG, and labeled PBR terminals. All 3 analyses suggest that ⅓-½ of RSD terminals are PBR, the rest being CG. The surprisingly large number of PBR terminals and their location amongst retinal inputs suggest a stronger than previously thought role for them in gating retinogeniculate transmission. Conversely, the location and number of CG terminals suggests a smaller role than formerly thought for this input. (Supported by USPHS Grant EY03038.)

631.9

DIFFERENCES IN SYNAPTOLOGY OF RELAY CELLS BETWEEN LAMINAE A AND A1 OF CAT LGN. S.C. Van Horn, A.Erişir, and S.M. Sherman*. Dept. Neurobiology, SUNY, Stony Brook, NY 11794-5230.

The A-laminae of the cat LGN are often viewed as matched structures differing only by ocular input: the contralateral and ipsilateral eyes innervate lamina A, and the ipsilateral eye, lamina A1. However, few studies have compared details of circuitry between these laminae. We thus used the EM to investigate synaptology in these laminae, sampling 1191 terminals in 9 sections from 6 brains. We analyzed the distributions for each section of RLP (retinal), RSD (mostly cortical), and F (local inhibitory) terminals, and we used postembedding immunocytochemistry for GABA to distinguish relay cells (GABA-) from interneurons (GABA+). Compared to lamina A, Lamina A1, showed significantly more RSD terminals (57.4±1.8% vs 49.9±1.9%) and fewer RLP (16.8±1.1% vs 19.1±0.6%) and F (25.8±2.8% vs 30.9±1.7%) terminals. Since these sections also contained terminals anterogradely labeled from visual cortex, we were able to determine that the interlaminar difference in RSD terminals was due to a difference in cortical terminals, which display RSD morphology. However, inputs to interneurons were statistically similar (lamina A vs A1: 35.6±2.5% vs 33.9±2.7% RSD; 37.0±1.8% vs 37.1±0.7% RLP; and 26.3±1.2% vs 28.9±2.4% F), and included significantly more RLP and fewer RSD inputs than did relay cells, so the interlaminar differences were limited to relay cells (lamina A vs A1: 53.2±1.7% vs 60.7±1.7% RSD; 15.3±0.9% vs 13.9±1.1% RLP; and 31.7±1.5% vs 25.4±2.8% F). Thus relay cells in these laminae received equivalent ratios of RLP inputs, but in lamina A they received significantly more F and fewer RSD inputs than in lamina A1. Other data indicate that relay X cells receive more F and less RSD input than do relay Y cells (Wilson et al., *Proc. Roy. Soc. B.*, **221**:411-436, 1984), so our observations are consistent with other evidence (Colby, *J. Neurophysiol.*, **59**:1783-1797, 1988) that lamina A1 contains an additional population of Y cells. That is, relay Y cells include a subset that feeds corticotectal circuitry, and while lamina A1 includes this subset, lamina A does not; this subset for the contralateral eye instead relays through lamina C. In any case, our results indicate subtle difference in synaptology between laminae A and A1, and these differences are limited to relay cell inputs. (Supported by USPHS Grant EY03038.)

631.10

EVIDENCE FOR ACTIVATION OF FEEDFORWARD GABAERGIC CIRCUITRY IN CAT LGN VIA A SPECIFIC METABOTROPIC GLUTAMATE RECEPTOR. D.W. Godwin*, Q. Zhou, and S.M. Sherman, Dept. Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Our prior evidence implicates a metabotropic glutamate receptor (mGluR) in feedforward inhibition through F2 terminals of interneurons in the cat LGN (Zhou et al., *Soc. Neurosci. Abstr.* **20**:133, 1994; Godwin et al., *Soc. Neurosci. Abstr.* **21**:659, 1995): the mGluR agonist ACPD applied *in vitro*, in addition to depolarizing LGN relay cells, produces in some cells a bicuculline sensitive decrease in input resistance. (R_N) Immunocytochemical localization shows that mGluR1 receptors on relay cell dendrites are likely responsible for the depolarization via activation from cortical terminals and that mGluR5 receptors on F2 terminals postsynaptic to retinal inputs are likely responsible for the decreased R_N. We have now performed a preliminary *in vivo* investigation to extend this interpretation of the inhibitory pathway by studying the effect activating mGluR5 receptors with *trans-azetidine-2,4-dicarboxylic acid* (tADA), reported to be a selective mGluR5 agonist. The effect of tADA application is fundamentally different from application of ACPD, which generally depolarizes all LGN relay cells and promotes tonic firing instead of burst firing. Application of tADA to most relay X cells *suppressed* the sustained portion of the response to flashed spots, with less effect on initial peak responses; tADA in some X cells produced nearly total inhibition. In contrast, tADA application had very little effect on relay Y cells or cells thought to be interneurons. Relay X cells are richly innervated by retinal and F2 terminals in triadic arrangements suggestive of feedforward inhibition, since the retinal terminal contacts both the X cell dendrite and the F2 terminal, which in turn, contacts the same X cell dendrite. In contrast, neither relay Y cells nor interneurons receive significant innervation from F2 terminals. Our data are thus consistent with the conclusion that retinal inputs produce feedforward inhibition in relay X cells (but not Y cells or interneurons) via activation of mGluR5 on F2 terminals. Since mGluRs may require high frequency afferent input for activation, this inhibitory circuit may be evoked mainly when retinal firing rates are high, and this could serve to increase the dynamic range of LGN relay X cells. (Supported by EY03038.)

631.11

DORSAL RAPHE AND TUBEROMAMMILLARY NUCLEI MODULATE VISUAL RESPONSES AND FIRING MODE OF LGN RELAY CELLS. J.-T. Xue, J.K. Harting & D.J. Uhlrich* Dept. of Anatomy, University of Wisconsin Medical School, Madison, WI 53706.

Ascending visual signals are actively gated in the thalamic lateral geniculate nucleus (LGN) under the control of extrinsic modulatory systems. Prior studies have focused on the effects of the brainstem cholinergic projection to the LGN, but *in vitro* pharmacology suggests that other systems should have an important effect as well. Thus, we have examined the effects on cat LGN visual responses of activation of the serotonergic dorsal raphe nucleus (DRN) and the histaminergic tuberomammillary nucleus (TMN) of the hypothalamus. We used the same *in vivo* extracellular recording methods that we used in studying the cholinergic projection to the LGN (J. Neurophysiol. 73:2428, 1995).

TMN activation usually resulted in an enhancement in visual responses of LGN X and Y cells. If these cells exhibited the burst mode of firing, TMN activation promoted them to the tonic mode. We examined the effects on spatial tuning properties, and found the greatest enhancement at high spatial frequencies. In contrast, DRN activation resulted in a pronounced inhibition in the visual responses of most LGN cells, and their firing behavior was switched from the tonic to the burst mode. Spatial tuning curves showed a reduction at all frequencies, often greatest at low spatial frequencies. These results, together with our previous cholinergic data, provide evidence for a continuum of modulatory effects in the LGN. This supports the idea that the LGN is a dynamic spatial and temporal filter whose tuning characteristics are under the control of extrinsic modulatory systems.

Supported by NIH grants EY-06610 and EY-01277.

631.12

BOTH NMDA AND $GABA_B$ SYNAPTIC MECHANISMS CAN ACCOUNT FOR CORTICOFUGALLY-INDUCED CROSS CORRELATIONS IN dLGN OF THE CAT. C. D. Brody*. CNS Program, California Institute of Technology, Pasadena CA 91125

Sillito et. al. [Nature 1994, **369**: 479-482] reported significant cross-correlations (typically centered at 0 ms and of approx. 80 ms width) between the firing of pairs of dorsal lateral geniculate nucleus (dLGN) cells whose receptive fields were 1 to 4 degrees apart in the visual field. The cross-correlations were dependent on intact feedback from visual cortex. Analysis of this data shows that significant cross-correlations exist both when the dLGN cells fire tonically and when they fire in bursts. A model of dLGN cells is used to investigate the biophysical mechanisms necessary to account for the cross-correlations. Computational simulations using model cells indicate that inputs which are common to both cells and which have time courses on the order of tens of milliseconds or longer can generate cross-correlations with a time width similar to those observed *in vivo*. The net synaptic strength required for the strength of the cross-correlations between simulated cells to be similar to that observed *in vivo* lies in a physiologically plausible range both for NMDA-type synapses and $GABA_B$-type synapses. These may account for cross-correlations during tonic firing. Low-threshold calcium channel-mediated spikes have also been shown to be a mechanism capable of causing the observed cross-correlations (see Kirkland and Gerstein, this volume), and are likely significantly involved during burst firing.

Funded by NSF Cooperative Agreement No. EEC-9402726

631.13

DEVELOPMENTAL CHANGES IN SYNAPTIC EFFICACY IN THE RAT RETINOGENICULATE PATHWAY. F-S Lo* & W. Guido. Anatomy & Neuroscience Center, LSU Medical Center New Orleans, LA 70112.

Little is known about the synaptic properties of LGN relay neurons and the prevalence of long-term changes in synaptic efficacy during the development and refinement of connections in the mammalian retinogeniculate pathway. Using intracellular whole cell recordings and extracellular field potentials, in an isolated (in vitro) brainstem preparation, we examined the nature of synaptic transmission in the LGN of rats between postnatal day (PND) 0-32. At all ages tested, field potentials in LGN and EPSP(c)s in relay cells were recorded following electrical stimulation of optic tract (OT) fibers. During the time when connections are still developing (PND 0-4), OT stimulation produced long-lasting (400-800 ms) and purely excitatory responses in LGN. The duration of these were voltage-dependent and APV sensitive. After PND 5, EPSPs were *always* coupled with an IPSP (EPSP/IPSP pair). The IPSP component had a short duration (40 ms) and was bicuculline sensitive. An additional IPSP emerged by PND 7 that was long-lasting and bicuculline insensitive. Long-term changes in synaptic efficacy were also observed throughout development. Between PND 0-7, tetanic stimulation (6 1-sec trains of 50 Hz pulses at 30-sec intervals) of OT fibers produced strong (>50%) long-term (≥60 min) depression (LTD) of synaptic activity (whole-cell recordings and field potentials). After PND 7, tetanus led to a mixed response: periods (10-40 min) of depression coupled with potentiation (5-15 min). By PND 30, tetanus produced a reliable, but moderate (10-20%) long-term potentiation (LTP). Thus many aspects of synaptic transmission are subject to developmental regulation, including the onset and prevalence of NMDA and GABA activity, and long-term changes in synaptic efficacy. These changes may contribute to the activity dependent refinement of retinogeniculate connections seen during development. *Support by NSF 9396270 (WG).*

631.14

DECREASED GABAERGIC INHIBITION IN THE ADULT CAT VISUAL SYSTEM AFTER SENSORY DEAFFERENTATION AS REVEALED BY IN SITU HYBRIDIZATION FOR GAD_{65} AND GAD_{67} L. Arckens[1,2*], E. Van der Gucht[1,2], U.T. Eysel[3], G.A. Orban[2], F. Vandesande[1] [1]Lab. Neuroendocr. & Immunol. Biotechnol., [2]Lab. Neuro- and Psychophysiol., Medical School. Katholieke Universiteit Leuven, B-3000 Leuven, Belgium; [3]Dept Neurophysiol., Medical School, Ruhr-Universität Bochum, D-44770 Bochum, Germany.

The effect of partial central retinal lesions on the GAD_{67} and GAD_{65} mRNA levels in the dorsal lateral geniculate nucleus (dLGN), the perigeniculate nucleus (PGN) and the visual cortex of the adult cat was investigated by in situ hybridization. Three days post-lesion, a decrease in the number of GAD_{67}-expressing cells became apparent in the deafferented dLGN, persisted uptil 12 months post-lesion and became more pronounced with longer survival times. This decrease in GAD_{67} mRNA was mirrored by a decrease in GAD-immunoreactive cells. GAD_{65} mRNA levels were low in the dLGN of control cats and remained very low after retinal lesioning. In all the retinal lesion cats we investigated both GAD_{65} en GAD_{67} mRNA levels were decreased in a restricted region of the PGN, specifically the region of the PGN above the deafferented portion of the dLGN. In the lesion-affected visual cortex, no changes at the mRNA level were observed for either GAD_{67} or GAD_{65} although changes in GAD immunoreactivity have been described (Rosier *et al.*, J. Comp. Neurol. 359:476-489, '95). Hence, in the dLGN, the PGN and the visual cortex, different mechanisms seem to be effective that might lead to decreased GABAergic inhibition in response to sensory deafferentation.

This work was supported by the Belgian Queen Elisabeth Medical Foundation, the National Fund for Scientific Research and the Belgian program of Inter-University Poles of Attraction (n° 22 - Vision and Memory).

631.15

AN OPTICAL FRACTIONATOR ANALYSIS OF NEURONAL CHANGES IN THE LATERAL GENICULATE NUCLEUS OF MONKEYS WITH EXPERIMENTAL GLAUCOMA. E.A. Nimchinsky[1*], J.C. Vickers[3], W.G. Young[4], R.A. Schumer[2], R.F. Wang[2], S.M. Podos[2], J.H. Morrison[1], and P.R. Hof[1,2] Depts of [1]Neurobiol. and [2]Ophthalmol., Mount Sinai Sch. Med., New York, NY 10029, [3]Dept of Pathol., Royal Hobart Hospital, Australia 7000, [4]Scripps Research Institute, La Jolla, CA 92037.

Degenerative alterations and cell loss occur in neuronal subsets in the retina and the visual pathways of monkeys with experimental glaucoma. In the present study, pathologic changes were quantified in the lateral geniculate nucleus (LGN) of macaque monkeys with different degrees of glaucoma severity. Glaucoma was induced in one eye by laser photocoagulation of the trabecular meshwork. After a variable period of sustained elevated intraocular pressure, the monkeys were killed and their brains prepared for immunohistochemistry with antibodies to neurofilament protein (NFP) and parvalbumin (PV). Stereologic unbiased estimates of total neuronal numbers were obtained using the optical fractionator in the ipsi- and contralateral magno (M)- and parvocellular (P) layers of the LGN, separately. Results indicated that both M and P layers receiving from the affected eye were severely affected in glaucoma with neuron loss as high as 63%. NFP-containing neurons were less sensitive in the M layers than in the P layers (25 vs 64% loss), whereas PV-positive cells were equally affected in both (50%). Monkeys with early signs of glaucoma had very mild changes in both M and P neuron counts. Comparison of the ipsi- and contralateral P to M total neuron count ratios provided a reliable indicator of glaucoma severity in each monkey, and revealed that PV labeling was less preserved overall than NFP labeling. Since our estimates of total neuron numbers suggest that these proteins are largely colocalized in M and P neurons, a decrease in PV-positive cells may be due in part to a decreased PV expression, as the relative loss of PV-positive cells exceeded in some cases that of NFP- and Nissl-stained cells. These data show that in glaucoma, severe neuron loss in the LGN may occur independently of the presence of NFP and PV, and without clear selectivity for the M or P layers.

Supported by NIH HBP grant MHDA52154, RPB, AHAF, and the Rudin Foundation.

631.16

EFFECT OF METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION ON RAT THALAMOCORTICAL NEURONES *IN VITRO*. J.P. Turner* and T.E. Salt. Dept. Visual Science, Inst. Ophthalmology, UCL, London, U.K.

The activation of postsynaptic metabotropic L-glutamate receptors (mGluRs) results in membrane potential depolarization, an increase in input resistance, action potential broadening and a decrease in spike frequency accommodation, owing to the inhibition of the potassium conductances contributing to the leak, the M and the calcium-dependent afterhyperpolarization (AHP) currents, and activation of a non-specific cation conductance. Previously, it has shown that the prototypical mGluR agonist 1S,3R-ACPD depolarizes the membrane potential of thalamocortical (TC) neurones by reducing a potassium conductance (McCormick & von Krosigk, 1992). Here, the effects of mGluR activation on the action potential, its AHP and firing patterns, together with the type of mGluRs responsible are addressed.

The application of 1S,3R-ACPD (50-300μM) to slices of rat dLGN maintained *in vitro*, caused a concentration-dependent depolarization of membrane potential (2-14mV), associated with an increase in input resistance. As a result, TC neurones were more excitable in the presence of 1S,3R-ACPD when tested with positive current pulses, and the action potentials elicited were larger in amplitude with a smaller late AHP. In addition, the firing of TC neurones was transformed from a regular pattern in control to an accelerating one in the presence of 1S,3R-ACPD. The type I mGluR agonist (RS)-3,5-dihydroxyphenylglycine (50-100μM) produced similar effects to 1S,3R-ACPD, including depolarization of membrane potential (3-11mV), while the type I mGluR antagonists (S)-4-carboxyphenylglycine (500-2000μM) and 1-aminoindan-1,5-dicarboxylic acid (200-500μM) depressed responses to 1S,3R-APCD (50-100μM) by up to 50%. In conclusion, the activation of postsynaptic mGluRs on TC neurones in the dLGN produces a number of effects consistent with an increase in neuronal excitability, and that these effects are mediated, in part at least, via type I mGluRs. (Supported by the Wellcome Trust)

631.17

SOMATOSTATIN DECREASES INHIBITORY POSTSYNAPTIC POTENTIALS IN KITTEN DORSAL LATERAL GENICULATE NUCLEUS IN VITRO. E. Asprodini*, Zs. Emri and V. Crunelli. Physiology Unit, School of Medical and Molecular Biosciences University of Wales Cardiff UK.

Immunohistochemical studies have revealed the presence of somatostatin immunoreactivity in cell bodies and primary dendrites of neurones in the nucleus reticularis thalami (A.M. Graybiel and R.P. Elde J. of Neurosci. 1983. Vol. 3 pp1308-1321.). In the hippocampus and septum where GABA and somatostatin are co-localized the modulation of GABAergic neurotransmission by somatostatin has been shown, but in the thalamus similar evidence is lacking.

We studied the effect of somatostatin (3-14) on spontaneous inhibitory potentials (IPSPs) recorded from dorsal lateral geniculate nucleus (dLGN) thalamocortical (TC) neurones of 5-10 days old kittens in vitro. In this age range TC neurones showed spontaneous $GABA_A$ receptor-mediated activity either as single IPSP or as bursts of IPSPs. Bath application of somatostatin (1-10 μM) did not change the passive properties of TC neurones (n=15) but reversibly decreased the frequency of the spontaneous events by 45%. The amplitude of the IPSPs were reduced by 25% and where bursts of IPSPs were present the number of IPSPs within the burst and the duration of the burst were also decreased (n=7). In 1 cell somatostatin (10μM) abolished all spontaneous activity.

These results indicate that somatostatin modulates GABAergic transmission in the visual thalamus.

Supported by the Wellcome Trust.

631.18

MULTI-COMPARTMENTAL MODEL OF A THALAMOCORTICAL NEURONE. K. Antal, Zs. Emri, T.I. Tóth* and V. Crunelli. Physiology Unit, Sch. Mol. & Med. Biosci., University of Wales Cardiff, Cardiff, UK.

An existing one-compartmental model (Tóth & Crunelli, *NeuroReport*, **3**, 65-68, 1992) of a thalamocortical (TC) neurone was extended to include dendritic regions. The geometry of this multi-compartmental model was chosen according to a representative soma-to-dendritic terminal path of an X cell of the cat dorsal lateral geniculate nucleus. The distances of terminals from the soma, and the number of dendritic branching along the path were based on the reconstruction by Bloomfield et al. (*J. Physiol.*, **383**, 653-692, 1987). The dendritic geometric parameters were calculated to yield the total surface area of the reconstructed dendrites, and the dendritic branches were further subdivided in order that all compartments had electrotonic lengths of less than 0.1, reaching thus a total of 25 compartments. The passive parameters and the specific conductances on the soma and on the dendrites were adjusted to produce the following characteristic activity patterns of TC neurones in response to constant injected current: tonic firing when the cell was depolarized above -45 mV, and pacemaker oscillation between -55 and -75 mV. The activity patterns of the multi-compartmental model with different distributions of non-linear ion channels on its dendrites were compared to those of *in vitro* electrophysiological measurements, and to the corresponding responses of the one-compartmental model. We found that the presence of voltage-activated non-linear currents on the dendrites was necessary to evoke pacemaker oscillation. Dendritic distributions of ion channels at which the multi-compartmental model reproduced the tonic firing and oscillatory activity with the same voltage-dependence as measured *in vitro* will be presented.

Supported by the Wellcome Trust.

631.19

INTRODUCING COMPUTER-GENERATED CONDUCTANCES INTO THALAMOCORTICAL NEURONES. S.W. Hughes, T.I.Tóth, S.R.Williams, A. Ceci* and V. Crunelli. Physiology Unit, School of Molecular & Medical Biosciences, University of Wales Cardiff, Cardiff, UK.

We describe a system based on the dynamic clamp technique which allows the addition of artificial conductances to thalamocortical neurones maintained *in vitro* using a combination of mathematical modelling and intracellular recording (J.Neurophysiol. 69: 992-995, 1993). The system is personal-computer-based and functions by simultaneously sampling (≥10kHz) the membrane potential (V_m) and injecting trans-membrane current (I), where I is related to V_m by a system of non-linear differential equations specifying the desired steady-state and kinetic properties of the conductance(s) to be added. In particular, the technique was used to investigate the effects of modulating the transient low-threshold calcium current (I_T) and non-inactivating mixed cation current (I_h) common to thalamocortical neurones. The equations used for I_T and I_h were adopted from a previously developed single-compartment model of a thalamocortical neurone (Neuroreport. 3: 65-62, 1992). We initially tested and refined our mathematical representations of these currents by introducing them with opposite sign into thalamocortical neurones. By fine parameter adjustment, we were then able to abolish the typical effects of their *in vitro* counterparts over an appropriate voltage range and obtain accurate descriptions of the currents. In the case of I_T, we demonstrated how a small increase in maximal conductance, and hence its steady-state window component, gave rise to novel input-output properties and oscillatory activity, and, in the absence of I_h, bistability of the neurone. We then blocked I_h by application of ZD 2788 (300μM) and after reproducing the control current-voltage relationship with artificial I_h, revealed how positive shifts in its activation curve affected the input-output properties of thalamocortical neurones. *This work was supported by the Wellcome Trust.*

631.20

SODIUM CURRENTS IN THALAMOCORTICAL NEURONES. H.R.Parri, N.Leresche* and V. Crunelli. Physiology Unit, School of Mol. and Med. Biosciences, University of Wales Cardiff, Cardiff, Wales, U.K.

Whole cell patch clamp recordings were made from rat thalamocortical (TC) dorsal lateral geniculate neurones in an intact thin slice preparation and an acutely dissociated cell preparation. Recording solutions were used that blocked K^+ and Ca^{2+} currents. Transient Na^+ currents were completely blocked by application of 1μM TTX. The threshold for activation was between -60mV and -50mV in both preparations. Maximum currents were observed around -30mV, and were -2.1±0.8nA (n=7) in the slice and -2.7±0.6nA (n=9) in dissociated neurones. Threshold responses in the slice were characterised by the sudden appearance of delayed large amplitude transient Na^+ currents. In dissociated neurones, responses to increasing step depolarisations displayed a gradual increase in the Na^+ current, permitting the construction of activation curves. Activation, from a holding potential of -120mV could be fitted with a single Boltzman function with V1/2= -40.2mV. Steady-state inactivation was also fitted with a single Boltzman, V1/2=-67mV. Recovery from inactivation at a holding potential of -120mV following a 10ms step to -10mV was rapid, with t1/2<5ms. These results show that Na^+ currents from TC neurones in an intact slice preparation display similar properties to those in acutely dissociated neurones. The enhanced space clamp properties of the dissociated neurones, however, permit more accurate kinetic analysis of these currents.
This work was funded by the Wellcome Trust

VISUAL CORTEX: STRIATE VI

632.1

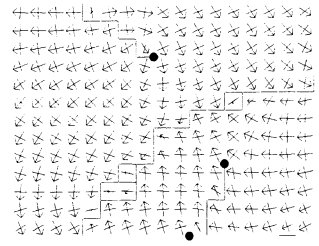
IN MACAQUE V1 LATERAL CONNECTIONS IN LAYER 4B HAVE A DIFFERENT TOPOGRAPHY THAN IN LAYERS 2/3, H.Asi, J.B.Levitt, and J.S.Lund* Dept. Visual Science, Institute of Ophthalmology, University College, London, U.K.

Pyramidal neurons in layers 2/3 of the adult macaque monkey V1 have laterally spreading axon collaterals making regularly sized and spaced patchy terminal fields (patches 227μm dia., 430μm center to center) in the same layers; these connections link points of similar orientation specificity and ocular dominance. Layers 4B and upper 4Cα also have laterally spreading intrinsic connections but unlike 2/3, contain directionally selective neurons and both spiny stellate and pyramidal cells. The topography of terminal sites within 4B/4Cα is not known. We have made small (300μm) iontophoretic injections of biocytin within layer 4B-upper 4Cα and sectioned the V1 tissue tangential to the pia to investigate the topography of connections within the region; interleaved sections were reacted for cytochrome oxidase(CO) to establish laminar boundaries. In adult and infant (4.5 wks.) the lateral connections establish parallel, bar shaped terminal fields immediately surrounding the injection site; bars are ~200μm wide, 700-2,000μm long, and spaced ~400μm center to center; these break into linear arrays of patches further from the injection site(~260μm dia., 545c/c) in the infant. The difference in connectional topography between layer 4B and layers 2-3 suggests that functional topography at the two depths may be different, perhaps relating to direction specificity. Supported by NEI-EY10021 and MRC G9408137.

632.2

A MODEL FOR THE CORTICAL MAP OF DIRECTION PREFERENCE. N. V. Swindale* *Department of Ophthalmology, University of British Columbia, 2550 Willow St., Vancouver, BC., Canada V5Z 3N9*

Recent optical recording data have suggested that cortical maps of direction preference in cat area 18, ferret area 17, and area MT of owl monkey, have similar properties. The maps are characterized by the presence of half-rotation point singularities in the orientation preference vector, and line singularities in direction preference. The line singularities subdivide regions with similar orientation preference into regions with opposed direction preferences. As observed experimentally (Malonek et al., 1994; Weliky et al., 1996), and suggested on the basis of topological arguments (Swindale et al., 1987), line singularities in direction preference originate in orientation singularities and join them via relatively short routes. These observations suggest that the orientation map is primary, and contrains the self-organization of the map of direction preference.



I present here modelling results in which an orientation map develops according to previously described principles of local continuity and global completeness, and a second direction preference vector is weakly constrained to be (a) locally continuous and (b) orthogonal to the orientation preference vector. These contraints conflict: the direction map can only be locally smooth if it is non-orthogonal to orientation preference while enforcing orthogonality leads to a large number of 180° reversals in direction preference. When a balance is struck, the resulting maps resemble the experimental data. The figure shows a computed map in which the spots mark orientation singularities and lines indicate discontinuities in direction preference. Non-orthogonality between orientation and direction preference is most marked close to the orientation and direction discontinuities. *Supported by NSERC 82020 and MRC 91400.*

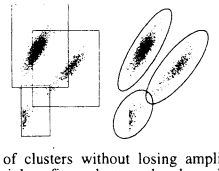
632.3

ROTATED HYPER-ELLIPSES IMPROVE MULTI-UNIT SPIKE SEPARATION. P. A. Hetherington*, P. Zakarauskas, & N.V. Swindale. Dept. of Ophthalmology, Univ. of British Columbia, 2550 Willow St., Vancouver, BC., Canada V5Z 3N9

Plots of waveform parameters from extracellularly recorded multi-unit spikes typically show clusters (Figure), where each cluster is likely to correspond to the firing of a single neuron. These clusters are often elongated, especially when recorded from cells that fire in bursts, and often overlap when lower-impedance stereotrodes or tetrodes are used.

Separating overlapping and elongated clusters typically involves manually establishing upper and lower parameter boundaries (Figure, left), and often requires examining many pairs of parameters before a satisfactory separation is achieved. This is a tedious, subjective, and inexact process. An automated method such as hierarchical cluster analysis is ideal, but impractical when many thousands of spikes are considered. K-Means analysis is much faster, but requires pre-specifying the number of units, and is inadequate when clusters are elongated. Hybrid methods combining hierarchical and K-Means (e.g., Mitra et. al., *S.N.A.*, **21**, 51.4) are good alternatives, but still split elongated clusters. Computing ratios between the spike amplitudes on two channels ("phase angles") removes elongated clusters, but adds additional parameters and loses absolute spike amplitudes.

We describe a new clustering algorithm that combines hierarchical and K-Means, and which also respects the natural elongated and rotated nature of spike clusters in a multidimensional space. Clusters are either manually or automatically computed from a small sample (N ≈ 500 - 10,000) of spikes, and multidimensional, rotated ellipses are established as the cluster boundaries using Principal Component Analysis (Figure, right). These hyper-ellipses are computed within seconds, and can be used to classify remaining spikes. The hyper-ellipse method: (1) respects the rotated and elliptical nature of clusters without losing amplitude information, (2) provides a more natural and tighter fit to clusters, thereby reducing cluster boundary volume and overlap, and (3) uses parameter covariances to significantly improve new spike assignment and automated cluster linkage. *Supported by NSERC 82020 and MRC 91400.*



632.4

A CORTICAL AMPLIFIER MODEL OF DIRECTION SELECTIVITY IN STRIATE CORTEX SIMPLE CELLS. Gary R. Holt*, Rodney J. Douglas, and Christof Koch, Computation and Neural Systems, Caltech, Pasadena, CA 91125; Institute of Neuroinformatics, ETH/UZ, Gloriastrasse 32, CH-8006, Zürich.

The majority of excitatory input to layer IV spiny stellate cells in primary visual cortex comes not from thalamic axons but from other nearby cortical cells. Yet most models so far have ignored the short range excitatory cortico-cortical connections.

Our model consists of two spatially offset populations of integrate-and-fire neurons; each population has 320 excitatory and 80 inhibitory cells. Excitatory cells are connected to other excitatory cells in the same population with the same receptive field, so a small thalamic input is amplified by feedback. Excitatory cells also connect to inhibitory cells in the same population, whereas inhibitory neurons connect to all types of cells in both populations. All model cortical cells, excitatory and inhibitory, are direction selective because in the null direction inhibition from the other population is sufficient to cancel out thalamic input. Although the model has a high gain and NMDA-like synapses with a slow time course, it does not have a long network time constant because adaptation effectively cancels out the slow parts of synaptic input.

This simple model can reproduce the bandpass velocity tuning curves in the preferred and null direction observed by Saul and Humphrey (1992), as well as the phase advance at high contrast reported by Carandini and Heeger (1994). Unlike the normalization model of Carandini and Heeger, there is only a small change in input conductance in response to stimuli in the null direction because only a small amount of inhibition is required to offset the small thalamic input.

Supported by grants from the Sloan Foundation, ONR, and the Engineering Research Center at Caltech.

632.5

STIMULUS SELECTIVITY OF NEIGHBORING CELLS IN MONKEY STRIATE CORTEX. T-D. Shou[2]*, Y-C. Wang[1], M.T. Schmolesky[1] and A. G. Leventhal[1]. [1]Dept. of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT 84132. [2]Dept. of Biology, USTC, Hefei 230027, P.R.China.

It has been reported that direction (D) and orientation (O) sensitive cells are segregated from D and O insensitive cells in monkey striate cortex. Cytochrome oxidase rich regions (blobs) are said to contain D and O insensitive, wavelength sensitive cells while cytochrome oxidase poor regions contain D and O sensitive, wavelength insensitive cells (Livingstone and Hubel, 1984). This organization is difficult to reconcile with reports that most monkey striate cortical cells are selective concomitantly for stimulus attributes such as orientation, direction and color (Leventhal et al. 1995).

In this study we compared the D and O sensitivities of striate cortical cells located within 60 - 300 μm of each other. Stimuli consisted of drifting sinusoidal gratings of various spatial frequencies. Our findings indicate that cells located within 120 μm of each other tend to prefer similar orientations and either similar or opposite directions of motion. This tendency is weaker in the upper than in the lower layers of V1. The degree of D and O sensitivity exhibited by wavelength sensitive and broad band cells does not differ. However, neighboring broad band cells exhibit a stronger tendency to prefer similar directions and orientations than do neighboring wavelength sensitive cells.

Our findings do not support the idea that wavelength sensitive and broad band cells are segregated in V1. However, the relatively poor local organization of D and O sensitivity exhibited by wavelength sensitive cells suggests that local regions rich in these cells should respond to a relatively wide range of stimulus parameters. Thus, field potentials, optical recordings and multiunit recordings from wavelength sensitive regions should yield relatively weak D and O sensitive responses. Supported by EY04951 to A.G.L.

632.6

CHARACTERIZATION AND LOCATION OF SPATIALLY STABLE PHASE SEGMENTS IN STIMULUS-RELATED SENSORY CORTICAL EEGs FOR THE DEMARCATION OF PERCEPTUAL EPOCHS

J.M. Barrie and W.J. Freeman*, Dep't of Molecular and Cell Biol., University of California at Berkeley, Berkeley, CA 94720-3200

Arrays of 64-electrodes (8x8 at 0.56, 0.79 mm interelectrode spacing) were chronically implanted onto the epidural surface of the visual, auditory, somesthetic, and prepyriform cortices of the NZW rabbit. Forty records of EEGs were recorded at 6 sec epochs from a series of classical conditioning experiments where the animal had to discriminate between a CS- and CS+ stimulus randomly delivered in time and sequence. Previous experiments had demonstrated that spatial patterns of normalized EEG amplitude could be classified as either a CS- or CS+ pattern by a process of arbitrary interval pattern reconstruction and subsequent cross-classification. In order to more precisely locate EEG segments corresponding to endogenous perceptual activity an analysis technique was designed to locate stable phase patterns. With use of data from previous experiments, spatiotemporal patterns of amplitude and phase were reconstructed by FFT decomposing 128 ms segments (at 4 ms intervals) of EEGs. Each phase pattern was 2D-filtered and fit with a conic surface. Stable phase segments were defined as those where the apex of the regressed cone moved less than 0.75 mm per 4 ms step, where the frequency of the FFT was between 20-80 Hz, where the residual of the regression of less than 20%, and where all of the above criteria were satisfied for at least 40 ms. The apex of each phase cone was a random variable per segment indicating the lack of any pacemaker driving the formation of the EEG spatial patterns. The modal phase velocity was 1-2 M/sec and the modal diameter (commonality or 'binding' of the phase signal within $\pm$ cos. 45°) was 0.5-1.0 cm. Using the onset and duration of each stable phase segment to parse the EEG amplitude patterns for classification yielded significantly superior spatial pattern separation compared to previous results, in which segment selection was arbitrary. Funded by the National Institute of Mental Health - MH06686.

632.7

MECHANISMS UNDERLYING CONTEXT DEPENDENT INFLUENCES IN PRIMATE VISUAL CORTEX. H.E. Jones* and A.M. Sillito. Dept. of Visual Science, Inst. of Ophthalmology, London EC1V 9EL, UK.

Orientation and direction context dependent effects can be seen at subcortical and cortical levels in the visual system (Knierim and Van Essen, *J. Neurophysiol.* **67**: 961-980, 1992; Sillito et al, *Exp. Brain Res.* **93**:6-16, 1993; Sillito et al, *Nature* **378**:492-496, 1995; Cudeiro and Sillito, *J. Physiol.* **490**:481-492, 1996; Jones et al, *JOVS* **37**:S1058, 1996). Here, we consider evidence from cells in V1 that provide insight into these effects. The data suggest 4 mechanisms. Firstly a core mechanism of variable strength for all cells that exerts a non-orientation specific orientation contrast influence, minimising the output of the cell for extended iso-orientation contours. This generates the suppressive surround and depends on coactivation of centre and surround mechanisms. It was seen in non-oriented 4Cβ cells, and in some oriented cells when the inner/outer stimulus boundary was well within the confines of the classical receptive field (CRF). Subcortical mechanisms may contribute to this. Secondly, in orientation tuned cells, a mechanism that seems to be best explained as a cross oriented (with respect to the cell's optimal orientation) disinhibitory influence that appears to diminish the effect of an inner stimulus encroaching on the suppressive surround. Thirdly, a facilitatory influence capable of exerting effects over a long distance, driven by orientation and direction contrast. This might also be brought into the disinhibitory category if the inhibitory mechanisms generating the suppressive surround extend through the centre of the field so that even an optimal stimulus drives them. Fourthly, two overlapping processes underpinning orientation tuning, the elongation of the central excitatory field and excitatory connections between iso-orientation columns. The dependence of the suppressive surround on coactivation of centre and surround suggests an "and" type mechanism for the underlying inhibition and is why diminishing centre contrast can make a surround suddenly seem excitatory (because it actually impinges on the CRF). The cross-oriented disinhibition seems to be exerted by a cross oriented stimulus outside the CRF field or one overlying it, hence in some cases the effectiveness of a cross oriented (with respect to optimal) central stimulus and iso-oriented surround stimulus. *Supported by the MRC*

632.8

THE ROLE OF CORTICOGENICULATE FEEDBACK IN MEDIATING CONTEXTUAL EFFECTS IN PRIMARY VISUAL CORTEX AND IN PSYCHOPHYSICS. Valentin Dragoi and Robert P. Erickson*. Department of Psychology: Experimental, Duke University, Durham, NC 27708.

Even though it is well known that the primary visual cortex (V1) projects back to the LGN outnumbering thalamocortical projections by a factor of about 10, most models of orientation tuning and context effects neglect the influence of the massive corticogeniculate feedback (CGF), relying instead on feedforward and lateral connections. This study describes a large-scale model that investigates the role of CGF projections, in addition to feedforward excitation and short and long-range horizontal connections, in explaining context-dependent distortions in the orientation tuning (Gilbert & Wiesel, 1990) and firing pattern (Knierim & Van Essen, 1992) of cells in V1, as well as their psychophysical correlates demonstrated in the case of geometrical illusions. The model simulates parts of the static form pathway, i.e., LGN Parvo → V1a(layer 4C) → V1b(layer 2-3) → V1(layer 5-6) → LGN Parvo. We configure 31,752 LGN neurons arranged on an array of 21 x 21 locations, and 63,504 cortical neurons arranged on two layers that correspond to V1a and V1b. Both V1a and V1b cells develop short-range cross-orientation inhibitory connections. Long-range horizontal connections are made onto iso-oriented cells in V1b within a circular patch of radius 3 at the center. We show that in the presence of an inhibitory oriented surround the long-range horizontal connections contribute to the disinhibition of the orientation detectors along a direction away from the surround orientation, an effect amplified by the CGF projections that control the transmission of information through the LGN. In order to evaluate the combined effect of corticocortical feedback (CCF) and CGF loops, we added recurrent iso-oriented excitatory projections from V1 (layer 5-6) to V1a. Computer simulations showed that although small ratios between the strength of CCF and CGF projections did not disrupt the context effects, as the strength of the excitatory CCF increases further both surround-dependent orientation shift effect and geometrical illusions reduce drastically in magnitude. Our simulations also showed that if we reduce the strength of CGF projections in favor of long-range horizontal connections both surround-dependent orientation shift effect and geometrical illusions diminish in magnitude. The present study suggests that CGF plays a crucial role in shaping the orientation tuning and firing rate of individual neurons in V1 in the presence of a suppressive surround, and that these physiological effects may be connected with psychophysical phenomena such as geometrical illusions. Supported by the Dept. of Psychology: Experimental, Duke Univ., and by the National Academy of Sciences.

632.9

A MODEL OF PERCEPTUAL GROUPING PROCESSES IN PRIMARY VISUAL CORTEX, P. Mineiro and D. Zipser*. Department of Cognitive Science, UCSD, La Jolla, CA 92092.

Research (Lamme (1994) *J. Neuroscience* **15**:2) has indicated that cells in primary visual cortex show changes in their firing patterns when their classical receptive fields fall within a region of the image which is perceptually grouped into a figure versus when it is perceived as part of a homogenous background. This is true even when the input to the receptive field of the cell is held constant. The modulation only significantly affects cells whose receptive field input is stimulating and begins approximately 100ms after initial response to the stimulus, strongly suggesting the firing assymetry is due to recurrent interactions with the rest of the visual brain.

We constructed a neural network model of complex cells interacting via lateral connectivity and optimized it to segment figure from ground for stimuli similar to those used by Lamme. This model shows steady-state activity modulation similar to that found *in vivo* and demonstrates that some perceptual grouping computations can be preformed efficiently using local lateral connectivity.

PM is supported by an NSF Graduate Student Fellowship.

632.10

A TOPOLOGICAL INTERPRETATION OF FIGURE-GROUND SEGREGATION. S. Barash*, Department of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel.

Visual segregation of figure from ground links local with global aspects of vision, low with high levels. Nevertheless, "figure" remains a primitive, undefined concept. There is no overall understanding of the essence of the process of segregation -- let alone of the underlying physiology.

Here I suggest the following: first, segregation is essentially a topological operation. Second, figures are closed, connected regions in a specific topology that is embodied in the activity of LGN and visual cortex neurons. Third, segregation is the process by which a well-defined topological interpretation emerges out of the noisy, ill-defined visual input.

One way to define a topological structure is by specifying a particular basis for a topology. Here we will consider three structures -- the LGN, layer 4 of V1, and the cytochrome-oxidase-rich blobs of V1 -- as the processing stages in which the basis for the perceived topology is being defined. In this context, the large increase in receptive field density of layer 4 (relative to the LGN) acquires a specific significance.

The topological analysis suggested here is dynamic. Processing is reinitiated with every new visual scene, probably for each fixation. Processing may end before completion -- depending on context (task). Cognitive context probably modulates the topological analysis via top-down connections. These qualifications do not, however, alter the core of the segregation process that remains, I suggest, as illustrated above.

Supported by the McDonnell-Pew Program in Cognitive Neuroscience.

632.11

COMPONENT-PERIMETRY: A NEW METHOD TO REVEAL DIFFERENT TYPES OF VISUAL DISORDERS CAUSED BY CORTICAL LESIONS. G. Bachmann[1], S. Henke-Fahle[2]* & M. Fahle[1]. [1]Sektion Visuelle Sensorik, [2]Dept. of Ophthalmology, University Eye Clinic, D-72072 Tübingen, Germany.

Striate and extrastriate lesions of the cerebral cortex usually lead to visual field defects or selective deficits in one submodality of visual perception which are often not consciously experienced by the patient. Our aim is to develop a new perimetric method that test different visual tasks or components separately (color, motion, temporal resolution, orientation, and stereoscopic depth) and that examines the visual field in parallel (simultaneous perimetry). Perimetric patterns were generated by a Macintosh computer and presented on a 20"-color monitor. We examined a visual field area of approximately 50° radius. 37 patients with circumscribed cortical lesions had to scan the different stimuli with their 'minds eye' while fixating steadily. If they perceived local differences of the stimulus they outlined these areas on the screen. Ten of 14 patients with extrastriate lesions perceived no local differences, i. e. no defect. Four patients with extrastriate lesions subjectively detected contralesional local differences within their visual field, but only for some of the components tested. The 23 remaining patients with lesions at or below the level of V1 subjectively experienced their visual field defects for all components tested. Three patients of this group experienced areas of local differences in some stimuli that were larger than the visual field defect obtained with conventional perimetry. Component perimetry is thus a new method to detect different types of visual disorders on very different levels of the visual system. A fast screening of the visual field is thus possible not just for the detection of defects in contrast sensitivity-as in conventional perimetry-but for other submodalities of vision and higher visual functions, too.

632.12

BORDERS OF HUMAN STRIATE CORTEX. AN INTEGRATED PET AND CYTOARCHITECTONIC STUDY. Zoltán Vidnyánszky[1,2], Balázs Gulyás[1], Per E. Roland[1,*], Karl Zilles[3], Thorsten Schormann[3], and Jan-Erik Litton[4]. [1]Division of Human Brain Research, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden; [2]Laboratory of Neurobiology, 1st. Department of Anatomy, Semmelweis University Medical School Budapest, H-1094; [3]Department of Anatomy, Heinrich-Heine University Düsseldorf, D-40225 Düsseldorf; [4]Department of Psychology, Karolinska Hospital, S-171 76 Stockholm

The main objective of the present study was to explore on the basis of various functional, anatomical, and neurochemical landmarks, the extent of area V1 in the human brain. With the help of a computerised brain atlas (HBA, Roland et al., Human Brain Mapping 1(1994):173-184), we have standardised in shape and size (1) the MR images of five normal autopsy brains, Nissl-stained for determining the V1/V2 border on the basis of quantitative cytoarchitectonic criteria, (2) MR and PET images of five normal subjects whose $GABA_A$-benzodiazepine receptor distribution (B_{max}) was measured by ^{11}C-flumazenil (and displayed a clear density difference between striate and extrastriate visual cortex), (3) MR and PET images (made with ^{15}O-butanol, a flow tracer) of ten normal subjects, undergoing visual stimulation which strongly activated the primary visual cortex, and (4) FDG-PET images of seven normal subjects (displaying a strong metabolic difference between striate and extrastriate visual cortices).

The area borders between V1 and the neighbouring extrastriate cortex, defined by metabolic, blood flow, receptor- and cyto-architectural landmarks, are in close agreement with each other.

Acknowledgement: EU - HCM Grant (CHRX CT930261)

632.13

FUNCTIONAL ORGANIZATION OF AREAS V1 AND V2 IN THE PROSIMIAN PRIMATE GALAGO REVEALED BY OPTICAL IMAGING. W.H. Bosking*, L.E. White, V.A. Casagrande, D. Fitzpatrick. Dept. of Neurobiology, Duke Univ. Medical Center, Durham, NC 27710 and Dept. of Cell Biology and Psychology, Vanderbilt Univ., Nashville, TN 37232.

Optical imaging of intrinsic signals was used to assess the organization of orientation preference in V1 and V2 of the galago. Polar maps of orientation preference were constructed by vector summation of data acquired during presentation of 4 stimulus angles. As in the macaque, the map of orientation preference was largely continuous and contained regions over which orientation preference changed along a straight line (linear zones) and point discontinuities around which orientation preference cycled in a radial fashion (pinwheels). In V1, the average size of iso-orientation (+/-45 deg) domains was approximately .12 mm^2 and the density of pinwheels was approximately 7 pinwheels/mm^2. In V2, a similar pattern of orientation preferences was seen, but with larger iso-orientation domains (.2 mm^2) and lower pinwheel density (4 pinwheels/mm^2). Despite this difference in the scale of the orientation preference maps, orientation preference was mapped continuously across the V1/V2 border.

We compared optical imaging data with the pattern of cytochrome oxidase (CO) staining in V1 and found no consistent relationship between the distribution of CO blobs and any feature of the orientation preference map. For example, blob and interblob regions could not be distinguished on the basis of pinwheel density or magnitude of orientation selectivity. These data are consistent with single-unit recordings in the galago which show little difference in the orientation selectivity of individual neurons in blob and interblob regions. Thus, neither the cytochrome oxidase compartments in V1, nor the V1/V2 border, disrupt the continuous mapping of orientation preference in V1 and V2. Supported by EY01778 and EYO6821.

632.14

OPTICAL IMAGING OF TREE SHREW AREAS V1 AND V2: MAPS OF ORIENTATION PREFERENCE AND RULES OF INTERCONNECTIVITY. M. L. Pucak*, W. H. Bosking, and D. Fitzpatrick. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Optical imaging of intrinsic signal was combined with anatomical techniques to examine the map of orientation preference in V2 and its relation to the patterns of connectivity with V1. The structure of iso-orientation domains was examined using both orientation difference maps, generated by subtracting the response to orthogonal stimuli, and vector maps, generated by vector summation of the maps derived for four different stimulus orientations. Iso-orientation domains revealed by difference maps were similar in diameter (approximately 250 μm) to those in V1. Vector maps of V2 also were comparable to those observed in V1: orientation preference was found to change in a largely continuous manner, forming linear zones and pinwheels. The similarity between the maps in V1 and V2 was especially apparent at the V1/V2 border, where individual iso-orientation domains often crossed in an uninterrupted fashion.

Injections of the anterograde tracer biocytin into identified iso-orientation domains in V1 demonstrated that, like the horizontal projections within V1, feedforward projections to V2 terminate as discrete patches of labeled boutons. Some patches are continuous across the V1/V2 border in a manner analogous to the continuity displayed by orientation domains. Comparison of the distribution of labeled boutons with the orientation map in V2 reveals that the majority of boutons lie in domains corresponding to the preferred orientation at the injection site. In contrast, feedback projections linking V2 with V1 terminate in a more diffuse manner that shows little correspondence to the map of orientation preference. These observations demonstrate that feedforward projections, like horizontal connections in V1, exhibit specificity for orientation preference, whereas feedback projections are less constrained by this response property. Supported by EY06821.

632.15

DIRECTION SELECTIVITY AND HORIZONTAL CONNECTIONS IN LAYERS 2/3 OF FERRET PRIMARY VISUAL CORTEX (V1). L.E. White*, W.H. Bosking, M. Weliky, and D. Fitzpatrick. Department of Neurobiology, Duke University Medical Center, Durham NC 27710.

Optical imaging of intrinsic signals in ferret V1 has been used to demonstrate the presence of "iso-direction domains", which represent the activity of clusters of neurons that respond best to the same direction of motion (Weliky M, Bosking WH, Fitzpatrick D, 1996, *Nature* 379:725-728). In this study, we investigated how horizontal connections in layers 2/3 are distributed in relation to the organization of direction selectivity in ferret V1.

Intrinsic signals were recorded while animals viewed gratings moving in one of two opposite directions orthogonal to the orientation of the grating. Vector summation of data acquired from each stimulus direction was then used to generate maps of direction preference, which guided the placement of small extracellular injections of the anterograde tracer, biocytin, into identified iso-direction domains. The direction selectivity of the target site was confirmed with electrophysiological recording through the micropipette prior to injection. The distribution of labeled synaptic boutons in tangential sections through V1 was plotted and aligned with the optical maps; the selectivity of the horizontal connections, with respect to direction preference, was evaluated quantitatively.

Close to the neurons of origin (<750 μm), the horizontal connections in layers 2/3 show little selectivity. Further away from the injection site (>750 μm), however, long-range horizontal connections preferentially interconnect iso-direction domains. Moreover, domains that share the same orientation preference, but respond best to opposite directions of motion, are not heavily interconnected at these distances. Thus, the specificity of these long-range horizontal projections provides a framework for understanding how these connections might contribute to the elaboration of the direction preferences of neurons in layers 2/3 of ferret V1. Supported by EY06729 and EY11488.

632.16

MULTIPLE ACTIVITY MAPS OF PRIMATE VISUAL CORTEX PRODUCED BY SUSTAINED AND TRANSIENT EXPRESSION OF THE IMMEDIATE-EARLY GENES *ZIF268* AND *C-FOS*

A. Chaudhuri[1]* and J. Nissanov[2]

[1]Department of Psychology, McGill University, Montreal

[2]Biomedical Engineering & Science Institute, Drexel University, Philadelphia

Long-term changes in the CNS brought about by alterations of sensory input are thought to be orchestrated by various immediate-early genes, such as *c-fos* and *zif268*. The linkage of transcriptional processing of these genes to membrane depolarization provides an opportunity to visualize activated neurons by detecting the accumulation of their mRNA or protein products. We report here the results of a double-labeling strategy that exploits the different time course of mRNA and protein induction of these genes.

We have used immunostaining and *in situ* hybridization histochemistry (ISHH) to detect both protein and mRNA products in striate cortex of adult vervet monkeys following monocular deprivation (MD) and reverse-occlusion (RO) procedures. In MD animals, one eye was occluded with an opaque patch for three hours. In RO animals, the patch was switched to the other eye after this period for 30 minutes. In all cases, chair-restrained awake animals were used. An ocular dominance (OD) pattern was evident with staining for products of both genes in RO animals. A striking OD pattern was also seen in MD animals with *zif268* staining but not with *c-fos* staining. These findings are consistent with the notion that *c-fos* expression requires a period of neural quiescence prior to activation whereas *zif268* expression is regulated by the magnitude of ongoing neural activity.

The sustained and transient nature of *zif268* and *c-fos* expression, when coupled with the different temporal characteristics of their mRNA and protein induction, provides an opportunity for producing multiple activity maps of the brain in response to separate stimulation conditions.

Supported by MRC Canada (MA12685) and NIH P41RR01638.

632.17

ABNORMAL RETINOFUGAL PROJECTIONS AND VISUAL CORTICAL MAPS IN A MUTANT WITH UNILATERAL MISROUTING AT THE OPTIC CHIASM. Dale Hogan,[1]* Preston E. Garraghty,[2] and Robert W. Williams.[1] [1]Dept. Anatomy & Neurobiology, Univ. Tenn., Memphis,TN and [2]Dept. Psychology, Indiana Univ., Bloomington, IN.

We are studying a mutation in dogs that increases the uncrossed projection at the optic chiasm. This report concerns a mutant in which most axons from the right eye fail to cross midline, but 30% of the axons from the left eye cross. An injection of WGA-HRP into the left eye shows that the left (monocular) LGN is unlaminated and uniformly labeled. Surprisingly, the binocular LGN is also poorly laminated. Lamination is only seen in the caudodorsal tail. The superior colliculus contralateral to the injection receives binocular input and label is patchy. However, the left colliculus, presumably innervated only by the ipsilateral eye, showed extremely sparse label concentrated in one restricted area. Anterograde label reveals an additional failure of axon guidance. A bundle of axons extends rostral from the right LGN in dorsal thalamus. Multiunit recording in visual cortex showed globally appropriate topography, with regional anomalies. In lower visual space, contralateral receptive fields were recorded from 25° azimuth and progressed into the ipsilateral field. Ipsilateral fields were plotted to 45° azimuth, well into the monocular field of normal dogs. This organization is similar, but opposite in polarity, to that found in Boston-type Siamese cats. Near the horizontal meridian, mirror image reversals in field azimuth were mapped. This implies the existence of hemiretinal dominance columns similar to those found in the cortex of albino cats. We also mapped a cell with bilateral receptive fields in mirror image positions across the vertical meridian. Most units were not orientation selective and responded well to all orientations. This mutant had impaired visual function, including congenital nystagmus and failure to make saccades, demonstrating the functional limits of plasticity in dealing with a decussation defect of this magnitude. We also now know that there may be additional defects of axon guidance associated with this mutation. Supported by NRSA EY06560 (DH) and NIH EY06627 (RWW).

632.18

SPATIOTEMPORAL CORRELATED FIRING OF V1 NEURON: SIMULTANEOUS RECORDINGS FROM 16 ELECTRODES. D. J. Warren, N. D. Hadfield, P. J. Rousche*, R. A. Normann. John A. Moran Laboratory in Applied Vision & Neural Sciences, University of Utah, Salt Lake City, UT 84112.

Neurons at all stages of the visual pathways manifest periodic firing, even in the lack of apparent stimulation. In development, this activity has been shown to be associated with "pruning" of the dendritic arbors and creation of specific neural pathways. This periodic firing may serve to maintain these pathways in adult animals. If so, one should see correlated firing of neurons that make up these local circuits in the absence of visual stimulus.

To pursue this question, we have used a 100 electrode array to record single and multi-unit neural activity simultaneously from groups of neurons in V1 of cats. The electrodes have a 1.1 mm length and are arranged in a regular 10 by 10 grid with 0.4 mm spacing between adjacent electrodes. Subsets of 16 of the 100 electrodes are monitored simultaneously. Data reported herein were obtained from dark adapted animals who had been on a stable anesthetic level for 8 hours.

Periodic firing was seen on all functional electrodes. The period of the firing varied from animal to animal and within an animal. The firing lasted between 1 and 4 minutes and recurred anywhere from 1 to 10 minutes. The temporal sequence of the firing suggested that it was not associated with waves of activity sweeping across the cortex. Crosscorrelation indicated that neurons separated by 0.8 mm could show highly correlated activity (1 millisecond wide bins). The degree of correlation varied: periods of highly correlated firing were separated by periods of uncorrelated activity. Both correlated and uncorrelated activity can occur within a single firing episode.

While the function of this periodic behavior is unclear, it may serve to maintain the synaptic interconnections that lead to the complex receptive field properties that characterize this cortical region.

This work is supported by the Whitaker Foundation and NSF Grant IBN 94-24509.

VISUAL CORTEX: STRIATE VII

633.1

BASAL DENDRITIC FIELDS OF LAYER III PYRAMIDAL CELLS IN MACAQUE VISUAL AREAS V1, MT, MST AND LIP. G. N. Elston, M. G. P. Rosa, M. B. Calford* and D. Pow. Vision, Touch & Hearing Res. Centre, The University of Queensland, AUSTRALIA. 4072

Morphology of layer III pyramidal cells was examined in functionally distinct compartments of the primary visual cortex (V1), as well as in the middle temporal (MT), the medial superior temporal (MST) and the lateral intraparietal (LIP) areas of the macaque. Neurones in fixed (4% paraformaldehyde) flattened cortical slices (250μm) were injected with Lucifer Yellow and processed for a permanent diaminobenzidine reaction product. Dendritic field areas and spine density were compared. Pyramidal cells located in layer IIIC (IVB of Brodmann) of V1 had significantly larger basal dendritic fields (n, mean ± s.d.; 25, 35.8 ± 5.5 x 10^3 μm^2) than those located within the blobs (93, 26.9 ± 10.6 x 10^3 μm^2) of layers IIIA and B ($t = -4.04$ $p \leq 0.0001$). Furthermore, pyramidal cells located within the blobs had larger basal dendritic fields than those of the interblobs (43, 20.1 ± 10.6 x 10^3 μm^2) within layers IIIA and B ($t = 4.0$ $p \leq 0.0005$). Thus, dendritic fields of pyramidal cells in V1 reflected the relative proportion of magnocellular input. Spine density was similar for all pyramidal cells in layer III of V1. Pyramidal cells of layer III in MT had significantly larger basal dendritic field areas (34, 83.8 ± 10.6 x 10^3 μm^2) than those in layer IIIC of V1 ($t = -20.7$ $p \leq 0.0001$) , as did those in MST (40, 85.5 ± 23.6 x 10^3 μm^2,$t = -10.3$ $p \leq 0.0001$) and LIP (40, 88.1 ± 16.0 x 10^3 μm^2, $t = -15.7$ $p \leq 0.0001$). However there was no significant difference in basal dendritic field area of layer III pyramidal cells between MT, MST and LIP ($p > 0.05$). Pyramidal cells in MST had the greatest spine density; that of cells in LIP and MT was similar and slightly greater than that in V1. Whilst these findings demonstrate that basal dendritic fields of layer III pyramidal cells in extrastriate areas are larger than those in V1, we found no progressive increase in the rostral areas of the motion pathway.

633.2

VIRAL ANTEROGRADE TRANSNEURONAL TRACING OF THE LAMINAR TARGETING OF VISUAL THALAMOCORTICAL AND CORTICOCORTICAL CONNECTIONS IN MOUSE M.D. Cassell*, C-J. Shi, N. Sun & S. Perlman, Depts. of Anatomy, Pediatrics & Microbiology, Univ. of Iowa, Iowa City, IA 52242

By using the viral anterograde transneuronal tracing technique, the present study investigated the laminar organization of potential recipient neurons of visual thalamocortical and intracortical projections. Herpes simplex virus type-1, strain H129 was injected into the mouse vitreous body of the eye and virally infected neurons were detected using in situ hybridization. Infection of contralateral visual cortex first appeared at day 5-6 following intraocular inoculation. In striate cortex (area Oc1), infected neurons were first present in layers 4 and 5. The labeling in layer 5 was always less intense than that of layer 4 and often disassociated from it, indicating it was targeted by different afferents. Subsequently, labeling appeared in layers 2 and 3 and finally in layer 6. In peristriate cortex, infected neurons were first detected in layer 5 of lateral area Oc2L and this labeling started, in most cases, at the same time as that in striate cortex. Later, labeling extended into layers 6 and 4 and finally all cellular layers exhibited heavy infection. In contrast, area Oc2M was usually infected at a later stage and had no obvious laminar sequence of infection. In temporal cortex, caudal area Te2 was infected simultaneously with area Oc2L and exhibited an identical laminar pattern and sequence of labeling as that of Oc2L. Area Te1 and lateral entorhinal cortex had infection of in layers 6 and 5 respectively when all cellular layers of striate and peristriate cortices were heavily infected. These results suggest that thalamocortical projections differentially target layers 4 and 5 of striate cortex and layer 5 of peristriate cortex, including areas Oc2L and Te2. In striate cortex, layer 4 and/or layer 5 may specifically innervate supragranular layers (2 and 3) which in turn project to layer 6 neurons. In contrast, thalamic recipient neurons of peristriate cortex innervate adjacent layer 6 and 4 neurons and the latter projects to supragranular layers.(Supported by NS24401 and DC01711).

633.3

ESSENTIAL AND ARCHAIC ANATOMICAL FEATURES OF MAMMALIAN VISUAL CORTEX, C.Tyler*, J.Lund, S.Dunlop, A.Harman, J.Dann, R.Lund and L.Beazley. Institute of Ophthalmology, University College, London and Depts. of Zoology and Psychology, Univ. of Western Australia, Perth.

Intrinsic organisation of visual cortex has been compared in a number of species, including eutherians and marsupials, to help determine which features are common elements, and therefore essential components, as opposed to features unique to individual species, and therefore less key to basic function. Despite a 100 fold difference in area and 3 fold difference in depth of V1 between species, the individual pyramidal neurons vary little in size. Layer 4 always includes spiny stellate neurons. Specific patterns of apical dendritic arborization of layer 6 pyramidal neurons correlate with stratification of thalamic axon terminals. A common set of interneuron classes are present in at least the superficial layers; of these, only the spatial scale of the large basket neuron axons varies. Superficial layer pyramidal lateral patchy axon connections are present in most species yet appear to be absent in those species with narrow arbor basket neuron axons and V1 of small area; overall area devoted to V1 as well as spatial relationship between pyramid dendritic field and basket axon may play a role in patch system development. Despite size and phylogenetic diversity there is conservation of specific V1 features that raise intriguing questions as to functional constraints. NEI-EY10021, MRC, NHMRC, British Council.

633.4

ELECTROPHYSIOLOGY OF THE HORIZONTAL CONNECTION OF KITTEN PRIMARY VISUAL CORTEX. Y. Yoshimura*[1], H. Sato[1,2], K. Imamura[1,3] and Y. Watanabe[1,3]. [1]Dept. Neurosci., Osaka Biosci. Inst., Osaka 565, [2]Fac. Health & Sport Sciences, Osaka Univ., Osaka 560, [3]Subfemtomole Biorecognition Project, JRDC, Osaka 565, Japan.

To assess the function of the horizontal projections in layer II/III of the kitten primary visual cortex, we performed a combined study of extracellular recording of visual responses *in vivo* and whole-cell recording of postsynaptic events of cells *in vitro*. Receptive field positions and response properties of cells were mapped in a few locations in the area 17. To label horizontally connected cells, a fluorescent retrograde tracer was injected into the recording site. After an appropriate survival period, slices of the visual cortex were prepared for the *in vitro* experiments. Focal electrical stimulation applied to single cells, including labeled cells, evoked monosynaptic excitatory postsynaptic potentials (EPSPs) in layer II/III pyramidal cells located at the dye injection site. The hot spot was often found in a vicinity (<150 μm) of the recorded cells. Stimulation applied 300-1200 μm away from the recording site also evoked the EPSPs via long range horizontal connections. Both proximally and distantly elicited EPSPs showed similar amplitudes and time courses. Concurrent activation of horizontal and vertical afferent inputs to the layer II/III cells showed a linear spatial and temporal summation of respective EPSPs. Temporal summation of EPSPs was also found when two successive activation of the horizontal input were applied at the inter-stimulus interval of 10-50 ms. These horizontal connections were visualized by intracellular staining of some of the stimulated cells with neurobiotin.

(Supported in part by JSPS Research Fellowship to Y.Y.)

633.5

USING LATERAL CONNECTIONS FOR POPULATION CODING. K. Zhang,[1]; A. Pouget[2] and P. Dayan*[3]. [1]The Salk Institute, La Jolla, CA. [2]Georgetown Institute for Computational and Cognitive Sciences, Washington D.C., and [3]Brain and Cognitive Science dpt, MIT, Cambridge, MA.

Coarse codes are widely used throughout the brain to encode sensory and motor variables. Methods designed to interpret these codes, such as population vector analysis, are either inefficient, i.e., the variance of the estimate is much larger than the smallest possible variance, or biologically implausible, like maximum likelihood. Moreover, these methods attempt to compute a scalar or vector estimate of the encoded variable. Neurons are faced with a similar estimation problem, i.e., they must read out the responses of the presynaptic neurons, but, by contrast, they typically encode the variable with a further population code rather than as a scalar. We show how lateral connections in a recurrent network can be used to perform statistically efficient estimations whilst using a coarse code. In the presence of gaussian noise, the variance of the network estimate matches the Cramer-Rao bound, the smallest achievable variance. This work suggests that lateral connections in the cortex may be involved in averaging out uncorrelated noise among neurons representing similar variables. (Supported by a training grant from the McDonnell-Pew foundation to A.P.).

633.6

STIMULUS-ELICITED NEURONAL RESPONSES IN STRIATE AND INFERIOR TEMPORAL CORTICES ARE WELL-DESCRIBED BY A GAUSSIAN DISTRIBUTION. E.D. Gershon, P.E. Latham, G–X. Jin* and B.J. Richmond. Lab. of Neuropsychology, NIMH, and Lab. of Developmental Neurobiology, NICHD, Bethesda, MD 20892.

Both the dynamic range and the variability of stimulus-elicited neuronal responses determine the ability of a neuron to transmit information. Knowing the variability of neuronal responses would allow analytic calculation of transmitted information and channel capacity. We took single-unit data from previously described experiments carried out in V1 and IT cortices of awake, fixating rhesus monkeys (Richmond et al., 1990; Eskandar et al., 1992) and compared them to Gaussian, Poisson and boxcar distributions. In the experiments, the neurons were stimulated using 32 or 128 two-dimensional black and white patterns each presented many times for 320 ms. We constructed histograms of the spike counts for each stimulus and made the best fit to each histogram for the three distributions. The Gaussian distribution was best for every cell.

We calculated the stimulus-related transmitted information using the response probabilities derived from the Gaussian fits. These information values in both V1 and IT are in close agreement with the values obtained previously using an artificial neural network (Heller et al., 1995); the average difference was 20% for V1, and 10% for IT. It is reasonable to estimate the channel capacity of these neurons using Gaussian-based models.

Support: IRP/NIMH/NIH and IRP/NICHD/NIH.

633.7

COMPLEX CELL RESPONSES COULD ARISE DIRECTLY FROM CENTER-SURROUND INPUTS: THE SURPRISING POWER OF INTRA-DENDRITIC COMPUTATIONS.

B. W. Mel, D. L. Ruderman, Dept. Biomed. Engin., USC, MC 1451, Los Angeles, CA 90089. & E. Niebur*. Krieger Mind-Brain Institute, Johns Hopkins Univ., Baltimore, MD, 21218.

The classical Hubel-Wiesel model for complex cell responses in V1 involves a hierarchy in which center-surround inputs drive Simple cells, which in turn drive Complex cells. Under typical modeling assumptions, complex cell output can be expressed as a second-order polynomial over the input pixel intensity values [Ohzawa et al., *Science*, v. 279, 1990]. In previous work, one of us has studied the nonlinear computational properties of neocortical cells, and has shown that second-order polynomial functions of this general kind can be naturally implemented by dendritic trees that contain certain classes of excitatory membrane mechanisms, including NMDA-type synapses, and voltage-dependent Na^+ and Ca^{++} channels [Mel, B.W., *J. Neurophysiol.*, v. 70, 1993]. Here we explore the hypothesis that nonlinear shift- and contrast-invariant complex cell responses could arise *de novo* in a dendritic neuron that receives only center-surround inputs, i.e. without an intervening layer of simple cells. Using a simple abstract model of a dendritic tree, we demonstrate the feasibility of strong orientation tuning coupled with both shift- and contrast invariance. We are currently extending our simulations to biophysically-realistic conditions using NEURON. This work was funded by the NSF.

633.8

INFLUENCE OF GABAERGIC INHIBITION ON STRUCTURE AND DYNAMICS OF RECEPTIVE FIELDS IN THE CAT VISUAL CORTEX. J. Pernberg*, K.-U. Jirmann and U.T. Eysel. Dept. of Neurophysiol, Ruhr-Universität Bochum, D-44780 Bochum, Germany.

Cortical cells in area 17 and 18 were investigated electrophysiologically in anesthetized adult cats. Extracellular recordings and iontophoretic application of bicuculline were performed with 3-barrelled micropipettes. A reverse correlation method (DeAngelis et al., J. Neurophysiol. 69:1091, 1993) was used with brief (50 ms) stationary light and dark bars for spatial and temporal receptive field analysis. Stimuli (0.4°x 4°) were presented with preferred orientation as determined previously by moving bars.

ON and OFF excitatory receptive fields of simple and complex cells were documented before, during and after microiontophoresis of bicuculline. Under the $GABA_A$ receptor blocker both, the stimulus-induced and the resting discharge frequency increased and ON and OFF fields changed significantly in space and/or time in a reversible manner. In space, bicuculline widened the receptive field, whereas in time, it *shortened* the duration of the excitatory cell response. ON and OFF receptive fields separated in space *and* time (simple cells) or time (complex cells) became less isolated or even superimposed.

Most interestingly intracortical inhibition appears to contribute to the late phase of the *excitatory* response in simple and complex cells. This could be due to postinhibitory activation on the cellular level or activity dependent multisynaptic effects in the cortical network.

This work was supported by the Deutsche Forschungsgemeinschaft Ey 8/23 and SFB 509 - TP C4.

633.9

THREE DISTINCT TYPES OF GABAERGIC NEURONS IN RAT PRIMARY VISUAL CORTEX. Y. Gonchar and A. Burkhalter*. Dept. of Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

In the cortex inhibition is mediated by GABAergic neurons. Although all of these neurons serve a similar purpose it was shown recently that they are immunologically and physiologically heterogeneous (Kubota et al., 1994; Kawaguchi, 1995) suggesting functional diversity. As a first step to understand how different types of inhibitory neurons are integrated into cortical circuits we have examined whether GABAergic neurons can be subdivided into distinct groups. For this purpose we have double-stained rat visual cortex (V1) with antibodies to GABA, parvalbumin (PV), calretinin (CR), somatostatin (SOM), calbindin (CB) and nitric oxide synthase (NOS). The results show that the majority of PV (100%), SOM (94%) and CR (88%) staining neurons are GABAergic. PV staining neurons constitute a distinct group (5% of PV cells colocalize CB) that show no overlap with CR, SOM and NOS expressing cells. PV cells account for 51% of all GABAergic neurons. A second group of SOM expressing neurons accounts for 18% of GABAergic cells. None of these cells colocalize PV or CR, but 5% of SOM neurons stain for NOS and 86% show CB immunoreactivity. The third distinct group of CR expressing cells accounts for 15% of GABAergic neurons. All of these cells are PV, CB, SOM and NOS negative. CB expressing neurons represent a heterogeneous group that includes GABAergic and non-GABAergic cells.

Our findings indicate that GABAergic neurons in rat V1 fall into three separate groups that can be identified by the expression of PV, CR and SOM. These cells account for 83% of all GABAergic neurons. These results confirm observations in rat frontal cortex (Kubota et al., 1994) and suggest that previous classifications of GABAergic neurons into two distinct groups of PV and CB expressing cells (Celio, 1990) do not apply to rat visual cortex. Supported by NIH Grants EY05935 and AG11455.

633.10

DARK-INDUCED SUBCELLULAR REDISTRIBUTION OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II IN RAT VISUAL CORTEX. N. Liu and N. G. F. Cooper*. Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292

Calcium/calmodulin-dependent protein kinase II (CaM kinase II) is enriched in the nervous system and plays an important role in synaptic transmission. The expression of CaM kinase II immunoreactive protein and mRNA in the primary visual cortex has been shown to be affected by prolonged ocular deprivation (Hendry and Kennedy, 1986; Benson et al., 1991). The purpose of this study was to determine the changes of CaM kinase II protein in rat visual cortex during short-term dark-adaptation. Primary visual cortex from forty-two-day-old male Sprague-Dawley rats which had been raised in a 12 hr light / 12 hr dark cycle or in the dark for 24 hr before sacrifice was collected, homogenized in 0.32 M sucrose-containing buffer, and subjected to subcellular fractionation. Immunoreactivities of CaM kinase II α and ß subunits in the crude tissue homogenates, crude synaptic membrane, and cytosol have been examined with western blotting and enhanced chemiluminescence. No significant differences in immunoreactivities of CaM kinase II were found between the crude tissue homogenates of normal and dark-adapted rats. However, both CaM kinase II α and ß subunits displayed significant increases in immunoreactivities in the crude synaptic membrane fractions and corresponding decreases in the cytosolic fractions when the rats were dark-adapted. This study indicates that the total protein level of CaM kinase II was not significantly altered but a translocation of this kinase from the cytosol to the synaptic membrane occurred during short-term dark-adaptation. This suggests a post-translational regulation of CaM kinase II expression mediated by neural activity. Supported by grant NSF-EPSCoR 994025.

633.11

MOLECULAR AND NEUROCHEMICAL CHANGES OCCURING IN GLUTAMATE RECEPTORS AND TYPE II CALCIUM-CALMODULIN-DEPENDENT PROTEIN KINASE IN ADULT MONKEY VISUAL CORTEX . B. Tighilet and E.G. Jones*. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Glutamatergic receptors play a crucial role in development and plasticity of the visual cortex. *In situ* hybridization histochemistry and immunocytochemistry were used to study activity dependent plasticity in the adult monkey visual cortex. Adult monkeys were monocularly deprived for periods ranging from 4 to 7 days by tetrodotoxin injections (TTX 15 µg) into one eye. After the deprivation period the animals were perfused and sections of brain processed for cytochrome oxidase, in situ hybridization and immunocytochemistry for α, β, δ, and γ subunits of Ca^{2+}/calmodulin-dependent protein kinase II, NMDA receptor subunits (NMDAr1, NMDA 2AB), AMPA receptor subunits (Glur1, Glur2/3, Glur4) and kainate receptor subunits (Glur5, 6, 7). The results showed a down regulation of both NMDAr1, Glur2AB , Glur1, Glur4, Glur5,6,7 and CaM II β protein particularly in deprived columns of layer IVC of the primary visual cortex. When changes were observed at the gene expression level they consisted of a down regulation except for CaM II kinase α mRNA which was up regulated in deprived columns of layer IVC. These data indicate that unilateral suppression of visual inputs induce plasticity in the adult monkey visual cortex. Molecules such as glutamate receptors and type II calcium-calmodulin-dependent protein kinase may have a crucial role.

Supported by Fondation Fyssen and NH grant NS21377.

633.12

DYNAMIC CORTICAL RECEPTIVE FIELD CHANGES: NEURONAL ADAPTATION OR CONNECTION PLASTICITY?

Jonathan A. Marshall* & George J. Kalarickal, Department of Computer Science, CB 3175, University of North Carolina, Chapel Hill, NC 27599-3175, U.S.A.

The size, shape, and position of neuronal receptive fields (RFs) change dynamically in response to artificial scotoma conditioning (Das & Gilbert, 1995; Pettet & Gilbert, 1992), retinal lesions (Darian-Smith & Gilbert, 1995), and intracortical microstimulation (ICMS) (Recanzone et al., 1992). We have formulated and tested a neural network model using the "EXIN" learning rules (Marshall, 1995) that exhibits similar RF dynamics and predicts some new experimentally testable RF properties. Two other models – another based on connection plasticity (Sirosh & Miikkulainen, 1995) and one based on neuronal adaptation (Xing & Gerstein, 1994) – were tested for comparison. The EXIN model uses both Hebbian and anti-Hebbian learning rules. In artificial scotoma conditioning, random stimulation in the whole visual field except in a "scotoma" region was followed by whole-field stimulation. In the novel *complementary scotoma* conditioning, stimulation of two adjacent regions was alternated repeatedly. In ICMS, only postsynaptic neurons were stimulated. The model produced the following effects, corresponding closely to the reported neurophysiology: (1) "iceberg" expansion of RFs in the scotoma center (DeAngelis et al., 1995); (2) asymmetric, centrifugal expansion and increased response of RFs inside the scotoma but away from the scotoma center; (3) the greatest expansion for RFs close to the scotoma boundary; (4) RF expansion that exceeded the scotoma boundary for RFs close to scotoma boundary; and (5) RF contraction during normal stimulation but not in the absence of stimulation. ICMS of the model produced (6) no size changes of ICMS-site RFs; (7) RF substitution by ICMS-site RFs; (8) RF expansion and centripetal shift of near-ICMS-site RFs; and (9) centrifugal shift of RFs farther from the ICMS site. The model predicts (10) RF expansion for neurons whose initial RFs were on *either* side of the scotoma boundaries during complementary scotoma conditioning and (11) less RF change during cross-orientation artificial scotoma conditioning than during iso-orientation artificial scotoma conditioning. The EXIN model, based on connection plasticity, most closely describes the observed dynamic RF changes under the artificial scotoma, lesion, and ICMS conditions.

Supported in part by ONR grant N00014-93-1-0208.

633.13

USE-DEPENDENT RECEPTIVE-FIELD PLASTICITY IN THE VISUAL CORTEX OF ADULT CATS. D. Eyding, G. Schweigart, U.T. Eysel*. Dept. of Neurophysiology, Ruhr-Universität Bochum, D-44780 Bochum, Germany.

Single cells in layers II/III of the cat visual cortex (area 17 and 18) were extracellularly recorded in anesthetized adult cats. The excitatory receptive field (RF) was determined and we attempted to elicit heterosynaptic associative plasticity by local synchronous visual stimulation (ON/OFF co-activation) of the RF and an adjacent unresponsive region on one side. A specific enlargement (S) into the costimulated adjacent region was observed in 15 of 33 cells, in 9/33 cells unspecific (U) enlargements into the co-stimulated and the unstimulated opposite side of the RF were found, and in 9 cases the coactivation failed to elicit any effect (N).

The response amplitude during co-activation turned out to be the most important factor determining the outcome. Low activity during co-stimulation rarely elicited any effect. Higher activity increased the probability of RF size changes - specific or unspecific. However, unspecific RF-enlargements always reflected a stimulus-independent general increase in excitability of a cell, and the increase of RF size was directly correlated to the increase of activity. The recovery-times were in the range of minutes to hours and correlated positively with the increase of responsiveness in the new, formerly unresponsive RF-area in the group with specific RF enlargement (S), whereas no such correlation was observed in the U group. Spontaneous transitions from N or S to U were only observed in one case each.

We conclude that synchronous stimulation of a RF and its adjacent region can lead to specific changes in RF size if a sufficient postsynaptic activity is evoked during co-activation. This supports the view that synapses in the adult cat visual cortex are capable of specific use-dependent plasticity based on LTP-like mechanisms *in vivo*. Unspecific RF enlargements seem to be based on different mechanisms like global intrinsic modulation of synaptic efficacy.

Supported by Deutsche Forschungsgemeinschaft Ey 8/23-& SFB 509 - TP C4.

633.14

MULTIPARAMETRIC ANALYSIS OF INTRACELLULAR RECORDINGS IN THE CAT STRIATE CORTEX *IN VIVO* REVEAL FOUR DISTINCT CELL CLASSES. R. Azouz*, L. Nowak, D.A. McCormick and C.M. Gray. Center For Neuroscience, University of California, Davis, CA 95616 and Yale Univ. Med. Sch. 333 Cedar St. New Haven CT 06510.

We have previously demonstrated that cortical cells can be divided into four electrophysiological types 1) Regular spiking; 2) Intrinsic bursting; 3) Fast spiking; and 4) Chattering. In order to determine if this classification represents distinct cell groups or variation along a continuum of cellular properties, we performed intracellular recordings from neurons in cat striate cortex and classified the cells using discriminant analysis of a wide range of parameters extracted from the cellular responses to visual stimuli and intracellular depolarizing pulses. The variables tested came from five broad aspects of physiological properties, (a) the interspike interval distribution, (b) the properties of burst firing, (c) action potential characteristics, (d) membrane potential fluctuations, and (e) the degree of rhythmic activity. Using subjective criteria, our sample of 72 cells consisted of 33 chattering cells, 18 regular spiking cells, 17 intrinsic bursting cells and 4 fast spiking cells. Discriminant analysis confirmed this classification for 91%, 100%, 100% and 75% (mean = 94%) of the chattering, regular spiking, intrinsic bursting and fast spiking cells, respectively. Further analysis, utilizing only those parameters obtained from the neuronal spike trains, achieved 91% correct classification. These results demonstrate the presence of at least four well differentiated cell classes. This classification can be achieved on the basis of intrinsic membrane properties or the patterns of spike trains that occur in response to visual stimulation.

Supported by NEI (CMG) and NSF (DAM, CMG).

633.15

ADVANCES IN THE CHRONIC IMPLANTATION OF THE UTAH INTRACORTICAL ELECTRODE ARRAY. N.D. Hadfield, E.M. Maynard, R.A. Normann*. John A. Moran Laboratory in Applied Vision & Neural Sciences, University of Utah, Salt Lake City, UT 84112

Previous attempts at chronically implanting the Utah Intracortical Electrode Array (UIEA) in feline cortex have had mixed success (Rousche, submitted). These experiments demonstrated that although it is possible to record neural activity using the UIEA for periods in excess of a year, individual neurons were not consistently present for longer than 2 weeks. This instability has been attributed to the encapsulation of the array and its gradual extrusion from the cortex and/or micromotion associated with adhesions between the implant and the dura or bone flap.

To improve the recording stability of the UIEA, we have modified the surgical technique used by Rousche. By "sandwiching" the dura between a 0.5 mil layer of non-porous FEP Teflon (Dupont) and a 0.1mm layer of porous Teflon (Preclude, W.L.Gore & Assoc.) cut to the size of the craniotomy, we hoped to eliminate adhesions to the electrode array. The craniotomy was filled with a silastic elastomer and covered with dental acrylic. To date, this technique has been applied to chronic implants in four cats (11 electrodes each) and one monkey (22 electrodes). Gross histological studies of two implants in cats has shown an absence of adhesions to the UIEA. In addition, single unit recording studies conducted in two of the cats have shown a significant number of the neurons (24% and 34%) to be present for 41 and 108 days, respectively. While single unit data from the monkey is incomplete, identified units are still present after three months. Our preliminary conclusion is that the use of these thin Teflon films prevents the formation of adhesions to the electrode array which minimizes the amount of electrode movement. The consistent presence of neurons with large amplitudes (100 µV p-p) further suggests that these electrodes are not being extruded in the manner of Rousche.

Portions of this work were supported under NSF Grant IBN 94-24509, the Center for Neural Interfaces a Utah State Center of Excellence, and the W.M.Keck Foundation.

633.16

THE SHAPE OF STIMULATION-INDUCED INTRINSIC OPTICAL SIGNALS IN RAT CORTICAL SLICES DEPENDS ON CORTICAL AREA. K. Holthoff*[1], K. Zilles[2] and O.W. Witte[1]. [1]Neurologische Klinik der Heinrich-Heine-Universität,[2]Institut für Hirnforschung, Moorenstr. 5, 40225 Düsseldorf, Germany.

In rat cortical slices columnar-shaped intrinsic optical signals (IOSs) could be induced by afferent stimulation. The spatial extent of IOSs correlates with the spatial extent of electrical activity (Holthoff et al. (1994), Neurosci. Lett. 180: 227-230). In this study we investigated whether the activated columns differ depending on the brain area involved.

IOSs were studied using near-infrared darkfield microscopy. Experiments were done on coronal cortical slices (between 5 mm and 6 mm behind bregma) of juvenile male Wistar rats (14 days old). IOSs were induce by afferent electrical stimulation in layer VI. By subsequent stimulations IOSs were induced in different cortical areas. After reconstruction, cortical area determination was done using a brain atlas.

The shape and intensity distribution of IOSs induced in the same cortical area were highly reproducible. Crossing a border between different cortical areas yielded a remarkable change in shape and intensity distribution of IOS. In the occipital cortex area 1 (Oc 1), the primary visual cortex, the maximum of optical intensity was obtained in layer IV. In the adjacent lateral occipital cortex area 2 (Oc 2 L) the maximum of intensity was shifted to cortical layer II/III. In the agranular retrosplenial cortex (RSA), optical signals appeared twice as wide as in all other cortical areas. In the granular retrosplenial cortex (RSG) optical signals were restricted to lower cortical layers.

The present data show that IOSs could be used to study the area specific spread of excitation in different cytoarchitectonic areas.

Supported by SFB 194/B2

634.1

PLASTICITY IN MACAQUE VISUAL CORTEX: COMPARISON OF LEARNING-RELATED EFFECTS IN V1 AND V2. G. Bertini*, A. Karni, P. De Weerd, R. Desimone and L.G. Ungerleider. NIMH, NIH, Bethesda MD 20892.

Psychophysical studies suggest that plastic changes at the earliest stages of visual cortical processing underlie improvements in performance following training on some simple tasks. We previously reported that training a monkey to discriminate a target embedded in a background texture results in large, long-term performance gains, similar to those observed in human subjects. This learning is specific to the target's visual-field location and the orientation of the background texture elements. Furthermore, as a consequence of practice, the ability of V1 neurons to detect the presence of the target embedded in the trained background extends beyond the classical receptive field (RF) (Bertini et al. *Soc. Neurosci. Abs.* 1995).

To determine whether the learning-related effects in V1 are due to feedback from V2, we recorded the responses of 40 V2 neurons to trained vs. untrained stimuli. The animal fixated a spot and performed an irrelevant task in the opposite hemifield. Stimuli were composed of a background array of horizontal (trained) or vertical (untrained) line elements (no-target conditions), or identical arrays but with a target made of 3 oblique line elements presented either within the RF ("inside" targets) or at about 3° beyond it ("outside" targets). As in V1, most V2 neurons responded better to stimuli containing a target than to the no-target conditions. This response difference was taken as a measure of *target detection* ability and depended on the location of the target and on the orientation of the background texture elements. "Inside" targets were detected by V2 neurons when embedded in both trained and untrained backgrounds, with a small bias towards trained conditions. "Outside" targets were not detected by V2 neurons when embedded in the untrained background but, as in V1, a small yet significant degree of target detection was evident for "outside" targets in the trained conditions. This differential response occurred about 40 ms later than in V1.

These results confirm and extend our previous findings, indicating that the ability of early visual cortical areas to perform figure-ground segmentation extends outside the neurons' classical RFs as a consequence of training. Furthermore, the time course of the neuronal responses suggests that target detection by V1 neurons is not a result of feedback activity from V2. *Supported by NIMH-IRP.*

634.2

TEXTURE SEGMENTATION WITH ATTENTION IN AREA V4 OF THE MACAQUE. J Nicholas*, J Reynolds and R Desimone. Laboratory of Neuropsychology, NIMH, Bethesda, MD.

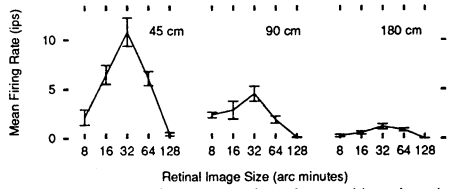
When two or more stimuli are located within a single V4 receptive field (RF), competition between the stimuli is biased in favor of the attended stimulus. We hypothesized that this attentional bias may modulate the interaction between texture-defined stimuli on textured backgrounds in the RF. To test this, neuronal responses in area V4 were recorded to sequences of texture-defined stimuli. The background was either a grey screen or a dense texture composed of line elements with an orthogonal orientation to those composing the stimuli. The degree of segmentation was varied by changing the length of the line elements in the stimuli and backgrounds. The monkey's task was to indicate when a target stimulus appeared in the sequence. One sequence appeared within the RF, and a second, simultaneous, sequence appeared in the opposite hemifield. The monkey's attention was manipulated by cueing it to perform the task at one location or the other.

With attention directed outside the RF, responses to texture-defined stimuli were greater than to a uniform texture background for about half the cells ("segmenting cells"). When segmentation was easy (long line elements), the response to the texture stimulus rapidly reached and sometimes exceeded the response to the same stimulus on a blank background. When segmentation was hard (short line elements), the response to the texture stimulus reached the level of response to the same stimulus on the grey background more slowly. With attention directed to the RF, responses to the texture stimuli defined by short line elements were enhanced, and the time course of the response was accelerated, but only for segmenting cells. There was no enhancing effect of attention for easily segmented stimuli. The results indicate that attention biases the interaction between figure and ground much the same way that it modulates the interaction between discrete stimuli. *Supported by NIMH-IRP.*

634.3

DISTANCE MODULATES RESPONSE MAGNITUDE IN V4: A POSSIBLE MECHANISM FOR SIZE CONSTANCY.
Allan C. Dobbins* Richard Jeo József Fiser John M. Allman
Division of Biology, Caltech, 216-76, Pasadena, CA, 91125

The majority of neurons in V4 are sharply tuned for stimulus size and shape with the preferred stimulus dimensions usually much smaller than the classical receptive field (Petersen, Baker and Allman, Brain Res., 1980; Desimone and Schein, J. Neurophysiol. 1987). In recordings from V4 in a macaque monkey trained to fixate a spot on a movable monitor, we have found that response magnitude is often strongly modulated by viewing distance. Size-tuned neurons generally have the same preferred retinal image size regardless of distance. Most frequently we have recorded from "nearness" neurons in which response magnitude increases with proximity to the monkey (illustrated here), but we have also recorded from "farness" cells in which response increases with distance.



In this cell, the distance modulation was dependent on binocular stimulation, but in others monocular input was sufficient for distance modulation. Absolute distance is a crucial parameter governing the responses of many V4 neurons, and distance modulation may underlie computation of size constancy. Supported by NSF grant IBN 9309393, Dell Webb and Sloan Foundations

634.4

COLUMNAR ORGANIZATION IN INFERIOR TEMPORAL CORTEX OF AWAKE MONKEY. M. Kato*, T. Uka and I. Fujita. Dept. of Cognitive Neurosci., Osaka Univ. Med. Sch., Suita, Osaka 565, Japan.

Area TE of the monkey inferior temporal cortex has columnar organization; i.e., neurons showing similar selectivities for visual features of objects are clustered in columns interspersed among columns of neurons with different stimulus selectivities. Evidence of this was obtained by extracellular neuronal recording and by optical imaging. However, both types of studies were performed in anesthetized monkeys; the columnar organization has not been demonstrated in awake monkeys.

A Japanese macaque monkey was trained to fixate on a small spot displayed on a color LCD monitor. During each fixation period, 3-6 different visual stimuli were sequentially presented. Five electrode guiding tubes were implanted perpendicularly to the lateral surface of area TE, which enabled repetitive access to nearly the same electrode tracks. First we recorded extracellular neural activities in the monkey anesthetized with N_2O/O_2 in order to determine track-specific stimulus features and to examine neural responses to these stimuli in addition to a fixed set of 40 stimuli. Two weeks later, neural responses to the same stimuli were examined along the tracks in the monkey performing the fixation task.

We recorded 62 multiple and single unit activities in each of the anesthetized and awake conditions. Correlation coefficients of normalized response magnitude were calculated among pairs of units in the same (CCs) and different (CCd) tracks. The average CCs in the awake condition was 0.30 while the average CCd was near 0 in both the awake (-0.001) and the anesthetized (-0.002) condition. In the anesthetized condition, the average CCs was small (0.14) but significantly larger ($p<0.001$) than the average CCd. Twenty-seven percent of the CCs's but only 2 % of the CCd's were larger than 0.5 in the awake condition. These results indicate a stronger correlation among cells within a vertical track than among cells in different tracks, suggesting that the neurons in area TE in the awake monkey are organized into columns. Supported by the Uehara Memorial Foundation.

634.5

ROLE OF GABAERGIC INHIBITION IN GENERATION OF VISUAL RESPONSE PROPERTIES OF INFERIOR TEMPORAL CORTEX NEURONS IN THE MONKEY. Y. Wang*, I. Fujita and Y. Murayama, Dept. Cognitive Neurosci., Osaka Univ. Med. Sch., Suita, Osaka 565, Japan.

Neurons in area TE of the monkey inferior temporal cortex respond to particular visual features of objects. Stimuli required for strong activation of most TE neurons are more complex than simple spots, bars or gratings. The examples include a T shape, a striped square, or a colored star. Little is known about where and how this stimulus selectivity is generated, although it has been suggested to be created in earlier cortical areas (V4 and TEO). In this study we applied bicuculine methiodide (BMI: a GABA-A receptor antagonist) by microiontophoresis with tungsten-in-glass/micropipette assemblies onto TE neurons to investigate how GABAergic inhibition in the TE contributes to the stimulus-selective response properties. A monkey (*Macaca fuscata*) anesthetized with a mixture of N_2O and O_2 was used.

Effects of BMI application on individual TE neurons were stimulus-specific. BMI administration augmented visual responses to some effective stimuli, but did not affect those to others. Moreover, in the presence of BMI, some cells began to respond to stimuli which did not evoke responses in them without BMI application. The latter effect was observed for a particular range of stimuli which often activated other neurons along the same or surrounding recording tracks. A general increase of neuronal excitability such as a lowered threshold does not explain these effects. Application of pH-matched vehicle did not affect the responses. We suggest that a generation process of the stimulus selectivity still goes on in the TE and that GABAergic inhibition contributes to this process.

Supported by grants from the Casio Science Foundation, and the Ministry of Science, Culture, and Education, Japan, to I.F.

634.6

LONG-TERM POTENTIATION AND DEPRESSION IN THE MONKEY VISUAL CORTEX. Y. Murayama*, I. Fujita, and M. Kato. Dept. of Cognitive Neurosci., Osaka Univ. Med. Sch., Suita, Osaka 565, JAPAN

Long-term changes in synaptic efficacy known as long-term potentiation (LTP) and depression (LTD) are possible cellular bases for learning and memory. To determine whether LTP and LTD are elicited in primates, the animal group having the most developed learning abilities, we examined three Japanese macaque monkeys anesthetized with a mixture of N2O and O2.

Extracellular field potentials were recorded in layer 2/3 of the inferior temporal cortex (TE) and of the striate cortex (V1) which are the final and first stages of the visual cortical pathway for object recognition. Stimulating electrodes were placed 0.5~1mm lateral to the recording site to activate horizontal axons in layer 2/3. After a high-frequency conditioning stimulus, the amplitude of field potentials evoked by a single shock in the TE gradually increased over 50-70 min to a maximum, and this potentiation lasted for more than 3 hours. An identical stimulus protocol did not potentiate field potentials in V1, but instead caused a depression which developed over 5-10 min and remained stable until the end of the recording. Steady state depression with a slow initial development suggests that this depression is a physiological phenomenon not caused by an experimental artifact such as a damage to the stimulating site. In both areas, field potentials evoked from an unconditioned pathway were not changed in their amplitude and wave form. These findings demonstrate that homosynaptic LTP and LTD are elicited in the adult primate neocortex, and suggest that the TE and V1 differed from each other in their synaptic plasticity. Supported by grants from the Uehara Memorial Foundation and the Inamori Foundation.

634.7

ATTENTION DEFICITS AFTER A COMBINED LESION OF AREAS V4 AND TEO IN A RHESUS MONKEY. P. De Weerd*, R. Hoag, R. Desimone & L.G. Ungerleider. Lab Psychology and Psychopathology & Lab Neuropsychology, NIMH, NIH, Bethesda, MD 20892.

Despite the anatomically well-established central position of areas V4 and TEO in the ventral pathway, the nature of the contribution of these areas to form and object perception remains uncertain. In one rhesus monkey, a complete removal of TEO in the right hemisphere was combined with a bilateral lesion in the dorsal portion of V4. While the monkey fixated a fixation target, visual discriminations in the intact upper quadrant of the right hemifield were compared with discriminations in the lower right (V4 lesion), upper left (TEO lesion), and lower left quadrants (V4+TEO lesion). Threshold performance on color, shape and orientation discrimination tasks was largely unaffected by those lesions. However, orientation thresholds obtained with a grating surrounded by three static luminance distractors (black or white solid disks), or by red distractors, rose significantly compared to performance without distractors, an effect that was absent in the control quadrant. A similar detrimental distractor effect was found during blue-green color discriminations in the presence of luminance distractors. The distractor effect was more pronounced in the V4+TEO quadrant than in the quadrants with the V4 and TEO lesions alone. Luminance distractors, however, did not raise signal-to-noise thresholds in a difficult direction-of-motion discrimination task. Thus, V4 and TEO resolve competition between a relevant oriented grating or color stimulus and irrelevant luminance or color distractors, all of which are predominantly processed by the ventral stream. The same areas, however, are not required to resolve competition between a motion stimulus and luminance distractors, probably because the former is mainly processed in the dorsal stream. This suggests that competition for attention between behaviorally relevant and irrelevant stimuli is maximal when both are processed within the same visual cortical stream. *(Supported by NIMH-IRP)*

634.8

DELAY ACTIVITY AND VISUAL RESPONSE TO THE SAME IMAGES CAN SHOW LOW CORRELATION. S Sobotka*, MD Diltz & JL Ringo. University of Rochester, Rochester NY 14642.

Single units were recorded from inferotemporal cortex (IT) and the inferior convexity of the frontal lobe (IC) while two monkeys performed a delayed matching-to-sample memory task using only a few highly-familiar images. In this pilot work, we have recorded 102 units. Twenty six percent of the units showed stimulus-selective delay activity during the (usually) 8 second delay, while 24% showed stimulus selective visual activity. Interestingly, the two groups of units were uncorrelated (Chi Square test χ^2=0.4, P>0.1). Analysis of the IC and IT units separately produced the same lack of correlation in each area alone (IC, χ^2=0.3, P>0.1 and IT, χ^2=0.01, P>0.1).

Responses were further analyzed by measuring the difference, for each cell, between the average delay activity following the image which elicited the greatest delay activity minus the average delay activity from that image which elicited the least. We then measured the difference, for each cell, between the average responses to the sample presentations of those same images. Across the 27 units with significant delay activity, the correlation between the delay activity measure and the visual response measure was low (r=-0.21, P>0.1). The correlations were also low when done separately for the IC units with significant delay activity (n=13, r=-0.40, P>0.1) or the similar IT units (n=14, r=-0.22, P>0.1).

The poor correlation between selective visual responses and selective delay responses was unexpected and contrary to at least some ideas which suppose delay activity to be a continuation of the initial visual response, occurring in an overlapping subpopulation. Supported by grant NS24217 from NINDS.

634.9

STIMULUS DEGRADATION HAS A SIMILAR EFFECT ON STIMULUS-RELATED INFORMATION IN INFERIOR TEMPORAL (IT) NEURONS AND ON THE MONKEY'S BEHAVIOR. M. Shidara*, Z. Liu & B. J. Richmond. Neuroscience Sect., Electrotechnical Lab., 1-1-4 Umezono, Tsukuba-shi, Ibaraki 305, Japan*, & Lab. of Neuropsychology, NIMH, Bethesda, MD20892, USA.

We recognize objects even when they are masked with visual noise. We studied the effect of noise on visual pattern recognition while we recorded from 26 lateral TE neurons in a monkey performing a sequential delayed match-to-sample task using 8 black and white 2-dimensional patterns. To add noise, we reversed the contrast of the pixel dots with probabilities of 5, 10, 15, 20, and 25%. As the noise increased, the monkey's rate of responding correctly decreased slowly, and above 5% noise the reaction time to bar release to the matching stimulus increased. Although neither the latency nor the peak firing rate of the neural responses to the matching stimulus changed consistently with increasing noise, the responses changed in both strength and pattern.

To quantify the utility of the responses for discriminating among the stimuli, we calculated the information about the stimuli carried in the neuronal responses (more information means that the response can be used to identify which stimulus appeared with greater certainty). Information rose more slowly and plateaued at lower levels as the noise level increased. The increase in behavioral response latency in the noise condition was closely paralleled by the increase in time necessary for the information carried by the neurons to rise significantly. Thus, these TE neurons generalize across stimulus degradation in a manner that closely parallels the monkey's ability to recognize the stimulus. Support: AIST/MITI and IRP/NIMH/NIH.

634.10

THE INFLUENCE OF BEHAVIORAL CONTEXT OF RESPONSES TO VISUAL STIMULI IN MONKEY INFERIOR TEMPORAL(IT) CORTEX. Zheng Liu, and Barry J. Richmond*. Lab. of Neuropsychology, National Institute of Mental Health, NIH, Bldg. 49 Rm. 1B80, Bethesda, MD 20892

It has been reported that neurons in monkey anterior-medial IT show decreased responses to the matching stimulus during a delayed match-to-sample task(DMS). We recorded 17 stimulus selective neurons from lateral TE (lateral to anterior medial temporal sulcus(AMTS)) while a rhesus monkey performed DMS using 8 black and white patterns. On average, the firing rates did not differ across the three conditions (sample, nonmatch, and match). When we used transmitted information to measure response dependent stimulus discriminability (more information means that the response can be used to identify which stimulus appeared with greater certainty). Stimulus-dependent information started to rise on average 100 ms after the stimulus appeared (80 ms was the earliest). 70% of the total information was transmitted in the first 120 ms of the information rise. These neurons carried 20% more information when a multivariate code (first 3 principle components plus firing rate) was used to evaluate the responses than when the firing rate alone was used. The amount information about the sample stimuli and about the match stimuli were exactly the same for the whole 500 ms period we examined. However, in the nonmatch condition, the information about the nonmatch stimuli plateaued at 73% of the amount carried in the other two conditions, even though information rose at the same rate during the first 100ms. The relation among responses across the sample, match, and nonmatch parts of the DMS task appeared to be different in the tissue lateral to AMTS than has been reported for responses of neurons medial to AMTS. Support: IRP/NIMH/NIH.

634.11

DIFFERENTIAL CORTICAL CONNECTIONS OF THE DORSAL AND VENTRAL SUBREGIONS OF THE MACAQUE INFEROTEMPORAL CORTEX.

K.S. Saleem[1]*, W. Suzuki[2] and K. Tanaka[1]; [1]RIKEN and [2]Osaka University.

We have recently found that connections with the perirhinal and entorhinal cortices are stronger in the ventral subregion of the anterior inferotemporal cortex (TEav) than the dorsal subregion (TEad). In this study, we examined the connections of TEad and TEav with the superior temporal sulcus (STS) regions and other paralimbic and limbic regions, by making focal injections of PHA-L and large injections of WGA-HRP into TEad or TEav in 8 Japanese monkeys.

Differential connections of TEad and TEav were found in the rostral part of STS. The labeled terminals were mostly limited to the upper bank in the rostral STS after injections into TEad, whereas, the terminals were mostly limited to the lower bank after injections into TEav. They extended to all the cortical layers in both cases. The distribution of labeled neurons in the rostral STS coincided with that of labeled terminals in both cases. They were equally distributed in the superficial and deep layers or confined to the deep layers.

Differential connections of TEad and TEav were also found with the TF/TH, cingulate cortex, insula, and hippocampus. Labeled neurons were found in TF/TH after injections into TEad but not after injections into TEav. Labeled terminals were also found in TF/TH in the TEad cases, but their distribution was more localized into patchy regions than the distribution of labeled neurons. Labeled terminals and neurons were found in the CA1 region of the hippocampus in the TEav cases but not in the TEad cases. Connections with the cingulate were only found in the TEav cases, and connections with the insula were stronger in the TEav cases than in the TEad cases.

These differential connection patterns of TEad and TEav, together with the previously reported differences in their connections with the perirhinal and entorhinal cortices, suggest the functional differentiation between the two subregions: for example, TEad may be more involved in cross-modal integration in STS, whereas TEav more directly interacts with limbic structures.

634.12

COMPARISON OF VISUAL RESPONSES OF CELLS IN VENTRAL AND DORSAL PARTS OF INFEROTEMPORAL CORTEX. H. Tamura[1,2]* and K. Tanaka[1], [1]Lab. for Neural Information Processing, RIKEN, Wako, Saitama, 351-01, Japan, [2]Lab. for Nervous Informatics, NIBH, Tsukuba, Ibaraki, 305, Japan.

The anterior part of the inferotemporal cortex (area TE) is thought to be important for the object recognition. Recent anatomical studies have shown differences in afferent and efferent connections between the ventral and dorsal parts of area TE (TEv and TEd, respectively). In this study, we examined responses of cells in TEv and TEd with a fixed set of 100 pictures composed mostly of color images of objects in awake macaque monkeys (*M. fuscata*) performing visual fixation task.

We have examined 113 cells recorded from TEv and 100 cells recorded from TEd. The proportion of responsive cells was significantly lower in TEv (58%) than in TEd (85%). The mean maximum firing rate of the visually responsive cells was lower in TEv (28 spikes/sec) than in TEd (40 spikes/sec). However, the stimulus selectivity of these responsive cells was comparable between the two areas. On the average, 9.3 stimuli evoked more than 50% of the maximum firing rate in the TEv cells, and 9.9 stimuli in the TEd cells. Highly selective cells showing more than 50% of maximum firing rate to less than 5 stimuli constituted 49% of the responsive cells in TEv and 46% in TEd.

We conclude that 1) visual responses are weaker in TEv than in TEd, and 2) the stimulus selecivity of the visually responsive cells is not significantly different between TEv and TEd, as far as responses are examined with pictures of objects in monkeys performing visual fixation task.

This work was supported by the Frontier Research Program, RIKEN.

634.13

INTERACTION BETWEEN SHAPES IN THE RECEPTIVE FIELD OF NEURONS IN THE INFERIOR TEMPORAL CORTEX. M. Missal*, R. Vogels and G.A. Orban. Lab. Neuro- en Psychofysiologie, KULeuven, B-3000 Leuven, Belgium.

Neurons in the inferior temporal cortex (IT) of the rhesus monkey (Macaca mulatta) have been found to respond selectively to shapes. However, shapes are usually not isolated but appear together with other shapes. We demonstrated previously that the response and shape selectivity of IT neurons are reduced when the shapes partially overlapped another one (Missal et al. 1995). In the present study, we determined in the awake monkey, the influence of the relative position of a second shape in the receptive field of the neuron on its response level. The response of shape selective units (n= 22) was measured first to a single shape presented at different positions and eccentricities. Responses were the strongest at the fixation position and in a part of the visual field contralateral to the recording site. A weaker response was evoked from a symmetric position in the ipsilateral visual field. Shape selectivity remained unchanged across the receptive field. Simultaneous presentation of the two shapes in the receptive field induced a reduction in response strength: 67 % reduction for the two shapes superimposed at the fixation position, 32 % if the second stimulus was contralateral and 11 % if it was ipsilateral with the first shape at the fixation position. These results show that the suppressive effect of a second shape in the receptive field is the strongest if the two shapes are at the same position and weaker if there is a distance between them. In the periphery, the suppressive effect of second shape is stronger in the contralateral vs ipsilateral hemifield.

Missal et al. *Soc. Neurosci. Abstr.*, Vol. 21, Part 1, p 19, 1995.

634.14

SAMPLE-SELECTIVE DELAY ACTIVITY DURING THE PERFORMANCE OF A DELAYED MATCH TO SAMPLE TASK IN THE MONKEY ENTORHINAL CORTEX. W.A. Suzuki*[1], E.K. Miller[2], and R. Desimone[1]. [1]Laboratory of Neuropsychology, NIMH, Bethesda MD, 20892 and [2]Dept. of Brain and Cognitive Sciences, MIT, Cambridge MA, 02139.

Neuropsychological studies in monkeys and rats suggest that both the entorhinal and perirhinal cortices are important for object memory. To investigate the neural mechanisms underlying object memory in the entorhinal cortex, we recorded from neurons in two monkeys performing a modified version of the delayed match-to-sample (DMS) task in which multiple and sometimes repeated stimuli could intervene between the sample and the match. To solve this working memory task, the monkey must (1) discriminate the stimuli, (2) maintain a memory of the sample stimulus during delay periods, and (3) evaluate whether a test stimulus matches the sample presented earlier in the trial. We previously reported that similar to cells in the perirhinal cortex, entorhinal cells may play a role in the first and third of these processes, in that they were stimulus selective and responded differentially to the test stimuli depending on whether the stimulus was a match or a nonmatch (Suzuki et al., Soc. Neurosci. Abstr., '95). We now report that entorhinal cells may play a role in the second process, in that some entorhinal neurons exhibit sample-selective activity during the delay intervals of the task and this selective delay activity is maintained across multiple intervening stimuli. By contrast, selective delay activity in perirhinal cortex is abolished by even one intervening stimulus (Miller et al., Soc. Neurosci. Abstr., '95). Preliminary evidence from a third monkey suggests that entorhinal cells also exhibit stimulus-selective activity during the delay intervals of a delayed match to place task. Thus, entorhinal cortex appears to maintain an explicit representation of the sample stimulus throughout the trial. Since the prefrontal cortex is the only other structure known to exhibit this property, these findings highlight an unexpected parallel between the mechanisms underlying working memory for objects and locations in both the entorhinal and prefrontal cortices. Supported by NIMH-IRP.

634.15

NEURAL CORRELATES OF VISUAL ASSOCIATIVE LEARNING IN INFERIOR TEMPORAL CORTEX OF THE RHESUS MACAQUE

C.A. Erickson*, and R. Desimone, Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892-4415.

To gain a better understanding of the neural basis of visual association learning, we first developed a task that allowed us to study implicit learning of visual-visual paired associates. Humans and monkeys were shown a brief cue stimulus at the start of the trial followed 1 second later by a go or nogo choice stimulus. Each choice stimulus was paired with a specific cue on 80% of the trials (valid trials). Typically, both humans and monkeys learned 8 concurrent pairs of stimuli, through trial and error, to a criterion of 90% in less than 200 trials. During probe trials, a cue that normally predicted a specific go or nogo choice stimulus was instead followed by a different choice stimulus, one that required either the same (neutral trials, 10%) or different response (invalid trials, 10%). Reaction times were fastest on valid trials (420 ms), slowest on invalid trials (478 ms) and intermediate on neutral trials (438 ms) for well-learned stimuli, indicating that subjects learned the valid predictor-choice pairings, even though some humans had no explicit awareness of the pairings.

Cells were recorded in inferior temporal cortex, a brain region important for learning visual associations, using multiple tetrodes. Consistent with the behavioral results, the cells' response latency to the choice stimuli were more than 10 ms faster on the valid trials, compared to the latency of the same stimuli on invalid and neutral trials. Thus, experience with temporally-linked stimuli can accelerate the sequential activation of neural representations. *Supported by NIMH-IRP.*

634.16

NEURONAL RESPONSES IN MACAQUE INFERIOR TEMPORAL (IT) CORTEX DURING LONG TERM LEARNING OF OBJECT SALIENCE.

B. Jagadeesh*, R. Desimone, M. Mishkin, Laboratory of Neuropsychology, NIMH, NIH, Bethesda, MD 20892.

When confronted with a scene containing many different objects, we tend to orient towards objects that are significant to us. In previous studies, we found evidence of neural activity that might account for this behavior: responses of IT neurons to arrays of stimuli are biased in favor of the stimuli that are associated with reward; the responses of cells that are tuned to the other stimuli in the array are suppressed. In these studies, the animals were rewarded for making an eye movement to a particular stimulus (target), which was either switched from trial to trial by presenting a cue at the start of the trial (a working memory paradigm; Chelazzi et al, *Nature* 1993) or was learned over the course of the session by consistent stimulus-reward pairings (Jagadeesh et al. *Neurosci. Abstr.* 1995). Since, in both cases, the particular target and nontarget stimuli (once learned) could be anticipated by the animal on each trial before the array was presented, the cells in IT may have been biased by feedback from other cortical areas involved in working memory. Alternatively, consistent target-reward pairings may have induced long-term changes in IT cortex, giving a competitive advantage to cells coding the target stimulus.

To help decide between these possibilities, we recorded IT responses in a monkey that was taught many concurrent target-nontarget discriminations. Because target and nontarget stimuli appearing in a given trial could not be anticipated, active working memory mechanisms were ruled out. When target and nontarget stimuli appeared together in the array the responses of the population of cells in IT were determined primarily by the target stimulus, as in the previous studies; the responses of cells selective for nontarget stimuli were suppressed. The effects were present only when the target and nontarget stimuli were paired; when the stimuli were presented alone, there was no effect of learning on the responses. The results suggest that learning induces long-term changes in the competitive interactions among stimulus representations in IT cortex, biasing responses in favor of important stimuli at the expense of others simultaneously in view. (Supported by NIMH-IRP)

634.17

COLOCALIZATION OF ACTIVITY IN HUMAN EXTRASTRIATE CORTEX TO FACES AND LETTERSTRINGS USING INTRACRANIAL FIELD POTENTIALS AND fMRI. A. Puce*, T. Allison, S.S. Spencer, J.C. Gore, D.D. Spencer, and G. McCarthy. Neuropsychology Lab., VA Medical Center, West Haven CT 06516, and Department of Neurology and Section of Neurosurgery, Yale University School of Medicine, New Haven CT 06510.

We have shown that: (i) in intracranial recordings faces and letterstrings evoke a negative field potential at about 200ms (N200) from discrete regions of extrastriate cortex (Allison et al 1994 Cerebral Cortex); (ii) the same region is activated by faces and letterstrings in fMRI studies in normal subjects (Puce et al, 2nd Int Conf on Human Brain Mapping June 1996). Here we address whether, in a given individual, the active regions as detemined by these two techniques are colocalized. We studied two epilepsy patients using potential recordings and fMRI to unfamiliar faces and letterstrings. In Pt1 faces produced fMRI activation in the fusiform gyri (FG). Face N200s from electrodes on the right and left FG colocalized with fMRI activation. Letterstrings produced little fMRI activation and no letterstring N200s. In Pt2 fMRI activation to faces occurred over a large extent of the right FG and colocalized with observed face N200s. A face N200 was also recorded from the left FG/OTS. While no letterstring N200s were seen, letterstrings strongly activated the left OTS in the fMRI study. As electrodes overlying a sulcus cannot readily record field potentials from a generator source within its walls, it is unlikely that letterstring N200s would be seen on surface cortex. Pt2 subsequently had an extensive right inferior temporal resection of her seizure focus. Post-operatively she had a left-visual field cut but was able to read words and numbers. She was prosopagnosic and could not match unfamiliar faces. The results provide evidence for perceptually based category-specific processing mainly in the right FG and left OTS for faces and letterstrings, respectively. (Supported by the Dept. of Vet. Affairs & NIMH grant MH-05286.)

634.18

MEANINGFUL IMAGES ENHANCE ACTIVATION IN OCCIPITAL AND LEFT TEMPORAL AND PARIETAL CORTICES. S. Vanni[1]*, A. Revonsuo[2], J. Saarinen[1] and R. Hari[1]. [1]Brain Research Unit, Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland, [2]Centre for Cognitive Neuroscience, University of Turku, 20520 Turku, Finland

Cortical magnetic fields were recorded with a whole-scalp neuromagnetometer to visual stimuli from 8 right-handed observers. The stimuli were either 'images' (pictures of 200 concrete objects or animals) or 'non-images' (generated from the images by randomly rotating circular areas within them), presented once every 3–5 s for 30, 45 and 105 ms in-between backward and forward maskers. The observers indicated their decision about the stimulus type (image vs. non-image) with index finger lifts. The parieto-occipital 8–13 Hz alfa rhythm was suppressed by both stimuli but the post stimulus rebounds were higher for non-images than images. Most observers (7 out of 8) showed evoked responses in medial occipital cortex, in lateral occipital cortex bilaterally (6) and in superior temporal lobe in or near the midsuperior temporal sulcus (8 left, 6 right), and in the left parietal lobe (6). The occipital and temporal sources peaked on average at 300–380 ms, followed by the left parietal activation around 500 ms. Images induced stronger activation than non-images in lateral occipital (left $p < 0.005$, right $p < 0.05$, 2-way ANOVA for each source; main factors image vs. non-image and presentation time), left temporal ($p < 0.001$) and left parietal ($p < 0.001$) cortices. The preferential activity for images in lateral occipital areas agrees with previous fMRI findings (Malach et al, PNAS 1995, 92: 8135). The right lateral occipital activity correlated positively with behavioral image detection ($r = 0.74$, $p < 0.001$), suggesting a strong involvement of this area in object detection. Supported by Academy of Finland.

634.19

THREE COMPUTATIONAL TRADEOFFS AFFECT OBJECT RECOGNITION IN A HIERARCHICAL, RECEPTIVE-FIELD-BASED VISUAL SYSTEM. J. Fiser, Dept. of Computer Science & B. W. Mel*, Dept. of Biomedical Engineering, MC 1451, USC, Los Angeles, CA 90089.

Some neurons in the primate inferotemporal cortex respond selectively to specific conjunctions of contour, texture, and color feature elements [Kobatake & Tanaka, *J. Neurophysiol.*, v. 71, 1994], but are invariant to substantial changes in stimulus position, size, and other viewing parameters [Ito, et al., *J. Neurophysiol.*, v. 73, 1995]. We consider three parameters that constrain the "design" of such conjunctive features, and which highlight algorithmic tradeoffs that influence recognition performance under different ecological conditions. These are (1) conjunctive *order*, i.e. the number of elemental features that make up a conjunction, (2) *locality*, i.e. the scale of spatial distribution of feature elements in the visual field that define a conjunction, and (3) *invariance* characteristics, i.e. those parameters, such as stimulus size and location, that may be changed without significantly altering the magnitude or selectivity of cell responses. We illustrate the performance ramifications of each of these tradeoffs in the context of typeset and handwritten word recognition.
This work was funded by the ONR.

634.20

DEVELOPMENT OF INVARIANT RESPONSES IN A MODEL OF THE INFEROTEMPORAL CORTEX EMBEDDED IN A BEHAVING REAL WORLD ARTIFACT. N. Almássy, O. Sporns, J. Wray* and G. Edelman. The Neurosciences Institute, 10640 John J. Hopkins Drive, San Diego, CA 92121.

Behavioral discrimination of shapes and patterns requires the ability to detect invariant properties of stimuli and to link the appropriate neuronal responses to motor output. We studied the development of neuronal responses and their linkage to motor structures in a model of the inferotemporal cortex. In order to take into account the role of ongoing behavior during the developmental period we embedded the neuronal model in a real world artifact called "NOMAD." Its environment contains numerous blocks that differ in the visual patterns on their surface and their conductivity, which serves as a value signal ("taste"). Visual input acquired by a video camera is transmitted to an area resembling V1, with units that respond selectively to horizontal or vertical lines, or "blobs" in the visual field. Omitting intermediate areas, V1 is linked directly to IT, which contains locally coupled excitatory and inhibitory units. V1-IT connections are widely divergent and modifiable according to a learning rule allowing for potentiation and depression. Initially, IT units send uniformly distributed connections to motor centers controlling behavior of NOMAD. Successive exposure to objects in the environment leads to changes in the response properties of neuronal units in IT. Before learning, IT responses are weak and show no selectivity for stimulus patterns. After the classification of about 90 blocks, IT units appear more sharply tuned, are selective for distinct stimulus patterns, respond invariantly with respect to their position, orientation or size, and units with similar response properties are spatially grouped together. Due to value-dependent strengthening of connections linking selective units in IT to motor units, NOMAD is able to behaviorally discriminate stimulus patterns based on their visual appearance. This approach allows the comprehensive analysis of both neural and behavioral data over a complete developmental history.
(Supported by Neurosciences Research Foundation)

VISUAL CORTEX: EXTRASTRIATE—DORSAL STREAM II

635.1

OPTIC FLOW DURING SIMULATED TURNS: SINGLE UNIT RESPONSES IN EXTRASTRIATE CORTEX. H. Sherk*, K.A. Mulligan, & J-N. Kim. Bio. Structure, U. Washington, Seattle, WA 98195

Vision provides essential information for the guidance of normal locomotion. When a locomoting observer turns, the optic flow field changes abruptly, and these changes might be detected by single neurons in motion-sensitive cortical areas such as MT in primates or LS (the lateral suprasylvian area) in cats. We searched for such neurons in LS in anesthetized cats. Our stimulus displays simulated a natural environment seen by a cat trotting both straight ahead and turning.

The large stimulus display (62x62 deg) represented an environment of small objects of variable contrast that covered the ground and also floated in air. The cat's simulated motion was initially straight ahead; after 200–350 msec it made a simulated turn of 15, 30, or 60 deg to the right or left lasting for 1 sec.

Of 404 neurons, 96 responded to turns, and gave only a transient response, or no response, during the preceding straight-ahead movement. Most commonly, neurons responded best during a turn of one size and direction (e.g., a left turn of 30 deg). Surprisingly, 25% of these neurons responded poorly or not at all to a whole-field texture stimulus that matched the speed and direction of image motion seen by the receptive field during an optimal turn. The texture stimulus differed from movies simulating turns in that it contained no optic flow cues, and was not preceded by whole-field motion in a different direction. Responses to turns frequently had significantly shorter latencies than responses to simple texture motion.

Supported by NEI RO1-4847

635.2

EXTRASTRIATE RESPONSES TO SALIENT OBJECTS IN NATURALISTIC OPTIC FLOW FIELDS. K.A. Mulligan*, H. Sherk, & J-N. Kim. Biological Structure, U. Washington, Seattle, WA 98195

Locomoting observers need to detect salient objects in the visual field that might represent obstacles to avoid, or landmarks for guidance. We sought to determine whether neurons in the cat's lateral suprasylvian area (LS) respond to such objects embedded in a large-field (62x62 deg), realistic optic flow simulation.

A preliminary study of 60 cells yielded consistently negative results. The salient object in this case was a black bar lying on a layer of small balls covering the simulated ground. We next tested optic flow movies containing a variety of larger and more naturalistic objects. All objects were variants of a bush with a simulated height of 36 cm. The ground was densely covered with the leaves of this bush, and objects were placed on the ground so that during the cat's simulated motion each object passed through the receptive field of the cell under study.

These objects proved to be highly effective stimuli. Of 404 cells tested, 220 responded well to one or more objects. Response levels were often comparable to those evoked by an optimal bar moving in the preferred direction. Even bushes whose texture and appearance closely resembled the leafy substrate often elicited strong responses. In general, large objects were more effective than identical ones of half height, even when the latter extended beyond the receptive field.

Because a given object was part of an optic flow field, its direction of motion depended only on its position in the simulated environment. Objects thus often moved in directions quite different from that preferred by a particular cell. Surprisingly, about 1/3 of cells responding to objects did so despite this kind of mismatch. Thus cells can respond well to a salient object in an optic flow context moving in a direction which, when tested with an optimal bar on a blank background, elicits a much weaker or no response Supported by NEI RO1-4847

635.3

LESION-INDUCED CHANGES IN VELOCITY AND DIRECTION TUNING, AND FIRING RATES OF CELLS ADJACENT TO LESIONS IN MONKEY MIDDLE TEMPORAL CORTEX. J. M. STARK, and D. S. Yamasaki*. Dept. of Anatomy, University of Wisconsin Medical School, Madison, WI 53706

Previous reports have shown that rapid recovery of smooth pursuit eye movements following lesions in the Middle Temporal (MT) area is dependent on the surviving cells in these areas. To test whether compensatory changes take place in the cells adjacent to MT lesions, we have recorded direction and velocity tuning curves, as well firing rates from single neurons in MT. Guide tubes were positioned chronically over MT, allowing us to record from the same sub-population of cells across several weeks. Also, smooth pursuit was recorded every recording day to monitor the behavioral deficits and the recovery times. One electrolytic lesion and two AMPA lesions were made in 3 hemispheres in two monkeys. In two hemispheres, cells showed preferred speeds higher than those seen before the lesion for a given recording site (> 32°/sec). There was a clear decrease in direction selectivity in a subpopulation of post lesion cells, however most of the cells did not change. At one recording site (3.5 mm from the lesion center), the range of directional indices (DI) was 1.13-0.52 before and 1.13-0.26 after the lesion. The half-height bandwidths (BW) ranged from 60.32°–143.64° prior to the lesion and 43.91°–360° post lesion. Similarly, there was an increase in both the spontaneous and maximum firing rates in a subset of surviving single units in all three hemispheres. The range of spontaneous rates in one hemisphere was 0.08-4.45 spikes/sec before and 0.06-10.42 spikes/sec after the lesion, while the maximum firing rates were 4.44-74.12 spikes/sec before and 2.70-100.92 spikes/sec after the lesion. All of these changes were observed during the pursuit recovery period. These results are consistent with a selective loss of inhibitory interneurons near the lesion, leading to an unmasking of latent inputs in the surviving cells. It remains to be shown whether these changes contribute to the pursuit recovery, though they are compatible with such a process. This work was funded by NIH EY09618.

635.4

ASYMMETRIC SURROUNDS ENABLE MACAQUE MT NEURONS TO SIGNAL DIRECTION OF TILT IN DEPTH. D.-K. Xiao , S. Raiguel*, V. Marcar and G.A. Orban. Laboratorium voor Neuro- en Psychofysiologie, KULeuven, Medical School, Campus Gasthuisberg, B-3000 Leuven, Belgium.

Our previous work has shown that the antagonistic surrounds of most MT cells exhibit speed-dependent spatial heterogeneity (Xiao *et al.*, Soc. Neurosci. Abstr. 21:663, 1995), a property which is useful for extracting 3D shape from motion cues (Buracas & Albright, Perception 22 suppl.:78, 1993). The present study investigates the selectivity of MT neurons for direction of tilt as defined by speed gradients.

Fifty-seven MT neurons were recorded in three anesthetized and paralyzed macaque monkeys. We presented tilt stimuli consisting of random dot fields moving in the optimum direction and covering both the classical receptive field (CRF) and antagonistic surround. The speed gradients specified planar surfaces tilted (inclined around the line of sight) in 8 different directions and at three different levels of slant (inclination in depth). Forty-two percent of the cells were selective for the direction of tilt in depth. This selectivity appears to be directly related to the presence of a speed-dependent asymmetric surround: There is a significant correlation between surround strength and the degree of selectivity ($n=50$ $\rho=0.63$, $p<0.0001$); masking the surround abolished the selectivity in all 7 cells so tested; the optimum direction of tilt and the direction of the line linking the center of the CRF and the center of the surrounding area evoking maximum inhibition tend to lie along the same axis ($n=19$, $\chi^2=4.26$, $p<0.05$). Selectivity for tilt could be explained by the speed tuning of the spatially heterogeneous surround in more than half of the cells ($n=9$) which were selective for direction of tilt and which had been tested with different speeds in the surround.

Our study demonstrates that the speed gradient of a tilted planar surface can modulate the response of cells in area MT via the antagonistic surround. Area MT is therefore capable of signaling the direction of tilt using depth from motion cues. This could be an initial stage in the extraction of shape from motion.
Supported by GOA 95/6 and BRA 6019 (Insight2).

635.5

NEURAL CORRELATE OF 3-D STRUCTURE FROM MOTION (SFM) PERCEPTION IN AREA MT G. C. Chang[1], D. C. Bradley[2], & R. A. Andersen[1,2*]. [1]Computation and Neural Systems & [2]Div. of Biology, Caltech 216-76, Pasadena, CA 91125.

Studies in our lab involving MT lesioning and motion-disparity integration suggested MT may play a role in SFM. To examine this possibility more directly we trained a monkey to report the perceived 3-D structure of a motion stimulus, and compared his perceptual reports with the activity of MT neurons. The animal viewed the orthogonal projection of a hollow, rotating cylinder composed of random dots. Even without disparity cues, the motion in the stimulus causes it to appear 3-D to human observers. However, without disparity cues, the depth assigned to the two oppositely moving surfaces is arbitrary, resulting in a bistable perception of the direction of rotation. If disparity is added to the display, the direction of rotation is no longer arbitrary. The animal's task was to report the direction of perceived rotation for cylinders with varying amounts of disparity. His performance was almost perfect at high disparity values and smoothly decreased to nearly chance performance at low disparities, suggesting that his reports matched his perception.

We recorded from 44 cells in area MT while the monkey performed this task. The cylinder stimulus was positioned in the receptive field such that one of the surfaces was at the preferred direction for the cell. For 18 cells it was found that the neuron's activity varied significantly for identical stimuli depending on the perceived direction of rotation for at least one of the disparities tested, including 0% (t-test, $p < 0.05$). Of these 18, 16 had responses which positively predicted the monkey's perceptual report. Thus, under low disparity and ambiguous (bistable) conditions, the activity of MT neurons often covaries with the animal's perceptual reports. These data support the hypothesis that area MT plays an important role in 3-D SFM perception.

Supported by HFSP, ONR, NIH, and NSF.

635.6

ACTIVITY DIFFERENCES WITH CORRECT AND FALSE DECISIONS IN AREAS MT, MST, AND STPP A. Thiele', S. C. de Oliveira, K.-P. Hoffmann, Dept. Zoology & Neurobiology, Ruhr-Univ. Bochum, 44780 Bochum, Germany

We investigated the neuronal activity of direction selective cells from areas MT, MST, and the upper part of STPp while two monkeys performed a direction discrimination task near contrast threshold. Stimuli consisted of evenly spaced bars, moving in one of the four cardinal directions. Stimulus onset, contrast and direction of motion were randomized. Upon stimulus perception, the monkeys were supposed to indicate the direction of motion by a hand movement to one of four touch bars. Stimulus contrast varied between 2% and 54%. Reaction time and error rate increased with decreasing contrast. To compare the activity between correct and false decisions the normalized population activity was calculated in 5 ms bins according to the direction of the decision. A binwise ANOVA was applied to the data, in order to see whether normalized population activity significantly differed dependent on the direction of the decision. In all three areas the activity was significantly reduced (about 20%) if the preferred direction was presented, but the monkey made an error. Nonetheless the population stimulated in preferred direction still exhibited significantly higher activity as compared to the population corresponding to the decision the monkey actually made.

Although the activity changes can be taken to predict the probability of error occurence, errors themselves cannot be explained by activity changes in one of these areas. If the monkey would rely on rate differences within these areas, an error would not occur, since the population stimulated in preferred direction always exhibits the highest activity.

The study was supported by DFG.

635.7

A MODEL OF THE NEURAL INTEGRATION OF MULTIMODAL SOURCES OF INFORMATION FOR SELF-MOTION: OPTIC FLOW, STEREO, AND EXTRARETINAL SIGNALS. M. Lappe* & A. Grigo, Dept. Zoology & Neurobiology, Ruhr University, D-44780 Bochum, Germany

For sensing and controlling self-motion in the real world humans can combine a number of different sources of information. Most models of self-motion computation have instead considered only the information available from the optic flow field, despite psychophysical studies that showed the inefficiency of optic flow alone under some circumstances. Moreover, recent years have seen first reports of neuronal modulations of visual flow responses in areas MT and MST by disparity, extraretinal eye movement and eye position signals, and even vestibular signals. We present a neural model of heading detection that integrates multiple signals at the single neuron level. A map of direction selective neurons, modelled after area MT, is used as an encoding of the optic flow field. These cells are assumed to average visual motion only within restricted disparity bands, thereby accounting for the recently observed disparity dependence of MT responses to transparent motion. The MT stage forms the input to a heading detection algorithm which models optic flow analysis in area MST. This visual heading detection scheme is supplemented by a set of visual tracking neurons, also located within area MST, which encode the presence of a slow eye movement by means of an extraretinal signal. The model makes a number of predictions about the modulations of visual responses of MST neurons by eye movements or stereoscopic displays. These predictions are compared to recent experimental data.

Supported by DFG SFB-509, LA952/1-1, and HFSP.

635.8

VESTIBULAR RESPONSES DURING LINEAR MOTION IN AREA MST OF THE MACAQUE MONKEY. M.Pekel, M.Lappe, M.Schmidt*, and K.-P. Hoffmann, Dept. Zoology and Neurobiology, Ruhr-University, D-44780 Bochum, Germany.

Area MSTd in the anterior part of the superior temporal sulcus has been shown to be involved in the processing of optic flow stimuli that simulate egomotion. Since vestibular information is usually present during real but not visually simulated egomotion we investigated the responses of single neurons in area MST of the macaque monkey to a real linear movement along the naso-occipital axes. This movement results in stimulation of the otolith organs which are sensitive to linear acceleration. The monkey chair was placed on a parallel swing. The monkey was trained to fixate a central spot of light (0.4 deg diameter). The duration of one cycle (back and forth) was 4s. The swing amplitude was 1.0 m and the vertical displacement was 0.03m. Each trial started with backward movement and lasted for one cycle. We tested the responses to sinusoidal movement in the darkness (the pure vestibular response). We also compared the single cell responses to real motion in the light with visual stimulation simulating identical egomotion. Half of the recorded cells showed clear responses to vestibular stimulation or clear differences between the real and simulated egomotion during visual stimulation. Cells responding to the pure vestibular stimulation often showed transient responses in the start and/or end phase. Phasic as well as tonic response differences over the whole movement period were observed when real and simulated egomotion were compared. We conclude that vestibular information resulting from linear acceleration is integrated with visual self- motion processing in area MST.

Supported by DFG SFB-509.

635.9

SHORT-LATENCY OCULAR FOLLOWING AND ITS ASSOCIATED NEURONAL ACTIVITY IN MEDIAL SUPERIOR TEMPORAL AREA (MST): DEPENDENCE ON OCULAR VERGENCE. Y. Inoue, A. Takemura, K. Kawano* and F. A. Miles. Electrotechnical Lab., Tsukubashi, Ibaraki, 305, Japan, and Lab. of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

The short-latency ocular following responses (OFR) elicited by motion of a large-field pattern are mediated at least in part by a pathway that includes MST, dorsolateral pons and ventral paraflocculus (Kawano et al, 1992, 1994; Shidara et al, 1993), and their magnitude is inversely related to viewing distance (Busettini et al., 1991, 1994). We now report on the dependence of OFR and the associated activity of MST neurons on the convergence angle of the two eyes in the monkey.

The animal faced a large tangent screen onto which two identical images were back-projected. Crossed polarizers in the projection paths ensured that each eye saw only one of the patterns and mirror galvanometers were used to control the horizontal and vertical positions of the images. At the beginning of each trial the image seen by one eye was slowly moved (horizontally) to a new position to induce the monkey to adopt a new convergence angle (distance to the plane of fixation ranged from 25 cm to infinity). Then, 50 ms after a centering saccade, both patterns were moved together at constant velocity (conjugate ramp) to the left or right to elicit OFR.

The rise in eye speed at the onset of OFR in response to conjugate ramps of a given velocity was directly proportional to the convergence angle. Of the neurons in MST that discharged in relation to OFR, 59% were sensitive to the convergence angle ($p<0.05$, t-test), some responding more vigorously with increases in convergence (36%) and the remainder responding less vigorously. However, the modulation with convergence [((max-min)/min) x 100%] was on average much lower for the neurons than for OFR: 43% vs 176%. These data suggest that vergence angle can be a major cue in the modulation of OFR with viewing distance, and that either MST neurons mediate only a portion of the OFR and/or most of the modulation of OFR with vergence angle results from changes downstream of MST.

Supported by the Human Frontier Science Program, the Japanese Agency of Industrial Science and Technology, and The National Eye Institute.

635.10

RESPONSE TO SPEED GRADIENTS IN AREA MST. **N. Ghani* and J-P. Roy. Montreal Neurological Institute, McGill University, Montreal, Quebec H3A 2B4, Canada**

When an observer moves in a direction different from her line of sight, the images of objects at different depth move at different speeds. We examine MST neurons for a sensitivity to such differential motion.

MST cells were presented with random dot stimuli containing motion at three speeds, over the same area of the visual field. The response to these stimuli were compared to a control stimulus with only one speed of motion, the average of the three speeds presented in the speed gradient. The disparity of the speed gradient and the control were always the same. We used three disparity conditions: fixation, 2 degrees preferred, or 1, 2 and 3 degrees preferred. In the last condition, the slowest speed was presented at the smallest disparity, the fastest at the largest. This is the expected correlation during observer motion in a static environment.

15/42 (37%) cells give a significantly (t-test, $p<0.01$) higher response to speed gradient stimuli as compared to the control containing only one speed. This response is not a consequence of the speed tunings of these cells. We measure speed tunings at the plane of fixation and in disparity at all values used for the speed gradient stimuli. The optimum response of an MST cell to stimuli containing one speed does not predict the response to stimuli containing multiple speeds. In addition, an analysis of variance shows no effect of disparity in cells which respond to speed gradient stimuli.

grant

635.11

THE DEVELOPMENT OF OPTIC FLOW SELECTIVITY IN MSTd NEURONS USING BACK-PROPAGATION NETWORKS S.A. Beardsley[1], L.M. Vaina[1], T. Poggio[2]* Boston University, Department of Biomedical Engineering, Boston MA[1]. Center for Biological and Computational Learning, MIT, Cambridge MA[2].

In neurophysiological experiments examining the responses of MSTd neurons to visual motion components of optic flow stimuli in monkeys, Duffy & Wurtz (1991) reported neurons with double-component (plano-radial and plano-circular) and triple-component (plano-radial-circular) selectivities while Graziano et al. (1994) reported neurons selective to spiral optic flows. Here we address the apparent disagreement of these reported findings under simulated experimental conditions. To examine the development of optic flow selectivities in the hidden units of a three layer back propagation network, we constructed two computational models by using different input layer formats. The first simulation used a sparse optic flow. The input format consisted of eight circular MT receptive fields placed in the MSTd receptive field such that the MT receptive fields were radially symmetric without overlap. Each MT receptive field corresponded to eight directionally selective MT neurons which equally divided the directional vector space. The second simulation used a dense optic flow. The input format consisted of 67 overlapping MT receptive fields of normally distributed diameter placed pseudo-randomly in the MSTd receptive field. Each MT receptive field corresponded to 16 directionally selective MT neurons which equally divided the directional vector space. The hidden units were classified as MSTd neurons whose receptive fields were coincident and encompassed the MT receptive fields from the input. The output layer of the network consisted of four MSTd neurons with the same receptive fields as the hidden layer. To minimize the effects of biasing, the output selectivities were designated as expansion, contraction, clockwise rotation, and counterclockwise rotation (consistent with MSTd selectivities found in both experiments). In both sets of simulations we found hidden units whose selectivities were consistent with the visual motion components of optic flow stimuli reported by Duffy & Wurtz and Graziano et al.

Supported by NIH grant EY-2R01-07861-06 to L.M.V.

635.12

DYNAMIC CHANGES IN VISUAL RECEPTIVE FIELD ORGANIZATION IN THE MACAQUE LATERAL INTRAPARIETAL AREA (LIP) DURING SACCADE PREPARATION. S. Ben Hamed, J-R. Duhamel*, F. Bremmer and W. Graf. CNRS-Collège de France, F-75270 Paris Cedex 06, France.

The spatial structure of the receptive field (Rf) of single visual neurons in area LIP was found to vary systematically depending on foveal attention or saccade preparation (Duhamel et al., Soc. Neurosci. Abstracts, 21, 1759, 1995). The present study examines the spatial and temporal characteristics of these effects. Responses were recorded extracellularly from 50 neurons in two hemispheres of one rhesus monkey who performed memory-guided saccades: during foveal fixation, a cue was turned on for 80ms at one of several possible positions inside, near or away from a given neuron's Rf; after a 2.3s delay the monkey was instructed to make a saccade to the cued location. Rfs were mapped during the delay period with rapid sequences of flashes randomly selected from a 9x9 array of predefined locations.

In about one half of the cells, the direction of the upcoming saccade did not modify significantly Rf characteristics or produced changes that were uncorrelated with eye movements. In the other half of the sampled neuron population, Rf activity was displaced in the direction of at least one saccade. This displacement could result from a global shift of the Rf, a shift of the location of the peak within the Rf, or an expansion of one of the Rf borders. Time-dependent changes in Rf structure were analysed by subdividing the delay period into three epochs to determine when these changes were at maximum in the interval between the sensory encoding of the saccade location and the execution of the motor response. Two main behaviors were seen: (a) the Rf remained fixed but the overall activity level changed over time, (b) there was a gradual shift of the Rf borders across the three epochs of the delay, while the overall activity was constant for a given saccade direction. Such dynamic reorganization of Rfs in response to changes in sensory, attentionnal and motor signals may be crucial for the encoding of visual space.

Supported by HCM grant ERBCHRXCT9930267 from the European Community.

635.13

STATIONARY SURROUNDS ENHANCE THE RESPONSE OF MSTl NEURONS TO MOVING STIMULI. S. Eifuku and R. H. Wurtz*. Laboratory of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20892.

We investigated the importance of foreground-background segregation on the response of monkey (Macaca mulatta) MSTl neurons to moving stimuli: whether the presence of a patterned background altered the response of the MSTl neurons to motion in their field center, and whether the neurons responded preferentially to motion of a patterned "object" or to motion of the same size patterned motion seen through an aperture.

Monkeys fixated on a tangent screen while random dot motion was projected onto the previously determined central receptive field of the neuron. Responses were measured for motion in the preferred and non-preferred directions for each cell. Object motion consisted of a random dot pattern (e.g. 10 deg in diameter) moving across the receptive field area; aperture motion consisted of the same pattern moving behind a window, (e.g. a window 10 deg in diameter). We then tested the effect of replacing the usually dark background with a patterned surround stimulus.

The neurons studied all were on the anterior bank and floor of the superior temporal sulcus as determined from an MRI, and their preferred stimuli were consistent with those of MSTl: typically large receptive fields but directionally selective responses given preferentially to small stimuli in the the larger field. We found that the response to the preferred stimulus motion was almost always increased when the normally dark surround was replaced by a patterned stimulus. This is in contrast to a general lack of such an increase in response in MT neurons when surround stimuli were added (Olavarria et al. 1992). We also found that many (but not all) of these neurons gave more vigorous responses to object motion. We conclude that stimulus factors that would alter the segregation of foreground from background change the response of MSTl neurons. Supported by the National Eye Institute.

635.14

RESPONSES OF LIP NEURONS TO CHANGES IN STIMULUS UTILITY. M.L. Platt and P.W. Glimcher. Center for Neural Science, New York University, New York, NY 10003.

We examined the effects of changes in stimulus utility on the responses of single LIP neurons in head-fixed, awake-behaving rhesus monkeys. First, the response field of each unit was measured at a 2° resolution using 100-400 trials of a delayed saccade task. Next, unit responses were recorded while animals performed a *Selection* task and a *Distributed Selection* task which differed only in the utility of a distractor stimulus. On both tasks, subjects initially fixated a central yellow LED for 200-800 msec. Two eccentric yellow LEDs were then co-illuminated for 200-800 msec, one above and one below the horizontal meridian. The fixation LED then changed color to either red or green, specifying either the upper or lower LED, respectively, as the saccade goal. On the selection task, fixation offset cued movement initiation, whereas on the distributed selection task distractor offset provided the initiation cue. This permitted us to examine the responses of the unit to a distractor both when it was irrelevant and when it cued movement onset. In both tasks, most cells responded about 50% more strongly when an LED at the center of the response field served as a saccade target than when the same LED served as a distractor. Overall, population selectivity for saccadic targets was unaffected by our manipulation of distractor utility.

We also examined the effects of changing the utility of a target stimulus by changing the probability that a particular target location would serve as the saccade goal in a series of delayed saccade trials. The response field of each unit was measured by varying the saccadic goal randomly throughout the response field of the unit under study when 1) all possible target locations were equally likely, and 2) when one particular location was much more likely to serve as the saccadic goal than all other locations. The response field properties of some units changed when target utility was altered in this manner.

Supported by EY06595-01 to MLP and a Whitehall Foundation Grant to PWG.

635.15

TOPOGRAPHIC ORGANIZATION OF CORTICO-TECTAL PROJECTIONS FROM AREA LIP OF MONKEYS JW Gnadt* and MF Kritzer. Dept. Neurobiology & Behavior, SUNY at Stony Brook, NY 11794.

Prior physiologic studies have failed to identify a coherent topographic map in area LIP. Anatomical studies show that area LIP links diverse visual and pre-motor inputs with cortical and subcortical oculomotor centers, including the superior colliculus (SC). Only one synapse away, the SC has a "motor map" that is is orderly and well established. Thus, we sought to determine area LIP topography in relation to its outputs for the generation of saccades via the superior colliculus.

We used standard microstimulation techniques to determine the topographic map of saccades in the intermediate layers of the SC. We then used this map to place multiple injections of distinguishable retrograde tracers (0.3-0.5 µl) into known positions of the tectal map. The resulting retrograde label in area LIP was then plotted separately for each tracer, and together on a common set of coordinates.

All collicular injections labeled pyramidal neurons in cortical layer 5. A consistent finding was that individual SC injections produced anterior/posterior bands of labeled cells along the sulcus. In some cases, labeling arched across the sulcus in a continuous band. In other cases the label was discontinuous, leaving open the possibility of interdigitation with other distinct populations, or for repeating patches of collicular representations along a line across the sulcus. Comparison of labeling *within subjects* revealed unique patterns of label for individual injection sites that were spatially overlapping in some cases. Comparison *between subjects* for injections at similar tectal loci revealed ideosynchracies in both the pattern and location of retrograde label. Future studies will emphasize intra-subject injection patterns placed along saccadic iso-direction and iso-amplitude lines within the superior colliculus. *Supported by EY08217 and a Sloan Foundation Fellowship to JWG, and a School of Medicine Dean's Research Award to MFK.*

635.16

NEURONAL SELECTIVITY TO RETINOTOPIC CENTERS OF MOTION IN AREA 7A OF THE BEHAVING MONKEY. J.F. McCollum* & R.M. Siegel. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Area 7a in the inferior parietal lobule is the apex of visuo-spatial processing in macaques. Optic flow stimuli (planar rotation and radial motion), which provide powerful cues for the analysis of egocentric motion (heading) and extra-personal space, have been found to activate neurons in area 7a. Selectivity to the retinotopic position of the flow field has been reported (Siegel & Read, 1994). The present study determines whether a retinotopic selectivity to the center of optic flow (*i.e.* axis of motion) can be found in the absence of changes in location of the border of the flow field. Such changes in the center of optic flow occur with variations in egocentric motion or eye position in the orbit. The activity of area 7a neurons was recorded during presentation of random dot optic flow fields that covered 50° of the visual field. The center of a flow field could be placed at 9 different retinal loci (3x3 array) without altering the position of the luminance border of the display. Monkeys performed a reaction time task in which they fixated a small square and attended to changes in the structure of the flow field. A two-way ANOVA was used to quantitatively determine the selectivity of neurons to the type of optic flow, the fraction of structure of the flow, the retinotopic center of the flow, and to the eye position.

Approximately 50% of the 77 neurons tested in one *Macaca mulatta* responded to at least one of the optic flow fields. As expected, some area 7a neurons were selective to the type of optic flow as well as its fraction of structure. Varying the retinotopic location of the center of optic flow altered the response of about 25% of the motion-sensitive neurons. These selective responses were modeled with linear and quadratic terms; maxima in the response were found both ipsilateral and contralateral to the recorded hemisphere. These data provide strong evidence that area 7a neurons specifically encode the center of optic flow, similar to that reported for the medial superior temporal area (Duffy and Wurtz, 1995). Variation of the eye position with constant retinal locus of the center of the flow field significantly altered the activity of 60% of the motion-sensitive neurons. In these responses, the linear and quadratic dependence on eye position is similar to that reported for area 7a with stationary bar stimuli (Andersen *et al.*, 1985). In summary, area 7a neurons appear to combine the retinotopic location of the center of optic flow with eye position signals to provide a representation of egocentric motion relative to extra-personal space.

Supported by NEI 9223 and ONR N00014-93-1-0334.

635.17

THE REPRESENTATION OF EYE POSITION IN THE PARIETO-OCCIPITAL SULCUS IN THE MONKEY. K. Nakamura*, H.H. Chung, M.S.A. Graziano, and C.G. Gross. Dept. of Psychology, Princeton Univ., Princeton, NJ 08544.

We recorded single neuron activity in the parieto-occipital sulcus of two monkeys *(Macaca fascicularis)* and found that for 47% of 141 neurons the neural activity depended on the position of the eyes during spontaneous eye movements even in the dark. Thirty-four of these eye-position dependent neurons were tested in a task in which the monkey was required to fixate a spot for 1.5 sec. On interleaved trials the fixation point was presented at 1 of 9 locations arranged in a square grid with 12 degrees between adjacent locations. All of the neurons fired more strongly when the animal was fixating at a peripheral location; none fired best at the central location. For some neurons the response profile was planar: they fired best at one edge of the grid and least at the opposite edge. Every one of the 34 neurons showed sustained firing throughout the 1.5 second fixation period. Thirteen of the eye-position neurons were tested in a saccade task. In this task, the monkeys were required to fixate a center spot and to make a saccade toward 1 of 8 peripheral locations. All of the neurons changed their activity only *after* the eye reached the peripheral location.

Histological examination of the brain of one monkey indicated that the eye position neurons were located either dorsal to Area PO or in the dorsal portion of Area PO, in the anterior bank of the parieto-occipital sulcus. These data suggest that neurons in this area represent eye position.

Supported by HFSP grant LT-716/94, Uehara Memorial Foundation and NIH grant EY11347-25.

AUDITORY, VESTIBULAR, AND LATERAL LINE: DEVELOPMENT AND REGENERATION

636.1

FIBROBLAST AND INSULIN-LIKE GROWTH FACTORS STIMULATE PROLIFERATION OF RAT UTRICULAR EPITHELIAL CELLS IN VITRO. J.L. Zheng*, C. Helbig and W.-Q. Gao. Dept. of Neuroscience, Genentech, Inc., South San Francisco, CA 94080.

Proliferation of supporting cells in the inner ear is the early, major event occurring during hair cell regeneration following acoustic trauma or aminoglycoside treatment. In the present study, we have examined the possible influence of 30 growth factors on the proliferation of pure rat inner ear epithelial cells in culture. Utricular epithelial sheets were separated and partially dissociated from early postnatal rats using a combined enzymatic and mechanical method. The cultured utricular epithelial cells expressed exclusively epithelial cell antigens but not fibroblast, glial or neuronal antigens. With tritiated thymidine incorporation assays, we found that several fibroblast growth factor (FGF) family members, insulin-like growth factor-1 (IGF-1), IGF-2, transforming growth factor-α (TGF-α) and epidermal growth factor (EGF) stimulated proliferation of the utricular epithelial cells. In contrast, neurotrophins and other growth factors did not elicit any detectable mitogenic effects. Of all of the growth factors examined, FGF-2 was the most potent mitogen. When FGF-2 was added as a combination with IGF-1 or TGF-α to the medium, combined effects were seen. These results were confirmed with BrdU immunocytochemistry. Therefore, the present culture system provides a rapid and reliable assay system to screen novel growth factors involved in proliferation of mammalian inner ear supporting cells. Furthermore, immunostaining revealed that the cultured utricular epithelial cells expressed FGF receptor and IGF-1 receptor and utricular hair cells produced FGF-2 in vivo. This work suggests that FGF-2 and IGF-1 may regulate the proliferation step during hair cell development and regeneration.

636.2

ESTABLISHMENT OF IMMORTALIZED UTRICULAR SUPPORTING CELL LINES USING A RETROVIRUS-MEDIATED GENE TRANSFER TECHNIQUE. W.-Q. Gao*, J. Valverde and J.L. Zheng. Dept. of Neuroscience, Genentech, Inc., South San Francisco, CA 94080.

Supporting cells in the inner ear sensory epithelium are most likely the progenitors for hair cells. To establish an in vitro model system of hair cell differentiation, we have transferred the tsA58 allele of the SV40 large T antigen oncogene into early postnatal utricular supporting cells using retrovirus. Utricular epithelial sheets containing supporting cells and hair cells were separated from early postnatal rats and plated on polylysine-coated culture dishes in serum supplemented medium. The cultured cells were transfected with a retrovirus containing temperature sensitive SV40 large T antigen. The transfected cells were subcloned and characterized with different markers. Immunocytochemical labeling confirmed that these cells were successfully transfected and expressed tight junction protein, an epithelial cell marker, but not fibroblast, glial or neuronal markers. The cell lines have been maintained for more than 9 months and grown for more than 20 passages. They exhibited similar features as those of the primary utricular supporting cells. For example, they grew in patches and assumed polygonal morphology. Although the immortalized cells grew rapidly in serum medium, their proliferation was limited when cultured in serum free medium. Several FGF family members, EGF and TGF-α promoted the proliferation of these immortalized cells cultured in serum free medium. Preliminary immunocytochemical labeling revealed that the immortalized cells have the capacity to differentiate and express hair cell markers. Therefore, the established utricular supporting cell lines could potentially provide an invaluable system for studying hair cell differentiation and regeneration.

636.3

IN VIVO GROWTH FACTOR (TGFα, IGF-1, RETINOIC ACID) TREATMENT OF AMINOGLYCOSIDE-DAMAGED UTRICLES SIGNIFICANTLY INCREASES THE RATE OF HAIR CELL RENEWAL. R. Kopke[1], R. Gabaizadeh[1], H. Staecker[1], B. Malgrange[2], P.P. Lefebvre[1,2], T.R. Van De Water[1,2]*. [1]Albert Einstein College of Medicine, Bronx, N.Y. 10461; [2]University of Liege, Liege, Belgium.

Vertigo and disequilibrium are common clinical problems that often share a common etiology of a loss of hair cells from the vestibular sensory receptors of the inner ear. We have developed a model for studying vestibular hair cell loss in vivo, which is a gentamicin-induced loss of utricular hair cells in the guinea pig. The inner ear is exposed to gentamicin by injecting a vestibulotoxic dose of this drug into the middle ear cavity. Delivery of growth factors to the inner ear is accomplished via direct infusion of a growth factor solution into the vestibular perilymphatic space over an 8 week period by an Alzet miniosmotic pump. Infusion of a combination of growth factors (i.e. transforming growth factor alpha, TGFα; insulin-like growth factor one, IGF-1; and retinoic acid, RA) over a period of 2 months significantly enhanced (i.e. a 3 fold increase) the renewal of the utricular hair cell population over that observed for either untreated or saline infused vestibulotoxin-exposed animals. Comparison of vestibulotoxin-exposed, untreated utricules to vestibulotoxin-exposed, growth factor treated utricles has shown that while there is some attempt at repair in the untreated utricles (i.e. a sparse population of renewing hair cells) in contrast the hair cell density in the growth factor treated utricles had returned to a near normal population density. These results suggest that growth factor therapy may one day be used in the treatment of inner ear disorders of balance.

Supported by a grant from The Hearing Research Fund of the Communication Disorders Institute, Montefiore Medical Center to TRV.

636.4

DIFFERENTIAL LECTIN BINDING REVEALS A SUBPOPULATION OF SENSORY HAIR BUNDLES IN AVIAN VESTIBULAR ORGANS M.E. Warchol* Dept. of Otolaryngology-HNS, University of Virginia, Charlottesville VA 22908.

Lectin binding studies have identified subclasses of sensory hair cells as a result of differing glycosylation of ciliary bundles. For example, peanut agglutinin (PNA) selectively labels the ciliary bundles of type II hair cells in the vestibular organs of chicks [R. Goodyear and G. Richardson, J. Comp. Neurol. 345: 267 (1994)]. Here I report that lectin from *Griffonia simplicifolia* -Isoform B4 (GS-IB4) labels a subpopulation of hair cells in the chick utricle, but does not label hair cells in the mature cochlea.

Utricles and cochleae from post-hatch chicks (P10-P20) and utricles from E12 chick embryos were fixed and processed for lectin-binding histochemistry. Video microscopy was used to quantify labeled ciliary bundles in whole mounts and in plastic sections. PNA- and GS-IB4-labeled cells were counted in selected 10,000 μm^2 regions of sensory epithelia. Quantitative results were obtained from the extrastriolar region (cotillus) of utricles, which lacks type I hair cells [J.M. Jørgensen, J. Morphol., 201: 187 (1989)].

PNA labeled nearly every cilia bundle in the cotillus of mature utricles, with a density of 172.1±6.2 PNA^+ bundles/10,000 μm^2 (mean±sem, n=40). In contrast, GS-IB4 labeled only a fraction of ciliary bundles in the cotillus (15.2±1.3/10,000 μm^2, n=50). When viewed in whole mounts, GS-$IB4^+$ ciliary bundles appeared smaller than the PNA-labeled bundles. The density of GS-$IB4^+$ hair cells was higher in embryonic specimens. Utricles from E12 embryos contained 88.7±5.0 GS-$IB4^+$ cells/10,000 μm^2 (n=32). No GS-$IB4^+$ hair cells were observed in mature cochleae (n=10 cochleae).

Avian vestibular organs continually produce hair cells during post-embryonic life. The observation that GS-$IB4^+$ ciliary bundles are smaller and less numerous than PNA^+ bundles, in combination with the high level of GS-IB4 labeling that was observed in embryonic utricles, suggests that GS-IB4 might selectively label the bundles of immature hair cells.

(Supported by grant DC 02291 from the NIDCD)

636.5

LOCALIZATION OF GAMMA-GLUTAMYL TRANSPEPTIDASE IN THE CHICK INNER EAR SENSORY EPITHELIA. J. Kil*, M.H. Hanigan[2], P.T. Taylor Jr.[3] and G.T. Hashisaki[1]. Depts. of Otolaryngology-HNS[1], Cell Biology[2], and Obstetrics and Gynecology[3], UVa Med School, Charlottesville, VA 22908.

Gamma-glutamyl transpeptidase (GGT) is a surface bound enzyme that cleaves gamma-glutamyl bonds such as those occuring in glutathione and glutathione bound conjugates. When GGT activity is irreversibly inhibited with acivicin, a glutamine analog, the destruction of renal proximal tubule cells normally induced by cisplatin treatment is prevented (Hanigan et al., 1994, Cancer Res, 54:5925-5929). Given the similarities in aminoglycoside and cisplatin ototoxicity and nephrotoxicity, we have examined whether GGT is also present in the inner ear sensory epithelia using an affinity purified polyclonal antibody against human GGT (AP5.1).

Inner ear sensory organs were dissected from three week-old White leghorn chicks and fixed in 4% paraformaldehyde/.1 M PBS for periods of one hr. Tissues were placed in 90% MeOH/0.3% H_2O_2 for 15 mins (x2), incubated in a series of aviden and biotin blocking solutions for 15 mins, followed by a 1.5% goat serum solution for 10 mins. A 1:1000 dilution of primary antibody was added to the tissues for 45 mins at room temperature. Tissues were incubated with a biotinylated secondary antibody for 10 mins at room temperature followed by an aviden binding complex solution for 10 mins. Specimens were stained in diaminobenzidine/H_2O_2 for 3 mins. Under light microscopy, GGT immunoreactivities were observed along the cellular membranes of cochlear and vestibular hair cells. Greater concentrations of label appeared along the luminal surface of these cells as compared with the basolateral membrane. Control inner ear sensory epithelia and kidney sections were devoid of label. Results demonstrate that AP5.1 can be employed to label cells within the chick inner ear sensory epithelia and kidney.

636.6

SELECTIVE INNER HAIR CELL LESIONS REDUCE THE SPONTANEOUS AND DRIVEN DISCHARGE RATE OF CHINCHILLA AUDITORY NERVE FIBERS. R.J. Salvi*, J. Wang, D. Ding and N. L. Powers. Hearing Research Lab, SUNY University at Buffalo, Buffalo, NY 14214.

Current theories assume that outer hair cells (OHCs) provide the inner ear with its exquisite sensitivity, sharp tuning and several of its nonlinear responses whereas inner hair cells (IHCs) are mainly responsible for transmitting this information to the central nervous system through the auditory nerve. To further evaluate this model, we selectively destroyed 25-35% of IHCs in the chinchilla using the antineoplastic agent, Carboplatin (50 mg/kg). Surviving OHCs were functionally intact as evidenced by normal distortion product otoacoustic emissions and cochlear microphonic potential. Auditory nerve fibers that synapse on surviving IHCs had low thresholds, sharp tuning, and two-tone suppression equivalent to those from normal controls. These results are consistent with current theories. However, the spontaneous discharge rate and maximum discharge rate to tones were reduced significantly. These deficits are presumably due to subtle pathologies in the surviving IHCs or their afferent synapses.

Supported by research grant R01DC00166-13 from the National Institute on Deafness and Other Communicative Disorders, NIH.

636.7

DISTORTION-PRODUCT OTOACOUSTIC EMISSION AMPLITUDE CHANGES IN THE AGING C57 MOUSE. A.M. Jimenez[†‡], B.B. Stagner[‡], G.K. Martin[†‡], and B.L. Lonsbury-Martin*[†‡]. [†]Neuroscience Program, [‡]Univ. of Miami Ear Inst., Miami, FL 33101.

Mutant strains of mice have proven to be very helpful in elucidating normal and abnormal mechanisms of hearing. Based upon the time course of hair-cell degeneration, the C57 (C57BL/6J) inbred mouse has been proposed as a model of early-onset presbycusis. In order to better understand and characterize this animal model, the basic features of distortion-product otoacoustic emissions (DPOAEs), at $2f_1$-f_2, $2f_2$-f_1, and $3f_1$-$2f_2$, were studied at 3,7,8,9,10,11, and 12 months of age. DPOAE level as a function of geometric-mean (GM) frequency from 4-16 kHz (DP-grams) were obtained at three stimulus levels (L_1=L_2=55,65,75 dB SPL). Additionally, growth of DPOAE level [input/output (I/O) functions] at a constant GM frequency (5.6,8.0,11.3,16 kHz), as a function of systematic 5-dB increases in primary-tone level, from 25-85 dB SPL, were also obtained, along with DPOAE level at a fixed f_2 frequency (f_2=8,11.3,16 kHz), as a function of primary-tone ratio (f_2/f_1=1-1.5). The major finding was that DPOAE amplitude for DP-grams, I/O functions and ratio functions systematically decreased with increasing age, for frequencies >8 kHz. These data support the notion that the C57 mouse shows gradual and systematic degeneration of cochlear outer hair cells with age. (Supported in part by the Public Health Service: DC00613, ES3500, GM16153 and the Neuroscience Program, Univ. of Miami Sch. of Med.)

636.8

WITHDRAWN

636.9

THE AUDITORY RECEPTOR OF THE EPILEPTIC HAMSTER STRAIN GPG/VALL. R.Cantos (1), R. Riquelme* (2), N. García-Atares (3), M.L. Sala (1), M.D. López (2) and J. Rueda (1). (1) Dpto. Histol., Fac. Medicina., Instituto de Neurociencias., Univ. Alicante; (2) Dpto. Biol. Cel. y Patol., Fac. Medicina., Univ. Salamanca; (3) Dpto. Anat. Fac. Medicina. Univ. Valladolid. SPAIN.

In the present work, basic morphological techniques have been used to study the auditory receptor of the hamster strain GPG/Vall, which shows genetic audiogenic seizures. Nothing is known about the auditory structures altered in such animals. The present results dealt with the description of the peripheral end of the auditory pathway, as a first attempt to characterize its changes in the mentioned animals.

In six hamsters (Mesocricetus auratus) of the GPG/Vall strain, the cochleae were removed, fixed with a mixture of alhehydes and embedded either in glycolmetacrilate or Epon. The cochleae of three other animals were studied by means of SEM. Hamsters of the same species were used as controls.

The overal morphology of the cochlea was not modified in the epileptic hamster. However, a number of changes were observed in both the organ of Corti and the spiral ganglion. The sensory epithelium appeared flattened in its lateral portion, where the outer hair cells disappeared except, occasionally, in the basal coil. The supporting cells of this area also showed an abnormal morphology. Inner hair cells were present along the cochlea, except in the very apex. The tectorial membrane appeared slightly swollen, and vacuoles were observed in the interdental cells, suggesting changes in the molecular composition of the membrane or in its hydration status. The spiral ganglion had a lower density of neurons than in normal animals.

These data suggest that, in absence of the outer hair cells, the auditory pathway could be overstimulated, participating in the genesis of the seizures.

(Supported by Spanish Government grants, FIS 94/1354 and FIS 94/1403)

636.10

GDNF PROTECTS BOTH COCHLEAR HAIR CELLS AND AUDITORY NEURONS AGAINST OTOTOXINS. E. Magal*, R.Kuang, G.Hever, F. Collins and J-C Louis. Department of Neuroscience, Amgen Inc., Thousand Oaks, CA 91320

Sensorineural hearing loss results from the degeneration of hair cells and/or auditory neurons (spiral ganglion neurons) in the cochlea of the inner ear. Aminoglycoside antibiotics, such as neomycin (Lerner et al, N. Engl. J. Med. 302: 1106-1109, 1980), and anti-neoplastic drugs, such as cisplatin (Strauss et al., Laryngoscope, 143: 1263-1265, 1983), are known ototoxins, that cause damage to hair cells and auditory neurons in humans and animals. We tested the effects of GDNF on the survival of cochlear hair cells and auditory neurons exposed to ototoxic treatments. Explants of P3-P4 rat cochleas were cultured floating in medium. Under control conditions, there was no significant decline in the number of hair cells after 4 days *in vitro*. Treatment with ototoxins for three days, starting 24 hours after plating, caused marked hair cell death: only 21.2 ± 6.0% hair cells survived the exposure to 35 µg/ml cisplatin and 7.4 ± 4.7% were found after neomycin (0.6 mM) treatment. GDNF (1-10 ng/ml), administered together with the ototoxins, increased the number of hair cells surviving cisplatin and neomycin toxicity to 46.7% and 37.4%, respectively. In dissociated cultures of 4-week-old rat cochleas, the number of auditory neurons declined to reach 48.5 ± 4.5% of their initial number after 4 days in culture. GDNF (1-10 ng/ml) increased the number of surviving neurons to 71.6 ± 7.4%. After exposure to cisplatin (5 µg/ml given 24 hr after seeding), only 6.1 ± 1.2% auditory neurons survived after 4 days. However, when GDNF (10 ng/ml) was given together with cisplatin, the number of surviving auditory neurons increased to 32.8 ± 1.0% of their initial number. These data demonstrate the protective effects of GDNF for both hair cells and spiral ganglion neurons and suggest that GDNF may be useful for treating sensorineural hearing loss. (supp:AMGEN)

636.11

DIFFERENTIAL DISPLAY IDENTIFIES UPREGULATION OF A MITOCHONDRIAL GENE DURING HAIR CELL REGENERATION IN THE CHICK AUDITORY SYSTEM. C. Helbig*, J.L. Zheng, A. Goddard and W.-Q. Gao. Dept. of Neurosci. and Molecular Biology, Genentech, Inc., South San Francisco, CA 94080.

Although it is well established that hair cells can regenerate successfully in chicks, the molecular biological mechanisms for this regeneration remain unknown. In the present study, we have employed the differential display technique in order to identify genes involved in or triggering hair cell regeneration in chicks following aminoglycoside treatment. Differential display is a rapid method to identify differentially expressed genes with small amounts of tissue.

Hair cell death was induced by daily injection of gentamicin (50 mg/kg, i. m.) into 3 days old white leghorn chicks for 9 days. Animals were sacrificed and the cochlear sensory papillae were dissected. Total RNA was extracted and differential display was performed using a primer kit from Display Systems. Of the one hundred and three upregulated bands identified, 35 bands were reamplified, cloned and sequenced. To confirm their upregulation, we employed RT-PCR and slotblot hybridization. Using RT-PCR and GAPDH as an internal control, the mitochondrial gene NADH-ubiquinone oxidoreductase was upregulated in the cochlea collected from gentamicine-treated chicks. Similar results were obtained using slotblot hybridization. These results suggest that mitochondrial activity is enhanced during the regeneration of hair cells and imply that agents promoting mitochondrial activity may facilitate hair cell repair and regeneration.

636.12

AUDITORY BRAINSTEM RESPONSE AS AN INDICATOR OF THE FUNCTIONAL CONSEQUENCES OF AMINOGLYCOSIDE OTOTOXICITY IN FISHES. H.Y. Yan* and T.N. Kenyon. T.H. Morgan School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225.

The ototoxic effects of aminoglycoside antibiotics are a serious limitation to their effective use in fighting infection in humans. At least one species of fish, the oscar cichlid, *Astronotus ocellatus*, suffers similar ototoxic reactions (i.e., selective damage to striolar hair cells of the inner ear) to gentamicin (GE), making the oscar a possible candidate for a wide range of ototoxicity studies. An additional useful aspect of the fish auditory system is that unlike most mammalian examples, damaged hair cells in fishes are capable of at least morphological regeneration. However, no data exists documenting the functional consequences of observed ultrastructural effects in fishes, largely because GE-dosed fishes suffer severe side effects, particularly edema due to GE nephrotoxicity, making them unresponsive to behavioral paradigms. Hence, we have employed auditory brainstem response (ABR), a method previously underutilized in lower vertebrates but widely applied in similar studies with birds and mammals, as an indicator of functional changes associated with known ultrastructural changes.

ABR waveforms in the oscar were obtained via implanted electrodes in response to tone burst stimuli. Relative frequency-specific ABR thresholds followed behavioral thresholds closely in untreated animals. Waveform amplitude decreased, and auditory thresholds increased after four daily injections of 20 mg/kg gentamicin. Ultrastructural evidence indicates loss of hair cell ciliary bundles in the striolar regions occurring 5-10 days post GE treatment, and subsequent regeneration of ciliary bundles occurring 10-20 days post treatment. ABR recordings indicate a decrease in waveform amplitude before visible signs of structural damage, with continued decreases through the tenth day post-treatment. These results also provide the first clear evidence of an important striolar contribution to auditory function in fishes.

(Supported by the Deafness Research Foundation)

636.13

DIFFERENTIATION AND THE EXPRESSION OF S-100 IMMUNOREACTIVITY IN SENSORY HAIR CELLS OF THE VERTEBRATE EAR. P.J. Lanford, J.C. Presson and A.N. Popper* Univ. of MD, College Park, MD 20742.

We are investigating the differentiation of sensory hair cells in the ear of the goldfish, *Carassius auratus*. In the goldfish saccule, hair cells in the rostral end of the epithelium stain positively with the antibody against S-100, a calcium binding protein (Saidel, et al. Br. Behav. Evol. 46: 362-370). It has been suggested that calcium binding proteins like S-100 may act as a means of buffering intracellular calcium in hair cells. The timeframe at which new hair cells begin expressing S-100 antigenicity may thus be an important developmental "moment". In this study, immunohistochemistry and autoradiography were used to determine the timeframe after S-phase at which new hair cells express S-100 immunoreactivity. The status of hair cell morphology at this timeframe was then assessed.

Post-embryonic goldfish were injected with a single dose of ^{3}H thymidine and allowed to survive 2, 3, 4, and 5 days post-injection. The saccules were dissected, fixed in paraformaldehyde and then processed as whole tissues for S-100 immunohistochemistry. The tissues were then embedded in epoxy resin, sectioned, mounted on glass slides, and dipped for autoradiography. After 10-14 days incubation time, the slides were developed and then assayed for the presence of double-labeled (S-100/^{3}H thymidine) hair cells. The results show that new hair cells first stain positively for S-100 at four days post-injection with ^{3}H thymidine. At the light level, the shape of these new cells is elongate, unlike surrounding mature hair cells which are more rounded or pear-shaped. However, apical structures such as the cuticular plate and a kinocilium are present in the new cells. We are currently using transmission electron microscopy to determine the status of ultrastructural development in these new cells and to elucidate the possible relationship between S-100 and hair cell function. (Supported by NIDCD training grant 5 T32 DC-00046-02.)

AUDITORY SYSTEMS: CENTRAL PHYSIOLOGY—PRIMATE CORTEX

637.1

CORRELATION CODING IN A STOCHASTIC NETWORK MODEL OF AUDITORY BINDING. R. Ritz* and T.J. Sejnowski. The Salk Institute, Computational Neurobiology Laboratory, POB 85800, San Diego, CA 92186, USA.

DeCharms, Schreiner, and Merzenich (Soc. Neurosci. Abstr. 21:1177, 1995) have provided evidence for stimulus–dependent changes in the correlations between spike trains of simultaneously–recorded pairs of neurons from the auditory cortex of marmosets even when there was no change in the average firing rates.

Most of the characteristics of these experimental observations can be reproduced by a simple model based on neurons having leaky integration, fire–and–reset spikes and with Poisson–distributed, balanced input. The source of synchrony in the model was common sensory input. Spike frequency adaptation was implemented by sensory–driven, delayed inhibition. The outputs of neurons in the model appear noisy (almost Poisson) owing to the stochastic nature of the input signal, but there is nevertheless a strong central peak in the correlation of the output spike trains.

The experimental data and this simple model clearly demonstrate how even a noisy–looking spike train can convey basic information about a sensory stimulus in the relative spike timing between neurons.

We address the binding problem and show why synchrony without periodicity might be advantageous in representing multiple objects at the same cortical site simultaneously. However, this binding scheme depends on different cells receiving common input which may restrict it to limited populations of neurons. In this context we discuss lateral interactions between the cells as well as active processing in the dendritic trees.

Supported by DFG (grant Ri 821/1-1) and The Howard Hughes Medical Institute.

637.2

SELECTIVITY FOR CLICK RATE IN A CENTRAL REGION OF MARMOSET PRIMARY AUDITORY CORTEX P. Bedenbaugh*, S. Cheung, C.E. Schreiner and M.M. Merzenich, *Department of Otolaryngology and Keck Center for Integrative Neuroscience, Univ. of California at San Francisco, San Francisco, CA*

We examined how the responses of cortical neurons to simple stimuli parametrically varied for frequency, intensity and modulation rate relate to their responses to natural vocalizations and other spectro-temporally complex stimuli. In the primary auditory cortex of the common marmoset (*Callithrix jaccus*) we mapped multi-unit responses to pure tones, entrainment to periodic click trains, and responses to marmoset vocalizations and other complex stimuli. Sharpness of tuning was measured by Q30dB. Tuning for click rate was assessed by vector strength, a normalized measure of phase locking of neural responses to click stimuli. Entrainment to the vocalizations and complex stimuli was assessed by R_e, the area in a 1/2 Hz wide bin centered at the stimulus envelope best repetition frequency (Wang *et. al.* J. Neurophysiol.,1995).

In one pentobarbitol anesthetized monkey (95 recording sites) we found a central region of A1 extending across the entire tonotopic axis where the vector strength was larger for click rates near 8 - 12 Hz than for slower or faster click rates. The responses in or near this click-rate tuned region were more likely to be non-monotonic with respect to intensity and had some tendency sharper tuning (higher Q30dB). In this region, adjacent penetrations found consistently high entrainment to vocalizations and other complex stimuli.

As in many species, responses of marmoset A1 neurons are tuned sound frequency, intensity and modulation rate. Our results suggest that there may be a map of temporal response properties on the medio-lateral axis, orthogonal to the tonotopic axis.

Supported by NIH DC00144 and NS10414, and by Hearing Research Incorporated.

637.3

HARMONIC COMPLEX TONES ELICIT RESPONSES DEFINED BY SPECTRAL COMPOSITION RATHER THAN PITCH IN PRIMARY AUDITORY CORTEX (A1) OF THE AWAKE MONKEY. Y. I. Fishman, D. H. Reser, S. Seto*, J. C. Arezzo and M. Steinschneider., Albert Einstein College Of Medicine, Bronx, NY 10461.

Neuromagnetic studies in humans and single-unit studies in monkeys have engendered conflicting views regarding the role of A1 in pitch encoding. While the former support the notion that A1 exhibits a topographic organization based on the pitch of complex tones, the latter have failed to provide evidence for the existence of pitch selective cells. In contrast, single-unit studies support the classical tonotopic organization of A1 defined by the spectral composition of the stimulus. It is unclear whether the incongruity of these findings is due to spatial resolution limitations of scalp recordings or whether the discrepancy genuinely reflects pitch representation based on population encoding, a dimension of analysis that eludes single-unit recording. To bridge the gap between these experimental approaches, we examined neuronal ensemble responses in A1 using auditory evoked potential, multiple unit activity, and current source density techniques.

Pure tones and harmonic complex tones missing the fundamental frequency (3rd, 4th, and 5th harmonics of 100, 250, 500, 1000, 1500, and 2000 Hz) were delivered at 60 dB SPL to the ear contralateral to the hemisphere from which recordings were obtained. Laminar response profiles in A1 reflected the spectral composition rather than the pitch (missing fundamental frequency) of the compound stimuli. Consistent with single-unit data, these findings indicate that cochleotopic organization is preserved at the level of A1. Thus, it appears that pitch encoding of complex sounds is more complicated than suggested by noninvasive studies based on the assumption of a single dipole generator within the superior temporal gyrus that corresponds to the fundamental frequency. Supported by DC00657 and MH06723 and the Institute for the Study of Music and Neurologic Function of Beth Abraham Hospital.

637.4

AMPLITUDE MODULATED TONES: EFFECTS OF CARRIER FREQUENCY ON PSYCHOPHYSICAL DISCRIMINATION AND RESPONSES OF PRIMARY AUDITORY CORTICAL NEURONS IN THE OWL MONKEY. R.E. Beitel*, C.E. Schreiner, X. Wang, S.W. Cheung, and M.M. Merzenich. Keck Center, UCSF. San Francisco, CA 94143.

Several distinct calls in adult owl monkeys contain amplitude modulated phrases with energy concentrated in the spectral region below 3-kHz. We have investigated the ability of monkeys to discriminate changes in the cadence of amplitude modulated (AM), low frequency tones (AM=4 to 40-Hz; carrier=0.5 to 2.0-kHz) that simulated temporal and spectral features that occur naturally in their vocalizations. Stable threshold and suprathreshold performance was obtained with carriers of 0.5-kHz and 1.0-kHz, but the monkeys were unable to discriminate changes in cadence of a 2.0-kHz carrier. Following training, the 0.3-kHz to 3.0-kHz region of primary auditory cortex was mapped with metal microelectrodes in the anesthesized monkeys. Multiunit neuronal responses (number of spikes, vector strength, interspike intervals) were analyzed as functions of AM and carrier frequencies. Preliminary results indicate that: 1) the number of spikes at recording locations with best frequencies (BFs) between 1.7-kHz and 3.0-kHz was greater than the number of spikes for locations with BFs between 0.3-kHz to 1.2-kHz ; 2) the proportion of interspike intervals (ISIs) that were grouped around the period of the AM envelope was smaller for locations with BFs between 1.7-kHz and 3.0-kHz; and 3) the signal to noise ratio (S/N=# periodic ISIs / # nonperiodic ISIs) was relatively low for locations with BFs between 1.7-kHz and 3.0-kHz. The results suggest that the proportion of periodic ISIs varied with carrier frequency and may have influenced the monkeys' ability to discriminate changes in the cadence of AM signals. (Supported by NIH Grant NS-10414 and ONR.)

637.5

RESPONSES TO COMPLEX SOUNDS IN THE LATERAL BELT AREAS OF AUDITORY CORTEX IN AWAKE RHESUS MONKEYS. B. Tian* and J. P. Rauschecker, Lab. Neuropsychology, NIMH, Bethesda, MD 20892 and Georgetown Institute for Cognitive and Computational Sciences, Washington, DC 20007

The posterior part of the superior temporal gyrus (STG) in anesthetized rhesus monkeys contains neurons that respond to various types of complex sounds, such as bandpassed noise (BPN) bursts, frequency modulated (FM) sweeps, and monkey calls. On the basis of BPN best center frequencies (BFc), three areas in this lateral belt region can be identified, which have been termed AL, ML, and CL (Rauschecker et al., Science 268, 1995). We now examine the responses of neurons in these areas to complex sounds in awake restrained animals to eliminate any effects of anesthesia.

The existence of three maps of BFc along the lateral fissure was confirmed in two awake monkeys with BPN bursts. The use of chronic recording techniques permitted more complete mapping in each animal. As in the anesthetized monkey, neurons in all three areas responded generally better to BPN and FM stimuli than to tone bursts ($p<0.05$; paired t-test). Most neurons were tuned to specific center frequencies as well as bandwidths. Best bandwidth (BBW) generally increased when the electrode was moved more laterally. Neurons in awake monkeys also were specific for the rate and direction of FM sweeps. The degree of specificity and the overall preference of neurons in the three areas did not differ from that in anesthetized animals. Neurons in AL preferred FM rates around 50 Hz/ms, whereas ML preferences were twice as high. Overall, 66% (46/70) of the neurons were FM-direction selective. When monkey vocalizations were used for stimulation, temporal and/or spectral combination-sensitivity was found in 7 out of 34 neurons. In 15 out of 32 neurons the response to the original vocalization was at least twice as good as the same vocalization played backwards, which indicates a fair amount of selectivity for monkey calls.

Thus far, the present results from awake monkeys fully confirm the findings from isoflurane-anesthetized monkeys. The existence of neurons sensitive to specific combinations of call elements indicates that the lateral areas may be involved in the processing of communication sounds. Similar neural mechanisms in the posterior STG of the human brain may be used for the phonological decoding of speech.

637.6

DISCHARGE PATTERNS OF AUDITORY CORTICAL NEURONS EVOKED BY SPECIES-SPECIFIC VOCALIZATIONS AND SYNTHETIC COMPLEX STIMULI IN ALERT *MACACA MULATTA*. M.J. Tramo*, B.F. Bellew, & M.D. Hauser. Depts. of Neurobiology, Neurology, Psychology, and Anthropology, Harvard University, Cambridge, MA 02138

Lesions of auditory cortex in humans and non-human primates can profoundly impair the perception of vocal communication sounds with relatively subtle effects on elementary psychoacoustic functions. Neurons in monkey and cat auditory cortex often exhibit greater sensitivity, response magnitude, and/or response complexity over time when vocalizations and synthetic complex stimuli are presented than when steady-state pure tones are presented, especially in awake preparations. We are investigating the relationship between these neuronal response properties and the acoustical features of vocalizations along the depth of microelectrode penetrations into the dorsal surface of the posterior STG containing neurons with pure tone BFs below 2 kHz at 70-90 dB SPL. Our preliminary observations suggest that patterns of neuronal excitation and inhibition unfold over time in relation to the envelope of the acoustic waveform and the "instantaneous" spectral energy distribution of the vocalization. The temporal pattern of excitation and inhibition over tens to hundreds of milliseconds, rather than, or in addition to, the magnitude of excitation at one or more points in time, may be an important element in the neural code used to represent different vocalizations. Constancy of the temporal response pattern in the face of minor variations in spectral content may confer neuronal response invariance within a given vocalization class.

Supported by DC00071 (MJT) & NSF-YIA SBR-9357976 (MDH)

637.7

COMPARATIVE ANALYSIS OF COMPLEX SOUND ENCODING IN AUDITORY CORTEX OF MONKEYS AND HUMANS. M Steinschneider*[1], I Volkov[2], D Reser[1], M Ollendieck[2], Y Fishman[1], H Damasio[2], J Arezzo[1] and M Howard, III[2]. Albert Einstein College of Medicine[1], Bronx NY, 10461 and University of Iowa College of Medicine[2], Iowa City IA, 52242.

Caution must be exercised when using animal data to identify neural mechanisms important for human speech and music perception. We tested the relevance of animal data by examining auditory evoked potentials (AEP) intracortically recorded from a multicontact electrode implanted within the right auditory cortex of a human patient. We compared these responses with laminar profiles of AEPs, multiunit activity and current source density recorded from primary auditory cortex of awake monkeys. Stimuli were synthetic syllables that varied in their consonant place of articulation (POA) and voice onset time (VOT), click trains, and 2-note music intervals.

Similar response patterns were observed in both species. POA was reflected by larger initial "on" responses that varied with the tonotopic specificity of the recording sites. VOT was reflected by single "on" responses for voiced consonants, and "double on" responses for unvoiced consonants. Categorical-like behavior of the "double on" response was observed across a 20-40 msec boundary for both the syllables and the 2nd pulse of click trains. Repetitive stimuli elicited phase-locked activity at rates of 100 Hz. Dissonant music intervals elicited a low frequency "roughness" in the evoked responses that was diminished in the responses to consonant music intervals. Cross species similarities in early auditory cortical response patterns evoked by speech and music stimuli suggest that animal models are relevant for clarifying initial auditory cortical mechanisms involved in human complex sound processing. (supported by Beth Abraham Hospital Institute for Music and Neurologic Function[1] and grants DC00120[2], DC00657[1] and MH06723[1])

637.8

SIMULATIONS OF SELF-ORGANIZING FEATURE MAPS IN PRIMARY AUDITORY CORTEX. N.N. Ge, C.E. Schreiner, H.L. Read*, Coleman Laboratory, Keck Center for Integrative Neuroscience, UCSF, San Francisco, Ca 94143.

Auditory cortical map has a functional and structural patchiness that is inherent to self-organized, topology-preserving projections. A cochleotopic representation is along one surface axis of primary auditory cortex (AI) and the bandwidths of single-neuron and multiple-neuron tuning curves are systematically arranged along the orthogonal axis. The intensity and the binaural coding were also indicated to be oriented orthogonally to isofrequency contours. Self-organizing feature maps suggest a process in which there is a decomposition of independently varying dimensions in the input space, which are mapped onto an output space. In this study we experimented with different simulation models using Kohonen associative learning rules to study the resulting feature maps. We used a 5 dimensional independently varying input space with the parameters uniformly distributed within a certain range to map to a plane, and the neighborhood size was defined as a function of the number of training epochs. When all inputs were randomly selected from within the same range and started at the same time, the resulting decomposed feature maps assumed randomly varying spatial features and relationships to each other. However, when we varied the dynamic range, delay of onset, and slope of rise or fall in the five input parameters in accordance with a few basic aspects of the maturation of auditory cortical response properties (J.J. Eggermont, ARO Vol. 19, p. 113, 1996) the resulting feature maps showed reproducible properties and interrelationships similar to those seen in physiological experiments. Our results suggest that the Kohonen learning rule can be used to capture the key characteristics of AI cortical feature maps, and specific features of the maps, such as, different degrees of patchiness, spatial frequency, and interrelationships, may be partially understood as consequences of early maturational processes.

Supported by the Office of Naval Research (N00014-94-1-00547).

637.9

ELECTRICAL STIMULATION OF HUMAN AUDITORY CORTEX. M.A. Howard*[1], I.O.Volkov[1], M. Ollendieck[1], H. Damasio[2], P.Abbas[3], B.Gantz[3]. [1]Div. of Neurosurgery,[2]Dept. Neurology,[3]Dept. Otolaryngology, University of Iowa School of Medicine, Iowa City IA 52242.

Useful insights concerning sensory cortex function may be gained by studying the effects of electrical stimulation in awake humans. In the current investigation, hybrid clinical-research depth electrodes (HDEs) were placed into the non-dominant (right) Heschel's gyrus of four epilepsy surgery patients (2 acute intraoperative, 2 chronic) as part of their clinical treatment plan. In addition to clinical EEG data, microelectrode recording and electrical stimulation data was gathered using the HDEs without placing patients at increased risk. A wide variety of electrical stimulation induced auditory perceptions were described. Most often patients "heard" these perceptions in the contralateral (left) ear. The quality of most percepts were similar to those described previously by Penfield and others (e.g. "propeller like", "whooshing", "sound of a fast jump rope"). In one patient, stimulation of lateral Heschel's gyrus resulted in prolonged suppression of hearing, or "muffling", that involved both hemifields and persisted beyond the duration of electrical stimulation. A new finding was noted in one patient whereby pure tone perceptions of differing frequency were consistently evoked from a small region of Heshel's gyrus. The critical experimental variables appear to be; the exact anatomical (MRI) and electrophysiologically defined location within Heschel's gyrus, stimulus waveform, frequency and polarity. These findings demonstrate a highly complex organizational pattern within the human auditory cortex.

(supported by Carver Trust, Hoover Foundation, and DC00120)[1]

637.10

TWO-DIMENSIONAL SOUND LOCALIZATION OF BROAD-BAND AND BAND-PASSED NOISE IN NORMAL HUMAN SUBJECTS. D.C. GUARD, M. L. PHAN, & G.H. RECANZONE* Center for Neuroscience, Dept. of Neurobiol., Physiol., and Behav., and Neurosci. Grad. Group, U. C. Davis, Davis, CA 95616.

Previous studies have shown that humans are fairly accurate in absolute localization of broadband noise stimuli (Makous and Middlebrooks, JASA 87:2188-2200, 1990). We have extended these studies to include 1-octave band passed stimuli with different center frequencies in order to determine the relative contributions of different frequency components to absolute sound localization ability.

All experiments were conducted on normal hearing subjects (aged 21-26 yrs.) in a darkened sound booth with echo-attenuating foam. Subjects were required to orient their heads within 2 deg of directly forward (0,0) to initiate a trial. Head position was measured using a magnetic search coil. A 200 msec (5 msec rise/fall; 60 +/- 3 dB SPL) free-field acoustic stimulus was presented from one of 125 possible locations (+/- 30 deg in azimuth and elevation), and the subjects was asked to orient their heads directly toward the perceived location of the sound. Stimuli consisted of broadband noise, low frequency (750-1500 Hz), mid-frequency (3-6 kHz) and high frequency (5-10 kHz) noise. Localization accuracy was measured as the difference between the real location and the mean final head position. Localization precision was measured as the vector strength calculated by defining a vector with the base at (0,0) and the tip at the final head position for each trial.

Accuracy was similar for broadband and high pass noise stimuli. Mid-range noise accuracy was moderate and low-pass noise localization was least accurate. Precision was also best for broadband noise and high-pass noise. Mid-range noise showed the lowest precision, with low-pass noise only slightly better.

These data indicate that the highest frequencies (5-10 kHz) provide the most salient cues for absolute localization in both azimuth and elevation, and that these frequencies can account for much of the accuracy and precision of sound localization to broadband noise stimuli. Funded by PHS DC-02371 and the Alfred P. Sloan Foundation.

637.11

ABSENCE OF AUDITORY N1 IN CHILDREN. H.J. Gould[1], D.F. Rose*[2], R.E. Greenblatt[3], AND M.A. Pousson[1]. [1]Univ. Memphis and [2]Univ. Tenn. Memphis TN 38105, and [3]Source/Signal Imaging, San Diego CA 92102.

The N1 is a consistent feature of the auditory evoked response (AEP) in adults, occurring approximately 100 msec following stimulus onset. We compared AEPs from 10 normal adults (ages 20-30) and 20 normal children (ages 5-12) and observed clear differences between adults and children in the 75-150 msec latency range.

880 hz 30 msec Blackman-windowed tone bursts were presented unilaterally (ISI randomly varying between 0.5 and 1 second), using insert headphones (Etymotic). The data were recorded continuously from 62 scalp electrodes (Electrocap) + 2 eye movement electrodes, digitized at 200 sec^{-1} (Neuroscan SynAmps) and filtered from DC to 40 hz. Data were averaged off-line following artifact rejection and baseline correction, and analyzed using source estimation (EMSE, Source/Signal Imaging).

All adult Laplacian maps showed a prominent N1 maximal sink in the midline frontocentral scalp region with a latency of 100-115 msec, corresponding to a voltage negativity, when appropriately referenced. In contrast, the children's Laplacian maps show a prominent frontal source in the latency range 95-120 msec, corresponding to a voltage positivity. The estimated generator for this peak has a contralateral temporal location similar to the adult, but the children's principal generator orientation is opposite that of the adult. After 175 msec a frontal negativity in children was inconsistently present and differed in distribution from the adult N1.

The absence of auditory N1 in children under these stimulus conditions and its consistent presence among adults may be a useful marker of cortical maturation. This work was funded in part by an NIMH grant to REG and a Herbert and Mary Shainberg Neuroscience Program grant to DFR and HJG.

637.12

TEMPORAL ORDER JUDGMENTS AND RELATIVE SOUND LOCALIZATION ABILITY IN NORMAL HUMAN SUBJECTS. M.L. PHAN*, P.G. GEIGER, D.C. GUARD, AND G.H. RECANZONE Center for Neuroscience, Neuroscience Graduate Group., Dept. of Neurobiol., Physiol., & Behav., U. C. Davis, Davis, CA 95616.

The functional role of the auditory system is similar to that of the visual system, namely to determine the spatial location and to resolve the characteristics of the stimulus. To characterize the relationship between these two abilities, normal hearing subjects (21-26 yr.) were asked to detect either a change in location ("where") or temporal order ("what") of tone-pip sequences on randomly interleaved trials.

All experiments were conducted in a darkened sound booth lined with echo-attenuating foam. Stimuli were four tone-pip sequences presented in the free field every 600 msec (56 +/- 3.5 dB SPL). Each stimulus was identical for 3-8 presentations before either changing location or temporal order. Pip duration and inter-pip interval was held at a constant ratio of 3:5 for each four-pip sequence, but the pip duration varied randomly from 15 to 45 msec within a trial. For the temporal order task, subjects had to detect a change in the order of the frequency of the four tone pips from a comparison sequence (standard-high-low-standard) to a target sequence (standard-low-high-standard) using 1, 2 and 4 kHz as standard frequencies. For the localization task, these same stimuli were presented initially at 32 degrees to the right and then moved to one of 5 other locations toward the midline. On 1/2 of the trials, the subjects received a cue to indicate whether the stimulus would change in location or temporal order; on the other 1/2 of the trials, the subjects did not receive a cue.

Sound localization performance for all subjects was dependent only on the standard frequency. Temporal order performance was dependent on pip duration, pip number, and the difference in frequency between the pips. Performance when only the middle two pips were presented was better than when four tone pips were presented. There was no significant effect of directed (cued) or divided (non cued) attention on either task. These results suggest that human subjects are able to process both types of information independently, indicating that independent neuronal mechanisms may underlie these perceptual abilities. Funded by the Klingenstein Foundation.

637.13

FREQUENCY AND LOCATION GROUPING EFFECTS IN PERCEPTION OF TONE SEQUENCES, K. N. O'Connor* and M. L. Sutter. Center for Neuroscience, University of California, Davis CA 95616.

The capacity of the auditory system to resolve distinct events comprising an acoustic stream is limited by the stream's spectral and temporal structure. Studies of this ability have typically required observer identification of order or pattern in rapidly cycling sound sequences (*auditory streaming*). We have used a variant of this technique to quantitatively examine human detection for reversal in order of two repeated target tones as a function of 1) varying one tone's frequency, 2) the presence or absence of a repeating background tone, and 3) the spatial separation between target and background. The background and constant target tone were 1 and 1.03 kHz, respectively; the other target tone varied between 1.045 and 8.50 kHz over trials. Tones were 50 ms in duration (5-ms rise/fall), presented in the free-field at 48 +/- 2 dB, at 50-ms intervals.

Performance in the absence of the background tone was nearly errorless at all but the smallest target frequency difference (Δf). In the presence of the background, reversal detection was described by an inverted U-shaped function that approached symmetry when plotted against log Δf. Specifically, performance: 1) was close to chance levels at the smallest Δf value (15 Hz), 2) rose rapidly with increasing Δf, reaching a maximum of about 90% correct in most subjects at about 100 Hz (range = ~50-250 Hz), and 3) declined slowly with further increases in Δf, reaching a minimum again close to chance at Δf = ~4-8 kHz. Preliminary results indicate that reversal detection was improved by spatially separating the location of the target and background.

These results are consistent with the views that 1) detection of change in the temporal organization of an acoustic stream follows known principles of auditory segregation and grouping, and 2) that free-field source location may play an important role in segregating distinct elements within a stream.

Work supported by Sloan Foundation and NIDCD Grant DC02514-01A1.

637.14

NEURONAL PROCESSING OF PERIODICITY PITCH MAY EXPLAIN THE DIFFERENCE BETWEEN 'RELATIVE' AND 'ABSOLUTE' PITCH PERCEPTION G. Langner* and Stefan Bleeck. Zoological Institute of THD, 64287 Darmstadt, Germany.

Acoustic signals with the same periodicity have the same pitch, nearly independent of waveform or spectral content. While only few people with 'perfect' or 'absolute' pitch are able to determine without effort the pitch of harmonic sounds according to musical notation, most people have relative pitch- the ability to identify or even name tonal intervals. A theory based on mechanisms of temporal periodicity analysis in the auditory midbrain (Langner, Hearing Res.60, 1992), provides an explanation for these complementary percepts, and is supported by new psychophysical experiments. The model makes use of a temporal representation of frequencies of fundamentals and their harmonics and includes internal reference periods provided by intrinsic neuronal oscillations. Due to their oscillatory and integrative properties, neurons in the cochlear nucleus produce synchronized and delayed responses to periodic envelopes which coincide on neurons in the auditory midbrain. The model may explain the percept of the missing fundamental and the pitch shifts elicited by anharmonic amplitude modulations. For relative pitch neuronal processing may be described in the following way. An integrating circuit which generates spike intervals multiple to the period of a harmonic is triggered by each envelope cycle. Provided the signal periods match, the resulting spikes coincide with spikes from the intrinsic oscillator which are triggered one envelope period later. The corresponding coincidence neuron thus may indicate which harmonic relates to a certain fundamental frequency ('binding'). However, the coincidence relation is ambiguous (many-valued), because it may be fulfilled by a number of (harmonically related) fundamentals. These ambiguities in temporal processing are necessary for relative pitch perception. In the case of absolute pitch intrinsic oscillations provide a reference for the measurement of signal periodicities. Oscillations synchronized 1:1 to fundamentals are compared by coincidence detection to appropriate multiples of the periods of harmonics. Since the tuning of intrinsic oscillations removes the ambiguity and prevents a response to more than one fundamental, this mechanism is inappropriate for the detection of harmonic relationships and may account for difficulties of subjects with absolute pitch in relative pitch tasks.

637.15

AUDITORY EVOKED POTENTIALS IN IDENTICAL QUADRUPLETS, S. A. Curtis and L. J. Achor*. Dept. of Psychology and Neuroscience, Baylor Univ., Waco, TX 76798-7334

Auditory evoked potentials were recorded in identical 19-year-old female quadruplets to assess consistency in evoked potentials. Brief tones were presented through headphones at 1/second for fifty trials. Recordings were obtained from the vertex using linked mastoids as reference. The EEG was amplified and filtered with a Grass P-15 amplifier. A signal averager sample the EEG (one msec dwell time) with a 36 msec pre-stimulus period and a 476 msec post-stimulus period. The averaged data was downloaded to a microcomputer for baseline adjustment and off-line analysis.

Morphology of the waveforms from the four subjects was very similar with distinct positive peaks at about 60 msec (P1) and 150 msec (P2) post-stimulus. Less distinct negative peaks were evident at approximately 80 msec (N1) and 215 msec (N2). Amplitude varied substantially between the four individuals. Latencies for N1 and N2 were difficult to determine as the peaks were broad and irregular. Latencies for P1 and P2 could be determined more readily as the peaks were sharply defined. Across the four subjects P1 and P2 varied within a very small range (59-62 msec for P1 and 147-150 msec for P2).

The small range of latency values for P1 and P2 are suggestive of a genetic basis for processing speed.

Supported by a university sabbatical and University Research Committee award to L. J. Achor from Baylor University

637.16

INDEPENDENT COMPONENT ANALYSIS APPLIED TO AUDITORY EVOKED GAMMA BAND AND 40 HZ STEADY STATE FIELDS. K. H. Knuth*. Kresge Hearing Research Lab. Louisiana State University Medical Center, New Orleans, LA, 70112, B. J. Schwartz. MEG Laboratory, Scripps Research Institute, La Jolla, CA 92037.

The magnetic fields from auditory click evoked gamma band responses (AEGBRs) and 40 Hz auditory evoked steady state responses (SSRs) driven by a 40 Hz click train were examined using a 37-channel biomagnetometer. Our past work has suggested that the AEGBRs are generated by two independent sources and that the SSR is mostly likely generated by a nonlinear superposition of the transient AEGBRs evoked by the individual clicks in the click train. In this study we use independent component analysis (ICA) to separate the independent sources responsible for the responses. The results from the ICA are compared to the results obtained from the equivalent current dipole fits to obtain a better understanding of the behavior of the generators of these responses.

Work supported by NIH NIDCD 5 T32 DC00007, Kresge Hearing Research Laboratory, Scripps Research Institute, and Biomagnetic Technologies Inc.

637.17

INTRACRANIAL EVOKED POTENTIAL AND SINGLE UNIT RESPONSES TO AUDITORY STIMULATION RECORDED IN HESCHL'S GYRUS. C.L. Wilson*, R.J. Staba K.A. McDonald, I.Fried. Brain Research Institute, Depts. of Neurology, Psychiatry and Div. of Neurosurgery. UCLA Center for Health Science. Los Angeles, CA 90024.

Studies of primary auditory cortex in lower primates show a tonotopic organization in which of high and low frequencies occupy spatially distinct areas (Merzenich and Brugge, Brain Res.1973,50:275-296). In humans, the primary auditory cortex (area 41) occupies the middle part of the anterior transverse gyrus and a portion of the posterior transverse gyrus, but there is little direct evidence concerning its receptive properties.

A patient with intractable partial complex seizures underwent bilateral depth electrode implantation in six areas of the temporal and frontal lobes in order to electrographically localize the origin of his seizure onsets. Because transient loss of hearing was sometimes associated with his seizures, electrodes were placed in right and left transverse gyrus. Electrode contacts were spaced at 6mm intervals spanning the mediolateral extent of the superior temporal gyrus. Nine platinum microwires (40µm diam) extended a few mm beyond the tip of each electrode. Clicks and tone bursts (90 dB) were delivered at rates from 14.3 to 1.1 Hz and 10 to 20 msec tone bursts were tested at 10 frequencies, ranging from 250Hz to 8kHz.

Evoked responses were most prominent in the gyrus contrateral to the ear stimulated, and in the more medial contacts of the electrodes. Onset latencies ranged from 16 to 22 ms, with a major negative peak at 60 msec. Of 6 microelectrodes in right transverse gyrus, 3 recorded cells responsive to auditory stimuli. Differential response to bilateral vs unilateral stimulation, frequency specificity of deep vs shallow electrode placements and properties of cells responsive to auditory stimulation will be presented. Supported by NIH grant NS02808

637.18

PHYSIOLOGICAL CORRELATES OF BINAURAL CLICK TRAIN FREQUENCY ILLUSIONS IN MACAQUE AUDITORY CORTEX. DH Reser*, YI Fishman, JC Arezzo, and M Steinschneider Albert Einstein College of Medicine, Bronx, NY 10461

Psychophysical investigations of interaural switching time (IST) in humans have shown that at frequencies greater than 10 Hz, subjects consistently underestimate click rate by 35-50% when component clicks alternate between subjects' ears vs. presentation to one ear alone. This limit on system performance may affect auditory streaming, particularly the sequential integration of stream components. Streaming seems to be critically important for processing time variant stimuli (e.g. speech and music), however, the underlying physiological processes are not known. Using a multichannel intracortical electrode in awake macaques, we recorded auditory evoked potentials, multiunit activity, and derived current source density, in response to 500 msec click trains presented at 60 dB SPL. Stimuli ranged in frequency from 5 to 1000 Hz, and were separated by 500 msec.

Phase locked activity (PLA) in response to single ear, binaural, and alternating presentations accurately reflected the stimulus frequency below 10 Hz. Above 10 Hz, PLA in binaural and single ear conditions matched the click rate, while PLA in response to alternating clicks was approximately ½ of the stimulus frequency. This effect was confirmed by Fourier analysis of digitized waveforms.

These results indicate that the upper frequency limit for accurate encoding of laterally alternating click trains in monkey auditory cortex is on the order of 10 Hz, which is in agreement with human behavioral data. This frequency limit may be important for assigning individual sounds to perceptual streams and for auditory encoding of stimulus movement. The ability to model these parameters in nonhuman primates will allow physiological investigation into the role of binaural interaction in extracting individual sound sources from complex auditory environments. Supported by DC00657 and MH06723, and by the Institute for the Study of Music and Neurologic Function of Beth Abraham Hospital.

637.19

MONAURAL AND BINAURAL SOUND LOCALIZATION IN HEMISPHERECTOMIZED PATIENTS. N. Lessard*1, F. Leporé1, P. Poirier2, J. Villemagne1 and M. Lassonde1. 1. Groupe de Recherche en Neuropsychologie Expérimentale, Univ. de Montréal, Canada, H3C 3J7. 2. Dept. of physiology, Kansas Univ. Med. Ctr., Kansas City, KS, 66160.

The present study was carried out to evaluate how subjects having unilateral cortical damage localize sound in three dimensional space. Response accuracy to monaurally and binaurally presented auditory targets on the horizontal plane was thus examined in hemispherectomized subjects (Hs). It is possible with these subjects to evaluate not only their ability to use binaural cues with their residual auditory cortical structures but also to compare the relative contribution of the ipsilateral and controlateral pathways to localization performance. The latter aim was examined using monaural stimulation. Three Hs and ten control (Cs) subjects were asked to localize broad band noise bursts (BBNBs) of fixed intensity presented on the horizontal plane in an anechoic chamber. BBNBs were delivered randomly through 16 loudspeakers, which were mounted at 10° intervals on a calibrated perimeter frame. Two conditions were tested: i) localization of a fixed-sound; ii) localization of the beginning and the end of a simulated moving sound. Listeners had to report the apparent stimulus location by pointing with the index finger its perceived position. Results indicated that Hs were as accurate as the Cs in binaural localization, indicating that one hemisphere and/or subcortical structures are sufficient to analyze the binaural cues to localization. Under monaural testing conditions, Hs performed as well as Cs when using the ipsilaterally projecting pathway but responded significantly better than Cs when using the controlateral pathway. This suggests not only that the ipsilateral pathway is quite adequate by itself to use the spectral information to localization sound but also that Hs learn to better utilize this information projecting to the normal cortex via the contralateral pathway, possibly to compensate for the lack of the supplemental contribution to localization which would normally be assured by the absent hemisphere.

637.20

INTERHEMISPHERIC BINDING OF ACTIVITY IN THE AUDITORY CORTICES A.A. Ioannides[1,2,*], L.C. Liu[1], J. Gross[1], J. Dammers[1], H.-W. Müller-Gärtner[1], Institute of Medicine, Research Centre Jülich, D-52425 Jülich, Germany[1]; Physics Department, The Open University, Milton Keynes MK7 6AA, U.K.[2].

The present study examines how cell assemblies of the bilateral auditory cortices in humans interact during sound perception. A 1KHz, 50 ms long tone was presented, in separate runs, to the left, right or both ears of 5 male, right handed subjects. The magnetic field was recorded over the left and right temporal areas using the twin (2x37 channel) MAGNES probe of BTi. Estimates of the electrical activity in the superficial areas of each auditory cortex were computed msec by msec, for a one second period beginning 200 msec before the onset of the tone. A separate estimate was computed for each one of the 120 tone presentations in each condition. During the prestimulus interval no temporal correlation was evident between the activity peaks in the left and right auditory cortex. The arrival of the stimulus established a temporal correlation, which we have quantified in terms of the delay time between peaks of activity in the left and right auditory cortices. For each condition an exponential form factor with a characteristic decay time, τ, described the distribution of trials at different interhemispheric delay times. Similar but not identical values for τ were appropriate during different post-stimulus periods. The mean and standard deviation of τ across the five subjects during the dominant M100 period was 16 ± 7 (mean$\pm$SD), 16 ± 4, and 23 ± 18 msec for binaural, left and right ear delivery respectively, when the left auditory cortex led, and 19 ± 6, 23 ± 12 and 16 ± 3 msec when the right auditory cortex led. The data show that the activity evoked by an external stimulus establishes a co-activation of distant, and in our case homologous areas, with a characteristic binding time of a few tens of msecs.

This study was supported by the Research Center Jülich GmbH.

638.1

RELATIVE CONTRIBUTIONS OF THE C1 AND C3 ZONES OF THE CEREBELLAR CORTEX TO EYEBLINK CONDITIONING IN THE FERRET

M. Ivarsson*, G. Hesslow and P. Svensson. Dept. of Physiology and Neuroscience, Lund University, Sölvegatan 19, S-223 62 Lund, Sweden.

The aim of the experiments was to study the contribution of different cerebellar cortical areas controlling eyeblink to the generation of a conditioned eyeblink response. Ferrets, decerebrated rostral to the superior colliculus and the red nucleus were trained in a classical conditioning paradigm. The conditioned stimulus (CS) was a train of electrical stimuli (15 pulses, 50 Hz , 1 mA) applied to the forelimb and the unconditioned stimulus (US) was a train of electrical stimuli (3 pulses, 50 Hz, 3-4 mA) to the periorbital region. The EMG was recorded from the orbicularis oculi muscle. The animal received CS-US trials (ITI 20-60s) until conditioned eyeblink responses (CRs) occurred on virtually every trial. Eyeblink related areas in the cerebellar cortex were localised by recording surface potentials on periorbital stimulation. A c3 area and a tentative c1 area were localised. A small lesion restricted to the c1 area abolished the CRs, while a much larger aspiration of the c3 area had no effect on the CRs. A filter paper (1x1 mm) soaked in lignocaine was placed on the different zones and the effect on the CRs was studied. Application of the filter paper on the c1 zone had a marked effect on the CR. This was not observed when applying the filter paper on the c3 zone.

In conclusion, the eyeblink related area in the c1 zone seems to be of greater relative importance than the c3 area in the generation of a conditioned eyeblink response.

Acknowledgement: The Swedish Medical Research Council (project no. 09899), the Knut and Alice Wallenberg foundation.

638.2

EFFECT OF CONDITIONED STIMULUS (CS) FREQUENCY ON THE LATENCY OF THE CONDITIONED EYEBLINK RESPONSE USING FORELIMB AND MOSSY FIBRE STIMULATION AS CS.

P. Svensson*, M. Ivarsson, G. Hesslow. Department of Physiology and Neuroscience, Lund University, Sölvegatan 19, S-223 62 Lund, Sweden.

The conditioned eyeblink response has a latency to peak that coincides with the onset of the unconditioned stimulus (US). If the interval between the conditioned stimulus (CS) and the US is increased, the CR peak is delayed so that it tends to occur at the new US onset. This makes functional sense because it maximizes the protective effect of CR. The neuronal basis underlying this timing mechanism is unknown.

The aim of the present study was to analyze the role of the cerebellum in the temporal delay of the CR. Decerebrate ferrets were classically conditioned with forelimb train stimulation as CS and periorbital stimulation as US. The CRs were recorded as EMG of the orbicularis oculi muscle. When the CRs appeared in each trial and the CR latency was stable, forelimb stimulation was replaced by stimulation of the brachium pontis (BP). This stimulation evoked true CRs. When the frequency of the BP stimulus train was increased (from 50 Hz to 100 Hz) both the CR onset- and peak-latencies were immediately decreased. When the CS frequency was reset, the latencies returned to initial values. Interestingly, when the CS frequency was kept high during a longer period the CR latency gradually shifted back to the original values. Identical results were obtained upon increasing forelimb stimulation intensity (from 1 mA to 2 mA).

These observations support the view that the cerebellum or/and the cerebellar output pathways are responsible for the delay of the CR.

Financial support: The Swedish Medical Research Council (project no. 09899) and the Knut and Alice Wallenberg foundation.

638.3

CRITICAL ROLE OF MOSSY FIBRES IN TRANSMITTING CONDITIONED STIMULUS INFORMATION IN CLASSICAL EYEBLINK CONDITIONING IN DECEREBRATE FERRETS.

G. Hesslow*, M. Ivarsson and P. Svensson. Dpt of Physiology and Neuroscience, Sölveg 19, S-223 62 Lund, Sweden.

Several lines of evidence suggest that a major site of synaptic plasticity for classical eyeblink conditioning is located in the cerebellum. It has been suggested that the conditioned stimulus (CS) information is transmitted to the cerebellum via the mossy fibres whereas the unconditioned stimulus (US) activates climbing fibre afferents, causing plastic changes to occur in the cerebellar cortex and/or nuclei. We tested the first part of this hypothesis in ferrets which had been decerebrated rostral to the superior colliculus and red nucleus. The animals were trained with a 300 ms, 50 Hz cutaneous electrical train stimulation of the forelimb as the CS and and periorbital electrical stimulation as the US. When the animals had learned to respond reliably with an eyeblink conditioned response (CR) to the forelimb CS, this was replaced with direct 50 Hz train stimulation of mossy fibres in the middle cerebellar peduncle (brachium pontis). Such stimulation immediately, i.e. without any further training, evoked characteristic CRs. If the response to the forelimb CS was extinguished by unpaired CS presentations, the mossy fibre stimulus no longer evoked a CR. When unpaired presentations of the mossy fibre CS were given until CRs to this CS had extinguished, the forelimb CS also failed to evoke a CR. These observations suggest that the mossy fibre stimulation activates the CS pathway and that the conditioned stimulus is transmitted via the mossy fibres. The results thus provide strong support for a cerebellar mechanism of conditioning.

This study was supported by grants from the Swedish Medical Research Council (project no. 09899) and the Knut and Alice Wallenberg foundation.

638.4

DIZOCILPINE (MK-801) BLOCKS HARMALINE-INDUCED TREMOR AND IMPAIRMENT OF LEARNING IN RABBITS.

W. Du*, V.J. Aloyo and J.A. Harvey. Div. of Behavioral Neurobiology, Dept. of Pharmacology, The Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19129.

Harmaline is known to produce tremors and retard learning as measured by acquisition of the rabbit's nictitating membrane response. Both of these effects have been attributed to harmaline's ability to increase the rhythmic bursting of cells in the inferior olive which give rise to climbing fibers that project directly onto Purkinje cells in cerebellar cortex. However, the precise receptor systems involved in harmaline's actions remain unknown. This study examined the role of the NMDA receptor in harmaline's actions. Harmaline (10 mg/kg, s.c.) produced intense tremors and severely retarded learning. Both of these effects of harmaline were significantly blocked by the prior administration of the noncompetitive NMDA channel blocker, dizocilpine (DIZ, 0.01 mg/kg, s.c.). This dose of DIZ had no effect on CR acquisition when given alone. A higher dose of DIZ (0.1 mg/kg) completely blocked the tremorogenic effects of harmaline. We found that NMDA receptors are present in the inferior olive of the rabbit as reflected by the specific binding of [^{3}H]dizocilpine to washed membranes and this binding was reduced by harmaline. Since, NMDA receptors occur in the inferior olive but not on Purkinje cells, the sole input of climbing fibers in cerebellar cortex, we conclude that harmaline's ability to activate the inferior olive and thus produce tremor and retardation of learning requires the normal activity of the NMDA receptor. Supported by MERIT award MH16841.

638.5

INFLUENCE OF THE CEREBELLUM ON THE COUGH MOTOR PATTERN.

Fadi Xu, Donald T. Frazier , Zhong Zhang, and Roger Shannon* Dept. Physiology, Univ. Kentucky, Lexington, KY 45036, and Dept. Physiology, Col. Med., Univ. So. Florida, Tampa, FL 33612.

The cerebellum modulates respiratory muscle activity, in part via influence on the central respiratory pattern generator (JAP 77:1232, 1994; Brain Res 705:53, 1995). Since coughing requires well-coordinated respiratory muscle activity, studies were conducted to determine if the cerebellum influences the centrally generated cough motor pattern. Phrenic and lumbar efferent neurograms (PN and LN) were monitored in decerebrated, paralyzed and ventilated cats. Fictive coughing was produced by mechanical stimulation of the trachea. Decerebellation (n=5) resulted in a significant decrease in the cough frequency without any consistent change in PN and LN amplitudes. Areas within the cerebellum known to have respiratory involvement (the fastigial nucleus, FN; and the interposed nucleus, IN) were selectively ablated to determine their potential role in the cough response. The cough frequency and LN amplitude were not altered by ablation of the FN (n=7), but were significantly reduced by lesion of the IN (n=7). PN amplitude was not affected by either FN or IN lesions. The baseline cardio-respiratory variables were not markedly changed following FN or IN lesions or decerebellation. These results suggest that the interposed nucleus is a putative site involved in regulation of the coughing reflex. (HL 49813 and HL 40369)

638.6

MODULATION OF SOMATOSTATIN INDUCED CEREBELLAR TOXICITY WITH GROWTH HORMONE IN SPRAGUE-DAWLEY RATS,

J.T.Robicheau, C.D.Balaban and J.D.Porter* Dept of Pharmacy and Therapeutics and Otolaryngology, Schools of Pharmacy and Medicine, University of Pittsburgh, Pittsburgh, PA 15260

Somatostatin (SRIF) is an endogenous neurotransmitter/modulator and regionally selective neurotoxin. Small intracerebroventricular doses of SRIF (20-40 µg) selectively damage sagittal Purkinje cell (PC) bands in the cerebellar anterior lobe, posterior lobe and hemisphere. Since SRIF participates in the regulation of growth hormone (GH) secretion, we tested the hypothesis that centrally administered GH may modulate SRIF toxicity in the cerebellum. Adult male SD rats were anesthetized and implanted stereotaxically with osmotic minipumps (Azlet® #2002 infusion rate, 0.5µl/hr) for the 14 day icv infusion of 1.8 ng or 18ng/0.5µl/hr. On the tenth postoperative day, animals were given an icv injection of SRIF bolus (30 µg/5µl) via the implant. Rats were euthanized on post-SRIF day 4 with a pentobarbital overdose (100mg/kg i.p.). The brains were stained for degeneration with a modified cupric-silver stain. Acute barrel rotation (BR) after the SRIF bolus was prevented by GH. The incidence of BR was 2/13 in GH-treated rats and 5/5 in aCSF treated rats (Yates Corrected χ^2 and Fisher's Exact Test, $p > 0$ .01). The appearance of PC degeneration was scored as mild (damage confined to lobule I-III), moderate (additional damage to damage lobules IX-X) or severe (additional hemispheric damage) or the absence of tissue damage. Animals that did not display BR had absent or mild damage. Conversely, animals that showed BR had severe damage. Rats which had pronounced SRIF-induced postural effects, but without BR displayed mild to moderate damage. These results suggest a neuroprotective effect of the GH infusion against SRIF-induced cerebellar toxicity in rats. (Support: University of Pittsburgh, Internal Funds)

638.7

EFFECTS OF SEROTONIN ON CEREBELLAR GRANULE CELL ELECTRICAL PROPERTIES Huo Lu* and L.J. Larson-Prior. Dept. of Neuroscience & Anatomy, Penn State Univ. College of Med., Hershey, PA 17033.

Serotonin has been hypothesized to play a role in state dependent setting of motor output in the cerebellum. Immunocytochemical and autoradiographic studies have been shown that all layers in the rat cerebellum receive serotonergic input. Serotonin has been shown to affect a transient outward potassium current, I_A and an inwardly rectifying current, I_h in cerebellar Purkinje cells (Wang *et al., Brain Res.* (1992) 571:345; Li *et al., Brain Res.* (1993) 617:87). Since these two currents exist in the cerebellar granule cell (GC), serotonin may affect GC processing in a similar manner.

Thin slices from rat vermal cortex were used to examine the effect of bath applied serotonin (1 μM) on GC electrical properties. Using patch clamp recording in the whole cell configuration, GC electrical responses were investigated under current and voltage clamp. Serotonin produced a minor increase on firing rate to depolarizing current injection, consistent with a reduction in I_A. This effect was confirmed under voltage clamp. Changes in the voltage response to hyperpolarizing current injection in the presence of serotonin were consistent with a reduction in I_h.

These observations were further investigated using our recently developed compartmental model of a cerebellar GC (Lu *et al., Soc. Neurosci. Abstr.* (1995) 21:916), by totally or partially removing either one or both of these potassium conductances from the somal compartment where those channels are located. Under these conditions, the response of the soma to hyperpolarizing and depolarizing current injection mirrored changes seen in whole cell recordings under current clamp. Thus, serotonergic effects on granule cell processing are partially mediated by changes in potassium conductances.

Supported in part by Penn. State Univ. , NS 30759 and IBN-9514844 (LLP).

638.8

CORTICOTROPIN RELEASING FACTOR (CRF) IN THE EMBRYONIC MOUSE CEREBELLUM, T. L. Overbeck,* G. A. Bishop and J. S. King Dept. of Cell Biology, Neurobiology and Anatomy and The Neuroscience Graduate Program, The Ohio State University, Columbus, OH 43210

CRF is present in climbing and mossy fibers in the cerebellar cortex of several mammalian species where it functions to modulate the responsiveness of Purkinje cells to both excitatory and inhibitory inputs. This peptide also is present during early stages of cerebellar development; however its function is unknown. We propose to use transgenic mice to block the expression of CRF or its receptor. Before these studies can be carried out, the developmental history of this peptide in the mouse cerebellum must be determined. An antibody to CRF, provided by W. Vale, was used to localize CRF immunoreactivity. The current data indicate that CRF is present as early as embryonic day (E)15. At E15 and E17, CRF is present in two distinct fiber bundles that traverse the junction between the brainstem and cerebellum. Immunoreactive varicosities are abundant at these same ages and are located throughout the cerebellum, primarily within the nascent Purkinje cell layer, and the intermediate zone of migrating cells. A few varicosities are present in the external granule cell layer (EGL) but none are seen in the ventricular layer. CRF immunoreactive cell bodies are present within the cerebellum at E15 in the intermediate zone, the subventricular zone and the ventricular layer especially in the medial regions of the cerebellar anlage. At E17, CRF cell bodies extend to the lateral regions of the developing cerebellum and are present primarily in the intermediate zone of migrating cells. CRF positive cell bodies are no longer present in the cerebellum after postnatal day 3. These data represent the first account of a transient expression of CRF in the developing cerebellum. A riboprobe for the CRF_1 receptor was used for *in situ* hybridization and revealed that the mRNA for the CRF_1 receptor is present in the EGL, the nascent Purkinje cell layer and the intermediate zone at E17. These data support our hypothesis that CRF regulates early developmental events in the cerebellum including cell survival and differentiation. (Supported by the Medical Scientist Program, The Ohio State University College of Medicine and NS 08798).

638.9

CORTICOTROPIN RELEASING FACTOR IN THE CEREBELLAR NUCLEI OF THE CAT. Georgia A. Bishop* Dept. of Cell Biology, Neurobiology and Anatomy and Neuroscience Program. The Ohio State University, Columbus, OH 43210

Corticotropin releasing factor (CRF) has been localized throughout the cerebellar nuclei of the adult cat (Cummings, JCN 289:657,1989). In this study the brainstem nuclei which give rise to CRF afferents to the cerebellar nuclei are identified. Fluorescent-labeled microspheres were injected into the nucleus interpositus (IP) of the cat to retrogradely label neurons that project to this area. The same sections were then processed for CRF immunohistochemistry using the PAP technique. Double labeled cells, (i.e. neurons that are both immunoreactive and retrogradely labeled) were localized using fluorescent microscopy. Using Berman's terminology, the following nuclei were found to give rise to CRF afferents to the IP: 1) the inferior olive; 2) the central inferior nucleus; and 3) the locus coeruleus. In addition, preliminary studies have been carried out to determine the physiological effects of CRF on nuclear neurons. To date, iontophoretic application of CRF increases the firing rate of both spontaneous and amino acid-activated nuclear neurons. CRF also has been applied to neurons that were activated by stimulation of the inferior cerebellar peduncle (ICP). Activation of this pathway produces a depression in the overall firing rate of nuclear neurons. If CRF is applied during the period of stimulation the firing rate increases, although it does not return to control levels. Likewise, if CRF is iontophoresed onto nuclear cells prior to activation of the ICP the extent of stimulation-induced suppression is reduced. In conclusion, these data indicate that CRF afferents to the cerebellar nuclei are derived from brainstem sources that also give rise to climbing fibers, mossy fibers, and a beaded plexus of fibers in the cerebellar cortex of the cat. At present it is not known if the nuclear input is derived from collaterals of afferents continuing on to the cortex or from a unique population of neurons within the nuclei of origin. The physiological data indicate that the amino acid-or stimulation-induced output of the cerebellum can be modulated if the peptide CRF is present in the local micorenvironment of nuclear neurons. (Supported by NS18028)

638.10

THE ORIGIN OF CALCITONIN GENE RELATED PEPTIDE (CGRP) WITHIN THE MOUSE INFERIOR OLIVARY COMPLEX, A. C. Peltier, K. V. Gregg, J. S. King* and G. A. Bishop. Dept. of Cell Biology, Neurobiology & Anatomy, and The Neuroscience Graduate Program, The Ohio State University, Columbus, OH 43210.

Neurons within the inferior olivary complex (IOC) are the source of climbing fibers to the cerebellar cortex. The activity of neurons in the IOC is regulated by both excitatory and inhibitory amino acids derived from various afferent systems. This neuronal activity may be modulated by peptides released by one or more of the same afferents to the IOC. The peptide CGRP is present in small varicosities which have an extensive distribution throughout the IOC as well as a population of larger profiles which have a more restricted distribution (Gregg et al. 1994 Neurosci.Abst.20:1759). CGRP has been shown to modulate olivary activity (Gregg & Bishop, Neurosci.Abst.21:1192). The intent of this study is to determine which brainstem nuclei give rise to CGRP varicosities within the IOC. Fluorescent labeled microspheres were injected into the caudal IOC to identify neurons which project to the nucleus. Sections from these cases also were processed for immunohistochemistry using a primary antibody to CGRP to identify neurons which contain the peptide. Retrogradely labeled neurons were located in all nuclei previously shown to give rise to olivary afferents. CGRP labeled cells were present in several brainstem nuclei including three which are known to project to the IOC, namely the locus coeruleus (LC), subparafascicular nucleus (SPF), and the zona incerta/Fields of Forel. Double labeled neurons were frequently seen in the ventral aspect of the LC, bilaterally. In addition, a few scattered double labeled neurons were present in the SPF. The afferents from the LC may distribute as the small CGRP-positive varicosities located throughout the olivary complex. CGRP derived from the SPF may distribute as the larger varicosities which have a more restricted distribution. The transmitter of the SPF is unknown. However, the LC is the primary source of norepinephrine (NE) to the cerebellum and other areas of the neuraxis. Future studies will be designed to determine if CGRP is co-localized with NE in olivary afferents or if the projection arises from an independent population of LC neurons. (Supported by the Roessler Foundation, OSU College of Medicine and NSF INT9319449).

638.11

MAPPING ESTRADIOL-SENSITIVE OSCILLATING NEURONAL CLUSTERS WITHIN THE INFERIOR OLIVE. R.S. Markowitz*, R. C.-S. Lin, J.K. Chapin, and S.S. Smith. Dept. of Neurobiology and Anatomy, Medical College of Pa. and Hahnemann Univ., Philadelphia, PA 19102-1192.

Ongoing studies from this laboratory have demonstrated that estradiol can enhance rhythmic discharge recorded from neuronal clusters within the inferior olivary (IO) complex during rhythmic behavior. In the present study we have examined the topographical localization of these estradiol-sensitive clusters. Towards this end, female rats were implanted with bundles of 15- 16 microwires (50 μ, NB Labs, Denison, TX) within the IO. Olivary discharge was then recorded from as many as 32 neurons simultaneously during spontaneous whisking behavior before and after administration of estradiol (2 μgs, i.p. in oil for 2 days) using software from Spectrum Scientific (Dallas, TX). At the end of the recording session, brains were removed, fixed in formalin, sectioned at 50 μ and stained with Cresyl violet for verification of electrode placement. Under control conditions, rhythmic olivary discharge was observed in phase with rhythmic whisking (8-9 Hz) in 20- 30% of the neurons recorded. Estradiol increased the spatial extent of synchronized, oscillations by an average of 150 μ ± 45 μ ($P<0.0001$ versus control) as well as the frequency ($P<0.05$ versus control) in medial and caudal portions of the MAO and DAO, with almost no effect within the PO. As gap junctions within the IO have been shown to play a role in synchronizing oscillations, a second experiment was conducted to test for the possibility that hormone treatment increased gap junction formation as has been demonstrated in uterus and brain, using connexin43 as a marker protein (Zymed Labs). Treatment with estradiol increased IO levels of connexin43 above control values, which suggests that increases in circulating levels of estradiol expand clusters of rhythmically discharging olivary neurons, possibly mediated via increases in dendro-dendritic gap junctions. This effect of the hormone may mediate reported effects of estradiol on facilitation of rapid movements. (Supp. by USAF93-1-0316 to SSS; NS23722, ONR95-1-0246 to JKC.)

639.1

CHOICE REACTION TIME IN CEREBELLAR DISEASE, S.E. Grill*. Dept. of Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD, 21205.

Simple reaction times are prolonged in human subjects and animals with cerebellar lesions and disorders. In the present study, reaction times of 7 normal subjects and 11 patients with cerebellar degenerations were compared during choice reaction time tasks with varying stimulus-response compatibility.

Subjects initially depressed a "home" button. After a random interval, a visual stimulus appeared indicating which of 7 buttons arranged in a semicircle around the home button should be pressed, as rapidly as possible. For each trial, the number of active buttons (choices) varied randomly from 1 to 7.

Four tasks with different stimuli but the same response (button press) were done: 1) All buttons initally red; subjects press button which turns green; 2) Each button a different color; subjects press button corresponding to color presented just above home button; 3) Each button a different color; subjects react to word spelling the color; 4) Each button a different color; subjects presented with list of 3 letter names associated with a color and react to presentation of a name. Seventy trials for each task were run.

Simple reaction times of patients (1047 +/- 524) were greater ($p < 0.02$) than those of normal subjects (383 +/- 33). However, slopes of the relations between reaction time and number of choices were similar for the two groups for each task including the task with greatest stimulus-response compatibility (task 1). Patients with cerebellar disease are able to take advantage of stimulus-response compatibility.

(Supported by the National Ataxia Foundation)

639.2

MODIFICATIONS OF POSTURAL RESPONSES DURING PERTURBED STEP INITIATION IN CEREBELLAR SUBJECTS. D. Timmann* and F.B. Horak. R.S. Dow Neurological Sciences Institute, Portland, OR 97210 and Dept. of Neurology, University of Essen, Hufelandstr. 55, 45122 Essen, Germany.

The aim of the present study was to investigate both the possible role of prediction of perturbation amplitude and the involvement of the cerebellum in the mechanisms involved in postural adaptation for voluntary movements. Nine cerebellar subjects and nine control subjects were instructed to either maintain stance or step forward in response to predictable and unpredictable amplitudes of backward surface translations. The size of the suppressed postural response was the same independent of prediction of perturbation amplitudes in both control and cerebellar subjects. Cerebellar subjects were able to suppress their initial postural responses to the same amount as normal subjects when instructed to step forward as soon as they feel the platform move backward, despite their hypermetria.

In conclusion, the dynamic interaction between automatic postural responses to an external perturbation and anticipatory postural adjustments for step initiation seems independent of prediction of perturbation amplitude and the integrity of the cerebellum. (Supported by NIA grant R01-AG06457 and Deutsche Forschungsgemeinschaft Ti 239/1-1)

639.3

POSTURAL RESPONSES TO CHANGING TASK CONDITIONS IN CEREBELLAR PATIENTS. P. Mummel, U.W.H. Krause, D. Timmann, R.A.W. Galuske*, H. Schicks, D. Böring[1], A.F. Thilmann[1], H.C. Diener and F.B. Horak[2]. Depts. of Neurology, University of Essen and [1]Fachklinik Rhein/Ruhr, 45122 Essen, FRG; [2]Posture Control Lab., R.S.D. Neurological Sciences Inst., Portland, OR 97209.

The aim of the present study was to investigate the role of the cerebellum in postural adaptation for both changes in the width as well as the direction of the support surface displacements. A total of 24 patients with chronic, isolated lesions of the cerebellum and 21 sex- and age-matched controls were tested. Both control and cerebellar subjects showed an immediate change in the response amplitude of the medial gastrocnemius and anterior tibial muscles when the direction of perturbation changed from a translation to a rotation and vice versa. Both control and cerebellar subjects also changed postural strategies with changes in the width of the support surface. A similar proportion of cerebellar patients and normals modified their postural strategies to include more hip strategy control when balancing across a narrow beam.

In conclusion, the integrity of the cerebellum does not seem to be critical for adaptation of postural synergies to changing task conditions. The present results confirm previous findings suggesting that the cerebellum's main role in automatic postural responses may be gain control.

639.4

TIMING OF MENTALLY SIMULATED MOVEMENTS IN PATIENTS WITH CEREBELLAR DISORDERS. Florian A Kagerer [1]*, Vlastislav Bracha[2], George E Stelmach[1], James R Bloedel[2]. [1]Motor Control Laboratory, Arizona State University, Tempe, AZ 85287, [2]Barrow Neurological Institute, Phoenix, AZ 85013.

Temporal features of mentally simulated actions were shown previously to correspond well to the temporal features of the same movement when actually performed. The purpose of this experiment was to examine whether mental simulation times (MSTs) reflect the increased movement times (MTs) resulting from the ataxia exhibited by patients with cerebellar pathology. Three patients with unilateral cerebellar lesions, 1 patient with cerebellar and cortical pathology, and 3 age- and sex-matched controls participated in the study. The *movement execution* part of the task required subjects to move a hand-held stylus horizontally on a digitizing tablet along a specified movement path (two conditions: pathwidth 3.5" and .75"); movement direction between start and endpoint changed three times. Subjects were instructed to move as straight as possible and at a comfortable speed. The *mental simulation* component required subjects to imagine the arm movement along the path without performing it. Subjects "started" the simulated movement in response to a tone and reported vocally when they had finished. 16 trials of mental simulation and 16 trials of movement execution were alternated; performance and simulation with both hands were assessed. Results show that 1) both the patients and normal subjects showed an increased MT as well as MST when executing or simulating movements through the narrower path, 2) cerebellar patients had substantially greater MTs and MSTs than controls, and 3) both the MTs and the MSTs of the patients were greater on the affected side than on the unaffected side. The findings suggest that the cerebellar pathology present in these patients did not disrupt their ability to simulate mentally their movements. Furthermore, their MSTs reflected the time required to execute their ataxic movements.

Supported by the Flinn Foundation, NIH Grant RO1 NS21958 and NIH Grant PO1 NS30013

639.5

SPEECH PERCEPTION AND PRODUCTION IN CEREBELLAR DISORDERS: THE CONTRIBUTION OF TIMING

S. Graeber*, H. Ackermann, I. Hertrich, I. Daum Dept. of Neurology and Inst. of Medical Psychology, Univ. of Tuebingen, Hoppe-Seyler-Str. 3, 72076 Tuebingen and Gartenstr. 29, 72074 Tuebingen, FRG

A role of the cerebellum as an "internal clock" has been proposed for a variety of motor and perceptual tasks, nevertheless an involvement in the processing of durational parameters of the perceived and produced acoustic speech signal has not yet been found (Gopal and Ivry, 92). In order to further investigate this issue we studied the performance of cerebellar patients (CERE) in a speech perception and production task. The results were also compared to matched groups of normal controls (NC) and patients with parkinson disease (PD). In the perception task the paradigm of categorical perception was applied using German words as minimal pairs which were manipulated with respect to the duration of voice-onset-time (VOT), vowel length (VL) and duration of closure (DCL). In the production task minimal pairs were spoken by the subjects and the duration of the parameters VOT and VL was analyzed.

The CERE group showed specific deficits in perception as in production tasks. The production of the VOT was significantly more variable compared to NC. In the perception task the CERE group did not show deficits in the perception of either VOT or VL. But there were clear deficits in the perception of the DCL. These results indicate that the cerebellar patients are not impaired in measuring time intervals in speech if they can utilize additional cues for discrimination (for example, acoustic energy). However, deficits are observed when these additional cues are not available and explicit temporal computation is required. In contrast, the performing of the PD group in the perception and production tasks differed markedly from that of the CERE group. These experiments suggest that there are specific cases of timing phenomena in oral language that specifically involve the cerebellum as a timing device.

Supported by the German Reseach Society (DFG; SFB 307/B10)

639.6

CADHERIN EXPRESSION IN PARASAGITTAL DOMAINS OF THE DEVELOPING CHICKEN CEREBELLUM. K. Arndt* and C. Redies. Institute for Biology III, University of Freiburg, Schänzlestr. 1, D-79104 Freiburg i. Brsg., Germany.

The adult cerebellar cortex consists of functional compartments which have been characterized physiologically and biochemically. These functional domains form parasagittal stripes and emerge during development.

By immunohistochemistry and in situ hybridization, we show that, in the embryonic chicken cerebellum, three cadherins (R-cadherin, cadherin-6B and -7) are transiently expressed in parasagittal domains at embryonic day E11, E15, and E19. Cadherins, a family of calcium-dependent cell-cell adhesion molecules, are known to be involved in morphoregulatory processes. The position of the cadherin-expressing domains in the embryonic cerebellum overlaps with that of functional domains in the adult: E.g., R-cadherin is expressed in domains A, C, E and F (see Arends and Zeigler, J. Comp. Neurol. 306:221-244). Efferents of these domaines terminate in deeper cerebellar nuclei which also express R-cadherin.

Cell type specificity of cadherin expression changes from E11- E19. At stage E11, all three cadherins are expressed by Purkinje cells and granule cells. At stage E19, cadherin-6B expression is restricted to Purkinje cells and cadherin-7 expression to the internal granular layer. R-cadherin is expressed in a subpopulation of cells in the internal granular layer.

These results suggest that cadherins are involved in the formation of the functional cerebellar architecture, possibly by providing transient adhesive cues during development.

(Supported by the Max-Planck-Society and the Land Baden-Württemberg.)

639.7

THE MOLECULAR MECHANISM OF CEREBELLAR GRANULE CELL DEVELOPMENT IN MOUSE. M. OZAKI*, T. HASIKAWA** and R. YANO Lab. for Cellular Information Processing, **Lab. for Neural System, Frontier Research Program, RIKEN, Saitama 351-01, JAPAN.

The cerebellar granule cell represents one of the most amenable classes of CNS neurons for developmental studies. A key feature of granule cell development, revealed by fate mapping of the cerebellar cell population, is that the granule neuron arises from a germinal zone that gives rise to only one class of neuron and then migrates to localize internal granule layer (IGL). The granule cell completes the development by makeing synaptic connection with mossy fiber. Studying this system characterized well phisiologically means revealing the basical concept of neurogenesis in CNS from the aspect of molecular biology. Indeed it's thought that some gene expression are required for processes of migration from external granule layer (EGL) to IGL and of innervation by mossy fiber. Herein we focused on neuregulin (also called NDF, heregulin, GGF and ARIA) and it's receptor because neuregulin is thought to be critical factor for innervation in neuromuscular junction. Neuregulin is a member of the EGF family which induces growth and differentiation of epithelial, glial and muscle cells in culture. Furthermore neuregulin can bind erbB. To know the destribution of each gene of neuregulin and the receptor's family, *in situ* hybridization was carried out. In addition we are studying also innervation by mossy fiber to granule cell by neural staining. We will report the relationship between innervation of mossy fiber and gene expression here.

639.8

SELECTIVE PERTURBATION OF THE VESTIBULOCEREBELLUM IN TRANSGENIC MICE. J. Oberdick[3], D. Redd[2], F. Bian[3], M. Ostrowski[2], and A. S. Berrebi*[1]. [1]Departments of Otolaryngology and Anatomy, West Virginia Univ. School of Med., Morgantown, WV 26506; [2]Departments of Molecular Genetics and [3]Cell Biology, Neurobiology, and Anatomy, and the [3]Neurobiotechnology Center, The Ohio State University, Columbus, OH 43210.

In order to elucidate the role of the ras pathway in terminal differentiation of neurons, a dominant-negative form of the ras GTPase has been overexpressed in cerebellar Purkinje cells. The mutated gene Ha-ras-N17 which encodes a form of ras believed to act by competing with the wild-type protein for binding to GDP exchange factors, was linked to the Purkinje cell-specific promoter, *pcp-2*(L7). Eight founders, each carrying greater than ten copies of the transgene, were obtained, none of which showed any overt signs of ataxia. One male founder never produced offspring and another showed a considerable delay in reproductive capacity. This delay correlated with high expression of transgenic RNA and the mutated ras protein with respect to three other founders that were good sires. The founder male that never produced young, and mice from the line that was established after a considerable lag, showed an obvious decrease in size of the paraflocculus clearly visible in whole mount upon dissection of the brains. Upon histological analysis, it was found that both the flocculus and paraflocculus were affected. These regions are characterized by thinner than normal molecular and granule cell layers, and the paraflocculus in particular by a change in the pattern of foliation. Purkinje cells in these regions appeared qualitatively normal by expression of a number of marker proteins. However, aberrant dendritic morphologies were observed by the Golgi method. Interestingly, calretinin+ cells seem to be missing from the more caudal aspect of the paraflocculus and flocculus. *In situ* hybridization analysis clearly shows uniform expression of the transgene throughout the cerebellum, at least after the first postnatal week. These data suggest either an early critical period of ras involvement in cerebellar development or a selective role for ras in vestibulocerebellar development. (Supported by NSF IBN-9309611 to JO; NIDCD-N02266 to AB; NIH CA 53271-08 to MO).

639.9

SINGLE-UNIT ACTIVITY OF CEREBELLAR PURKINJE CELLS IN THE AWAKE GENETICALLY DYSTONIC RAT. M.S. LeDoux*[1], and J.F. Lorden[2]. Dept. of Neurology[1], Univ. of Tennessee Coll. of Medicine, Memphis, TN 38163; Dept. of Psychology[2], Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The genetically dystonic (dt) rat, an animal model of generalized idiopathic dystonia, develops a motor syndrome by Postnatal Day 12 that progresses rapidly. Lesion studies have established cerebellar output as critical for the expression of the dt rat motor syndrome. In the awake dt rat, cells in the deep cerebellar nuclei (DCN) exhibit an abnormal pattern of bursting activity. The amount of bursting increases with age and the severity of the motor syndrome. Single-unit recordings from cerebellar Purkinje cells were obtained from awake dt (N = 29) and normal (N = 23) rats at Postnatal Days 12 to 26 to determine the cerebellar cortical correlates of abnormal DCN activity. Simple-spike frequency (mean ± SEM) was 40.43 ± 2.86 Hz for normal (39 cells) and 45.25 ± 4.74 Hz for dt (35 cells) rats. Complex-spike frequency was significantly higher in normal (1.48 ± 0.07 Hz) than in dt (0.88 ± 0.09 Hz) rats. Simple-spike frequency increased with increasing postnatal age in both normal and dt rats. Abnormal cerebellar output in the dt rat may be a consequence of its low Purkinje cell complex-spike firing rate or a loss of DCN sensitivity to Purkinje cell activity. (Supported by NIH K08 NS01593-01 and the Dystonia Medical Research Foundation)

CEREBELLUM: ANATOMY

640.1

CONVERGENT CEREBRAL CORTICAL AND SOMATOSENSORY INPUT TO THE PRIMATE BASILAR PONTINE NUCLEI. G.A. Mihailoff*, Departments of Anatomy and Neurology, University of Mississippi Medical Center, Jackson, MS 39216-4505.

Although regarded primarily for its role in the transfer of signals from the cerebral cortex to the cerebellum, recent studies from our laboratory and others have indicated that the basilar pontine nuclei (BPN) receive afferent projections from a much more diverse array of cell groups than was previously recognized. For example in the rat, earlier studies revealed that the BPN received a substantial projection from the dorsal column nuclei and the terminal fields of this projection overlapped with the terminals of sensorimotor corticopontine axons. Subsequent experiments in rats using electrophysiological recording revealed that single BPN units received convergent input following activation of deep and cutaneous receptors on the forepaw in combination with input from the forepaw representation in the sensorimotor cortex. Electron microscopic studies using double labeling methods confirmed that axons from the dorsal column nuclei and the forelimb sensorimotor cortex formed synaptic contacts with single BPN projection neuron dendrites. In the present experiments, we seek to investigate whether such circuitry also exists in the BPN of non-human primates (*Macaca fascicularis*). Previous studies in the monkey have shown that (1) axons course ventrally from the medial lemniscus to reach the subjacent dorsal regions of the BPN where they form multiple, small foci of terminal aggregates, and (2) corticopontine axons from SI cortex, premotor cortex, and parietal areas 5 and 7 also terminate throughout this same dorsal BPN region. When SI corticopontine axons are labeled by orthogradely transported biotinylated dextran amine (BDA) and dorsal column nuclear axons are caused to degenerate by ablative lesions of the cuneate and gracile nuclei, electron microscopy reveals that both types of axons form synaptic boutons with single dendrites in the BPN neuropil. Further, both types of afferent synaptic terminals participate in serial synaptic arrangements where they always form the presynaptic element in contacts with other vesicle containing profiles. Thus in primates as in rodents, BPN input from the dorsal column nuclei appears to converge with the input from sensorimotor cerebral cortex. This finding highlights the integrative functional role of the BPN. *Supported by USPHS grant NS12644-16*

640.2

DIFFERENTIATED CORTICONUCLEAR PROJECTIONS FROM FORELIMB-AREAS IN CEREBELLAR C1-C3 COMPARTMENTS. A. Rosina[1]*, W. Marcotti [1], S.Morara[1], and L. Provini[1,2]. [1]Ist. Neuroscienze Bioimmagini CNR, and [2]Ist. Fisiol. Gen. Chim. Biol. Universita', Milano, Italy.

Purkinje cells (Pc) in the cerebellar C1-C3 compartments, receive climbing fiber (CF) afferents from the rostral part of the accessory olives, and project their axon to the interposed nuclei. Olivocerebellar fibers design, within these compartments, multiple cortical body maps, and recent tract-tracing studies have shown that maps are linked by axon collaterals of individual olivary neurons. Neurons in the deep nuclei were also found to project back to the cortex following this pattern of somatotopic topography and axon collateralization. Our study was aimed at ascertaining how homologous areas of the CF projection maps are translated, through the Pc projections, to the neuronal groups within the deep nuclei, and hence to the interposed output stations, the red nucleus (RN) and the motor thalamic (VA-VL) nuclei. Retro-anterograde markers were injected into the face-forelimb related (FL) areas of the cortical maps, in compartments C1-C3 of the anterior lobe, paramedian and ansula lobules. Anterogradely labeled Pc axon terminals were analysed in the interposed nuclei, and direct comparison was made with the topography of retrogradely labeled olivary and deep nuclear neurons, projecting to the injected cortical areas. Pc projections from homologous cortical areas were found to converge only in part on the same FL nuclear neurons, always leaving significant adjacent patches of non overlapping terminal areas. These results are taken to indicate that: I) Pcs in individual FL areas of the cortical body maps in the intermediate cerebellar compartments have a finely differentiated pattern of projections to specifically localized neurons in the FL nuclear groups; II) this differentiated projection pattern is likely to be reflected in the cerebellar output to RN and VA-VL nuclei.

640.3

ELECTROPHYSIOGICAL IDENTIFICATION OF CLIMBING FIBRE SAGITTAL ZONES IN THE CEREBELLAR ANTERIOR LOBE OF THE RAT. C.-F. Ekerot*, H. Jörntell, M. Garwicz and X.-L. Lou. Dept. of Physiology and Neuroscience, Lund University, Lund, Sweden.

The investigation was performed to elucidate the zonal organization and information carried by climbing fibres to the cerebellar anterior lobe of the rat. Climbing fibre field potentials evoked on electrical stimulation of the skin were recorded with microelectrodes in barbiturate anaesthetized rats. The microelectrode penetrations were made in the sagittal plane usually starting in lobule VI and aimed at the rostral part of the anterior lobe.

In the hemivermis the a, x and b zones were identified. The a zone in lobule II-V received a short latency, topographically well organized climbing fibre projection mainly from the hind part of the ipsilateral body. Caudally to this projection the a zone received a long latency bilateral projection from the forelimbs. The x zone, characterized by a short latency input from the ipsilateral forelimb, seemed to form a caudal extension of the short latency projection to the a zone. The b zone in the lateral vermis received a long latency, often bilateral, climbing fibre projection.

In the pars intermedia the c1 and c2 zones were identified. The c1 zone received a short latency climbing fibre from the ipsilateral side of the body. The distal forelimb was represented in lobule VI-IV and the hindlimb further rostrally. In the rostralmost parts in lobule II and III there was a representation of proximal and axial structures. The c2 zone received a bilateral input dominated by input from the contralateral forelimb. In the narrow remaining area lateral to the c2 zone there were no short latency climbing fibre responses suggestive of a c3 zone.

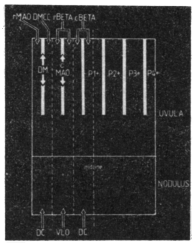
The detailed somatotopical organization in the a zone is an important new finding suggesting that vermis controls not only posture but also distal movements.

This work was supported by the Swedish Medical Research Council (project no. 8291), the Medical Faculty at the University of Lund, Greta and Johan Kocks Stiftelser, Thorsten and Elsa Segerfalks Stiftelse.

640.4

COINCIDENCE OF OLIVOCEREBELLAR CLIMBING FIBER ZONES AND ZEBRIN-POSITIVE AND -NEGATIVE PURKINJE CELL ZONES IN NODULUS AND UVULA OF THE RAT. J.Voogd*and T.J.H.Ruigrok. Dept.of Anatomy, Erasmus University Rotterdam, Box 1738, 3000 DR Rotterdam, The Netherlands.

Axonal transport of Phaseolus vulgaris in climbing fibers(cf) was combined with immunostaining with an antibody against zebrin-I, a marker for a zonally distributed subpopulation of Purkinje cells (Hawkes and Leclerc, 1987, J.Comp.Neurol.,256:29). Purkinje cells of nodulus (NOD) and rostral half of ventral uvula (UV) are all zebrin-positive. In UV a zebrin positive midline band (P1+) and three bands P2/4+, separated by narrow, zebrin-negative slits P1/3- are present. Three complex cf zones can be distinguished; their borders are located in the middle of P1/3+.The medial zone is innervated by caudal group beta, the middle zone by rostral beta, lateral zone by dorsomedial cell column (DMCC) and rostral medial accessory olive (rMAO). In NOD medial and lateral zones merge with dorsal cap (DC)-innervated zones, middle zone with cf from ventrolateral outgrowth (VLO). Zebrin-negative slits P2- and P3- are innervated by caudal MAO and dorso- medial cell group (DM).



Funding: FGG EUR ANA 110209

640.5

Descending hypothalamic projections to the basilar pontine nuclei studied with light and electron microscopy in the rat. Hao Liu* and G. A. Mihailoff Department of Anatomy, Univ. Mississippi Medical Center, Jackson, MS 39216

The projections from a variety of hypothalamic nuclei to the basilar pontine nuclei (BPN) in the rat were traced utilizing the anterograde transport of biotinylated dextran amine (BDA). Those hypothalamic nuclei with projections to the BPN included the anterior hypothalamic nucleus (AH), dorsomedial hypothalamic nucleus (DMH), lateral hypothalamic nucleus (LH), lateral mammillary nucleus (LMN), medial mammillary nucleus (MMN), premammillary nucleus (PMN), posterior hypothalamic nucleus (PH), supramammillary nucleus (SMN), tuber cinereum (TC), and ventromedial hypothalamic nucleus (VMH). Light microscopic observations revealed that the LMN, MMN, PH, and the caudal 1/3 of LH give rise to densely labeled terminal fibers that reach the medial and dorsomedial BPN. A few labeled terminals from the TC and PMN were also seen in the BPN, but no labeled fibers reached the basilar pons from the AH, DMH, SMN, and VMH. The labeled hypothalamic axon terminals were distributed bilaterally in the BPN with an ipsilateral predominance. With electronic microscopy, almost all labeled axon terminals contained round synaptic vesicles while a few contained a mixture of round vesicles and dense-core vesicles. The labeled terminals formed asymmetric synaptic junctions with dendrites of various sizes including a characteristic structure in the BPN neuropil, the glomerular synaptic complex. Very few synaptic contacts were seen with BPN neuronal somata. The present studies indicate that the hypothalamus projects predominantly to the medial portion of the BPN, and may play an excitatory role in activating pontocerebellar neurons. Supported by USPHS Grant NS12644

640.6

OPTICAL IMAGING OF THE PARASAGITTAL ORGANIZATION IN THE RAT CEREBELLAR CORTEX EVOKED BY ELECTRICAL AND NATURAL STIMULATION OF THE FACE. G. Chen*, C. Hanson, T.J. Ebner. Depts. of Neurosurgery & Physiology, Univ. of Minnesota, Minneapolis, Minnesota 55455

There is growing evidence that cerebellar afferents, Purkinje cells, and their efferent projections are compartmentalized parasagittally. Using optical imaging and immunostaining techniques, we demonstrate in this study that both electrical and tactile stimulation of the rat face evoke responses consisting of parasagittal bands in the cerebellar cortex, and that there is a close spatial correspondence between these patterns and the parasagittal zonation revealed by anti-zebrin immunostaining.

Crus I and II in anesthetized rats (ketamine/xylazine) were exposed and stained with the pH sensitive dye, neutral red. After washout, images of the cortex were acquired before, during, and after ipsilateral facial electrical or tactile stimulation with a Ling Dynamic probe. A statistical analysis based on z-scores yielded a spatial and temporal map of the pattern of activation in Crus I and II. Following the optical recording, immunostaining of zebrin II was carried out by incubation of the cerebellar sections with anti-zebrin II (a gift from Dr. R. Hawkes), and perioxdase-conjugated secondary antibody. Immunoactivity was revealed by using a peroxidase substrate. The optical responses to face electrical stimulation consisted of 2-7 parasagittal bands of change in fluorescence. The bands varied in width from 100-500 μm and generally extended antero-posteriorly across the entire field of view. Ipsilateral tactile stimulation of the face evoked parasagittal bands similar to those produced by electrical stimulation. Zebrin positive bands unveiled by immunostaining techniques corresponded with the bands defined by the optical imaging techniques.

The present study demonstrates that functional parasagittal compartments exist in the rat cerebellar cortex and that the Purkinje cell zebrin positive subgroups may be anatomically related to this functional organization.

Supported by NIH grants PO1 NS 31318 and RO1 NS 18338

640.7

FUNCTIONAL CORRELATION OF PARALLEL FIBER ACTIVITY AND PARASAGITTAL BANDS ELUCIDATED BY SINGULAR VALUE DECOMPOSITION OF OPTICAL IMAGES C. Hanson*, G. Chen, T.J. Ebner Graduate Program in Neuroscience and Departments of Neurosurgery and Physiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455.

The highly regular cellular lattice and circuitry of the cerebellar cortex and its afferent systems are organized around several spatial plans. Optical imaging can be utilized to accurately map the patterns of activity in the cerebellar cortex. In this study, we used singular value decomposition (SVD) to extract spatial patterns of activity from optical images of rat cerebellar cortex evoked by either, direct cortical or, electrical and/or mechanical peripheral stimulation. Further, this method of analysis allowed us to investigate functional correlation, and connectivity between these varied spatial patterns.

Optical signals from stimulus evoked fluorescence changes in Crus II were recorded with a frame transfer equipped, digital, CCD camera in anesthetized rats (ketamine / xylazine). The cortex was stained by superfusion (2.5mg/ml) and intravenous injection (10mg/ml) of the pH sensitive dye, neutral red. Stimuli consisted of direct cortical stimulation to evoke parallel fiber activity, or electrical and/or mechanical stimulation of the face to elicit parasagittal banding.

SVD of the images obtained with direct cortical stimulation revealed spatial patterns of activity consistent with activation of a band of parallel fibers, while SVD images of bipolar electrical or mechanical stimulation of the ipsilateral face demonstrated patterns of parasagittal banding. Remarkably, facial stimulation evoked "parallel fiber" like patterns of activity in the higher order components. Similarly, during direct cortical stimulation "parasagittal" like patterns were detected. Additionally, some components showed both "parallel fiber" and "parasagittal" like spatial patterning. Therefore, SVD offers the potential to not only separate out various sources of fluorescence change, but also to quantify these changes and analyze how various sources of signals correlate temporally and spatially. Supported by NIH grants PO1 NS 31318 and T32 GM08471.

640.8

SOMATOTOPICAL ORGANISATION WITHIN THE INFERIOR OLIVE PROJECTION TO THE POSTERIOR LOBE OF THE RAT CEREBELLUM. **M. J. Atkins and R. Apps. (SPON: Brain Research Association).**
Dept. of Physiology, School of Med. Sci., University Walk, Bristol BS8 1TD, UK.

The cerebellar cortex of the cat can be divided into a number of olivocerebellar zones, however it is not established whether a similar zonal organisation exists in the rat. To test this possibility both electrophysiological and anatomical techniques were used to map the olivocerebellar projection to the posterior lobe of the rat cerebellum.

Using electrophysiological techniques, in male rats anaesthetised with sodium pentobarbitone (Sagatal, 60mg/kg), three different areas were identified within the Paramedian Lobule (PML: areas 1-3) and two areas within Copula Pyramidis (CP: areas 4-5). Each area was characterised as follows according to the site of peripheral electrical stimulation that evoked the largest climbing fibre responses within that area: area 1 (contralateral face), area 2 (ipsilateral forelimb), area 3 (ipsilateral and contralateral forelimbs), area 4 (tail) and area 5 (ipsilateral hindlimb). The olivocerebellar connections of these areas were then investigated by placing single, small (150-200nl) injections of retrogradely-transported fluorescent microspheres into the centre of each electrophysiologically defined area. After 6-10 days survival time, the rats were transcardially perfuse-fixed and the brainstem sectioned and analysed for cell labelling in the inferior olive.

The results suggest that from mediolateral in PML: area 1 has input from caudal Medial Accessory Olive (cMAO); area 2 has input from medial rostral Dorsal Accessory Olive (rDAO) and rMAO; area 3 has input primarily from rMAO, but also rDAO. In CP, from medial to lateral: area 4 has input from the lateral most part of middle DAO and area 5 has input primarily from a rostro-caudal column of cells in lateral DAO. These results suggest at least zones A, C1 and C2 may be present within the posterior lobe of the rat cerebellum. (Funded by The Wellcome Trust.)

640.9

RECONSTRUCTIONS OF SINGLE CLIMBING FIBER COLLATERALS IN THE DEEP CEREBELLAR NUCLEI OF THE RAT. I. Sugihara*, H. Wu and Y. Shinoda Department of Physiology, Tokyo Medical and Dental University School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan

The olivocerebellar climbing fiber (CF) system, especially its innervation of the deep cerebellar nuclei (CN), was investigated in the rat with reconstructions of single axons labeled with biotinylated dextran amine (BDA). A small number of CFs were stained by making a small and localized injection of BDA in the inferior olive nucleus (IO). This enabled us to trace single identified axons and their branches from serial sections throughout the cerebellum and medulla oblongata. In all of five CFs reconstructed so far collaterals terminating in the CN were given off from stem axons projecting to the cerebellar cortex. The number of nuclear collaterals per CF ranged from one to six. These collaterals bifurcated one to several times to give rise to terminal branches. Primary nuclear collaterals and terminal branches were much thinner than CF stem axons innervating cortical Purkinje cells, and bore en-passant and terminal varicosities as reported (Van der Want et al., 1989). When many CFs were labeled collaterals of many CFs made dense plexuses. The dense plexuses suggest significant convergence of CF fibers on cerebellar nuclear cells, while no convergence of CF fibers occurs at the level of a Purkinje cell in the cerebellar cortex. The area in which nuclear collaterals originating from a single CF made terminal branches with varicosities was relatively localized (about 100-300 μm in width in either direction within the CN). This suggests the precise topographical relationship in the olivocerebellar system between subnuclei of the IO, subdivisions of the CN and cerebellar cortical zones. Supported by Grant-in-Aid for Scientific Research from The Ministry of Education, Science and Culture of Japan to I. S. and Y. S., by Research Grant from Narishige Foundation to I. S. and by Research Grant from Sasagawa Medical Foundation to H. W.

640.10

ORGANIZATIONAL FEATURES OF THE DENTATO-THALAMO-CORTICAL PROJECTION. R.A.West*[1] and P.L. Strick[2]. [1,2]Research Service, VA Medical Center and [2]Departments of Neurosurgery and Physiology, SUNY Health Science Center, Syracuse, NY, 13210.

We used anterograde transneuronal transport of the H129 strain of the herpes simplex virus type 1 (HSV1) to determine the pattern of cerebello-thalamocortical projections. Injections were placed into a caudal and ventral portion of the dentate nucleus in cebus monkeys (*cebus apella*, n = 2). Following a five day survival period, discrete patches of "second order" neurons were labeled in area X and ventralis lateralis pars caudalis (VLc) of the thalamus. The patches had a mean diameter of approximately 380μm and some patches extended up to 1mm rostrocaudally. "Third order" neurons were labeled in several cortical regions, but were primarily found in areas 6 and 9. These neurons were most often located in layer III. In one animal, several dense 'columns' of labeled neurons were found in the portion of area 6 which lies medial to the superior limb of the arcuate sulcus. The labeled neurons in these columns were most concentrated in the middle cortical layers, but could extend from layer VI to layer II. Columns had a mean width of approximately 480μm and measured 0.5 to 1mm rostrocaudally. These observations indicate that portions of the dentate nucleus project to an assembly of rostrocaudally oriented rods in the thalamus. These rods in turn innervate clusters of oriented columns in specific areas of cortex. Support: NRSA Postdoctoral Fellowship NS09766-02 (RAW), VA Medical Research Service (PLS), and USPHS 24328 (PLS).

640.11

THE EFFERENT PROJECTIONS OF THE INTERPOSED NUCLEUS OF THE CEREBELLUM: A PHA-L STUDY IN THE RAT. J.M. Lockard* and D.G. Lavond. Dept. of Biol. Sci., University of Southern California, Los Angeles, CA 90089.

From previous anatomical data it has been suggested that the Interposed Nucleus (IP) is merely an output relay from the "spinocerebellar" cortex. Connections with the brainstem motor relay nuclei and motor thalamus have been emphasized (Allen & Tsukahara, 1974; Chan-Palay, 1977; Asunama et al., 1982). However, others have demonstrated connections to centers concerned with planning and execution (Uchida et al., 1983; Vaudano & Legg, 1992), with nociception (Basbaum & Fields, 1978), and a variety of autonomic centers and affective behaviors (Haines et al., 1990; Ikai et al., 1992). It is for these reasons that we decided to do a systematic reexamination of the IP efferents using the sensitive anterograde tracer PHA-L. PHA-L was stereotaxically injected into 30 rats to describe the overall pattern of efferent projections from the IP.

Here we report projections to the following: Spinal Cord, Rostral Linear and Parafascicular Raphe' Nuclei, N. Prepositus, Med. and Sup. Vestibular N., Med. Terminal N., Edinger-Westphal N., N. of Darkschewitsch, Red N., Inf. Olive (da, ma, po), VTA, Zona Incerta, Snc, Interstitial N. of Cajal, Mesencephalic Reticular N., PAG, Sup. Colliculus, Post. Hypothalamus, N. Post. Commissure, Lat. Hypothalamic Area, Pons, VM, VAL, VPL, VPM, Central Medial, Rhomboids, Medial Dorsal, Paracentral, and the Parafascicular Thalamic Nuclei. The well-established cerebellar cortical projection was also observed, in addition to a commissural projection to the contralateral IP.

Judging from its projections, the IP might coordinate or modify many functions of the nervous systems in much the same way as it has been shown to regulate muscular activity.

This work supported by NIMH RO1 MH51197 to D.G. Lavond, and a Sankyo grant to R.F. Thompson.

640.12

A RETROGRADE TRANSPORT STUDY OF THE CONNECTIONS BETWEEN THE CEREBELLAR NUCLEI AND THE MEDULLARY RETICULAR FORMATION IN THE CAT. M.-J. Rho*, T. Cabana and T. Drew. Depts. of Physiology and Biological Sciences, U. Montréal, Québec, Canada.

Previously we have examined the functional organization of the connections between the pericruciate cortex and the reticular formation of the brainstem (Rho et al. Soc. Neurosci. Abstr. Vol 20: p985) with the goal of better understanding the neural mechanisms that may underlie the integration of posture and movement. We have now extended these studies to examine the pattern of projections from the cerebellar nuclei to the medullary reticular formation (MRF). Small injections (0.15-0.6μL) of either Texas Red or Fluorescein were made into restricted regions of the MRF in 5 cats, and labelled cells in the cerebellar nuclei were identified using fluorescent microscopy. In contrast to the projections from the pericruciate cortex to the MRF, which are largely bilateral in origin, the majority of labelled cells in the cerebellar nuclei were located contralateral to the injection site (mean = 93±4% of the total). Although there was appreciable labelling in the contralateral dentate nucleus (mean = 19±9% of the total) the majority of the labelled cells (mean = 56±19% of the total) were localised within the fastigial nucleus. It is suggested that this unilateral projection from the fastigial nucleus to the MRF might be largely involved in the modification of muscle tone in response to feedback signals from the limbs. This is in contrast to the corticoreticular projections which we suggest would provide a bilateral feedforward signal to the MRF. (Supported by the MRC, FRSQ and FCAR).

640.13

THE CYTOSKELETON OF UNIPOLAR BRUSH CELLS OF THE MAMMALIAN CEREBELLUM. **Maria R. Dino*, Gabriela Sekerkova, Shane Cunha, Lester Binder[1] and Enrico Mugnaini[1]** Northwestern University Institute for Neuroscience and [1]Dept. of Cell and Molecular Biology, 320 E. Superior Street, Chicago, IL 60611-3010

Unipolar brush cells (UBCs) are a special type of neuron newly discovered in the granular layer of the mammalian cerebellar cortex. These small neurons are intermediate in size between Golgi cells and granule cells and display several unusual cellular features. UBCs usually have a single, stout dendrite that terminates in a brush-like bunch of dendrioles and a thin axon. The dendrioles receive an extensive and powerful synapse from a single mossy fiber rosette and establish dendrodendritic synapses with granule cells, of which they represent the presynaptic element. Despite being only 8-12 μm in soma diameter, UBCs are particularly rich in neurofilaments. These peculiarities suggest that their cytoskeleton is highly differentiated.

By fluorescence and electron microscopy we show that UBCs possess an extraordinary postsynaptic array of actin filaments and their somato-dendritic compartment is rich in tubulin, MAP 2, MAP 1B and tau-1. While actin indicates a high degree of motility in the dendrioles, the well developed microtubular apparatus may be essential for somato-dendritic transport of synaptic proteins directed to postsynaptic and presynaptic sites. *Supported by grant NS 09904 and AG06969*

640.14

UNIPOLAR BRUSH CELLS ARE PRESENT IN CEREBELLAR GRANULE CELL CULTURES. R.Anelli, R.E. Kettner* and E.Mugnaini Northwestern University Insti. for Neuroscience and Dep. of Physiology*, 320 E. Superior Street, Chicago, IL 60611-3010

A type of neuron, termed the *unipolar brush cell* (UBC), has recently been characterized in the granular layer of the mammalian cerebellar cortex with several procedures including light and electron microscopic immunocytochemistry. The UBCs possess many distinctive features that make them easily distinguishable from other cerebellar neurons. The cell body usually emits a thin axon and is provided with a single, large dendritic trunk which terminates with a bunch of dendrioles synapsing with a mossy fiber rosette. UBCs are densely immunostained with antisera to calretinin, secretogranin II, high-molecular-weight neurofilament protein, and glutamate receptors, especially GluR2/3. In primary cultures of cerebellar granule cells derived from rats of different postnatal ages (P2-P8), we observed three neuronal cell types, presumably granule cells, Golgi cells and UBCs, that demonstrate calretinin immunoreactivity after 3-12 days in vitro. The granule cell body is moderately stained. Golgi cells are moderately stained in the somatodendritic compartment, while UBCs are entirely strongly stained in a Golgi impregnation-like manner. The data suggest that the peculiar configuration of the UBC is largely cell-autonomous and confirm that calretinin is a useful cell population marker both in vivo and in vitro. In vitro, granule cells are densely stained by antisera against NF-H, and these are not useful to differentiate UBCs from other cerebellar neurons, contrary to the situation in situ. We are in the process of expanding these data by immunostaining with antisera used to identify UBCs in the adult cerebellum, as well as by standard electron microscopy and co-colturing with neurons that may provide mossy fiber afferents.

Supported by NIH grant NS 09904

640.15

CHARACTERIZATION OF GOLGI INTERNEURONS IN THE RAT CEREBELLUM. S. Dieudonné and J.S.Kehoe*. Lab. de Neurobiologie, Ecole Normale Supérieure, Paris, France.

We have performed a patch clamp study of Golgi cells in thin slices of the young rat cerebellum. The Golgi cells, first identified visually by the large size of their cell body, were filled with biocytin during patch clamp recordings for later histological evaluation. Recorded cells had the characteristic features of Golgi cells: a large and branching axonal plexus, small and stout basal dendrites in the granular layer and one or two apical dendrites crossing perpendicularly the Purkinje cell layer to form an ascending tree into the molecular layer up to the pia. They had a triexponential capacitive transient corresponding to a capacity of 30 to 100 pF (first two components). Some cells had lost most of their axon and could be identified by the small size of the third capacitive component. In the cell attached configuration, cells were spiking regularly at 1 to 10 Hz.

Excitatory currents with NMDA and non-NMDA components could be evoked either by electrical stimulation of the parallel fibers in the molecular layer or by granule cell stimulation. The unitary AMPA current had an amplitude of 20 to 100 pA (at -70 mV) and a decay time of about 1 ms. The total charge necessary to evoke an action potential in current clamp was equivalent to that of 20 to 100 such excitatory events. GABAergic inhibitory currents were evoked by stimulations applied in the vicinity of the Purkinje cell bodies, suggesting that basket cells can inhibit Golgi cells. Glycinergic currents were recorded in frontal slices when the Purkinje cell layer was stimulated, suggesting that glycinergic axons travel perpendicular to the sagittal plane.

We acknowledge the support of the CNRS (URA 1827) and of the Institut Lilly.

640.16

AN ANALYSIS OF NEUROBIOTIN-FILLED STELLATE CELL AXONS IN THE RAT CEREBELLUM. C. Pouzat* and S. Kondo. Arbeitsgruppe Zelluläre Neurobiologie, Max-Planck-Institut für Biophysikalische Chemie, Am Faßberg, D-37077 Göttingen, Germany.

Stellate cells located in the upper molecular layer of the cerebellar cortex are known to inhibit Purkinje, basket, and stellate cells. They are usually separated into two classes, the short-axon type, supposed to mediate local (on-beam) inhibition and the long-axon type supposed to mediate distal (off-beam) inhibition. In order to understand their relative contribution to on-beam versus off-beam inhibition, especially for the long-axon stellates, we performed a quantitative description of their axonal arborization. Stellate cells from slices of rat cerebella (between 21 and 34 days postnatal) were filled with neurobiotin during tight-seal whole-cell recording, and stained with horseradish peroxidase/diaminobenzidine enhanced with nickel ammonium. 7 stellate cells were recovered with a well-filled and intact axon. 5 cells were of the long-axon type and 2 of the short-axon type. On 4 of them varicosities could be clearly seen. Quantitative analysis showed that the total length of the axon was 970±370 µm (m±SD) for the long-axon cells and 520 and 930 µm respectively for the two short-axon stellates. For the long-axon type the length of the main branch of the axon was 290±64 µm and the number of collaterals and their extension decreased markedly with the distance from the soma. Varicosities were homogeneously distributed along the axon. The average interval between varicosities was 6.8±2 µm. Homogeneous distribution of varicosities along the axon and condensed collateral distribution around the soma suggest, in contrast to the prevailing view, that the main role of long-axon stellate cells is on-beam rather than off-beam inhibition.

S.K. is a recipient of HFSP fellowship (LT385/95).

640.17

DIFFERENTIAL EXPRESSION OF GAT-1 AND GAT-3 IMMUNOREACTIVITY IN THE RAT CEREBELLAR CORTEX. S. Morara[1]*, A. Rosina[1], N. Brecha[2], and L. Provini[3]. [1]Istituto Neuroscienze Bioimmagini, CNR, Milano, Italy, [2]Dept. Neurobiology, CURE, UCLA, Los Angeles, CA, [3]Ist. Chim. Biol. Fisiol. Gen., Univ. Milano, Italy.

Specific Na^+- and Cl^--dependent, high affinity GABA transporters (GAT) are located on GABA-containing neurons and glial cells, and are thought to inactivate GABA's action by removing it from the vicinity of its receptors into presynaptic terminals and/or glial cells. Four cDNAs encoding GAT (GAT-1,2,3,4) have been cloned, and in rat, GAT-1 and -3 have been localized to neural cells. To detect the distribution and cellular localization of GAT-1 and -3 in the cerebellar cortex, double immunofluorescence experiments used GAT-1 and GAT-3 antisera with antibodies for synaptophysin or GFAP. High levels of GAT-1-IR were seen around Pcs, in cone-like structures that, due to position, shape and positivity to synaptophysin were identified as the *pinceau* formed by basket cells. GAT-1-IR fibers and terminal-like structures were also found in the molecular layer (ML). Lower levels of GAT-3-IR were found in the Pc layer, confined to GFAP-IR structures that were identified as Golgi epithelial cells. Punctate GAT-3-IR structures were also found in the ML. Very low levels of GAT-1- and GAT-3-IR were detected in the granular layer. Thus, GAT-1 and GAT-3 display a differential distribution in the cerebellar cortex, where GAT-1 is preferentially localized to neurons and GAT-3 is preferentially expressed in astroglia. Accordingly, previous observations indicated that in the cerebellum, a high affinity GABA uptake system is present only in interneuronal and glial cells. One can further speculate that the predominant role of GAT-1 is in terminating GABA's action at interneuronal inhibitory synapses, whereas the predominant role of GAT-3 is in regulating the extracellular levels of GABA.

640.18

SPECIFICATION OF CORTICAL AND SUBCORTICAL POPULATION OF NEURONS REVEALED BY MONOCLONAL ANTIBODY 8B3. A. F. Pimenta*, P. L. Strick and P. Levitt. Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854 and Depts. of Physiol. and Neurosurg., SUNY-HSC @ Syracuse and VA Med. Ctr. Syracuse, NY 13210.

We previously generated a monoclonal antibody 8B3 that recognizes a condroitin sulfate proteoglycan expressed on the surface of somata and proximal dendrites of a subpopulation of neurons. In cortical areas, the staining pattern is characterized by a single row of cells on the border of layer I/II that is present in old world monkey and human, but absent in new world monkey, cat and rat, suggesting an evolutionary specification. There is a scattered population of neurons and intense neuropil staining in deep layers V and VI that is continuous over all cortical areas. A variable number of neurons are stained with stellate morphology in layers II through upper V depending upon the analyzed area. A large number of interstitial neurons is observed in the white matter of adult primate and human, whereas very few are observed in cat and rat, consistent with the fate of subplate neurons in these species. In the visual cortex 8B3 clearly demarcate the borders of area 17/18. At the subcortical level, 8B3 stains spiny stellate neurons in the striatum clearly defining a compartment that may correspond to the matrix. Neurons in the reticular nucleus of the thalamus are intensely labeled. In addition, in the rostral portions of the ventrolateral thalamus labeled cells form clusters. At more caudal levels, a large number of neurons are labeled and these are more evenly distributed. In the cerebellum, 8B3 marks the deep nuclei with striking ventral to dorsal gradient in the dentate. In the cerebellar cortex, a subpopulation of neurons with Golgi morphology is stained in the upper granular layer. Few neurons are labeled in deeper aspects of the granular cell layer. Within regions, 8B3 appears to be a marker for specific subpopulation of neurons that correspond to functional domains. Supported by NIMH grant MH45507 and VA Medical Research Service.

HUMAN POSTURE

641.1

POSTURAL RESPONSES TO LATERAL SURFACE PERTURBATIONS. S.M. Henry,* J.Fung, F.B. Horak. R.S. Dow Neurological Sciences Institute, LGSHMC, Portland, OR 97209.

The purpose of this study was to compare and contrast automatic postural responses to lateral and anterior/posterior (A/P) surface perturbations in narrow and wide stance in order to examine the neural and biomechanical factors characterizing postural strategies. Healthy adult subjects stood on a moveable platform with each foot on a force plate in both narrow and wide stance while undergoing 9 cm lateral and A/P surface translations at 35 cm/s. Bilateral 3-D ground reaction forces, joint positions, and lower limb and trunk muscle EMGs were recorded. For A/P translations, the EMG activation pattern was distal to proximal with a trunk flexor or extensor added early. In contrast, for lateral translations, the proximal tensor fascia latae muscle was activated first, followed by cocontraction of the distal muscles in the loaded leg. The EMG activation pattern did not change from narrow to wide stance, but the EMG amplitude was decreased in wide stance. The forces exerted by the feet against the surface during both lateral and A/P translations were primarily vertical. With A/P translations, there was loading/unloading of the A/P part of the foot whereas with lateral translations, there was loading/unloading of the right/left foot. For A/P and lateral translations, the kinematic corrective response was a shank, thigh, trunk pattern, allowing maintenance of a relatively erect trunk position. Thus, the trunk orientation goal is achieved using vertical contact forces and similar kinematic patterns via very different EMG synergies that are specific to the biomechanical constraints of A/P and lateral movement in order to maintain equilibrium. *Supported by NIA (RO1-AGO6457), NIDCD/ NASA (P60-DC02072), and the American Physical Therapy Association.*

641.2

POSTURAL CONTROL IN A PATIENT WITH TOTAL BODY SOMATOSENSORY LOSS. F.B. Horak, Y. Lamarre, J.M. Macpherson*, C. Jones, S.M. Henry. R.S. Dow Neurological Sciences Institute, LGSHMC, Portland, OR 97209.

The critical role of somatosensory information for postural control was apparent in a patient (GL) with complete large fiber neuropathy below cranial nerve VIII. The patient could stand unsupported up to 30 seconds only by using visual feedback in a mirror to prevent knee collapse. Bursts of muscle activity in the legs and neck in response to surface translations and rotations were directionally specific, although significantly late (270-800 ms). With eyes closed and auditory cues masked, however, postural responses were absent. Anticipatory postural adjustments prior to rapid arm raising were also absent. These results demonstrate that somatosensory information is essential for both reactive and predictive postural activity, although voluntary responses to perturbations can partially compensate using visual or auditory cues. Body tilt in response to bipolar, galvanic vestibular stimulation was opposite to the normal direction suggesting that somatosensory information is also critical for interpreting vestibulospinal disruptions of orientation of the body in space. Supported by NIA (RO1-AGO6457), NIDCD (R01-DC01849), and NIDCD/ NASA (P60-DC02072).

641.3

FLEXIBILITY IN POSTURAL COORDINATION: THE EMERGENCE OF TASK-SPECIFIC POSTURAL PATTERNS. J. J. Buchanan* and F.B. Horak. R.S. Dow Neurological Sciences Institute, LGSHMC, Portland, OR 97209.

Normal subjects and patients with profound bilateral vestibular loss stood on a sinusoidally translating platform (disp. = 12cm) whose frequency of motion varied across trials from 0.1 Hz to 1.0 Hz. Arms were folded across the chest with the feet shoulder width apart. For each platform frequency, the eyes were either open or closed. Subjects were instructed to maintain their balance without using their arms or hands, but were not given specific instructions on how to accomplish this behaviorally. Two specific postural coordination patterns emerged as a function of platform frequency: (1) head fixed to moving surface; and (2) head fixed in space. For platform frequencies equal to or less than 0.5 Hz, subjects rode the platform and the head moved in-phase with the platform (pattern 1). At platform frequencies above 0.5 Hz, subjects fixed their head in space relative to the platform, while the hips, knees and ankles flexed and extended in various combinations (pattern 2). These observed changes in postural patterns were very similar with and without vision. Vestibular patients with their eyes opened produced postural patterns consistent with normal subjects, but marked differences with their eyes closed, i.e., large sway and stepping. Such results demonstrate that the CNS flexibly assembles task-specific postural coordination patterns as a function of displacement frequency and availability of sensory information. Supported by NIDCD/NASA (P60-DC02072).

641.4

VESTIBULAR LOSS PATIENTS SHOW ABNORMAL JOINT TORQUE RESPONSES TO FAST PERTURBATIONS.

CF Runge,[1,2] CL Shupert,* [4] FB Horak,[4] FE Zajac.[1,2,3] [1]Rehab. R&D Ctr., VA Palo Alto HCS (153), CA 94304, Depts. of [2]Mech. Eng. & [3]Funct. Restoration, Stanford Univ., CA 94305 & [4]R. S. Dow Neurol. Sci. Inst., Portland, OR 97209.

Normal subjects maintain balance during fast, rearward, support-surface translations by combining hip strategy with ankle strategy. We hypothesized that patients with bilateral vestibular loss, who are unable to use hip strategy to control balance on beams, should show abnormal responses to these translations. Patients with profound bilateral vestibular loss and age-matched controls underwent 20-cm rearward support surface translations with velocities ranging from 5-35 cm/s presented in random order. As previously shown in normals, early (<50 ms) head accelerations increased systematically with platform velocity, and the range of head acclerations was similar for patients and normals in this study. However, using Karhunen-Loeve expansion techniques on the torque responses to 30-35 cm/s perturbations, we show that the stabilizing net joint torques at the hip and/or knee were abnormal and uncharacteristic of hip strategy in the patients. In contrast, trunk muscle burst onset patterns were not consistently different in the two groups. We conclude that while the vestibulospinal system may not be solely responsible for triggering the observed muscle responses, the magnitude and pattern of force produced by the muscles depend at least partially on vestibular cues resulting from early head movements. Supported by NIDCD/NASA (P60-DC02072), NSF (94-21315), and the Rehab R&D Service of the DVA.

641.5

HEAD STABILIZATION IN LABYRINTHINE DEFICIT PATIENTS DURING HORIZONTAL AND VERTICAL PLANE ROTATIONS. K.J. Chen, E.A. Keshner, and B.W. Peterson*. Dept. of Biomed. Eng., Northwestern Univ. and Sensory Motor Performance Program, Rehab. Inst. of Chicago, Chicago, IL 60611

In previous studies (Keshner & Peterson 1995, Keshner et al. 1995) we found that both rotational frequency and instructional set influenced head stabilization. We have begun to study patients with bilateral labyrinthine loss. Head velocity and neck muscle EMG responses to 10° ramp and sum-of-sine (SSN) rotations of the trunk (0.025-3.15 Hz in yaw; 0.35-5 Hz in pitch) were examined in 3 patients and 8 healthy subjects. Subjects were seated in the dark and voluntarily stabilized their heads with (VS) or without (NV) visual feedback, or were mentally distracted (MA). In both planes, EMG responses to ramp rotations exhibited < 200 ms latencies. Neck muscle of healthy subjects exhibited activation that was compensatory to trunk motion, while patients exhibited no directionally consistent activation. In VS, SSN rotations in both planes produced compensatory head responses at low frequencies in both healthy subjects and patients. But in NV at low frequencies, normal subjects compensated for trunk rotations while patients could not. In MA, both normal subjects and patients showed no compensation during yaw rotations; in pitch, most normal subjects show reduced gains but compensatory phases, whereas patients' phases led compensation by 20°-40°. At high frequencies (>1 Hz), no differences were found between healthy subjects and patients in all paradigms. Yaw rotations caused increasing gains and decreasing phases of head velocity with resonance appearing between 1-2 Hz. Pitch rotations caused scattered gains and phases. Resonance occurred in the pitch plane in some subjects in the range of 2-5 Hz. EMG responses indicate that patients have diminished directional compensation of the muscles. During voluntary stabilization at low frequencies, patients required visual feedback in order to produce compensatory head motion. Variability at high frequencies in pitch may be due to individual system mechanics. *Supported by NS22490, DC02072, DC01125.*

641.6

SO WHAT HOLDS THE HEAD UP ANYWAY? G.C.Y. Peng*, T.C. Hain, B.W. Peterson. Northwestern University, Chicago, IL 60611

Holding one's head upright rarely requires any conscious effort. Yet, the head on the neck is intrinsically an unstable system. Particularly in the sagittal plane, the location of the head center of mass, anterior to the pitch axis, causes gravity to pull the head forward. Roberts (1967) postulated static positional reflexes act on the neck muscles, and suggested that these reflexes are driven by vestibular and proprioceptive inputs. We extended our sagittal plane head movement model, reported last year, to further explore these mechanisms. The extensions consisted of adding static otolith (sVCR) and positional neck (sCCR) reflex feedback to the existing dynamic feedback loops of the vestibulocollic (dVCR) and cervicocollic (dCCR) neck reflexes.

Our simulations demonstrated that without any reflex control, a smoothed 6° step input to the trunk caused the head to fall to 90°. This steady-state position is achieved when the moment due to the passive stiffness of the head cancels out the moment due to gravity. We found that either sVCR or sCCR control was individually capable of compensating for the effects of gravity. We tested our head holding mechanisms by changing the initial head and trunk posture to 30°. The head continued to remain aligned on the trunk, despite the additional gravitational torque at the offset angle. On adding the dynamic reflexes, head oscillations were significantly diminished, and caused the head with respect trunk movement to fall within the range of human frequency response data. Although the sVCR is an accurate gravity compensator, combining the sCCR provided a better fit to human data. Utilizing the sCCR in conjunction with the sVCR required the otoliths to only compensate for 70 percent of the gravitational torque. We speculate that the redundant effects produced by both sCCR and sVCR allow for adaptive adjustments in one system to compensate for any weakness of the other.
Supported by NS22490, DC02072.

641.7

DISTANCE-DEPENDENT SOMATOSENSORY DRIVE FOR HUMAN POSTURAL CONTROL: PRELIMINARY RESULTS. P. Ribeiro, J.J.Jeka, K.S.Oie, Department of Kinesiology, University of Maryland, College Park, MD 20742.

When a subject lightly touches a moving surface with merely the fingertip, whole-body entrainment to the moving somatosensory input is observed despite the fact that contact forces are inadequate to move the body. Small changes in contact force across the fingertip are thought to drive postural sway. The main focus in the present study was to investigate the influence of somatosensory cues at different locations relative to the body. The question was to determine if changing the distance and the height of a somatosensory stimulus relative to the body affects its influence on head and body sway.

Six subjects were tested in a heel-to-toe stance while touching a rigid metal horizontal plate positioned laterally (on the right side) to the subject. Subjects were required to maintain contact forces below one Newton throughout each trial. The plate moved sinusoidally, 4 mm peak-to-peak, at 0.2 Hz in the medial-lateral plane in each condition. The three experimental conditions had the plate positioned: 1) close to the subject at waist level (Low-Close); 2) close to the subject at shoulder level (High-Close); and 3) at arm's length from the subject at shoulder level (High-Far). The results showed strong coupling between the somatosensory stimulus and body sway in all conditions. However, in the High-Close condition, head gain was greater than in the other conditions, which showed equivalent gain levels. These results suggest that somatosensory input is interpreted in a similar manner as visual cues, that is, stimuli far away from the body are interpreted as smaller than those close to the body, even when the actual stimuli are of equivalent magnitude.
P. Ribeiro is supported by the Brazilian Government CAPES doctoral program.

641.8

FINGERTIP CONTACT SUPPRESSES THE DESTABILIZING EFFECTS OF LEG MUSCLE VIBRATION, J.R. Lackner*, P. DiZio, J.J. Jeka and E.Rabin. Ashton Graybiel Spatial Orientation Laboratory and Volen Center for Complex Systems, Brandeis University, Waltham , MA 02254.

Fingertip contact with a stationary surface greatly attenuates postural sway even at applied force levels (<100g) inadequate to provide mechanical stabilization. Sensory cues from the fingertip and proprioceptive information about arm configuration provide alternative spatial information about posture critical to stabilization (Jeka & Lackner, 1994. Exp Brain Res, 103:267-276). We studied here whether fingertip contact (<100g) could stabilize posture under conditions of instability induced by leg muscle vibration.

Subjects (N=10) were tested, eyes closed, in a heel-to-toe stance in four conditions involving either contact of the right index finger with a laterally placed surface at waist height (T) or no contact (NT), and with mechanical vibration at 120 Hz of the right peroneus brevis and longus muscles to elicit tonic vibration reflexes (PV) or without (NV): (NT NV), (T NV), (NT PV), (T PV).

Fingertip contact suppressed virtually completely the profoundly destabilizing effects of peroneus vibration on postural stability. Mean sway amplitudes and mean total powers of center of pressure and head showed the following pattern (NT PV) > (NT NV) > (T NV) = (T PV) with all differences significant ($p<.01$). In T trials, applied fingertip forces were always <100g. These observations point to the use of haptic cues in enhancing postural stability and locomotion in individuals with balance disorders.

Supported by the NASA Grant NAG9-4374, NAG9-4375; NAWC-TSD N61339-95-K-0005.

641.9

EVENT-RELATED POTENTIALS DURING THE EXECUTION OF VOLUNTARY AND COMPENSATORY REACH AND GRASP. W.E. McIlroy*[1], W.R. Staines, J.D. Brooke and B.E. Maki[1]. [1]Sunnybrook Health Science Centre, Toronto, Ontario, M4N 3M5, and Department of Human Biology and Nutritional Sciences, University of Guelph, Ontario, N1G 2W1, Canada.

Complex compensatory limb movements, such as grasping, are commonly executed in response to whole-body postural instability. It has been proposed that the control of such compensatory reactions, which are initiated very early after the onset of perturbation, is distinct from voluntary grasping. To address this issue, the present study explored the temporal and spatial characteristics of cortical activity associated with compensatory and voluntary grasping. Subjects sat in a chair on a movable platform. Grasping reactions of the right upper limb to a handle located in front were cued by a light or by movement of the platform (backward translation). Samples were also collected during trials featuring whole body movement with no arm reaction to evaluate the event-related potentials (ERPs) associated with the sensory array arising from whole-body movement. ERPs were recorded from standard international 10-20 sites (C3, C4, P3, P4, F3, F4, T3, T4, Cz, and Cz') referenced to the left earlobe. EMGs were collected from deltoid, biceps and extensor digitorum of the right arm. Latencies of the perturbation-cued reaction, measured from EMG and measured from ERP onsets, were far more rapid than light-cued reactions. This occurred even though subjects were instructed to react 'as fast as possible' under both stimulus conditions. In spite of these temporal differences the underlying pattern of ERPs was similar between the two task conditions suggesting similarities in the involved cortical loci. Are these the ERPs for a triggered reaction? Specific behavioural correlates of ERP spatial loci are presently being explored.

Supported by NSERC (Canada), Sunnybrook Trust

641.10

EFFECT OF TARGET DIRECTION ON REACHING MOVEMENTS IN YOUNG AND ELDERLY SUBJECTS. H. Chai and M.M. Gross*. Center for Human Motor Research, Division of Kinesiology, University of Michigan, Ann Arbor, MI 48109-2214

Decreased postural stability in the elderly is associated with increased risk for falling. Although the effect of reaching on postural stability has been described for young and elderly subjects in the forward direction, less in known about postural stability during reaching in lateral directions. It is important to understand the effect of reaching direction on postural stability because lateral reaching is a common activity of daily life. Further, the risk of hip fracture is increased in falls with impact on the side of the hip.

The purpose of this study was to examine the effect of target direction on reaching movements in young and elderly subjects. We hypothesized that reach distance would be maximum when reaching towards targets located in the direction, would decrease when reaching for lateral targets, and would be less in the elderly subjects than the young for all target directions. Healthy elderly (> 65 yrs) and young adults (20-30 yrs) participated in the study. Subjects were asked to reach as fast as possible in each of four target directions (0°, 45°, 90° and 120° from the subject's midline). Markers were placed on the hand, wrist, elbow, shoulder, hip, knee, ankle, and MTP joints, and kinematic data were recorded with a 3D video-based motion analysis system. Subjects stood on a force platform and center of pressure excursions were calculated from the ground reaction force data. Reach distance was calculated as the difference between maximum reach and arm's length reach for the wrist marker.

Age affected movement speed and maximum reach distance. For the elderly subjects, movement time was greater but reach distance was less than in the young subjects for all target directions ($p<.01$). The effect of target direction was the same for elderly and young subjects. Reach distance was maximum in the forward direction (0°) and minimum in the lateral direction 90° ($p<.01$). However, reach distance was greater in the posterolateral direction (120°) than in the lateral direction (90°). Results showed postural stability was decreased during reaching in lateral directions for all subjects. However, instability was greater for the elderly regardless of reach direction.

641.11

The Stability of Standing Perturbations in Elderly and Young adults: A Dynamic Systems Perspective. M. Hoffman*, R. M. Angulo-Kinzler & D. M. Kocejа, Motor Control Lab, Indiana University, Bloomington, IN 47405.

The control of the standing posture is essential for effective functional behavior. Humans are complex systems that demonstrate emergent solutions to motor problems. The preferred movement patterns that each individual demonstrates are called attractors. The stability of the attractor can be measured by its relaxation time after a perturbation. We investigated the stability of 9 elderly (age= 71.7 yrs) and 9 young (age= 27.2 yrs) adults' motor responses to perturbed standing. We elicited a 100% M-wave excitation (1 ms square wave pulse) in the left and right soleus while subjects were standing on a force platform with their eyes open. This perturbation produced a disequilibrium in the backward direction. Six trials were administered, but only the last four were included in the analysis. We analyzed the characteristics of the COP divided into three periods: pre-perturbation, active, and post-active. The active period was defined from the onset of the perturbation to the recovery of similar stability as in the pre-perturbation period. Significant differences were found in the duration of the active period between elderly and young adults ($p=0.014$). Furthermore, elderly show a tendency to converge to a different point in the state space after the perturbation. These results suggest that the standing strategy that elderly use is less stable than young adults and therefore, the effects of perturbations may place them more easily at risk.

641.12

CROSS-CORRELATION ANALYSIS OF THE HIP-KNEE COORDINATION STRATEGIES OF SQUATTING MOVEMENT IN HUMANS. A. Bengoetxea, J.P. Draye, B. Dan, M. Bourgeois, T. Pozzo and G. Cheron*. Lab. of Movement Biomechanics, Univ. of Brussels, ULB, Brussels, Belgium.

Nine normal human subjects were asked to perform rapid self-paced standing up movement from a initial squatting position. The whole body movements in the frontal plane were recorded by means of a 100 Hz optoelectronic ELITE system, that computed the coordinates of 11 small reflective markers placed on the principal joints. Electromyograph (EMG) patterns of 8 lower limb muscles on one side were recorded using telemetry, rectified and integrated. A four-link kinematic model was used to calculate body center of mass (COM) position in the sagittal plane. Angular velocity and acceleration signals of the hip, knee and ankle were obtained by digitally differentiating angular position signals. The goal of the study was to determine the strategies involved during the active phase of the standing up movement. For this purpose, a subset of each measured series of the angular acceleration were subjected to conjugate cross-correlation analysis. This analysis performed at the level of the hip, *h(t)*, and knee, *k(t)*, joints strongly indicates that there exist two common strategies denoted "in -phase" and "out-of-phase" strategy. For the "in-phase" strategy, recorded in 5 subjects, the cross-correlation function (CCF) between *h(t) and k(t)* presents a positive peak (the Z transforms of the CCF range from 0.6 to 1.15). Conversely, for the "out-of-phase" strategy, recorded in the 4 others subjects, the CCF presents a negative peak (from -0.45 to -1.7). Quantitative analysis of the first 100 ms of the EMG patterns (integrated area) of the mono- and biarticular muscles of the thigh and of the initial COM partition does not revealed any significant difference between the 2 strategies. Taken together these results suggest that the emergence of these strategies is not dependent on the initial conditions or the initial relative activation of the muscles but mainly reflects a more subtle time partitioning of the neural command.

641.13

MULTIVARIATE MEASURES OF POSTURAL CONTROL DURING ALTERED SENSORY CONDITIONS RA Speers[1], AD Kuo[2] and FB Horak[3]* [1]Program in Bioengineering and [2]Dept. of Mechanical Engineering and Applied Mechanics, The University of Michigan, Ann Arbor, MI 48109-2125; [3]R.S. Dow Neurological Sciences Institute, Portland, Oregon, 97209

Human postural sway is conventionally defined as a univariate measure of movement, usually of the center of gravity or center of pressure. We propose that sway is actually multivariate in character, such that more than one descriptor is necessary to fully characterize postural control during various sensory conditions.

The Sensory Organization Test (SOT) (EquiTest, Neurocom International, Clackamas, Oregon) examines postural response to six sensory conditions which alter the available visual and proprioceptive information. The SOT was used to collect data from a normal population of 12 young adults. Covariances of body kinematics during the SOT protocol were used as a general measure of multijoint sway, and are a superset of univariate COM or ankle sway. For multivariate sway, the covariances exhibit changes which a univariate measure such as COM variance might not detect. Multivariate sway descriptors based on the covariances were computed for each of the six SOT conditions for each of the subjects. Multivariate ANOVA found significant differences ($p < 0.05$) among the mean multivariate descriptors for the 6 SOT conditions. Discriminant analysis further identified contributions to postural response due to vision, platform sway-referencing, and visual surround sway-referencing, which univariate measures such as COM variance cannot capture. A computational model of sensorimotor integration and control supports these findings.

NASA Graduate Student Researchers Program, Claude Pepper OAIC at The University of Michigan, NIH grant 1R29DC02312-01A1 and the NASA/NIH Center for Vestibular Research grant P60-DC02072.

641.14

Modulation of soleus H-reflex with varying visual and auditory input. G.A. Novak and D.M. Kocejа*. Motor Control Laboratory and Program in Neural Science, Indiana University, Bloomington, IN. 47405.

Previous research demonstrates modulation of Hoffmann reflex amplitude with changes in visual input. However, we are unaware of any published studies investigating the modulatory effects of auditory and combined visual-auditory stimuli on H-reflex amplitude. In this study the effects of manipulating auditory and visual input were investigated in eight subjects (mean age = 24.4 years) under five experimental conditions: (1) control, (2) no visual input - no auditory input, (3) visual stimulus - no auditory input, (4) auditory stimulus - no visual input, (5) control. Subjects performed ten trials in each condition. The H-reflex was electrically elicited at the beginning of each trial by delivering a 1 ms square wave stimulation to the tibial nerve in the popliteal fossa of the right leg while standing. Average soleus background EMG, average tibialis anterior background EMG, (40 ms bin) and peak to peak amplitude of the soleus H-reflex were measured online for each trial (sampling rate = 2 kHz). A repeated measures ANOVA revealed significant decreases in H-reflex amplitude ($p < 0.05$) for all experimental conditions while background EMG remained constant. These results suggest that supraspinal mechanisms, possibly due to presynaptic or recurrent inhibition, modulate H-reflex amplitude across varying modes of sensory input.

641.15

RELATIONSHIP AMONG PRESYNAPTIC INHIBITION, H-REFLEX MODULATION, STATIC STABILITY, AND RECOVERY FROM POSTURAL PERTURBATION. R.G. Mynark[1,2]*, D.M. Koceja[1,2], and C.A. Lewis[1]. [1]Motor Control Laboratory, [2]Program in Neural Science, Indiana University, Bloomington, IN 47405

As the demands placed upon the body by movement increase, a subsequent decrease in the amplitude of the monosynaptic reflex response can be observed. Recent studies have correlated this modulation to changes in presynaptic inhibition of Ia afferent nerve fibers prior to their synapse onto α-motoneurons. It has been hypothesized that the modulation of the H-reflex may be related to the ability to maintain static stability and recover from acute postural perturbation. The purpose of this study was to correlate the supine to standing changes in presynaptic inhibition and H-max/M-max ratio with measures of static stability and recovery from acute perturbation of balance. Changes in presynaptic inhibition from supine to standing were measured in six subjects (4 females/ 2 males; age = 31.8 ± 6.7) using a heteronymous facilitation protocol as described by Hultborn et al. (1987). Modulation of H-max/M-max ratio between conditions was also measured. Static stability was tested during normal standing with eyes open on a Kistler force platform. Recover from acute postural perturbation was tested under the same conditions with perturbation elicited by a bilateral supramaximal tibial nerve stimulation. Results indicated a 46.9% increase in presynaptic inhibition, and a 13.0% decrease of the H-max/M-max ratio from supine to standing. Static stability testing yielded a mean sway area of 22.29 mm^2/s, while perturbation testing yielded a mean recovery duration of 1.81 seconds. Correlation analysis revealed a strong association between presynaptic inhibition and H-max/M-max ratio. Furthermore, results demonstrated a possible link between mechanisms of reflex modulation and the ability to recover from acute postural instability.

MECHANICS AND DYNAMICS

642.1

NEURAL COMPUTATIONS UNDERLYING THE EXERTION OF FORCE: A MODEL. B. Amirikian*, A. V. Lukashin, A. P. Georgopoulos. Brain Sci. Ctr., Dept. of Veterans Affairs Med. Ctr., Minneapolis, MN 55417; and Dept. of Physiology and Neurology, Univ. of Minnesota Med. Sch., Minneapolis, MN 55455.

We have developed a model that simulates possible mechanisms by which supraspinal neuronal signals coding forces could converge in the spinal cord and provide an ongoing integrated signal to the motoneuronal pools whose activation results in the exertion of force. The model consists of a three-layered neural network connected to a two-joint-six-muscle model of the arm. The network layers represent supraspinal populations, spinal cord interneurons, and motoneuronal pools. We propose an approach to train the network so that, after the synaptic connections between the layers are adjusted, the performance of the model is consistent with experimental data obtained on different organisms using different experimental paradigms: the stiffness characteristics of human arm; the structure of force fields generated by the stimulation of the frog's spinal cord; and a correlation between motor cortical activity and force exerted by monkey against an immovable object. The model predicts a specific pattern of connections between supraspinal populations coding forces and spinal cord interneurons: the weight of connection should be correlated with directional preference of interconnected units. Finally, our simulations demonstrate that the force generated by the sum of neural signals can be nearly equal to the vector sum of forces generated by each signal independently, in spite of the complex nonlinearities intervening between supraspinal commands and forces exerted by the arm in response to these commands.

Acknowledgments. This research was supported by contract N00014-94-1-0033 from the Office of Naval Research.

642.2

TRANSFORMATION OF SPIKING ACTIVITY OF MOTOR-CORTICAL CELLS INTO MOTOR OUTPUT OF AN ACTUATOR. A. V. Lukashin*, B. Amirikian, A. P. Georgopoulos. Brain Sci. Ctr., Dept. of Veterans Affairs Med. Ctr., Minneapolis, MN 55417; and Dept. of Physiology and Neurology, Univ. of Minnesota Med. Sch., Minneapolis, MN 55455.

Neurophysiological studies have shown that the direction of motor output (movement or isometric force) is markedly related to the activity of motor cortical cells. One problem in motor control concerns the mechanism whereby the central nervous system translates the motor cortical command encoded in temporal series of action potentials (spike trains) into balanced activation of limb muscles to produce precisely directed movement or force. The aim of the present report is to examine whether and how the *natural* spiking activity obtained from recordings in the motor cortex of monkeys while the animals performed a visuomotor task can be transformed into the motor output of a simulated actuator that mimics the primate's arm. The transformation is done by an artificial neural network using experimental spike trains as the input activity, whereas the output activity controls the contraction of actuator "muscles". The actuator responds to the motor cortical signals with surprising fidelity, both for the temporal and the spatial characteristics of the performance, often matching the performance of trained monkeys. Moreover, we show that the motor output at each instant of time may be controlled by spiking activity of as few as 15 motor-cortical cells. These results suggest a simple analog scheme for real-time biological signal processing.

Acknowledgments. This research was supported by contract N00014-94-1-0033 from the Office of Naval Research.

642.3

POSTURE DEPENDENT SENSITIVITY OF CHANGE IN GEOMETRICAL CHARACTERISTICS OF HUMAN ARM STIFFNESS ELLIPSE. H. Gomi 1 & R. Osu 2*. 1NTT Basic Res. Labs. Kanagawa, Japan, 2ATR HIP Res. Lab. Kyoto, Japan.

The sensitivities of the geometrical characteristics of arm stiffness at hand during force control in the horizontal plane were investigated, which are indexed by shape and orientation of stiffness ellipse and characterized by arm posture and ratios between shoulder, elbow, and double-joint stiffness. The subjects, four people 23-33 years old, were restrained by straps in front of a manipulandum controlled by a position servo. The hand was tightly coupled with the handle of the manipulandum and supported in the gravity direction. The subjects were requested to push the handle and keep a specified force (0, 5, 10, 15 N) in a certain direction (among 8 directions) assisted by a force vector on a computer monitor without cocontraction. To keep muscle activities constant through one condition, rectified and filtered EMG of six muscles were also monitored. The hand was slightly pushed and pulled back in randomized eight directions within a brief period (0.3 s) (24 times under each condition). The arm stiffness parameters under each condition were estimated by least square error method.

At the distal hand position, as previously studied, geometrical characteristics, shape and orientation of stiffness ellipse, derived from arm stiffness matrix, were not much altered under different requested conditions. At the proximal hand position, those characteristics, however, were much altered. On the other hand, the ratios between joint stiffness components were greatly changed according to the force direction at any hand positions, although double-joint stiffness values mainly contributed by double joint muscles were always lower than the elbow and shoulder single joint stiffness mainly contributed by single- and double-joint muscles. From sensitivity analyses, it has been revealed that the shape and orientation of ellipse are insensitive to the changes of the joint stiffness ratios at the distal hand position, and are sensitive to those changes at the proximal hand position. In other words, slight changes of geometrical characteristics of stiffness ellipse do not directly indicate slight changes of joint stiffness ratios. The experimental results partially support the opinions that the human can change not only size but also geometrical characteristics of stiffness ellipse by regulating joint stiffness ratios (Hogan 1985), and that joint stiffness values under force resisting conditions are related to joint torques (Gurevich 1993).

642.4

AFFINE DIFFERENTIAL GEOMETRY ANALYSIS OF HUMAN ARM TRAJECTORIES.

T. Flash,* A. A. Handzel. Dept. of Applied Mathematics and Computer Science, Weizmann Institute of Science, Rehovot, 76100, Israel.

A mathematical framework based on differential geometry, Lie group theory and Cartan's moving frame method was developed for the analysis of human hand trajectories. According to Lacquaniti et al. (1983) drawing movements obey the two-thirds power law: $A = KC^{2/3}$, A, C and K are the angular velocity, Euclidean curvature and the piecewise constant velocity gain factor. We show that within each segment the unimodular affine arc length ($\sigma = \int C^{\frac{1}{3}} ds$) is proportional to movement time. The trajectories of a one-parameter subgroup of unimodular affine transformations are conics or straight lines $x = x_0 G$; $detG = 1$. The affine curvature χ is conserved in this process and is constant along the trajectory and the curve has no inflection points. Thus, conics (the parabola, ellipse and hyperbola with $\chi = 0, > 0, and < 0$, respectively) are the orbits of some point under the action of $G = e^{\sigma v}$ where v is the Cartan matrix with zero trace that depends on χ which is the simplest non-trivial affine differential invariant. Parabolas are the unimodular affine geodesics. The time scaling property of human movements is equivalent to keeping the affine arc length invariant under speed changes. For an ellipse $L = 2\pi^2 A_r^{\frac{1}{3}} = KT$ where L, A_r and T are the ellipse's perimeter, area and movement duration, respectively. Georgopoulos et al., (1988) and Schwartz (1993) showed that the movement velocity vector is represented by N, the neural population vector. We show that a variational principle based on N and $\frac{dN}{dt}$ leads to the two-thirds power law and is consistent with a Kepler's like law whereby $(\frac{d\sigma}{dt})^3 = N \times \frac{dN}{dt} = K$. Affine differential invariants were used to examine movement segmentation and classification.

642.5

RECONSTRUCTION OF JOINT EQUILIBRIUM TRAJECTORIES DURING UNRESTRAINED ARM MOVEMENTS. M.L. Latash* and A.S. Aruin, Department of Kinesiology, The Pennsylvania State University, University Park, PA 16802, USA.

The framework of the equilibrium-point (EP-) hypothesis was used to reconstruct equilibrium trajectories (ETs) in the elbow and in the wrist during fast voluntary movements in one of the joints. Natural movement variability was used as the source of perturbations that are necessary to reconstruct ETs based on a series of trials at the same motor task. A second-order linear model was used to calculate the instantaneous position of the joint compliant characteristic at different times during the movement. Time patterns of active joint torques were calculated using the inverse dynamics techniques. ETs in the focal joint were N-shaped, similar to ETs described earlier for single-joint movements. ETs in the "postural" joint represented a single oscillatory cycle whose peaks coincided with the peaks of the N-shaped ETs in the focal joint. Peak-to-peak ET amplitude in the wrist during elbow movements could be of a similar amplitude or even bigger that peak-to-peak ET amplitude in the elbow. Note that the actual trajectory in the "postural" joint was an irregular, low-amplitude flapping while the actual trajectory in the focal joint was a typical, sigmoid curve. Reconstructed ETs corresponded well to the EMG patterns in the flexor-extensor pairs recorded during the same movements. We suggest an explicit relation among control variables to the two joints as the foundation of a simple synergy whose purpose is to preserve control over the trajectory of the limb's endpoint in the presence of joint coupling. Supported in part by a grant HD 30128 from the National Center for Medical Rehabilitation Research, NIH.

642.6

MUSCULOSKELETAL CONSTRAINTS ON THE NEURAL CONTROL OF REACHING. C. A. Buneo*, J. F. Soechting, and M. Flanders, Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

We have previously described a method for obtaining *in vivo* estimates of human shoulder muscle actions across a range of arm postures (Buneo et al., 1995). To completely characterize the variations in muscle action with changes in arm posture, a multiple regression analysis was performed. The analysis revealed that muscle actions depended linearly on the parameters describing three-dimensional arm posture; the inclusion of nonlinear (quadratic and/or cubic) terms did not substantially improve the fit to the data. For most muscles, the actions predicted by this analysis could be approximated by models of musculoskeletal geometry which included effective origins and insertions that remained fixed to the body and arm, respectively, during changes in arm posture. The actions of some muscles (e.g., Middle Deltoid), however, could only be adequately described by allowing for movement of the effective origins and insertions relative to their respective body segments. The results demonstrate that muscle lines of action do not move entirely with the arm, an important consideration in the design and interpretation of experiments pertaining to neural representations of arm posture. (Supported by NS-15018 and NS-27484.)

642.7

Origins of the Power Law Relationship between Movement Velocity and Curvature: The Effects of Muscle Mechanical Properties and Limb Dynamics.

Paul L. Gribble* and David J. Ostry, McGill University, Montreal, Canada.

When subjects trace patterns such as ellipses, the instantaneous velocity of movements is related to the instantaneous curvature of the trajectories according to a power law — movements tend to slow down when curvature is high and speed up when curvature is low. It has been proposed that this relationship is centrally planned. However, kinematics are the result of the interaction of central control with muscle mechanics and limb dynamics, and even under isometric conditions, muscle mechanical properties can affect the development of muscle forces and torques. Thus without a model which accounts for these effects, it is difficult to distinguish between kinematic patterns which are attributable to central control and patterns which arise due to dynamics and muscle properties and are not represented in the underlying control signals. In this paper we address the role of muscle mechanical properties and dynamics in the emergence of the power law. We use a mathematical model of arm movement control based on the λ version of the equilibrium-point hypothesis. We demonstrate that simulated elliptical and circular movements, and elliptical force trajectories generated under isometric conditions, obey the power law even though there was no relation between curvature and speed in the modelled control signals. We suggest that limb dynamics and muscle mechanics — specifically, the spring-like properties of muscles — contribute significantly to the emergence of the power law. This research was supported by grants from NSERC, Canada, and FCAR, Quebec.

642.8

MUSCLE PROPERTIES PREDOMINATE IN DETERMINING FORM OF DYNAMIC MOVEMENTS. A.M. Krylow, J.D. Given*, and W.Z. Rymer, Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611, and Department of Biomedical Engineering, Northwestern Univ., Evanston, IL, 60208.

In models of motor control, it is often assumed that muscles act as simple torque generators or conservative springs. Likewise, it has been suggested that reflexive inputs active during movement strongly modify muscle actuator properties. Such reflex modifications are often presumed to be so powerful that comparisons with mechanical properties observed in areflexive muscle preparations are irrelevant to movement control issues. We studied the influence of muscle properties in human subjects on the control of inertially loaded, single DOF movements at the wrist. Comparisons were also made to mechanical behavior of reduced animal preparations under similar loading conditions.

Two experimental protocols were performed. In the first, subjects moved a simulated inertial load as quickly as possible by flexing their wrist from an initial angle. In the second, subjects moved the loaded wrist between specified initial and final angles in the flexion direction. Additionally, they were required to match the peak decelerating torque to a predefined level (~20% of MVC). Loads ranging from 1 to 50 times the hand inertia were applied. Wrist angle, velocity, net torque, and electromyographic (EMG) signals from one primary wrist flexor (FCR) and extensor (ECRB) were recorded.

Results of the first protocol show characteristic torque time courses and peak values that vary substantially and systematically with load. Larger loads allow production of larger torques at the expense of movement speed. Very similar force profiles are produced in inertially loaded, isolated muscle using constant electrical stimulation. The second protocol shows that although time scaled angle and torque profiles, and normalized velocity profiles are nearly superimposable, agonist EMG amplitudes scale strongly with changes in load with matched torques. This scaling of EMG amplitude is absent in the antagonist. These findings indicate that 1) muscle mechanical properties like those seen in isolated preparations provide strong constraints on dynamic performance of intact subjects, and 2) the asymmetric muscle responses observed in isolated shortening versus lengthening muscle are clearly operating during normal movements, with little evidence of strong reflex modification. Funding: NIDRR H133P20016; AR40425.

642.9

COUPLING OF MUSCLE ACTIVATION IN SINGLE AND TWO JOINT PLANAR MOVEMENT ABOUT THE WRIST AND ELBOW. E. Oguni*, K.K. Maitra and J. D. Cooke, Appl. Health. Sci., Univ. Western Ont., London, ON, Canada N6G 1H1.

In this study we address the issue of whether the central nervous system compensates for reaction torque at the elbow due to wrist movement. We examined the relation between movement kinematics, dynamics and the phasic muscle activation in planar arm movements involving wrist and elbow. Movements were made with different loads (0 -1.0 kg) at the hand. Subjects made movements about only wrist (amplitude 10 - 50 deg), only elbow (amplitude 10 - 70 deg) or both in a step tracking paradigm. In movements involving only the wrist or elbow the other joint was not restrained.

Movement about the wrist alone caused small (<10 deg) transient and oppositely directed elbow movement. These were amplitude and load dependent. Movement about the elbow only caused wrist movement in the same direction (< 20 deg), the amplitude varying with elbow amplitude but not load. In two joint movement, wrist movement always started and ended ahead of elbow movement. In all cases agonist-antagonist activation pattern at the elbow and wrist were time locked with each other. However, magnitude of activation depended on the torque and task specification. Compensation for reaction torque was observed at the wrist but occurred at the elbow only during two-joint movement with load.

The data suggest that the CNS utilizes common programming for movements of any or all of the elbow-forearm-wrist-hand segments. EMG magnitudes are matched to the task and force requirement.

Supported by NSERC

642.10

SIMULTANEOUS ACTIVATION OF MUSCLES FOR TWO-JOINT ARM MOVEMENTS IN DOWN SYNDROME M. M. E. A. Inoue, G. L. Almeida, D. M. Corcos and Z. Hasan*. Universidade Estadual Paulista, SP, Brazil, Universidade Estadual de Campinas, SP, Brazil, and University of Illinois, Chicago, U.S.A.

Studies on individuals with Down Syndrome (DS) have suggested that their multijoint arm movements may involve an abnormal, distal-to-proximal sequencing of muscle activity.

We examined the kinematics and electromyographic (EMG) activity for horizontal-plane arm movements in 8 DS and 8 age-matched NN subjects. The arm rested on supports that could slide freely on the surface of a table. Subjects moved "as fast as possible" to a visible target, and returned immediately to the initial position. Four target locations were employed, for which the required elbow angle excursions were comparable, but the required shoulder angle excursion varied from 10 deg abduction to 75 deg adduction. EMG onsets were determined from pectoralis major, posterior deltoid, the biceps and triceps brachii muscles.

The movements of DS subjects were slower and less smooth than NN's; we did not, however, observe abnormalities at motion reversal such as those described for deafferented subjects. The EMG onsets for DS subjects were markedly different from those for NN subjects. For the latter, the EMG onset time difference for any 2 of the 4 muscles varied systematically with target location. The 6 pairs thus examined included muscles antagonistic to each other, and muscles acting primarily on different joints. DS subjects, in contrast, exhibited almost simultaneous activation of all 4 muscles, and the onset time difference for none of the 6 pairs of muscles varied with target location.

Our results do not support the idea that DS individuals use a distal-to-proximal sequence of muscle activation; instead, their coordination deficit is reflected in an inability to modulate the onset times of different muscles with changes in task requirements.

(Supported by N.I.H. grants NS19407, AR 33189, NS 28127, NS 01508 and CNPq proc. 3000587/95-3.)

642.11

AUTOSCALING OF PERCEIVED MUSCLE OUTPUT. G.I. Boorman, V.R. Edgerton, J.A. Hodgson, C. Goulet, A. Garfinkel*. Dept. Physiol. Sci. Univ. California,Los Angeles 90095-3149.

Perception of muscle force derives preferentially from central signals related to the output pathways. Most studies of force perception have examined either single output levels or narrow ranges of muscle contraction. Reports of experiments examining the full range of muscle output are contradictory when describing the shape of the relationship, with some reporting a linear form, and others reporting a curvi-linear relationship. The present study was undertaken to characterize perception of the full range of muscle output. Isometric muscle contractions were examined at both the ankle and elbow in 14 subjects who were requested to contract muscle groups at levels which were described as a percentage of maximal output. At each request the subject contracted the muscle group at an output level perceived to match the request. No feedback relating to the muscle contraction was given to the subject. The relationship between measured and requested outputs, as determined by examining actual torque and EMG outputs across the full range, was well fit by a line with slopes commonly close to unity. Moreover, when torque levels were reduced by altering the joint angles, the slopes and y-intercepts of the relationships were unchanged. These observations demonstrate that autoscaling of perceived muscle output occurs, such that the nervous system continually senses a constant range of muscle output. The simplest model which explains these findings is that, as suggested by others, force is perceived by way of a corollary discharge of the motor output. Additionally, these data examining the full output range demonstrate that the corollary discharge is an invariant proportion of the output signal, a scheme which might benefit higher motor centers by reducing the time required to rapidly appraise novel plans for changes in ongoing motor outputs.

Supported by NASA #199-26-12-09 and NIH, NRSA(DE07212) from NIDR.

642.12

THE CONTRIBUTION OF STOCHASTIC PROCESSES TO VARIABILITY IN CONTINUOUS FORCE PRODUCTION. A.B. Slifkin* and K.M. Newell. Depts. of Biobehavioral Health and Kinesiology, Penn State University, U. Park, PA 16802.

Traditionally variability in motor behavior has been viewed as a reflection of the stochasticity (noisiness) of perceptual-motor processes. The purpose of the present research was to provide an explicit test of the assumption that a positive correlation exists between variability and noise in force production. Accordingly, subjects (n = 10) produced force over continuous, extended periods (30 s) at different levels (3, 6, 12, 24 & 48%) of their maximum voluntary contraction (MVC). Here, measures of variability were the within-subject standard deviation (SD) and the coefficient of variation (CoV) for force production. Approximate entropy (ApEn) and the power spectrum (PS) were used to index the noise content of force output. For example, a pure sine wave, reflecting an absence of noise, has low ApEn and a PS with all power at a single frequency. On the other hand, random white noise, which has been taken as the signature of maximum stochasticity, has high ApEn and equal power at all frequencies of the PS. Results revealed that the SD increased exponentially and CoV of force output decreased exponentially as the criterion level of force increased. Despite increases in target force level, the PS remained sharply peaked with almost all power confined to a narrow bandwidth (1-5 Hz). Furthermore, ApEn was quite low and did not change across the range of force requirements. These findings suggest that the increment of force variability with level of force output is not due to greater noise as reflected in greater randomness of force output. The concepts of variability and noise are *not* synonymous in motor control.

Supported by NIH (NICHD) F32-HD07885-02.

642.13

THE RELATIVE IMPORTANCE OF OPEN-LOOP AND ERROR-DRIVEN MECHANISMS IN HUMAN LOAD HANDLING. P.R. Burgess*, C.F. Buhler, T.A. Cooper and L.F. Jones. Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108.

The relative importance of open-loop and error-driven mechanisms in normal motor behavior is a fundamental question which remains unresolved in spite of long-standing recognition of its importance. In this study, subjects raise a weight an unspecified distance and then lower it in their own time. The kinesthetic and visual feedback loops are then opened by making muscular force generation isometric and the precision and accuracy of the subject's force control is again evaluated. The kinesthetic and visual loops are not opened in the sense of being physically cut in the isometric situation but are dissociated from subsequent mechanical events; a motor feedback pathway is functionally open when it can no longer respond to changes in muscular force produced by the open-loop controller. Under isometric conditions, the subject can produce muscular force either with the aid of force feedback or with an unregulated open-loop model, whichever is most effective. Thus, this approach defines an upper limit for open-loop capability and will overestimate that capability if, as seems likely, force feedback enhances performance. In preliminary studies where the subject is to raise the lighter but not the heavier of two weights, force control under isometric conditions (weight not lifted) is relatively poor compared to force control under isotonic conditions (weight lifted). The current conclusion is that open-loop performance is poor compared to the force control required by the mechanics of load handling.

FIHC

642.14

MAXIMAL USE OF REFLEXES IN THE CONTROL OF THE FASTEST MOVEMENTS: REFLEX-DEPENDENT CHANGES IN THE ONSET TIME OF AGONIST ACTIVITY M. F. Levin*, S. V. Adamovich and A. G. Feldman, Institut de réadaptation de Montréal, Montreal, Qc H3S 2J4, Canada & Institute for Information Transmission Problems, Acad Sciences, Moscow

The convergence of descending and reflex pathways on common interneurons and motoneurons implies that active movements may result from changes in reflex parameters produced by control signals. We hypothesized that control systems may exploit rather than suppress reflexes during fast movements. Consequently, external perturbations may be ineffective in eliciting additional reflex modifications of EMG patterns unless they are relatively strong. This may account for the relatively weak effects of perturbations on the initial agonist EMG burst (Ag1) observed in fast movements. On the other hand, there may be robust reflex modifications of the timing and shape of Ag1 in response to strong perturbations. In control trials, subjects made fast unopposed 60° elbow flexions (peak velocity >500°/s) in response to an auditory signal. In random test trials, a brief (50 ms) torque of 8-15 Nm either assisting or opposing the movement was applied 50 ms after this signal. Subjects had no visual feedback and were instructed not to correct arm deflections. In all subjects, the onset time of the Ag1 depended on the direction of perturbation: about 37 ms less in opposing compared to assisting load conditions. Assisting torques caused a short latency (37 ms) additional antagonist EMG burst preceding the Ag1. Our results suggest that the direction-dependent changes in the onset and duration of Ag1 as well as the antagonist activation preceding Ag1 resulted from stretch reflex activity elicited by the perturbations rather than from a change in the control strategy. Results are consistent with the hypothesis that control systems exploit proprioceptive reflexes in the production of even the fastest movements.

Supported by Natural Sciences and Engineering Research Council of Canada.

642.15

NONLINEAR DAMPING PROPERTIES OF THE MUSCLE/REFLEX SYSTEM WITH INERTIAL LOADS. D.C. Lin* and W.Z. Rymer. Depts of Biomedical Eng. and Physiology, Northwestern Univ., Chicago, IL 60611.

The dependence of reflex response on length, velocity, and frequency has been well characterized in length controlled conditions. However, the maintenance of posture requires these properties to interact via a load, often dominated by inertia, and it is difficult to extrapolate how they combine to damp perturbations. Therefore the objectives were: (1) to identify special damping properties of the muscle/reflex system with an inertial load and (2) to determine if there is compensation for load variations.

Data were collected from the soleus muscle in a decereberate cat. A background force was generated by the crossed-extension reflex and a force impulse was then imparted on the load, stretching the muscle and resulting in an oscillatory motion. Both the magnitudes of the impulse and inertia were varied.

Damping within a given trial was measured by calculating the logarithmic velocity decrement of each oscillation cycle. For a given mass, the damping increased with the amplitude (both displacement and velocity) of oscillation. The force-length plot revealed that energy dissipation occurred almost exclusively during the shortening phase of the cycle, while the lengthening phase behaved like a mass-spring system.

Damping across masses was assessed with two methods. First the slope of the damping-displacement relationship should decrease as mass increases because velocity decreases as mass increases for a given amplitude. Yet the slope did not statistically decrease, implying that damping also increased with mass. Secondly, the calculated time constant of exponential decay decreased by approximately $m^{-1/3}$, compared to the m^{-1} decrease of a linear system.

The nonlinear damping properties of the muscle/reflex system are significant in determining the response of an inertial load to a perturbation. The amplitude dependent relationships provide more damping for bigger oscillations which can result from either a larger perturbation or inertia. In addition, the decrease in damping was not as sensitive to an increase in inertia as a linear system, indicating the reflex response is well tuned for compensating for load variation.

Work supported by AR40425, T32-HD07418, and the Faulk Trust.

642.16

INTERLIMB COORDINATION PATTERNS DURING ASSYMETRICAL REACHING MOVEMENTS. V. Hatzitaki[1] and P.A. McKinley[2]*, Dept of Physical Education[1], School of Physical and Occupational Therapy[2], McGill University, Montreal Canada, H3G 1Y5

The purpose of the present study was to examine intersegmental dynamics and muscle activation patterns of *discrete* asymmetric bilateral reaching movements. Our goal was to determine if torque production at the joint is scaled according to the level of asymmetry introduced by bilateral variations in movement amplitude as previously suggested (Marteniuk et al, Quart J Expt Psych 36A: 335-365, 1984; Walter & Swinnen, Brain & Cognit 14:185-200, 1990). Adult, right-handed male subjects (N = 10) executed bilateral reaching movements in the horizontal plane. Randomized block trials (n = 40) consisted of symmetrical full-reach movements and 2 asymmetrical movements where the preferred arm reach was reduced to 75% and 50% of full-reach respectively. EMG from the pectoralis, deltoid, triceps and biceps muscles was filtered (15-500Hz), collected online (1000Hz) and synchronized with kinematic data from shoulder, elbow and wrist(240Hz, Ariel systems). Inverse dynmamics were used to generate inertial, muscle and net joint torques which were expressed with respect to an interlimb decoupling coefficient (right:left integrated torque or *T*). During the acceleration phase, constrained limb antagonist muscle onsets were earlier and muscle *T* scaled according to the level of asymmetry at both the shoulder and elbow, across subjects. By contrast, agonist muscle onset was immutable, while inertial *T* was preferentially affected by only one of the levels of asymmetry, and was subject specific. During deceleration, degree of asymmetry was reflected primarily in inertial *T*. Taken together, these results suggest that decoupling is effected during the acceleration phase by a mutable onset of the antagonist muscles which scale the muscle torque and limb stiffness. This controlling behavior results in the inertial *T* differences observed in the deceleration phase. Supported by NSERC

642.17

CATS USE MULTIPLE COORDINATION PATTERNS TO ACHIEVE THE SAME MOTOR TASK UNDER DIFFERENT INERTIAL LOADS. K. D. Statler, E. A. Keshner*, S. L. Delp, and B. W. Peterson. Sensory Motor Performance Program, Rehab. Inst. of Chicago, Dept. of Biomedical Engineering and Dept. of Physiology, Northwestern University, Chicago, IL 60611.

Simultaneous video-fluoroscopic and neck muscle EMG data were recorded from one cat performing ±15° sinusoidal (0.25 Hz) head tracking movements in the sagittal plane. The cat performed the movement in a standing body posture with two different starting neck orientations and four different inertial loads. Radio-opaque markers were inserted into the anterior/posterior and lateral aspects of the occipital ridge and C_1-C_7 to measure vertebral displacement. Kinematic data of the head and neck were analyzed using a computer model. The model was used to characterize the limits of movement in the cervical spine and to estimate the moment arms of the neck muscles at different orientations of head-neck movement. Experimental results showed that for each initial neck orientation the cat utilized a distinct set of vertebral alignments, relative joint movements, and muscle activation patterns to achieve the same movement outcome. As inertial load increased, vertebral alignments and relative joint movements were constant, but muscle activation patterns varied. The model predicted that muscle moment arms would vary little for the different vertebral alignments chosen by the cat to complete the task. Increasing the mass on the cat's head was expected to produce a generalized increase in EMG responses. We found, however, that rectus capitis and splenius EMG gains increased with increased mass, but complexus EMG gains decreased with increased mass. Biventer cervicus and occipitoscapluaris EMG gains initially increased, but then remained constant as mass was increased. EMG phases were not affected by changing the mass of the system. The data indicate that different muscle activation patterns were used to maintain the same kinematic pattern when the cat completed the tracking task with increased inertial loads.
Supported by grants BNS9109705 and NS22490.

642.18

CHARACTERIZATION OF ARM MUSCLE ACTIVITY IN A FREELY MOVING OCTOPUS (*Octopus vulgaris*). **Y. Gutfreund[1], G. Fiorito[3], T. Flash[2], I. Segev[1] and Y. Yarom[1], B. Hochner*[1].** [1]Department of Neurobiology, The Hebrew University, Jerusalem, Israel. [2]Department of applied mathematics, The Weizmann Institute of Science, Rehovot, Israel. [3]Department of Neurobiology, Zoology Station, Naples, Italy.

The extreme flexibility of the octopus arm enables it to perform a large repertoire of different movements. Combining kinematic studies of arm movements with physiological studies of muscle activation provides an important step towards understanding of the neuronal control mechanism underlying the generation of these movements. In a previous study we described a basic invariant motor structure in arm extension - a bend in the arm that travels from its base towards its tip. In the present study we measured the muscle activation (EMG) that accompanied this movement. Stainless steel, electrodes were implanted in the arm musculature of an anesthetized octopus. Following recovery from anesthesia, a target was lowered into the water to initiate arm movements. The movements were recorded on videotape synchronously with muscle activity. The results show that the traveling bend is associated with a propagating wave of muscle activity. Maximal muscle activity was recorded slightly before the traveling bend reached the recording site and occasionally tonic activity was maintained afterwards. In order to correlate the level of activity to the movement velocity the EMG signals were rectified and integrated over various time windows. The integrated EMG measured at the time in which the bend passed the electrode site, was positively correlated with the peak velocity of the bend movement. The peak velocity usually occurred distally to the recording site. Based on this observation and on the invariance of the bend velocity profile we suggest that the velocity of the bend propagation is controlled, in a feed forward way, by adjusting the level and velocity of propagation of muscle activity.
Supported by grants from the U.S. ONR and from the Israel Science Foundation.

642.19

EMG PATTERNS: AGE DIFFERENCES IN A SELF INITIATED, SPEED CONSTRAINED MOVEMENT R. D. Seidler & G. E. Stelmach*, Motor Control Laboratory, Arizona State University, Tempe AZ 85287-0404.

The purpose of this investigation was to explore the ability of elderly adults to control braking force activity. The issue addressed was whether antagonist activity is improperly timed in the elderly. Constant amplitude, submaximal speed elbow flexion movements were performed under three different inertial load conditions. Seven young adults and seven elderly adults performed the elbow flexion movements through the horizontal plane while grasping a lever manipulandum. Lever movements were represented in real time by corresponding left (for elbow flexion) and right (for elbow extension) movements of a cursor on a monitor located in front of the subjects. Movement times were constrained via a metronome for both groups to the range of 650-950 ms for a 30 degree flexion movement; trials not meeting the duration criterion were excluded from further analysis. Fifty trials were performed for each of the load conditions. Results demonstrated that the elderly scaled both agonist (AG) and antagonist (ANT) activity to increasing load in a similar manner to young subjects. Despite this, the elderly did not consistently time the cessation of ANT activity such that the offset preceded the initiation of the AG1 burst. In addition to this temporal activation error, the elderly exhibited high tonic ANT activity throughout the movement, possibly to increase control over the manipulandum. This tonic ANT cocontraction combined with the incomplete ANT cessation prior to AG1 initiation resulted in velocity profiles that were flatter and more symmetrical for the elderly than for the young. Results are discussed in terms of implications regarding the aging motor control system.
Supported by: Flinn Foundation and NINDS NS17421.

CONTROL OF POSTURE AND MOVEMENT: KINEMATICS

643.1

KINESTHETIC CONTROL OF A THROWING MOVEMENT. W.G. Darling*, M. Pizzimenti, M. Treinen Department of Exercise Science , University of Iowa, Iowa City, IA 52242.

Recent studies on the role of proprioception in control of throwing-type movements by Cordo and colleagues (e.g.,Cordo; J Neurophysiol 64:161) have used a constrained task involving supported horizontal plane motion of the forearm at the elbow. However, when throwing under less constrained conditions the motions usually involve more joints and release time of the ball may be a function of the forearm position relative to an external axis such as gravity. It is well known that humans have an excellent static sense of forearm angle relative to gravity. Thus, the purpose of this study was to examine the use of kinesthetic information on forearm angle in space during dynamic throwing movements.

We investigated this issue by examining kinesthetically guided throwing movements. Seven subjects were instructed to move the arm in the sagittal plane and to release the ball when the forearm was horizontal. Six different experimental conditions involving manipulations of initial shoulder, elbow, and trunk/head angles during active throwing and in a passive task in which the forearm velocity was varied by attaching weights to the forearm were studied. Forearm angle at ball release averaged about 0.4 rad above horizontal and did not differ in the different conditions. Thus, subjects were not accurate in their estimation of horizontal forearm position in this dynamic task. Variability of forearm angle at release was similar in unconstrained throwing and when starting elbow and shoulder angles were manipulated. In contrast, manipulation of trunk angles increased such variability ($p < .05$). These data show that our dynamic perceptions of forearm angle relative to horizontal are rather poor and that manipulations of trunk/head angles strongly influence such perceptions.

643.2

VELOCITY PROFILES OF ARBITRARY DRAWING MOVEMENTS ARE WELL PREDICTED BY A MODIFIED MINIMUM-JERK PRINCIPLE. E. Todorov, M. Jordan, E. Bizzi*, Brain & Cognitive Sciences, MIT, Cambridge MA.

The trajectories of simple arm movements are well described by the minimum-jerk principle (Flash & Hogan '95), which states that arm movements constrained to pass through a set of via-points are maximally smooth (the mean squared jerk is minimized). However, it is not clear how to apply this principle to tasks where the spatial constraints are not specified as via-points. Furthermore, it fails to predict the observed arm trajectory in some single via-point tasks (Uno et al.'89). Here we demonstrate that in multiple via-point tasks the deviations from the minimum-jerk trajectory are rather large, and vary within and across subjects. We propose a modification that solves both problems: for a specified path of the movement, the tangential velocity along the path is such that the mean squared jerk is minimized. This modified principle cannot predict the path of the movement (which, we argue, is quite variable); it only predicts the velocity profile for an experimentally observed path. We present data from two experiments, in which subjects were asked to move a cursor, i) through a set of via-points; ii) along a completely specified path. We tested arm, wrist, and finger movements, and in all cases the modified minimum-jerk principle fit the observed velocity profiles well.

An alternative theory that predicts velocity as a function of path is the power law (Lacquaniti et al.'83), which states that tangential velocity is proportional to the curvature of the path, raised to the power -1/3. Here we analyze the power law, and show that in 2D and 3D it is mathematically equivalent to setting to zero the jerk along the normal vector. For movement paths with high curvature the power law is a computationally efficient approximation to minimizing jerk (since suboptimal velocity profiles produce high jerk along the normal). For many paths the velocity predictions of the power law and the modified minimum-jerk principle are indistinguishable, except for some specific position along the path. The observed velocities at such positions are much closer to the minimum-jerk prediction, suggesting that the power law may be a consequence of an underlying motor strategy to produce smooth movements.

643.3

HAND SPACE, JOINT SPACE AND PERCEIVED SPACE IN A VISUAL ENVIRONMENT. J.D.Cooke and K.K.Maitra. Appl. Hlth. Sc. Univ. of Western Ont., London, Canada N6G 1H1.

As part of the study to explore the hypothesis whether motion planning is organized in a visually perceived space, we studied two-joint planar arm movements involving the wrist and elbow under explicit visual guidance. Subjects were shown a joint space representation of their movements (wrist Vs elbow angle) on a computer monitor. They were asked to move naturally between two self chosen points in joint space with no instruction of path profile. In another session, they were asked to move between the two self chosen points in a straight line. In a third and fourth session, the subjects were shown a hand space representation of their movements and the two paradigms (i.e., to move naturally and to move in a straight line between two self chosen points) were repeated.

With visual feedback of joint space subjects did not naturally move in a straight path in a joint space. However, they did so when instructed to move in a straight line. This they did without changing the workspace region they used in the natural movements. Similarly, with visual feedback of hand space, they did not move naturally in a straight path in hand space rather produced extremely curved hand paths utilizing large area of hand space. When instructed to move in a straight line hand path, they utilized comparatively limited area of hand space and moved in a minimally curved hand path. Interestingly, this generated a straight path in joint space.

The results suggest that movement planning and organization are two different processes. In visually guided motion, planning is done in visual space where a superposition of visual perception and visuomotor transformation is necessary for proper trajectory formation. Such superimposition may be done by a third process which is instruction or intention dependent. On the other hand, the trajectory is organized in joint space. Support: NSERC, Canada.

643.4

HAND SPACE, JOINT SPACE AND PERCEIVED SPACE IN A NON-VISUAL ENVIRONMENT. K.K.Maitra and J.D.Cooke. Appl. Hlth. Sc. Univ. of Western Ont., London, Canada N6G 1H1.

Recent studies predict that motion planning can be made in either hand space or in joint space depending upon the space in which subjects perceive the motion. We examined this hypothesis by comparing two-joint planar arm movements involving the wrist and elbow in blindfolded subjects. In one session, subjects were instructed to make a "natural" movement of their hand at their own speed between two, self-chosen points. No instruction was given as to the path the hand should follow. In a second session they were also blindfolded and asked to move between two self-chosen points in a straight line.

When instructed to move naturally, the subjects utilized a large area of the hand work space. Handpaths were extremely curved while the joint paths (crossplots of wrist and elbow joint angles) were linear. In contrast, when the subjects were asked to move their hand in a straight line, they utilized a very limited area of the workspace and adopted a different strategy by altering the hand-forearm configuration. This approach produced a minimally curved hand paths and, once again, highly linear joint paths.

The data suggest that in the absence of visual information, the CNS organizes motion in joint space to produce a perceived motion in hand space. This implies that perceptual space and organizational space for motion planning are different. Superposition or dissociation of these two spaces may be task or intention dependent.
Supported by: Natural Sciences and Engineering Research Council of Canada.

643.5

INTER- AND INTRA-LIMB COORDINATION IN ARM TREMOR S. Morrison and K. M. Newell.* Pennsylvania State University, PA 16802.

Inter- and intra-limb coordination in arm tremor was examined in adult subjects under vision and no vision conditions using accelerometery techniques. Standard time and frequency domain analyses were performed as was a measure of the regularity of the acceleration time series using an approximate entropy (Ap En) measure. Data analysis was structured to examine the hypothesis that there is a functional compensatory relation between the motion (tremor) of the limb segments in the arm coordination postural pointing task.

The level of acceleration was shown to increase from proximal to distal within a single arm and was symmetrical across homologous arm segments. Analysis revealed no linkage *between* the arms, suggesting that the neuronal commands underlying normal tremor are not derived from a common central oscillator within the central nervous systrem but are organized in a parallel fashion. The strength of the *intra-limb* coupling varied according to the particular adjacent limb links with significant correlations and coherence being observed between upper arm and forearm, and, between hand and finger. The Ap En analysis revealed that there was more regularity to the upperarm and hand accelerometer signals than the forearm and finger signals. No effect for vision were observed.

The findings show that the intra-limb coordination of the arm links in a two-limb postural pointing task is effected by a compensatory synergy organized about the action of the wrist and shoulder joints. This compensatory synergy reduces the coordination of the 4 within-limb degrees of freedom (arm links) to in effect a single degree of freedom arm control task that is not coupled in organization to the motion of the other limb or the torso. It is proposed that this coordination solution reduces the degrees of freedom independently regulated for realization of the task goal but preserves independent body segment control in critical degrees of freedom for potential adaptation to postural perturbations.

643.6

SUPERPOSITION OF TWO FUNCTIONALLY INDEPENDENT SYNERGIES IN ARM-REACHING MOVEMENTS INVOLVING THE TRUNK. P. Pigeon*, L.H. Yahia, and A.G. Feldman. Centre de Recherche, Institut de réadaptation de Montréal, Montréal, Québec, Canada, H3S 2J4.

To resolve the redundancy problem in the number of degrees of freedom for reaching tasks involving the trunk, the nervous system may use two functionally independent synergies: one synergy involves only arm joints to produce an arm endpoint trajectory (the arm reaching synergy), while the other coordinates movements of the trunk and arm joints leaving the position of the arm endpoint unchanged (the compensatory synergy). To determine whether the two synergies are similarly superimposed during pointings with opposite trunk motions, elbow and shoulder angles were recorded during reaching tasks involving the trunk. Subjects made fast planar movements of the index finger (the arm endpoint) from a near to a far target situated in the contralateral workspace at a 45° angle to the sagittal midline of the trunk. The arm movements were performed alone (control movements) or combined with a forward (in-phase movements) or a backward (out-of-phase movements) sagittal motion of the trunk. The movements were repeated with vision blocked upon initiation of the arm endpoint movement. The elbow angle recorded during control movements was compared to the semi-sum of elbow angles recorded during reaching with in- and out-of-phase trunk motions. The same procedure was applied to shoulder joint traces. Linear regressions for each joint produced correlation coefficients ranging from 0.9868 to 1.000 for both visual conditions. This indicates that the coordination between joint angles produced by the compensatory synergy unfolds with a similar timing for in- and out-of-phase movements performed with or without vision. These results support the hypothesis that multi-joint reaching movements result from the superposition of movements produced by independent synergies, solving, in such a way, the redundancy problem. Supported by NSERC, MRC and FCAR grants.

643.7

DIFFERENTIAL EFFECT OF TASK CONDITIONS ON ERRORS IN DIRECTION AND EXTENT OF REACHING MOVEMENTS. J. Messier, Y. Lamarre* et J.F. Kalaska. CRSN, Dépt. de Physiologie, U. Montréal, Québec, Canada H3C 3J7

Invariant patterns in motor behaviour are presumed to reflect the general organizing rules and sensorimotor transformations by which the CNS plans and executes movements. Invariant patterns in the distribution of the end points of reaching movements have been used to suggest that two important movement parameters of reaching movement, direction and extent, are planned by two independent processing channels. This study examined this hypothesis by testing the interdependency of direction and extent variable errors in two task conditions. In task 1, subjects made planar horizontal reaching movements to 25 targets located at 5 different distances and directions on the horizontal workspace. Subjects closed their eyes before moving the arm and did not receive knowledge of their performance. In task 2, arm movements were made to the same target locations in the same horizontal workspace, but target location was displayed on a vertical screen in front of the subjects. Also, there was a scaling factor of 1:2.4 between the movement amplitude displayed on the screen and the amplitude of movement made by subjects. These changes imposed additional sensorimotor transformations in task 2. Subjects could not see their arm before movement in task 2, but they were shown their movement trajectory after each trial. For both tasks, variable errors of movement extent (on-axis error) were greater than for movement direction (off-axis error). Also, both on and off-axis variable errors increased with movement amplitude.The main difference between the results of these two tasks was that the off-axis/on-axis error ratio values remained constant at different movement amplitudes in the first task, while they increased linearly with movement amplitude in the second task. This result suggests that variable errors of direction and extent can be modified independently by changing task conditions. The modification of the relation of these variable errors to movement extent can be considered evidence that at some point in the planning of reaching movements, direction and extent are processed independently. Supported by MRC Group Grant in Neurological Sciences (JK), and FCAR studentship (JM).

643.8

THE EFFECTS OF ACCURACY CONSTRAINTS ON HAND-TO-MOUTH MOVEMENTS DURING FEEDING. R.M. Dorcy, A.M. Gordon, T.R. Kaminski* & A.M. Gentile. Teachers College, Columbia University, New York, NY 10027.

Feeding behavior in humans involves movement between the hand and mouth, which must be spatially accurate and temporally coordinated to achieve the task objective. The present study examined the coordination of hand and head movement during transport of a glass to the mouth under various constraints of accuracy (levels of fluid). Subjects were instructed to transport a 42 ml capacity glass to the mouth without spilling. The glass was placed at a standard height at two distances: near (40% of arm length), and far (80% of arm length). The three levels of accuracy constraint were: high (full glass), moderate (three-quarters full), low (one-quarter full). Our findings showed that the extent of hand and head displacement depended upon accuracy demands. With higher accuracy constraints (full glass), head displacement increased and hand displacement decreased. Remarkable within-subject consistency was observed in location of the hand-mouth point of coincidence, and in the direction and amplitude of hand and mouth movement within each accuracy constraint-level and distance. The degree to which trunk movement contributed to movement of the head varied between subjects in both the near and far distances, particularly during transport under high constraint. In the near condition, some subjects demonstrated a strategy involving forward excursion of the trunk while others used primarily head/neck movement to displace the head. However in the far condition, all subjects incorporated the trunk into the movement. These findings suggest that the transport phase of feeding requires precise, accuracy dependent specification of a common end-point parameter (in extrinsic space), which links the organization of the two movement subcomponents into a single action.

643.9

TASK DEMANDS AND MANUAL ASYMMETRIES IN REACHING. E.A.Roy*. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

The influence of task demands on manual asymmetries was examined in a reach and place task involving three conditions which increased in precision demands. Ten right-handed young adults participated in this study. In all conditions subjects reached for, grasped and picked up a small dowel. The subsequent movement defined the precision demands of the task. In one condition the dowel was tossed into a large receptacle. The other two conditions involved placing the dowel into either a small receptacle (small-place) about the same size as the dowel or a large receptacle (large-place). Subjects performed each task condition with each hand with the order of condition randomized for each subject. Movements of the arm (transport component) and the hand (grasp component) were recorded using an optoelectric analysis system (OPTOTRAK). The effect of precision demands on kinematic measures of the transport component (movement time, peak velocity and time in acceleration and deceleration) was most clearly seen in the second (move and place the object) as opposed to the first phase (reach and pick up the object) of the task. The small-place movements exhibited longer movement times, lower peak velocities and more time in deceleration than the large-place movements. Analyses of hand differences in performance revealed an advantage for the right hand only in the transport component and only in the second phase of the task, with the right hand exhibiting shorter movement times, higher peak velocities and less time in deceleration. These findings reveal that the magnitude of hand differences in performance depends on task demands with the hand differences being observed when the precision demands of the task are most apparent. The implications of this findings for a skill interpretation of handedness are discussed.

Research funded by grants from the Natural Sciences and Engineering Research Council of Canada and the Ontario Mental Health Foundation

643.10

SINGLE- AND MULTI-JOINT ARM MOVEMENTS: ARE THEY CONTROLLED THE SAME WAY? R. A. Scheidt, W. Z. Rymer* Northwestern Univ., Evanston, IL 60201, SMPP, Rehab. Inst. of Chicago, Chicago, IL 60611.

The objective of this study was to evaluate whether single-joint and multi-joint goal-directed, arm movements are controlled in a similar fashion (Hong, Corcos and Gottlieb, 1994). Two human subjects made movements in the horizontal plane supported against gravity via an air-bearing system. Two types of movements were compared: 1) single-joint, point to point movements of 60° extent (at the elbow) made in the presence of a mechanical constraint which disallowed translation of the elbow, and 2) two-joint, point-to-point movements between the same initial and final targets as in 1), yet in the absence of the constraint. The subjects were asked to make the blocks of 150 movements "from point A to point B as accurately as possible in 200 ms". Shoulder and elbow muscle electromyograms (EMGs) and limb kinematics were captured with an OptoTrak 3010 system. Kinematic and EMG metrics were examined to assess the performance and control behavior of the subjects as a function of constraint condition. Our data reveal that, with the exception of the obligatory curvature of single joint end-point paths, there were no systematic differences in the movement kinematics between the single- and multi-joint movements tested (ANOVA and Tukey tests; $p \leq 0.01$). One subject exhibited a small increase in shoulder antagonist activity with the addition of the constraint ($p \leq 0.01$); however, no systematic changes in the relative timing of EMG activity during single- and multi-joint movements were found. Constancy of EMG activity under very different biomechanical conditions is surprising since the addition of a shoulder constraint should allow the CNS to exploit modes of control not available in the unconstrained case. Specifically, the energetically efficient mode of minimal shoulder activity was not observed. This study suggests that single-joint, goal-directed arm movements may be considered constrained multi-joint movements and are controlled in a manner indistinguishable from the multi-joint case. Although it is conceivable that adaptation to the constraint may occur with prolonged exposure or practice, such adaptation effects were not evident within the two hour span we tested. NIH NS19331.

643.11

THE CURVATURE OF HAND PATHS IN MULTI-JOINT REACHING MOVEMENTS: DEPENDENCE ON ARM POSTURE AND MOVEMENT DURATION E. Nakano[1,2], H. Imamizu[1], R. Osu[1], Y. Uno[1], and M. Kawato[1] [1]ATR Human Information Processing Res. Labs., Kyoto, Japan [2]Graduate School of Science and Technol., Kobe Univ.

In multi-joint movements, possible trajectories to a given target are infinite, but actually have certain invariant features. It has been discussed whether trajectories of the human arm are planned in extrinsic (kinematic) space or in intrinsic (dynamic) space. Hand paths planned in the former are predicted to be always straight, while those in the latter are generally curved. Both Uno et al. (1989) and Osu et al. (1994) reported that actual hand paths tended to significantly curve for some specific arm postures, movement distances, and movement durations. We have extended the previous studies using various initial and final positions located within a workspace and examined if the curvature of a trajectory quantitatively varies with arm posture when humans make point to point reaching movements on a 2D horizontal plane. A hand position was measured by using a marker attached to a subject's hand. Curvatures of measured hand trajectories were linearly estimated from elbow and shoulder joint angles at initial and final positions. Movement durations and initial and final elbow joint angles significantly contributed to curvature. Then, in the second statistical analysis, curvatures were estimated from initial and final positions in Cartesian space and movement durations. However, initial and final positions in Cartesian space did not significantly contribute to curvature. In more detail, the trajectories were curved when an elbow was more extended at the initial position and more flexed at the target position. These results suggest that trajectory curvature depends on arm posture and is in accordance with predictions made under planning in the intrinsic space, rather than in the extrinsic space. Further, the result that a longer movement duration causes a larger curvature is in agreement with the predictions of Uno and Kawato (1994), in which a longer movement duration makes paths expand toward the outer side because of an effectively larger viscosity ratio.

643.12

Obstructed versus unobstructed reaching to stationary and moving targets in humans. B.J. McFadyen*, Dép. physiothérapie, Univ. Laval, Québec, G1K 7P4, Canada; H. Carnahan, Dept. Kinesiology, Univ. Waterloo, Canada.

The study of anticipatory strategies underlying obstructed reaching in humans has been limited. The present work details the three dimensional kinematics, moments of force, and mechanical powers in the upper limb during unobstructed and obstructed reaching to both stationary and moving targets. Electromygraphic activity of certain muscles was also recorded. Eight normal young subjects (mean age, 24.5 yrs.) were tested. Unobstructed reaching was initiated by either simultaneous control by the elbow and shoulder flexors, or elbow flexor control through a combination of a decreased shoulder flexor moment with an increased elbow flexor moment. Initial sole control by the elbow flexors predominated obstructed reaching with increased moments of force and powers over unobstructed trials and a greater phase disparity between elbow and shoulder patterns. This elbow strategy also provides initial passive shoulder flexion. The moving targets required greater reaching speeds, but initial reaching strategies were relatively unaffected. The control required while closing in on the target was more dependent on both environment and target characteristics. These results suggest a basic initial reaching behaviour that exploits intersegmental dynamics, and may parallel work presented for forelimb reaching in the cat (Martin et al., 1995). The greater emphasis on active control by a more distal joint during obstructed movement has similarities with patterns seen during the lower limb avoidance of obstacles in gait (McFadyen et al., 1991). Supported by NSERC.

643.13

THE INFLUENCE OF MOVEMENT SEGMENT DIFFICULTY ON MOVEMENTS WITH TWO STROKE SEQUENCE. M.K. Rand*, J.L. Alberts, J.R. Bloedel, G.E. Stelmach. Motor Control Laboratory, Arizona State Univ., Tempe, AZ, 85287; Barrow Neurological Institute, Phoenix, AZ, 85013.

Arm movements in the horizontal plane consisting of two segments were examined to determine whether the difficulty of the second segment influenced the kinematic characteristics of the first segment. Based on Fitts' law, the index of difficulty (ID) of the second segment varied between 3.0 and 4.83. The direction of the first segment was an elbow extension movement away from the trunk and remained constant. The direction of the second segment varied between elbow extension and flexion movements. All movements were recorded by a x-y digitizer.

Variations in the ID of the second segment produced kinematic changes in the performance of the initial segment. Movement duration lengthened when the ID was increased by reducing target size for both extension-extension sequence and extension-flexion sequence. Peak velocity decreased, time to peak velocity and deceleration time increased for higher ID targets. In addition, changing ID of the second segment influenced the time between the two segments (inter-segment interval). Context-dependent characteristics, defined as one movement segment influencing another, were stronger in the extension-flexion sequence than in the extension-extension sequence. These data suggest that planning processes consider the accuracy demands of both segments of a sequence.

Supported by the Flinn Foundation and the grant NINDS-NS17421.

643.14

REACHING ARM MOVEMENTS: THE PROCESS OF DIRECTIONAL SPECIFICATION IS NOT INFLUENCED BY CHOICE NUMBER. M. Favilla*. Istituto di Fisiologia Umana, v. Fossato di Mortara 17, I-44100 Ferrara, Italy.

We have previously found that extent and direction of hand trajectories aimed at visual targets located in a two-dimensional workspace can be independently specified. The specification of each parameter is not influenced by the need of concurrently specifying the other, at least as long as the number of the possible targets (choices) is maintained constant. We now study the effect of varying the number of choices on the time course (or rate) of response specification, while keeping constant the number of parameters (only one, direction).

Subjects moved a hand-held cursor on a digitizing tablet from a common start point; targets and a screen cursor were displayed on a computer monitor. Movements were initiated in synchrony with the last of a series of regularly interspaced tones; targets appeared at unpredictable times before the last tone.

Three series of experiments were run, in which 2, 4, 8 targets were presented in different directions, all at the same distance from the starting point. Response directions were correlated with target directions, within bands of progressively longer stimulus-response intervals and the time course of these correlations compared across the experiments. The time course of directional specification of reaching arm movements was uninfluenced by the number of possible choices. Subjects are able to specify responses to different numbers of targets at the same rate.
Source of support: Government grant from M.U.R.S.T.

643.15

A NEW VIEW ON HICK'S LAW. O. Bock*, Dep. Physiol., German Sports University, Köln, Germany.

Hick's law states that manual reaction time (RT) increases logarithmically with the number of response alternatives. Unfortunately, many studies claiming support for that law confounded two variables: as the number of alternatives increased, so did the area of space in which responses could occur. We undertook two experiments to deconfound these variables.

In Exp. A, possible response positions were *precued* by presenting: 5 discrete locations on a 90 deg arc about the starting point of the pointing finger; 3 locations on a 90 deg arc; 3 locations on a 45 deg arc. These precue types reduced RT in descending order, which suggests that both the number of alternatives and their spatial extent are relevant.

In Exp. B, precues were: a continuous arc of 60 deg an 30 cm radius; 60 deg and 7.5 cm radius; 15 deg and 30 cm radius. These precues reduced RT in descending order, which confirms Exp. A, and suggests that spatial extent is coded in polar rather than Cartesian coordinates.

We conclude that *RT depends both on the number of alternatives and on the range of possible response directions*, thus reflecting the response selection and the movement preparation stage of the motor system.

Supported by the German space agency DARA.

643.16

INDIVIDUAL DIFFERENCES IN OROFACIAL MOVEMENT COORDINATION WITH VARIATIONS IN SPEECH RATE. M.D. McClean*Audiology and Speech Center, Walter Reed Army Medical Center, Washington, D.C. 20307-5001.

To understand pattern generating networks regulating speech production, it is important to quantify orofacial movement coordination and assess the range of individual variation. Subjects produced a nonsense utterance ("a bad daba") numerous times at slow, normal, and fast speech rates. An electromagnetic system was used to transduce two-dimensional displacements of the upper lip, lower lip, tongue tip, and jaw in the midsagittal plane. Tangential velocity patterns were derived for each structure, and the peak velocities were measured for vocal-tract opening and closing gestures associated with the consonants /b/ and /d/. Within subjects, the magnitude and timing of peak velocities were strongly conditioned by speech rate, and the peak velocities of different structures were often well-correlated. However, the pattern of these associations differed markedly across subjects. For example, some subjects showed positive correlations between lip and tongue opening velocities, while others showed positive correlations between tongue-opening and tongue-closing velocities. Negative correlations between tongue and jaw opening velocities were seen in some subjects, indicating motor equivalence; but this pattern was not present in others. These results suggest that individuals use different pattern generating networks or network organizations to produce the same speech utterance.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CIRCUITRY AND PATTERN GENERATION V

644.1

INTERMITTENCY: A FEATURE OF RHYTHMIC MOVEMENTS IN INTACT ANIMALS. G.E. Gerstner*. Dept of Biol. & Mat. Sci., Sch. of Dent., Dept of Psychol., Univ. of Mich., Ann Arbor, MI 48109-1078.

Rhythmic movements, like mastication and gnawing, appear to be produced by central pattern generating circuits (CPG). Evidence suggests that in intact animals, rhythmic movements occur intermittently, i.e., in sequences of 1-3 s rhythmic bursts interdigitated with 0.2-1 s pauses. It has been hypothesized that catecholaminergic circuits play an important role in producing this intermittency. The current study further hypothesized that catecholaminergic drugs, that are typically used to elicit rhythmic behaviors, disrupt this intermittency by activating CPG or pre-CPG circuits for artificially long time periods. The current study was undertaken to test this hypothesis. Ten albino guinea pigs were videotaped while masticating, and six albino guinea pigs were given 0.5 mg/kg i.m. apomorphine and videotaped while gnawing. Software developed in the laboratory was used to score mastication and gnawing bursts and to characterize the intermittencies of these behaviors using an autocorrelation method. Results indicated that a 1-3 s intermittency was a feature of mastication: however, intermittency was not a feature of the apomorphine-associated gnawing. These results support the hypothesis that catecholaminergic drugs disrupt intermittency in rhythmic movements. A model will be presented as to how the intermitteney arises. and why catecholaminergic drugs and certain diseases disrupt the intermittency. This model posits that catecholaminergic pathways are involved in maintaining rhythmic movements; however, intermittency results from these pathways' inability to perpetuate output for > 3 s time spans without recurrent initiatory input from other circuits. Supported in part by NIH grant DE10625.

644.2

AN EXPECTATION-BASED MODEL OF ADAPTABLE AND FLEXIBLE PREY-CATCHING IN ANURANS. F. J. Corbacho*, K. C. Nishikawa, J-S. Liaw, M. A. Arbib. Ctr. Neural Eng., Univ. Southern Cal., Los Angeles, CA 90089 and Dept. of Biol. Sci., N. Arizona Univ., Flagstaff, AZ 86011.

Prey catching in anurans involves the coordination of several motor commands in a highly flexible and adaptable manner. Behavioral observations demonstrate that prey catching is guided by visual input about the prey and sensory feedback about the jaws and tongue. We have developed a simple model that accounts for a variety of data on modulation of prey catching by visual input and sensory feedback, as well as interactions between them. The model includes: 1) jaw muscles Depressor Mandibulae (DM) and Levator Mandibulae (LM); 2) tongue muscles; 3) corresponding premotor control circuits in the reticular formation (RF); 4) an efference copy of the motor command to DM through excitatory connectivity from premotor DM to an interneuron (INT) in RF; 5) sensory feedback from motion of the tongue through mechanoreceptors in the hypoglossal nerve (HG); and 6) sensory feedback from motion of the jaw through muscle spindles in the LM muscles, which inhibits INT. The model explains patterns of motor pattern selection based on parameters of the visual input. When frogs see large prey, INT receives low frequency inhibitory input from the optic tectum. When active, INT inhibits premotor LM, giving rise to jaw prehension. When frogs see small prey, INT receives high frequency inhibitory input from the optic tectum and is not active enough to reach its threshold. In this case, premotor LM is inhibited by HG instead of by INT, giving rise to tongue prehension. The model also replicates observations on motor learning in toads after hypoglossal transection. INT detects incoherences about mouth opening by subtracting the signal carrying the efference copy of the command to DM and the feedback signal from the muscle spindles in LM. The strength of inhibitory connections between HG and INT and between INT and premotor LM (Y) are both adapted during learning. The model accounts for variability across individuals (faster vs. slower learners) by varying Y based on past experience. (Supported by NSF grant IBN-9411503 to MAA & IBN-9507479 to KCN)

644.3

EFFECT OF INCREASING RESISTANCE ON MOTOR PATTERNS DURING SWIMMING IN CHICKS. A. Bekoff*, B. C. Hurtt and J. L. Ryan. EPO Biology Department and Center for Neuroscience, University of Colorado, Boulder, CO 80309.

We are interested in examining the role of sensory input in modulating rhythmic motor patterns. We analyzie the role of increasing resistance to leg movements during swimming in chicks because this is a simple, symmetrical locomotor behavior.

Previous studies in our lab have characterized swimming in chicks using both kinematic (Johnston & Bekoff, 1992. J. Exp. Biol. 171: 143) and EMG recordings (Johnston & Bekoff (J. Comp. Physiol. in press). In contrast to walking, swimming motor patterns are symmetrical: extension and flexion durations are similar. During walking extension duration increases with increasing cycle period while flexion duration does not. In swimming both extension and flexion durations increase to a moderate and similar extent.

In the present study, we alter resistance for both extension and flexion by having chicks swim in fluids of three different viscosities. Chicks are videotaped during swimming and markers are digitized to determine hip, knee and ankle joint angles. From these data, cycle period, flexion and extension at each joint, phase values, and joint excursions can be calculated. Results suggest that the motor pattern becomes increasingly asymmetric with increasing resistance. This suggests that extension is more readily modulated than flexion.

Supported by NIH Grant HD28247 to A.B and undergraduate research funds from Hughes Biomedical Support Grant to J.R.

644.4

EFFECT OF SENSORY INPUT DURING RHYTHMIC EMBRYONIC MOTILITY. A.A. Sharp* and A. Bekoff. EPO Biology Department, University of Colorado, Boulder, CO 80309.

The integration of sensory information into functional rhythmic motor patterns during development is not well understood. Hamburger et al. (1966, J. Exp. Zool.) demonstrated that sensory information is not required for maintenance of embryonic motility in chicks, but this does not mean that sensory-motor synapses, which first appear on the seventh day of embryogenesis (E7) (Lee et al., 1988, J. Neurosci.), are not utilized during embryonic motility. For example E7 embryos produce reflex responses to tactile stimulation and the reduction of buoyancy of E9 embryos causes a greater covariance of wing joints during rhythmic motility (Chambers et al., 1995, Exp. Brain Res.). We are using kinematic and EMG recordings to determine if localized sensory information provided by sustained perturbations of one leg are incorporated into rhythmic leg movements.

E9 chick embryos are prepared for recording, *in ovo*, by first gluing two paddles to the back. This prevents rolling of the embryo during motility. A third paddle is placed under the right foot such that the resting leg position is similar to that during post-hatching stance. This configuration allows the embryo to press against the pad with its right foot during extension of the leg and to lift the foot from the paddle during flexion. The paddles reduce the amplitude of possible leg extension. Kinematic measurements of the right leg reveal that the amplitude of joint excursion at both the knee and ankle are reduced, but that the hip is unaffected. The duration of ankle extension is increased resulting in a phasing delay for ankle flexion. This is similar to the response of a post-hatching chick to loading during walking (Johnston and Bekoff, 1992, J. Exp. Biol.). The duty cycle of hip and knee extension and flexion are unaffected. Synchronous kinematic and EMG recordings are being performed to determine if these changes are the result of active sensory-motor interactions or passive physical constraints. Supported by NIH grant HD28247 to A.B. and NRSA grant HD07884 to A.A.S.

644.5

TRANSIENT CONFIGURATIONS OF BRAINSTEM CARDIO-RESPIRATORY NEURONAL ASSEMBLIES: DETECTION AND DISPLAY OF REPEATED MOTIFS. B. G. Lindsey*, K. F. Morris, R. Shannon, and G. L. Gerstein. Dept. of Physiology & Biophysics, Univ. of South Florida Med. Ctr., Tampa, FL 33612 and Dept. of Neuroscience, Univ. of Pennsylvania, Philadelphia, PA. 19104.

We previously identified moments of synchrony in distributed assemblies (Soc. Neurosci. Abst. 21:145, 1995). Jumps in gravity particle condensation rates were defined and displayed as a set of vectors with a common origin. The direction of each vector in a plane identified the neuron pair represented; vector length indicated the aggregation velocity of corresponding particles. Because of the appearance of the vectors in animations of successive planes 'through time', these graphs are called 'spark' plots. Next we described a method to determine if particular patterns of impulse synchrony in a group of neurons recur more frequently than expected by chance (FASEB J., 10: A410, 1996). Here we report results of the combined application of these approaches to data sets of 8 or more spike trains recorded simultaneously in the medullary raphe nuclei and ventrolateral medulla of anesthetized, vagotomized, artificially ventilated cats. Spatiotemporal patterns of synchrony with a significant number of recurrences were detected in each of 3 animals. In one sample, a spark motif repeated exclusively during a period of increased baroreceptor stimulation and decreased respiratory drive; a second motif was confined to other times. The reiterated patterns were not apparent in either corresponding firing rate histograms or the conventional gravity representations from which the results were derived. These approaches may have a general utility in screening time series for evidence of transient 'states' of neuronal assemblies linked to sensory processing, motor control, and the induction and expression of memories (Morris et al., these proceedings). Support: NIH grant NS19814.

644.6

INFLUENCE OF MULTISEGMENTAL COORDINATING FIBERS ON RHYTHMICITY OF DISTANT SEGMENTS IN THE LAMPREY SPINAL CENTRAL PATTERN GENERATOR. W.L. Miller and K. A. Sigvardt*. Department of Neurology and Center for Neuroscience, University of California, Davis, CA 95616.

Little is known about the distribution and functional effects of propriospinal projections in the lamprey spinal cord. We are interested in how these projections contribute to intersegmental coordination. Our previous electrophysiological studies suggested that propriospinal neurons can influence the timing and variance of ventral root activity as many as 20 or more segments away. In the present experiments on the isolated silver lamprey spinal cord (*Ichthyomyzon unicuspis*), we combined mechanical entrainment of the ends of the preparation with blockage of local activity in the middle of the preparation, in order to further quantify the properties of the multisegmental coupling. Rostral and caudal entrainment frequency range, and rostro-caudal coherence and phase were measured for blocking lengths of 4-20 middle segments in 4-segment steps. In a second experiment, we separately measured the extent of ascending (rostrally-directed) and descending (caudally-directed) coupling.

Supported by an NIH IRSA Predoctoral Fellowship MH10590 (WLM), and NIH grants MH47150 and NS22360 (KAS).

644.7

ELECTRICAL AND L-GLUTAMATE STIMULATION OF THE PRECOMMAND NUCLEUS (PC) REVEALS EXCITATORY INPUTS TO PC AND COMMAND NUCLEUS IN GNATHONEMUS PETERSII. G. von der Emde*, J.C. Prechtl and C.J.H. Wong. Zool. Inst., Univ. Bonn, 53115 Bonn, Germany and Scripps Inst. of Oceanography, UCSD, La Jolla, CA, 92093.

Mormyrid weakly electric fish display extensive changes in the rhythm of electric organ discharges (EOD) associated with various stimuli and behavioral states. The EOD rate is determined by the bulbar command nucleus (CN) located in the caudal medulla. CN receives input from the pre-command nucleus (PC) which is a small nucleus located in the midbrain tegmentum, ventral and caudal of the posterior commissure. Injections of neurobiotin into PC retrogradely labeled cells in known afferent structures including the ventro-posterior nucleus of the torus. In addition, neurons in several other brain areas were labeled. There was also transsynaptic anterograde transport of tracer across up to three synapses into structures associated with the electric organ corollary discharge, indicating tight synaptic contact between pre-motor elements and corollary discharge pathways. Characteristic field potentials recorded within PC showed partial temporal overlap with CN field potentials. In addition, single units within PC fired spikes between EODs, but were silent during a period immediately following the generation of an EOD. Iontophoretic injection of L-glutamate with negative currents into PC for several seconds caused a tonic increases in EOD rate, similar to the effect elicited by long-term electrical stimulation of PC with depolarizing current. The amount of EOD acceleration depended on current strength up to a saturation level both in chemical and electrical stimulation. Turning off a longer period of stimulation with L-Glutamate caused a short, transient EOD acceleration that resembled the 'novelty response', a behavioral response to novel sensory stimuli. These data suggest that PC itself is in receipt of excitatory input and exerts an excitatory effect on the CN. Supported by grants to Walter Heiligenberg from NIH, NIMH, NSF, by the Director's Office Scripps Institution Oceanography, and by a Heisenberg stipend of the German Science Foundation (DFG) to GE.

644.8

DIFFERENCES BETWEEN THE PRODUCTION OF PRECISELY REPLICATING TRIPLETS AND DOUBLETS IN V1 CORTICAL CELLS. R. Lestienne*, Institut des Neurosciences, 9 quai St Bernard 75005 Paris.

Temporal patterning in visual cortical spike trains was assessed by measuring the number of replicating triplets (NT2) and the number of doublets present three times (ND3), in successive windows of duration 100 ms, when the maximum total duration of individual patterns was 25 ms and the precision of replication of each interval was <= .5 ms. Temporal organization of spike discharges was evaluated using the NT2/ND3 criterion, because of its relative insensitivity to changes in the cell firing rate (*Soc. for Neur. Abst. #18.1, 1994; Biol. Cybern. 1996, 74:55-61*).

Seven data samples were processed, each comprising between 38,000 to 157,000 spikes, from single unit and multi unit recordings in primary visual area of anesthetized and behaving animals. NT2/ND3, NT2/spike and ND3/spike were separately measured, as a function of firing rate, and compared to Monte-Carlo produced data, obeying a Poisson renewal process modified to introduce refractory periods.

In all samples, similar trends of NT2/ND3, NT2/sp and ND3/sp ratios were observed. At low firing rates (~30-100 Hz), discharges markedly deviate from prediction of the Poisson-like model, mostly because they contain an excess of precisely replicating triplets with respect to the latter, while the replicating doublets production remains closer to the level predicted by the model. In kittens, the number of replicating patterns rises quite rapidly with age, from 4-6 weeks to 9-13 weeks, at the same time as the average firing rate increases. NT2/ND3 ratios also show a tendency to increase with age.

Research supported by CNRS. The author thanks the international teams that generously provided their data samples, recorded with a timing precision of 0.1 ms. In area 17 of anesthetized cats and kittens: J.C. Beaux and M. Imbert, Paris; V. Bringuier and Y. Fregnac, Gif/Yvette; R. Siegel et al, Rutgers Univ. In V1 of anesthetized and awake monkeys: L. Nowak and J. Bullier, Lyon ; B. Richmond et al, Bethesda (data with timing precision of 1ms).

644.9

ANALYZING NEURONAL DISCHARGE; ELIMINATING FORMAT BIASING EFFECTS AND DETECTING CHANGES IN STATE. J. L. Johnson*. Dept. of Physiol. & Pharmacol., USD School of Medicine, Vermillion, South Dakota 57069.

Previously, it was shown that the reciprocal frequency (Hz) and interval (ms) formats were oppositely biased in their ability to appreciate variability in discharge sequences [Brain Research, 666 (1994) 125-127]. These format biasing effects (which are not mirror image distortions) must be overcome and eliminated entirely if we are ever to accurately detect changes of state in functioning neurons. It was discovered that, for any given discharge sequence, by plotting sum(INT; sequential interval data) vs sum (Hz; sequential frequency values for the same data) ALL format biasing effects were canceled out and changes in state could be readily detected. The slope of the regression line for any single state [sum (Hz)/sum (INT)] can be converted to an equivalent average interval value which is to be applied over the duration of the state. The regression coefficient for this regression equation reflects the state variability about the mean (slope) in the complete absence of any format biasing. If the above approach is not employed, the use of the interval format alone will amplify low frequency variability in a discharge sequence and the use of the frequency format alone will amplify high frequency variability in the discharge sequence. Such format biasing should only be included if the functional effect regulated by the discharge input also exhibits the property of format biasing. In conclusion, the sum (Hz) vs sum (INT) plot must be critically examined for its potential usefulness is assessing changes in discharge patterns in a manner that does not include any biasing distortions which are an integral property of reciprocal functions. The above specified method of utilizing both reciprocal formats (INT & Hz equivalent) together in the analysis of discharge patterns may enable us to more precisely relate changes in input patterning in an unbiased way to the regulated function.

644.10

OPTICAL STUDIES OF AN ENTERIC NETWORK: SIMULTANEOUS RECORDING OF ELECTRICAL ACTIVITY FROM SEVERAL INTERCONNECTED SUBMUCOUS GANGLIA WITH SINGLE CELL RESOLUTION. A.L. Obaid*, M.A. Farries, M.A. Kisley, T. Sakai, and B.M. Salzberg. Dept. of Neuroscience, Sch. of Medicine, and The David Mahoney Institute of Neurological Sciences, UPENN, Phila., PA 19104-6074.

The enteric nervous system is a self-contained network capable of generating complex behaviors in isolation. Its neurons are arranged in plexuses [submucous (SM) and myenteric] confined to distinct planes in the gut wall. Since the neurons of SM ganglia of the guinea pig small intestine lie in the same optical plane, they are particularly amenable to Multiple Site Optical Recording of Transmembrane Voltage (MSORTV). One problem that can be addressed using this technology is the identification of functional units within the SM network. Are these units the SM ganglia themselves, or are they distributed, supraganglionic networks that overlie each other and share the anatomical scaffold of the SM plexus? To answer this, we have begun to examine whether sets of functionally connected neurons are found primarily within single ganglia or are distributed among several ganglia. We use the technique of MSORTV [Salzberg et al., J. Neurophysiol. 40:1281, 1977; Grinvald et al., J. Neurophysiol. 45:829, 1981], the fluorescent potentiometric dye di-8-ANEPPS [Bedlack et al., Neuron 9:393, 1992], and a 40X, 1.3 n.a. objective (Olympus), to record simultaneously the action potentials from neurons of up to 5 interconnected ganglia. The data are acquired at 2 kHz, with 18 μm spatial resolution, using 32 selected outputs of a 464-element photodiode array mounted on an inverted microscope equipped for epifluorescence. The light source is a 150 W Xe short arc, and the filter combination is 530±25 nm/ 560/ OG570. Spike sorting analysis of the raw optical data is used to identify the active neurons and isolate their individual spike trains. Our records are not yet long enough to generate statistically significant conclusions, but quasi-synchronous bursts of activity from cells in separate ganglia suggest the existence of a supraganglionic organization. Supported by USPHS grant NS16824, and a travel fellowship (T.S.) from the Ministry of Education, Science and Culture of Japan.

644.11

DIAGONAL LIMB RESPONSE FOLLOWING SPINAL CORD COMPRESSION INJURY IN CATS.

W.M. Norman, F.J. Thompson, C.J. Vierck, Jr., D.R. Howland*, and D.K. Anderson. Dept. of Neuroscience, Univ. of Florida Coll. of Med. and VAMC Gainesville FL 32610.

A preliminary study was designed to determine if thoracolumbar spinal cord compression injury in adult cats alters the diagonal limb postural response (DLR). Cats were trained to stand on force detectors made from saline-filled bags containing pressure transducers. Rapid fluid removal from one of the bags initiates the DLR. The polarity and magnitude of the change in the vertical vector of the ground reaction force exerted against each paw was recorded. Normal cats load the diagonally opposing pair of limbs, while the stimulated limb and its diagonal, unload. Injured cats show a direction-dependent response. Hindlimb (HL) stimulation elicits a response from the expected diagonal limb pair in all injured cats. Although the pattern response was normal in the more moderately injured cats, the magnitude of the response was diminished. In the more severely injured cats, the DLR was either diminished or absent. In contrast, forelimb (FL) stimulation induced bilateral extension of the HL instead of invoking the DLR. These findings suggest that the injury disrupts normal neural networks underlying the DLR. The DLR of some of the injured cats to HL stimulation is likely mediated by neural circuits that survive the injury but which differ from the normal anatomical substrate. In addition, in the most severe injuries, perhaps passive, mechanical forces may account for some of the response pattern. We are beginning to perform more detailed analysis of this and other postural responses using force plates, EMG, and kinematic analyses. Supported by the Deptartment of Veterans Affairs and the C.M. and K.E. Overstreet Endowment.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XIII

645.1

THE STORAGE OF LONG-TERM MOTOR MEMORIES AT PARALLEL FIBER SYNAPSES ONTO STELLATE/BASKET CELLS IN THE CEREBELLAR CORTEX.

G.T. Kenyon* Department of Neurobiology and Anatomy, University of Texas Medical School, 6431 Fannin, Houston, TX 77030.

Climbing fiber-dependent plasticity at granule-to-Purkinje (Gr→Pkj) synapses in the cerebellar cortex has been implicated in several forms of motor learning. We use a mathematical model of the cerebellar cortex and its reciprocal interactions with the inferior olive to show how an additional site of plasticity at granule-to-stellate (Gr→St) synapses could facilitate the storage of long-term motor memories. It has been widely postulated that movements are initiated by brief pauses in Purkinje cell activity, and that climbing fiber inputs produce such pauses by selectively inducing long-term depression (LTD) in Gr→Pkj synapses active during the intended movement. Our results suggest that the selective induction of long-term potentiation (LTP) in coactive Gr→St synapses could also produce similar pauses in Purkinje cell firing. The present model depends on the following experimentally testable predictions: 1) Golgi cells experience an abrupt depolarization during the execution of a learned movement due to a release from tonic Purkinje cell inhibition. 2) Rapid depolarization produces a calcium-mediated pause in tonic Golgi cell firing. 3) Pauses in Golgi cell activity initiate bursts of granule cell firing. 4) Bursts of Gr→St synaptic activity promote the induction of LTP, while normal activity produces LTD. 5) Increased inhibition from stellate/basket cells can block the rapid depolarization of Golgi cells during the execution of learned movements, thus providing negative feedback. We conclude that rapid motor adaptation is mediated by climbing fiber-dependent plasticity at Gr→Pkj synapses but long-term storage results from the transfer of this plasticity to Gr→St synapse during continued reinforcement training (NIMH #1 F32 MH10683-01).

645.2

CLASSICAL CONDITIONING UNDER TEMPORAL UNCERTAINTY: SIMULATION OF RESPONSE STRATEGIES BY THE TD MODEL.

D.H. Brunzell, J-S Choi, and J.W. Moore*, Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Rabbits were trained in an eyeblink conditioning task in which the CS–US interval varied randomly from trial to trial, with CS duration held constant. A mixture of two CS–US interval (300 and 700 ms) results in bimodal CRs with amplitude peaks 300 and 700 ms after CS onset. A mixture of three CS–US intervals (300, 500, 700 ms) results in either of two response strategies: a *fail-safe* strategy in which the CR resembles a voluntary response or a *conditional expectation* strategy in which the CR consists of three successive peaks of increasing amplitude.

All three response strategies (bimodal, fail-safe, conditional expectation) can be described by a real-time version of Sutton and Barto's Time-Derivative (TD) learning rule which assumes that CS-US intervals can be decomposed into ordered sequences of time-tagged components. In the model, connection weights between one such component and the US are computed by the following equation: $\Delta V(t) = \beta[\lambda(t) + \gamma Y(t) - Y(t-1)] \times \alpha \bar{X}(t)$. The amplitude of the CR at t is given by $Y = V \times X$. λ represents the US, and X represent the on-off status of the CS component ($X = 1$, if CS on; else 0). $\bar{X}$ is the eligibility of the CS component for modification: $\bar{X}(t+1) = \bar{X}(t) + \delta[X(t) - \bar{X}(t)]$. α, β, δ, and γ are constant positive fractions. Response strategies can be simulated by various combinations of parameter values and assumptions about the structure and modulation of timing mechanisms.

http://www-unix.oit.umass.edu/~jwmoore/td.html

645.3

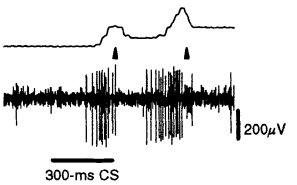
CEREBELLAR SINGLE CELL ACTIVITY RELATED TO BIMODAL CONDITIONED EYEBLINK RESPONSES.

J-S Choi* and J.W. Moore, Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Conditioned eyeblink responses (CRs) predict the timing of the US. Rabbits trained with a mixture of two CS-US intervals develop bimodal CRs with amplitude peaks at the loci of the US. Bimodal CRs are predicted by real-time computational models such as Sutton and Barto's TD model.

In the present study rabbits were trained with a fixed-duration tone CS of 300 ms and an eye-shock US. The two CS-US intervals were 300 and 700 ms. Only one US occurred per training trial. Recordings from single cerebellar cells showed activity highly predictive of both amplitude peaks of bimodal CRs.

The figure shows a typical bimodal CR (upper trace) and the firing pattern of a single cell in nucleus interpositus (lower trace) on a single CS-alone probe trial. The arrows mark the times of the US on training trials.



The firing of some Purkinje cells ceased in anticipation of each amplitude peak of bimodal CRs.

These observations are consistent with schemes that implement learning and CR timing within the cerebellum.

http://www-unix.oit.umass.edu/~jwmoore/td.html

645.4

TD MODEL OF CLASSICAL CONDITIONING: RESPONSE TIMING AND CEREBELLAR IMPLEMENTATION.

J.W. Moore, J-S Choi, and A.G. Barto*, Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Sutton and Barto's Time-Derivative (TD) learning rule can describe the timing and topography of conditioned responses, if the CS–US interval is decomposed into an ordered sequence of time-tagged components. Connection weights between one such component and the US are computed by the following equation: $\Delta V(t) = \beta[\lambda(t) + \gamma Y(t) - Y(t-1)] \times \alpha \bar{X}(t)$. The amplitude of the CR at t is given by $Y = V \times X$. λ represents the US, and X represent the on-off status of the CS component ($X = 1$, if CS on; else 0). $\bar{X}$ is the eligibility of the CS component for modification: $\bar{X}(t+1) = \bar{X}(t) + \delta[X(t) - \bar{X}(t)]$. α, β, δ, and γ are constant positive fractions.

The TD model has been particularly successful in describing classical eyeblink conditioning, a behavior that depends on the integrity of the cerebellum and associated brain stem circuitry. We present a scheme for implementing the TD model in the cerebellum. In terms of the model, X is represented by mossy fiber activity from the pontine nuclei, λ is represented by climbing fiber activity from the inferior olivary nuclei, $Y(t-1)$ is represented by mossy fiber activity from the spinal trigeminal nuclei, and $Y(t)$ is represented by mossy fibers from the red nucleus. The scheme assumes that ΔV is computed through LTD of parallel fiber synapses on Purkinje cells.

The scheme is consistent with known anatomy (Rosenfield & Moore, *Behavioral Neuroscience, 109,* 1106-1118). It gives rise to testable predictions, e.g., that inactivation of the red nucleus would not preclude first-order conditioning (consistent with Clark & Lavond, *Behavioral Neuroscience, 107,* 264-270), but it would prevent second-order conditioning.

http://www-unix.oit.umass.edu/~jwmoore/td.html

645.5

IMPAIRED CLASSICAL EYEBLINK CONDITIONING IN MUTANT MICE (STARGAZER) WITH LOCALIZED DEFICIENCY IN BDNF EXPRESSION IN THE CEREBELLUM. L. Chen*, S. Bao, X. Qiao, B. Knusel and R. F. Thompson. Neuroscience Program, Univ. of Southern Calif., Los Angeles, CA 90089-2520.

Gene knockout mice have been proven to be a powerful approach to study the biological basis of learning and memory. But, because the loss of the genes is global, it may be difficult to interpret the behavioral deficits. In the present study, we tested classical eyeblink conditioning in spontaneous mutant mice called stargazers (*stg*). The *stg* mice show deficient BDNF expression in cerebellar cortex during the entire life span. The BDNF expression in other brain regions and the expression of other neurotrophins appear to be normal. The cerebellar cytoarchitecture in adult *stg* mice also appear to be normal.

Adult male *stg* mutant mice (n=11) and normal littermates (n=10) mice underwent standard delay eyeblink conditioning. The eyelid muscle electromyograph was recorded to score CRs. The *stg* mice exhibited a severe impairment in the acquisition of delay eyeblink conditioning in comparison to their normal littermates. There was no difference between the mutant and wild-type mice in their sensitivities to the US or their UR to the periorbital shock. In addition, we tested the sensitivities of the two groups of mice to hot plate induced pain and startle responses to a loud 1kHz sound. The *stg* mutant mice showed no significant differences in either tests compared to normal littermates. Thus the impairment was not due to sensory deficits.

The *stg* mice also exhibit non-convulsive seizure. To test the possible effects of the seizure activities on classical eyeblink conditioning, we trained another type of mutant mouse called *tottering* (*tg*) for eyeblink conditioning. Despite seizure activity similar to *stg* mice, *tg* mice learned eyeblink conditioning as well as wild type littermates.

The fact that mice with localized cerebellar BDNF deficiency are impaired in classical eyeblink conditioning strongly supports the idea of essential involvement of the cerebellum in this type of learning. It also suggests that the neurotrophins may be important in learning and memory.

Supported by research grants from NSF (IBN-9215069), NIMH (MH52194), ONR (N00014-95-1-1152) and Sankyo Co., Ltd.

645.6

EYEBLINK CONDITIONING-RELATED NEURONAL MODEL IN LATERAL PONTINE NUCLEUS ARE DRIVEN BY CEREBELLUM. S. Bao*, L. Chen and R. F. Thompson. Neuroscience Program, Univ. of Southern Calif., Los Angeles, CA 90089-2520.

Inactivation of the ipsilateral cerebellar interpositus nucleus (i-IP) has been shown to abolish classical eyeblink conditioning induced neuronal models in several brain regions, such as the red nucleus, whereas inactivation of the red nucleus and brain stem motor nuclei did not abolish the model in i-IP. In the present study, we examined wheather the neuronal model in contralateral lateral pontine nucleus (c-LPN) is driven by the i-IP.

New Zealand white rabbits were surgically implanted with two stimulating electrodes in ipsilateral lateral reticular nucleus (i-LRN), two recording electrodes and a guide cannula in c-LPN, and in i-IP. After recovery, the animals were trained with delay classical eyeblink conditioning with LRN stimulation as the CS and airpuff as US. When they reached the criteria (9 CRs out of 10 trials), the CS was changed to tone and the animals were trained to criteria again. Then, muscimol was infused into i-IP, and the animals were tested with mixed training (50 tone/air and 50 LRN/air trials). After one day recovery and mixed training, the animals were infused with TTX into c-LPN and tested with mixed training. Finally, some of the animals received electrolytic lesions in the c-LPN and the others received lesions in the i-IP. After one day recovery, the animals were tested with mixed training. The multiple unit activities in c-LPN and i-IP were and recorded during training.

Inactivation of i-IP abolished CRs when the CS was tone or LRN stimulation. Inactivation of c-LPN only abolished CRs to tone, but not to the LRN stimulation. When LRN stimulation was used as the CS, inactivation of i-IP abolished the neuronal model in the pontine region, while inactivation of c-LPN had little effect on the neuronal model in i-IP. Thus, the learning induced neuronal model in c-LPN is very likely driven by i-IP. These results suggest that the lateral pontine nucleus is not a locus of memory storage for the conditioned eyeblink response, but simply acts as a sensory relay nucleus for acoustic stimuli. Supported by grants from NSF (IBN9215069), NIMH (MH52194), ONR (N00014-95-1-1152)and Sankyo Co., Ltd.

645.7

EFFECTS OF HIPPOCAMPAL AND INTERPOSITUS REVERSIBLE LESIONS ON LEARNING OF THE RABBIT CLASSICALLY CONDITIONED EYELID RESPONSE. Eliaszadeh, P., Tocco G., Thompson J. K.* & Thompson R.F. Neurosciences program, Univ. Southern California, Los Angeles, CA 90089-2520.

Reversible inactivation with either muscimol, lidocaine or DNQX was used to identify neural substrates which might mediate learning and/or consolidation of the rabbit's conditioned eyelid response. While the cerebellum is necessary and sufficient for the learning of the classical conditioning of the nictitating membrane, in a delay paradigm, the hippocampus is required for the learning of a trace paradigm when at least 300 ms separate the end of the CS from the US onset. While the interpositus nucleus is absolutely necessary for the performance of a previously learned trace conditioning task, its importance during the acquisition phase is not well understood. On the other hand the hippocampus, seems to be important during the acquisition phase and only for a short period of time after the learning In an attempt to clarify the role of these structures in the different phases of learning (acquisition, retention/consolidation?) reversible inhibition of these brain areas were performed with various drugs (muscimol, DNQX, lidocaine) either during of after conditioning in a trace paradigm.

Male New-Zealand white rabbits were implanted with one cannula in the both hippocampi and/or one cannula in the interpositus nucleus, ipsilateral to the stimulated eye. Rabbits were then trained in a trace paradigm and infused with inactivating drugs either during acquisition trials or in training session after they reached criterion.

Our results show that an intact cerebellum is necessary at all times for learning and consolidation of a trace paradigm while the hippocampus is only necessary for the acquisition of a learned response and for a short period of time thereafter.

Supported by NSF (IBN-9215069), NIH (AG05142), NIMH (MH52194), ONR (N00014-95-1152) and Sankyo Co., Ltd.

645.8

Reversible Inactivation of Red Nucleus With Cooling Has No Effect On Tone Evoked and Learning Related Unit Activity In Central Regions of The Lateral Pontine Nuclei. M.C.Cartford*, E.Gohl, M. Singson, E. Wang, L. Tasa, D.G. Lavond. University of Southern California, Depts of Psychology and Neurobiology, HNB 501, University Park, Los Angeles, California 90089-2520.

In simple delay classical conditioning of the rabbit nictitating membrane response conditioned stimulus (CS) tone information projects to the cerebellum via the lateral pontine nuclei (LPN). Steinmetz et al. have shown that stimulation of this region can itself serve as a CS (Bull. Psych Soc., 23, 1985, pp.245ff) and that LPN lesions abolish conditioned responses to a tone (Proc Nat'l Acad Sci, 84, 1987, pp.353ff).

Additional studies have shown that unit activity recorded in the pontine nuclei shows evoked and learning related responses when animals are trained with a tone as the CS. Reversible inactivation of the cerebellar interpositus nucleus (IP) abolishes this learning related unit activity in the LPN but has no effect on tone evoked neural responses (Gohl et al., Neurosci Abst, 19, 1993, p.1000).

We have now tested the effects of red nucleus cooling on evoked and learning related unit activity in LPN in rabbits trained to a tone CS. Our results indicate that cooling red nucleus abolishes learning and tone related unit activity throughout the pons except the mid level area of the lateral pons. This area is known to project to the IP (Steinmetz, et al., Behav. Neur. Bio, 60, 1992, pp.172ff) and to receive reciprocal projections (Watt et al., Neurosci Abst, 5, 1979, p.108). Together these data support the hypothesis that the IP participates directly in the expression of learning related unit activity in the LPN.

Supported by NIMH 1 R01 MH51197

645.9

INFUSION OF PROTEIN KINASE INHIBITOR H-7 INTO THE CEREBELLUM IMPAIRS THE ACQUISITION OF CLASSICAL EYEBLINK CONDITIONING IN RABBITS. G. Chen*, and J. E. Steinmetz. Dept. of Psychology/Neural Sciences, Indiana Univ., Bloomington, IN 47405.

Eyeblink conditioning in rabbits has been well studied and it has been demonstrated that cerebellum is critical to this simple associative learning. Yet, its molecular mechanism is still unclear. Previous studies have shown that protein kinases are involved in many non-associative and associative learning paradigms in Aplysia, Hemissenda and LTP in hippocampus. This study is an attempt to assess whether protein kinases may also be involved in the eyeblink conditioning and to verify whether the memory trace of this learning is located in the cerebellum.

Rabbits were implanted with cannula just dorsal to the interpositus nucleus (IP) bilaterally. After recovery, the rabbits received standard delay eyeblink conditioning for 8 days (60 trials each day). All animals received unilateral injection of H-7 (3 uL, 20 ug/uL) and a contralateral injection of the same amount of saline (0.9%) as a control during different period of the experiment. Injections were made either immediately before or during the training (at a rate of 3uL/15min). The sides and order of treatment were designed to let different sides of each animal be its own control and the possible transfer effect be counterbalanced between experimental and control groups. Well-trained control animals were also injected with H-7 to test its effect on performance or retention. The effectiveness of the cannula locations were verified with lidocaine or muscimol.

Our preliminary results from 4 rabbits show that rabbits injected with H-7 learned slower than control rabbits. It appears that H-7 may have some long-term effect on this learning. Our results also suggest that H-7 is unlikely to affect the performance or retention of the learned response after the animals are well-trained. These results indicate that protein kinase activity in the IP may be involved in the acquisition of eyeblink conditioning and implicate the IP as a site of plasticity. Supported by NIMH Grant #MH51178 to J.E.S..

645.10

HIPPOCAMPAL LESIONS DO NOT DISRUPT THE LEARNING-DEPENDENT TIMING OF CLASSICALLY CONDITIONED RABBIT EYEBLINK RESPONSES. M.T. Allen * & J.E. Steinmetz. Prog. in Neural Science, Psychology Dept. Indiana University. Bloomington, IN 47405.

Rabbits are able to discriminate between two different tone frequencies that are used as conditioned stimuli (CS) when paired with an air puff unconditioned stimulus (US) at a long or short inter-stimulus interval (ISI) (Mauk & Ruiz, Behavioral Neuroscience. 106. 1992). The timing of CRs in well trained animals is adaptive for maximal eyelid closure immediately prior to US onset for both the long and short ISi intervals. The hippocampus has been implicated in playing a role in the timing of the CR, so we were interested in testing the effects of ibotenic acid hippocampal lesions on the acquisition and retention of these differentially timed CRs. Rabbits in the acquisition groups were given either hippocampal or sham lesions during aseptic surgery prior to training. Rabbits were trained by using two different tone frequencies paired with the US at either a long (747 msec) or short ISI (249 msec) in a standard delay paradigm. Each rabbit received 96 paired presentations of a tone CS and an air puff US and 24 tone alone test trials in daily sessions. On half of the trials, a 249 msec period was allowed between CS and US onsets while on the other half of the trials, a 747 msec period was allowed. Criterion was defined as 75% CRs to both tone/ISI pairings with the mean peak latency within 100 msec of the actual ISI length on tone alone trials. Rabbits in the retention group were given either hippocampal or sham lesions following two sessions of criterion responding. Hippocampal lesions had no detrimental effects on the acquisition or retention of the timing of the long and short latency CRs. Hippocampal lesions did facilitate acquisition and led to larger amplitude CRs. Overall, it appears the hippocampus is not necessary for the precise timing in this differential ISI paradigm. Supported by NIMH grant #MH51178 .

645.11

CONTEXTUAL DISCRIMINATION DURING CLASSICAL EYELID CONDITIONING IN RABBIT. R.F. Rogers*, D.M. Shock & J.E. Steinmetz
Department of Psychology, Indiana University, Bloomington, IN 47401

Contextual discrimination of the conditioned eyeblink response (CR) was investigated. Animals were exposed (80 trials/d) to a single CS (tone; 350 ms) which was either presented alone or coterminated with a corneal airpuff (US; 100 ms). In conjunction with this discrete training, animals experienced two different contextual backgrounds during the intertrial interval and trial presentation. These contexts were defined by either the presence or absence of a diffuse oscillating chamber light. Consequently, for half the animals, paired CS-US trials were signaled by the presence of light and CS alone trials were signaled by darkness. The remaining animals were trained under the opposite contextual contingency.

The results indicate that in rabbits, conditioned eyelid responding to a single CS is subject to contextual modulation. Specifically, early in conditioning, animals developed CRs in both the reinforced and nonreinforced contexts, but with subsequent training, began to respond differentially (72% in reinforced and 27% in nonreinforced) to the CS as a function of the context. In order to further demonstrate contextual control over responding, a subset of animals were exposed to contextual extinction trials in which all trials occurred within their nonreinforced context. With the contextual contingency removed, animals came to respond equally on CS-alone (75% CRs) and paired (72% CRs) trials. Moreover, when the contextual contingency was reestablished, differential responding was again observed. This paradigm has several advantages for neurophysiological investigations into contextual processing during discrete motor learning, most prominent are, 1) the contextual information used by the animal has been restricted to a single sensory modality, and 2) contextual shifts are performed on a trial-by-trial basis, thus facilitating neural recording and allowing a within subject analysis of the data. Supported by NIMH Grant # MH51178 to J.E.S..

645.12

INTERPOSITUS LESIONS ALTER THE TIMING OF, BUT DO NOT ELIMINATE, CONDITIONING-RELATED SINGLE-UNIT ACTIVITY IN RABBIT CEREBELLAR CORTEX. D. B. Katz* & J. E. Steinmetz. Department of Psychology/Program of Neural Science, Indiana University, Bloomington, IN 47405.

The interpositus nucleus (INP) appears to be integrally involved in the production of conditioned eyeblinks in the rabbit. The involvement of the cerebellar cortex (CTX) has been more difficult to ascertain, despite theoretical work anticipating cerebellar cortical coding of motor learning. In the present study, 20 male albino New Zealand rabbits were trained to blink to a 1kHz tone paired with a 3 psi airpuff (the interval between the onsets of stimuli was 250 msec). Following attainment of conditioning criterion, rabbits received either kainic acid or sham lesions of the INP, after which training was resumed. Single Purkinje and granule cells were recorded during post-lesion training.

Lesioned animals failed to produce appreciable numbers of conditioned eyeblinks in post-lesion sessions (sham animals were unimpaired), but did demonstrate "conditioning-related" firing patterns in a subpopulation of cerebellar cortical neurons. Purkinje and granule cells showed patterns of activity that preceded the onset of the UCS by varying amounts. The temporal properties of peri-trial histograms were affected by the INP lesions, however: Complex patterns of activity, sometimes involving two distinct subperiods of excitation in the CS-period, could be discerned in the firing of cortical neurons in lesioned animals. In both lesioned and unlesioned animals, the same neurons that appeared conditioning-related in paired and CS-alone trials often responded phasically to the airpuff in UCS-alone trials.

Results suggest that the CTX supports conditioning-related activity independent of the INP, but that the timing of neural activity in relation to eyeblink activity may be a function of the entire cerebellar system.

Supported by NIH grant #MH51178 to JES.

645.13

PURKINJE CELL RESPONSES IN PONTINE STIMULATION-TRAINED RABBITS ARE SIMILAR TO THOSE FOUND IN TONE TRAINED ANIMALS. J. Tracy* and J.E. Steinmetz. Department of Psychology and Program in Neural Science, Indiana University, Bloomington, IN 47405.

Evidence of cerebellar involvement in eyeblink conditioning includes extracellular unit recordings of Purkinje cells which show learning-related, as well as stimulus evoked, discharge patterns. Responses correlated with conditioned behavior include changes in firing rates that precede the behavior by 20 to 100 ms (Bertheir & Moore, 1986). Peripheral stimuli typically used in this paradigm reach the cerebellum via mossy fibers emanating from the pontine nuclei. As such, pontine stimulation serves as a highly effective CS. Although pontine stimulation supports learning when used as a CS, and earlier studies have shown that unit responses in the cerebellar nuclei show the same patterns of activation as those evoked with a tone stimulus, it has not been established that the same holds true for the cerebellar cortex.

The current study examines the responses of cerebellar cortical cells to short-latency pontine stimulation in trained rabbits. Animals were surgically prepared by implanting bipolar stimulating electrodes in the dorsolateral region of the pons. A microdrive adaptor was implanted over the cerebellum. After a week recovery period, animals were trained to criteria using 25 ms, 200 Hz trains of pontine stimulation as the CS (10-100 uA pulses of 0.1 ms each) paired with airpuff (US).

Single unit recordings of Purkinje cells in trained rabbits reveal patterns of responses very similar to those seen in rabbits trained to a tone CS. Thirty six percent of all recorded cells show a significant change in firing frequency during the CS period, prior to onset of the US. In addition, many very short latency changes in simple spiking patterns are observed which appear to be directly related to mossy fiber activation from the pontine stimulation. Supported by NIMH grant #MH51178 to JES.

645.14

CEREBELLAR INTERPOSED NUCLEUS AND THE CLASSICALLY CONDITIONED AND AVOIDANCE LIMB WITHDRAWAL RESPONSES IN THE CAT K.B. Irwin, J.R. Bloedel, and V. Bracha*. Barrow Neurological Institute, Phoenix, AZ 85 013.

Previously, we demonstrated that inactivation of the cerebellar interposed nucleus (IN) blocks the limb flexion component of the classically conditioned forelimb withdrawal response (FWR). This finding supports suggestions of the critical role of the IN in the organization of learned anticipatory noci-fensive reflexes. To examine the generality of this notion, we tested IN involvement in the FWR in a paradigm which allowed the animal to avoid the unconditioned stimulus (US) by generating the conditioned response. FWRs were first classically conditioned using a cutaneous airpuff as a conditioned stimulus (CS) and electrical stimulation of the forelimb as an US. Then, IN sites critical for classically conditioned FWRs were identified by muscimol microinjections. Subsequently, the same animals were trained in an avoidance paradigm using the same CS and US. Finally, the animals were retrained in the classical conditioning paradigm. Inactivation of sites at which the drug blocked the classically conditioned FWR produced an appreciably smaller effect on the avoidance FWR. Surprisingly, injections at the same sites in cats retrained from avoidance to classical conditioning increased the conditioned responses. It is concluded that the classically conditioned and avoidance FWRs are subserved by different neural networks and that the effects of IN inactivation on classically conditioned responses are dependent on the previous experience of the animals.

NIH Grants NS 30013 and NS 21958.

645.15

CONDITIONED ANTINOCICEPTION IN SPINAL RATS: ASSOCIATIVE LEARNING VERSUS PROTECTION FROM HABITUATION. R.L. Joynes* & J.W. Grau. Dept. of Psychology, Texas A&M University, College Station, TX 77843.

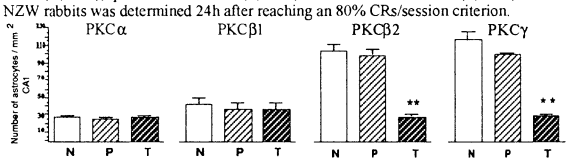
Conditioned antinociception can be demonstrated in spinalized rats by pairing stimulation (10-s, 1-mA shock) to one hind leg (the conditioned stimulus [CS]) with an intense (2-s, 3-mA) tailshock (the unconditioned stimulus [US]). After 30 CS-US pairings, tail-flick latencies are assessed during the CS. Relative to a CS that was presented in an unpaired fashion (the CS-), rats exhibit longer tail-flick latencies during the paired CS (the CS+) (Grau et al., *Behav. Neurosci., 104, 489*, 1990). The present study explores the mechanisms that underlie this learning.

Prior work has shown that our CSs elicit some antinociception prior to training and that presenting the CS alone attenuates (habituates) its antinociceptive impact. But when the CS is paired with the US, no habituation is observed. Does the US simply "protect" the CS from habituation, or does it support a simple form of associative learning? These two alternatives can be distiguished by varying the intertrial interval (ITI) and the number of training trials. If associative learning is involved, increasing the ITI and the number of CS-US pairings should strengthen the CS+/CS- difference, while a habituation account anticipates the opposite; increasing the ITI should reduce the CS+/CS- difference by decreasing habituation to the CS-, whereas increasing the number of CS-US pairngs should reduce it by facilitating habituation to the CS+. Our results indicate that protection from habituation underlies learning in our spinal paradigm. Supported by MH48994 to J.W.G.

646.1

DIFFERENTIAL CHANGES IN PKC-IMMUNOREACTIVITY IN ASTROCYTES AND NEURONS IN RABBIT CA1 AFTER ASSOCIATIVE LEARNING. E.A. Van der Zee*, M.A. Kronforst-Collins, and J.F. Disterhoft. CM Biol., Northwestern Univ. Med. Sch., 303 E. Chicago Ave., Chicago, IL 60611, USA

Protein kinase C (PKC) modulates neuronal activity in the hippocampus in relation to learning and memory, partly through the phosphorylation of K^+ channels. PKC is expressed in both neurons and astrocytes. A two-fold trace eyeblink conditioning-induced increase in the immunoreactivity for PKCγ, but not -α, -β1, or -β2, was found in principal neurons of the dentate gyrus, CA3 and CA1 (but not the subiculum) of the rabbit hippocampus (Van der Zee et al., 1995, Soc. Neurosci. Abstr. 21:1218). Here the number of PKC-immunoreactive astrocytes in the subiculum and CA1 of naive (N, n=7), pseudoconditioned (P, n=7) and trace conditioned (T, n=7) female NZW rabbits was determined 24h after reaching an 80% CRs/session criterion.



PKCα, -β1, -β2, and -γ are differentially expressed in astrocytes. A significant decrease in the number of PKCβ2 and -γ-positive astrocytes was found in the CA1 region, whereas no differences were found for PKCα or -β1. No alterations occurred in the subiculum. These results suggest a selective down-regulation of PKCβ2 and -γ in astrocytes in a brain region we have shown to be involved in associative learning. We speculate that the down-regulation of glial PKC relates to the K^+ buffering function of astrocytes. Down-regulation of PKC should reduce the phosphorylation of K^+ channels and enhance the efficacy of K^+ buffering in astrocytes of conditioned over naive and pseudoconditioned animals. (Supported by NIH RO1 MH47340 and AG08796)

646.2

DISCRIMINATIVE HIPPOCAMPAL NEURAL RESPONSES TO SPATIAL CUES IN RABBIT CLASSICAL CONDITIONING. M.A. Seager*, R.L. Borgnis, A.A. Kendall & S.D. Berry. Center for Neuroscience and Department of Psychology, Miami Univ., Oxford, OH 45056.

The predominant theory of hippocampal function in the rodent has been that this structure creates a cognitive representation or "map" of the animal's environment. Lesions to the hippocampus or its connections typically result in spatial learning and memory deficits. Another very different line of research has investigated the role of the hippocampus during classical conditioning in the rabbit. Pyramidal cells in this structure become highly engaged during learning and performance of NM/eyelid and jaw movement conditioned responses. Additionally, lesions to this structure produce behavioral deficits (e.g., disruptions in the timing and form of responses) during a subset of conditioning tasks.

It was our goal to bring these two disparate lines of research together. To accomplish this, we developed a spatial classical conditioning task. Rabbits were trained using a modified delay NM paradigm which incorporates a spatial dimension. A click train CS varied in location (either 45 degrees to the left or to the right of the animal). The click train coming from only one of the spatial locations was followed by a 100 ms air puff.

Hippocampal unit data was analyzed by averaging spike counts in the PRE-CS, CS, and US periods across trials in a session. These hippocampal unit averages revealed a clear discriminative response between the CS+ and the CS- trials, whether or not the animal was discriminating behaviorally.

646.3

HIPPOCAMPAL LESIONS IMPAIR SPATIAL LEARNING, BUT NOT REINSTATEMENT OF EXTINGUISHED APPETITIVE PAVLOVIAN CONDITIONING IN RATS. G.D. Fox[1], M. Gallagher[1], and M.E. Stanton[1]*[1], P.C. Holland[2]. Dept. Of Psychology, Univ. North Carolina, Chapel Hill, NC; [2]Dept. Psychology, Duke Univ., Durham, NC.

Male, Long-Evans rats with ibotenic acid lesions of the hippocampus and non-lesioned control rats were conditioned in a Pavlovian appetitive procedure in which a visual stimulus was paired with food. Conditioned responding was then extinguished. After extinction, one group of lesioned and one group of non-lesioned animals were given presentations of the food US in the original conditioning chambers. The remaining animals were placed in the chambers but did not receive US presentations. Finally, all rats received a test of conditioned responding to the light CS alone. Rats that received the post-extinction US presentations showed higher levels of conditioned responding than the rats that had only been placed in the chambers. This reinstatement of conditioned responding to the light CS was not effected by hippocampal lesions. A sample of lesioned animals from this study did, however, demonstrate impaired performance in a water-maze task known to be sensitive to hippocampal damage. These data are in contrast to those of Wilson, Brooks, and Bouton (Behav. Neurosci., 109, 828-836), who have demonstrated that reinstatement of aversively conditioned behavior is abolished with lesions of the fornix.

(This research was supported by NIMH grants 14277 and MH-53667, and by Human Frontier Science Program Grant RG-12/93B)

646.4

A ROLE FOR THE DORSOLATERAL STRIATUM IN EXPRESSION OF CONDITIONED ORIENTING RESPONSES. J. S. Han[1]*, R. W. McMahan[1], P. C. Holland[2], and M. Gallagher[1]. [1]Dept. of Psychology, Univ. of North Carolina, Chapel Hill, NC 27599, [2]Dept. of Psychology, Duke University, Durham, NC 27708.

Prior studies have shown that neurotoxic lesions of the amygdala central nucleus (CN) impair conditioned orienting responses (OR) that are acquired with conditioned stimulus (CS)-unconditioned stimulus (US) pairings. The integrity of an amygdalo-nigrostriatal pathway is critical for the development of conditioned ORs; aasymmetrical lesions of CN and dorsolateral striatum (STR), but not unilateral lesions of each of these structures, abolish conditioned ORs. Here we used a reversible inactivation of the STR combined with a contralateral CN ibotenic acid lesion to test whether the STR is necessary for the acquisition or only for the expression of conditioned ORs. Each session was comprised of 4 trials, consisting of a 10-sec light (CS) followed by 2 food pellets. The STR was inactivated with a lidocaine (1 µl) infusion before each session for 8 consecutive days, but then no infusions were made for 6 subsequent daily sessions. Rats in the asymmetrical group (ibotenic acid lesion of CN with STR inactivation contralaterally) failed to exhibit conditioned ORs during the inactivation sessions. However, these ORs emerged during the first session without inactivation and did not increase with subsequent training. Rats in the symmetrical group (ibotenic acid lesion of CN with STR inactivation ipsilaterally) showed normal conditioned ORs. These results show that the STR is critical for expression of a conditioned OR. Supported by an award from the Human Frontier Science Research Program, a Research Scientist Award (K05-MH01149) and MH53667 to MG.

646.5

PRETRAINING LESIONS OF THE INTERMEDIATE MEDIAL HYPERSTRIATUM VENTRALE IMPAIR SICKNESS-CONDITIONED LEARNING IN DAY-OLD CHICKS. T. A. Barber*, P. D. Howorth, and C.C. Cho. Department of Psychology, Dickinson College, Carlisle, PA 17013-2896.

The intermediate medial hyperstriatum ventrale (IMHV) is necessary for learning a one-trial passive avoidance task (Patterson, Gilbert, & Rose, 1990; Patterson & Rose, 1992). We examined the effects of pretraining IMHV lesions in one-trial sickness-conditioned learning, which is similar to passive avoidance since the chick must remember visual cues in order to respond correctly. However, the two tasks differ in type of aversive stimulus (sickness vs. bitter taste) and CS-US separation (30 min delay vs. none).

Day-old chicks were given either bilateral, unilateral or sham lesions of the IMHV. At training, chicks pecked a dry 3 mm chrome bead and 30 min later were injected (IP) with either saline or lithium chloride (LiCl; 0.1 ml 1.0 M). Chicks were tested four hours after training by presentation of a 3 mm chrome bead. Chicks that pecked the bead after receiving LiCl were considered amnesic for the association.

Bilateral IMHV lesions impaired sickness-conditioned learning, similar to results found for passive avoidance. Unilateral left or right IMHV lesions also impaired sickness-conditioned learning, suggesting that memory formation for this task is dependent on intact functioning of both left and right IMHV. These results are in contrast to those for passive avoidance in which only unilateral left IMHV lesions produced amnesia.

The IMHV appears to play a general role in memory formation in the chick; differences in lateralization may be related to the specific paradigm under study.

646.6

EFFECTS OF IBOTENIC ACID INDUCED LESIONS ON MEMORY FOR A VISUAL DISCRIMINATION TASK. R. A. Deyo*, P. A. Baukol, L. J. Edelbach, T. J. Finne and G. E. McKenney. Department of Psychology, Winona State Univ., Winona, MN 55987.

The pebble floor visual discrimination task is an economical method of screening nootropics in chicks that generalizes very well to other animal models, and more importantly to effects on human memory (e.g., Deyo & Hittner, 1995; *Neurobiol. Learn. Mem.*, **64**, 10-16). However, there still is very little known about which areas of the chick brain learn and remember visual discriminations. Thus, the present experiment was designed to study the localization of visual discrimination memory in the chick brain.

Five-day-old male chicks received 100 acquisition trials on the "pebble floor" visual discrimination task. Immediately after the last trial, chicks received 1 µL injections of 0, or 1 µg/µL ibotenic acid. All drugs were injected bilaterally within the intermediate hyperstriatum ventrale (IMHV) using a 10 µL Hamilton microsyringe that was fitted with PE tubing to insure an injection depth of 4 mm. The IMHV was selected on the basis of previous studies of avoidance learning in chicks that suggest the IMHV is required for learning and memory (e.g., Freeman, et al., 1995; *Neurobiol. Learn. Mem.* **63**, 291-295). Forty-eight hours after treatment, chicks received 20 retention trials. After the last trial, each brain was removed and fixed in a buffered formalin solution for at least two weeks. Histological analyses were completed following cresyl violet staining for cell bodies.

Ibotenic acid treated chicks made significantly more errors 48 hr after treatment. Analyses of the histology revealed significant cell loss in the IMHV. These data suggest that retention of the visual discrimination task is dependent upon the integrity of the IMHV.

646.7

LESIONS OF THE MEDIAL PREFRONTAL CORTEX AND APPETITIVE JAW MOVEMENT CONDITIONING IN THE RABBIT. Joselyn McLaughlin* & D.A. Powell. VA Medical Center and University of South Carolina, Columbia, SC 29209.

Sham and medial prefrontal (mPFC) lesioned rabbits received classical conditioning in which a tone served as the conditional stimulus (CS) and an intraoral 1 cc pulse of sugar water was the unconditional stimulus (US). Conditioned jaw movement and heart rate changes were concomitantly recorded. A non-associative sham control group received explicitly unpaired tone and sugar water presentations. mPFC lesions had no effect on jaw movement conditioning, but abolished the early-occurring decelerative heart rate changes obtained in sham-lesioned animals. Later-occurring increases in heart rate, associated with asymptotic jaw movement responding were, however, augmented by prefrontal lesions. These results are comparable to those obtained in a previous study, indicating that the early-occurring decelerative conditioned HR changes during appetitive classical conditioning are attenuated by mPFC lesions but the later occurring increases in heart rate are augmented. Thus both components of the HR CR are affected by mPFC lesions.

Supported by DVA Institutional Research funds

646.8

FUNCTIONAL CONNECTIVITY BETWEEN FRONTAL CORTICAL NEURONS DURING REVERSAL CONDITIONING IN THE DOG. A. Kozlov*, V. Shabaev, Pavlov's Physiol. Dep., Inst. Exp. Med., St.Petersburg, 197376, Russia

We studied spatiotemporal patterns of correlations between simultaneously recorded spike trains in the frontal cortical areas of the dog which performed a task of reversal conditioning. Using developed in our lab approach for the correlation analysis of neuronal firing we revealed that functional connectivity between cortical cells was organized in the "interspike interval-specific" manner. This type of temporal relations between spike occurrences in two impulse trains was expressed as a different probability of the spike generation by one unit to the interspike intervals (ISI) of various durations of another neuron. It was found that parameters of ISI-dependent correlations (it's "strength", duration of effective ISI) have varied among situations with different types of behavioral responding (rewarded vs. unrewarded conditioned reactions, maintenance set vs. behavioral set shifts). These findings indicate that reorganization of stimulus-response associations is accompanied by a shift in a tuning characteristics of neuronal networks as it was expressed in dynamic of the ISI-specific interneuronal connectivity.

646.9

EFFECTS OF BLOCKING THE PAVLOVIAN CONDITIONING OF TONES ON THE RAT AUDITORY SYSTEM MAPPED WITH CYTOCHROME OXIDASE HISTOCHEMISTRY. A. Poremba*, F. Gonzalez-Lima, and D. Jones, Dept. of Psych. and Inst. for Neurosci., Univ. of Texas, Austin, TX, 78712

The Kamin blocking phenomenon occurs when behavioral conditioning to a novel stimulus fails in the presence of a previously conditioned stimulus. Quantitative histochemistry of cytochrome oxidase (C.O.), a mitochondrial enzyme of oxidative metabolism, was used to evaluate cumulative changes in metabolic capacity resulting from the entire training experience of Pavlovian conditioned suppression of drinking. Rats in Group 1 (Blocking) received pairings of a light conditional stimulus (CS; flashing at 10 Hz) with a mild footshock to elicit conditioned suppression of drinking during Phase 1 training. Rats in Group 2 (Excitor) received random presentations of the light CS and shock stimuli. Both groups then received the same Phase 2 training consisting of a tone CS (1 - 2 KHz FM) and the light CS presented simultaneously, paired with footshock. The Excitor Group exhibited significant suppression of drinking ($p < .05$) to tone only presentations after training and as expected, the Blocking Group did not. Metabolic mapping results for the auditory system demonstrate significantly elevated levels of C.O. activity in the central nucleus of the inferior colliculus and the dorsal cochlear nucleus for the Blocking Group versus the Excitor Group, specifically in the region of the CS tonotopic representation ($p<.05$). These findings suggest that 1) distinct neural changes in the response to the tone CS can be attributed to the blocking phenomenon and 2) specific changes in C.O. activity can be linked to behavior. (Supported by NIH grant RO1 MH4333).

646.10

BRAIN IMAGING OF EXCITOR VS BLOCKED TONES: CONDITIONING EFFECTS ON AUDITORY PROCESSING OF IDENTICAL STIMULI. D. Jones* & F. Gonzalez-Lima, [1] Dept. of Psych., Univ. of Texas, Austin TX, 78712 USA.

Neural processing of a tone conditioned as an excitor was compared to processing of the same physical tone when excitatory conditioning was blocked by previous experience with a light. Fluorodeoxyglucose (FDG) autoradiography was used to assess changes in the auditory processing of the conditioned tone. Auditory regions (18) were compared in Long-Evans rats trained under a typical 3-phase blocking protocol (Kamin Blocking Effect). A conditioned suppression protocol was used. In phase I, Group Blocked received presentations of light (L) paired with footshock (US) and Group Excitor received unpaired presentations. Phase II training was identical for both groups, consisting of compound tone/light (TL) presentations followed by the US. The pairing of L-US in phase I results in decreased conditioning to T in phase II. Optical density of FDG uptake during exposure to T alone was measured. Significant group differences in 7 auditory regions were revealed using z-scores transformations. Changes in the patterns of intercorrelations of the auditory regions were also seen. Findings suggest that neuronal encoding of a stimulus changes the activity interrelations of auditory regions when the stimulus has acquired different meanings. To our knowledge, this is the first demonstration of changes in the patterns of auditory system processing of a tone due to excitatory vs blocked conditioning. (Supported by RO1 MH43353 and NSF grant IBN 9222075.)

646.11

CLASSICAL CONDITIONING RESULTS IN DENDRITIC EXCITABILITY AND OCCLUDES LONG-TERM DEPRESSION. B. G. Schreurs*, D. Tomsic, P. Gusev and D. L. Alkon. LAS, NINDS, NIH Bethesda, MD 20892.

Intradendritic recordings in Purkinje cells (n=89) from parasaggital slices of cerebellar lobule HVI obtained from rabbits given paired presentations of tone and periorbital electrical stimulation resulted in increased membrane and synaptic excitability relative to cells (n=63) from animals given explicitly unpaired stimulus presentations ($p's < .01$). The location of cells with low thresholds for local dendritic calcium spikes suggested that there are specific sites within lobule HVI where learning-related changes take place. These areas may correspond to learning "microzones" and are consistent with the locations of some learning-related changes in Purkinje cell activity recorded in vivo. Application of 4-aminopyridine, an antagonist of the potassium inactivating current I_A, reduced the threshold for dendritic spikes in slices from naive animals to levels found in slices from trained animals ($p < .01$). Following 20 pairings of a brief, high frequency train of parallel fiber stimulation (8 pulses, 100 Hz) with a brief, lower frequency train of climbing fiber stimulation (3 pulses, 20 Hz), Purkinje cell excitatory post synaptic potentials (EPSPs) underwent a long-term (> 20 min) reduction in peak amplitude (-24%) in cells (n=12) from animals given in vivo unpaired stimulus presentations but to a far less extent (-9%) in cells (n=20) from animals given in vivo paired training ($p <.01$). In fact, whereas 92% of cells from unpaired animals showed pairing-specific depression, 50% of cells from paired animals showed no depression and in several cases actually showed potentiation. The threshold level of stimulation required to elicit the 6-8 mV EPSP as well as a 4-mV EPSP (n=30) and a Purkinje cell spike (n=56) was found to be significantly lower in slices from animals given paired training than those given unpaired stimulus presentations. It is suggested that this enhanced synaptic excitability and not a learning-induced synaptic depression occludes pairing-specific long-term depression.

646.12

SENSITIZATION OF THE EYEBLINK RESPONSE IN THE RESTRAINED MOUSE.

M.A. Kronforst-Collins*, C. Weiss and J.F. Disterhoft, CM Biology, Northwestern University Med. Sch., 303 E. Chicago Ave., Chicago, IL 60611 USA.

Eyeblink conditioning is a popular paradigm used in the investigation of the neural substrates of learning and memory. With the recent increase of genetic manipulation capabilities, the mouse has become an appealing experimental animal for use in the study of learning and memory. A total of 60 mice were used to adapt the restrained rabbit eyeblink conditioning paradigm to the mouse. Balb/c, B6C3F1 and C57BL/6J mice were used to compare the effects of strain differences on eyeblink conditioning. Prior to training, a 2-56 x 5/8" machine screw was implanted on the skull with skull screws and dental acrylic to serve as a headbolt. During training subjects were restrained in plastic tubes with a stock over the neck to secure the body, and the headbolt was used to secure the head. Subjects were trained in either the Delay 250 or Trace 500 paradigms, or were used as pseudoconditioned controls. Subjects were trained in pairs in a sound-attenuated chamber. The conditioned stimulus (CS) consisted of an open-field 350msec (Delay 250) or 100msec (Trace 500) tone at 1, 6 or 10kHz and 70 - 90dB. The unconditioned stimulus (US) was a 150msec, 1.3psi airpuff directed at the cornea. Conditioned responses (CRs) were measured by an infrared reflective sensor placed in front of the eye. Conditioned subjects received 40 paired trials per day for 5 - 15 days. Pseudoconditioned subjects received 80 explicitly unpaired trials per day for an equal number of days as their conditioned cohort. The CS was manipulated between sets of mice in an attempt to maximize conditioning and minimize sensitization. Regardless of the manipulation, comparable levels of responding, as measured by percent CRs and area of the CR, were observed in both conditioned and pseudoconditioned subjects. Our data demonstrate the high level of sensitization that may result with a tone CS and the importance of pseudoconditioned controls in differentiating associative CRs from sensitized responses in the mouse eyeblink conditioning preparation. (Supported by NIH AG08796, NIH F32MH10837 and Alzheimer's Assoc. PRG94054)

646.13

Eyeblink Conditioning in the Freely Moving Mouse C. Weiss,* M.A. Kronforst-Collins, and J.F. Disterhoft. Dept. of Cell and Molecular Biology, Northwestern University Medical School, Chicago, IL. 60611

Eyeblink conditioning is a powerful paradigm for examining the neuronal mechanisms of learning and memory. Our current series of experiments uses the mouse to take advantage of recent genetic advances and other benefits related to long- term aging studies, i.e., smaller size, shorter life span and lower expenses.

Male C57BL/6 mice were anesthetized with ketamine and xylazine for the surgical implantation of eyelid EMG recording wires and a skull mounted connector which served to couple the light conditioning stimulus (CS), airpuff unconditioned stimulus (US) and EMG recording leads. Mice were randomly assigned to either a conditioning group which received paired presentations of the CS and US, or to a control group, which received explicitly unpaired presentations of the stimuli. The CS (a 350ms flash from an LED) and US (a 100ms puff of air) were presented in a delay paradigm, and the conditioned and unconditioned responses were analyzed from the rectified and integrated EMG activity.

The results indicate that the visual CS minimized sensitization and maximized associative learning. Mice which received paired presentations of the stimuli had a greater percentage of trials with conditioned responses than mice which received unpaired presentations of light and airpuff. The responses from conditioned mice were also greater in amplitude and area of response than the responses from mice which received unpaired presentations of the CS and US.

This experiment indicates that eyeblink conditioning in the freely moving mouse is a useful model system for the genetic analysis of age-related learning and memory impairments. Learning in this paradigm may also be correlated with learning in other tasks, e.g., spatial mazes, and with physiological and pharmacological data to obtain a more complete understanding of learning mechanisms. This combination of tasks is practical to do in the mouse, and should help to fill a void in the behavioral database that is developing as the genetic and molecular effects of age-related diseases, e.g., Alzheimer's disease, become more well known. Support: NIH AG08796, NIH F32MH10837 and Alzheimer's Assoc. PRG94054.

646.14

EFFECTS OF TRACE INTERVAL ON THE LEARNING OF CONDITIONED FEAR IN A CO2 PAVLOVIAN CONDITIONING PARADIGM: CONDITIONING FOLLOWING A ONE HOUR TRACE INTERVAL. D. L. Mongeluzi*, B.C. Caldarone, H. S. Stock, & R. A. Rosellini. The University At Albany, State University of New York, Department of Psychology, Albany, NY 12222.

Previous studies in our laboratory have shown that 30 s exposure to 100% CO2 can serve as an effective US in a Pavlovian conditioning paradigm. The present study began to determine the effective interval for trace conditioning with the CO2 preparation (i.e., either 0, immediate, 5, 15, 30, or 60 min). Results indicated that the shorter the trace interval during CO2 exposure, the higher was the level of conditioning as represented by conditioned freezing and tail-flick latencies. Trace intervals of immediate, 5, 15, 30, and 60 min produced elevated levels of freezing twenty-four hours following conditioning relative to controls. Moreover, there existed a positive monotonic relationship between trace interval and resistance to extinction as measured by freezing, ranging from relatively little resistance to extinction with a 30 and 60 min trace interval to a great deal of resistance with an immediate and 5 min trace interval. This positive and monotonic relationship between trace interval and level of conditioning to the context was also evident on the tail-flick measure. Immediate and five minute trace intervals resulted in the highest elevation of tail-flick latencies, a 15 min trace interval resulted in intermediate tail-flick latencies, and 0, 30, and 60 min trace intervals resulted in latencies equivalent to baseline. Supported in part by a SUNY Graduate Student Organization grant to DLM.

646.15

WHERE NO PAVLOVIAN LEARNING PARADIGM HAS GONE BEFORE. EYEBLINK CONDITIONING IN SPACE. R. J. Servatius [*1], T. J. Shors [2], R. Peterson [3], C. Amberboy [3], D. Grounds [3] & W. N. Tapp [1]. [1] Dept. Of Neuroscience, New Jersey Medical School, [2] Dept. Of Psychology, Princeton University, [3] Johnson Space Center, Houston, Texas.

Space travel remains a perilous adventure. The mental stresses of spaceflight are compounded by prolonged exposure to a microgravity environment. Our challenge for Neurolab, the shuttle mission dedicated to Neuroscience, is to measure nonassociative and associative learning in astronauts in terms of visceral and motoric responses. We have developed a compact, portable stimulus generation box (SGB) to accomplish these tasks. The SGB has an air pump (DC 12/16 NK Furgut Industries, distributed by MRM International) which fills an air filter resevoir (type 9933-11-BX filter tube, Balston Filter Products) through a check valve (#302PPb-3", Smart Products, Inc.). A solenoid valve with 0.089" diameter orfice (CS-1744, Clippard Industrial Laboratory, Inc) controls air puff outflow. A quick disconnect is the interface between the SGB and a headset (Model H10-56, David Clark Co.). The microphone armature on the headset has been adapted to deliver the airpuff. The SGB also has the capability to produce acoustic stimuli, white noise for tests of sensory reactivity (105, 90, 75 dB) and pure tones for two-tone discrimination (1500 and 800 Hz tones at 90 dB). To complete the system, electromyograph, electrocardiograph and respiration signals will be amplified and passed to a PCMCIA A/D card (DAQ 700, National Instruments). An IBM 755C notebook computer running Labview software (National Instruments) will control the delivery of stimuli and the acquisition of the physiological signals. We will present schematics for this equipment as well as a working model. This NASA-designed equipment is amenable for use in nontraditional experimental venues. Supported through NASA contracts to TJS and RJS.

646.16

A COMPARISON OF DELAY AND TRACE PAVLOVIAN EYEBLINK CONDITIONING IN DEVELOPING RATS. D. Ivkovich*[1] and M.E. Stanton[2], [1]Duke University, Durham, NC 27708-0086 & [2]U.S. EPA, RTP, NC 27711.

Classical eyeblink conditioning (EBC) may be a useful model system for studying the neural basis of learning and its disorders during ontogeny. In adult rabbits, acquisition of delay EBC, in which the CS and US overlap and coterminate, is dependent solely on cerebellar-brainstem circuitry. In contrast, trace conditioning, in which a temporal gap separates the CS and US, additionally involves the hippocampus. In the rat, both cerebellum and hippocampus are known to continue to develop postnatally, and acquisition of delay EBC develops between postnatal day 17 (PND17) and PND24 (Stanton, Freeman, & Skelton, 1992, *Behav. Neurosci.* 106, 657-665). The present study compares development of acquisition of delay and trace conditioning in PND23 and PND30 rats.

In standard delay conditioning, rat pups experienced a 380 msec, 2.8 kHz, 90 dB tone CS and 100 msec, 2 mA periocular shock US (Stanton et al., *ibid*). Trace conditioning consisted of the same tone and shock stimuli separated by a 500 msec trace interval. Animals were given 6 sessions of training, three 100-trial sessions/ day, over two days. Percent CRs increased significantly across the 6 sessions for all subjects with no significant effect of age or conditioning paradigm. For CR amplitude, responses were greater in delay than trace conditioning ($p<.01$) but did not differ significantly across age (although in trace conditioning responses tended to be greater for PND30 than PND23 rats). UR amplitude did not differ between training groups or age groups. These findings suggest that by PND23, rats have sufficiently developed cerebellar and hippocampal circuitry to support both delay and trace conditioning. SUPPORTED BY U.S. EPA

GENETIC MODELS: NATURAL MUTANTS

647.1

A PANEL OF CONGENIC MOUSE STRAINS FOR SYSTEMATIC, CONTROLLED STUDY OF A COMPLEX GENETIC TRAIT, EPILEPSY. M. E. Legare, W.N. Frankel*, The Jackson Laboratory, Bar Harbor, ME

In the EL/Suz and SWXL-4 mouse strains, several unidentified susceptibility genes produce recurrent, tonic-clonic seizures that resemble the most common human epilepsies. Quantitative trait locus (QTL) mapping in various intercrosses and backcrosses of EL with ABP (a low-seizure strain) have identifed four seizure frequency QTL on three different chromosomes. QTL mapping of SWXL-4 with ABP has identified at least two seizure frequency QTL on two different chromosomes. Major conclusions from these studies were that no single mapped QTL is necessary and sufficient for frequent seizures, and that the effects of some loci were unusually dependent on very specific allelic combinations.

We are simplifying the models by breeding high seizure alleles from EL or SWXL-4 onto an ABP background, using DNA flanking-marker selection. For El genes, introgression of *El1* (Chr 9), *El2* (Chr 2) or *El3* (Chr 10) led to three ABP.EL congenic strains, and one compound congenic (*El1*, *El4* plus *El2*). In homozygotes, significant trait differences were found so far: EL strain (0.72 seizure frequency) > *El1*,*El2*,*El4* (0.50), *El2* (0.35) > *El1*,*El4* (0.22) > *El3*, ABP strain (0.09). Crosses of *El1*,*El2*,*El4* and *El2* congenics are currently underway to determine whether the seizure frequency increase in the compound congenic maps to *El1* or *El4*. Testing of early generations of similar ABP.SWXL-4 congenics for *Szf1* (Chr 7) and *Szf2* (Chr 16) suggests that at least *Szf1* homozygotes will have a seizure frequency of ~0.30.

This panel of congenic strains with identical genetic backgrounds will offer a high level of control for multiple goals, including examination of genes that regulate or respond to introgressed seizure frequency loci, and better mapping (towards cloning) seizure frequency genes themselves. To this end, meiotic recombinants from within the *El2* interval are being tested. So far, with seizure frequencies of ~0.20, the distal half of the segment explains only part of the full congenic phenotype - suggesting that *El2* may itself be encoded by more than one gene.

(Supported by grant NS31348 and a Klingenstein Fellowship to WNF.)

647.2

ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL ANALYSIS OF DRUG- AND CONVULSION-NAIVE WSP AND WSR MICE. J.F. Buckman* and C.K. Meshul. Oregon Health Sciences Univ., Dept. of Behavioral Neuroscience and the V. A. Medical Center, Portland, OR 97201.

Withdrawal Seizure Prone (WSP) and Withdrawal Seizure Resistant (WSR) mice were selectively bred in replicate for differential ethanol withdrawal susceptibility, as measured by handling-induced convulsions (HICs). However, it has been established that some WSP mice exhibit HICs prior to drug exposure. This then raises the possibility that the selection process resulted in differences in general susceptibility to convulsions in addition to differential ethanol withdrawal responses. The activity of the glutamatergic system is thought to influence susceptibility to both ethanol withdrawal symptoms and seizure-related disorders. WSP mice exhibit a genetic propensity for both of these states suggesting that alterations in glutamate functioning may exist. Furthermore, a strong foundation for specific hippocampal involvement in seizure-related disorders has been established. Quantitative ultra-structural immunocytochemistry is useful in determining, at the synaptic level, the density of neurotransmitters within nerve terminals. Using the CA1 region of the hippocampus (CA1) and layer II of the somatosensory cortex (SSC), we determined that the density of intracellular glutamate immunoreactivity in the CA1, but not in the SSC, was significantly higher in both replicate lines of WSP mice when compared to WSR mice. These results suggest that WSP mice, as compared to WSR mice, may exhibit an increased susceptibility to convulsions due to an inherently higher level of glutamate in brain regions associated with seizure activity.

This study was supported by the Dept. of Veterans Affairs, National Institute on Alcohol Abuse grant 5T32AA07468 and a Tartar Trust fellowship.